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Metabolic Messengers: FGF21

Kyle H. Flippo[1,2,3], **Matthew J. Potthoff**[1,2,3,4,✉]

[1]Department of Neuroscience and Pharmacology, University of Iowa Carver College of Medicine, Iowa City, IA, USA.

[2]Fraternal Order of Eagles Diabetes Research Center, University of Iowa Carver College of Medicine, Iowa City, IA, USA.

[3]Iowa Neurosciences Institute, University of Iowa Carver College of Medicine, Iowa City, IA, USA.

[4]Department of Veterans Affairs Medical Center, Iowa City, IA, USA.

Abstract

As a non-canonical fibroblast growth factor, fibroblast growth factor 21 (FGF21) functions as an endocrine hormone that signals to distinct targets throughout the body. Interest in therapeutic applications for FGF21 was initially sparked by its ability to correct metabolic dysfunction and decrease body weight associated with diabetes and obesity. More recently, new functions for FGF21 signalling have emerged, thus indicating that FGF21 is a dynamic molecule capable of regulating macronutrient preference and energy balance. Here, we highlight the major physiological and pharmacological effects of FGF21 related to nutrient and energy homeostasis and summarize current knowledge regarding FGF21's pharmacodynamic properties. In addition, we provide new perspectives and highlight critical unanswered questions surrounding this unique metabolic messenger.

Fibroblast growth factors (FGFs) have various structures, and their functions can broadly be described as paracrine, autocrine or endocrine[1]. The FGF19 subfamily, which consists of FGF15/19, FGF21 and FGF23, comprises endocrine factors that are able to leave their tissue of origin because of a diminished affinity for heparan sulfate glyocosaminoglycans[1–3].

FGF21 was first characterized as a novel FGF expressed in the liver. It shares ~75% amino acid sequence identity between mice and humans[4] (Fig. 1). Although *Fgf21* mRNA can be detected in numerous tissues including the liver, pancreas, muscle and adipose tissues[4,5], circulating levels of FGF21 are now known to be derived primarily, if not exclusively, from the liver under physiological conditions in both rodents[6] and humans[7]. Although non-hepatic tissues, such as white and brown adipose tissues, may make negligible contributions to the circulating levels of FGF21, FGF21 derived from those tissues may

✉**Additional information Correspondence and requests for materials** should be addressed to M.J.P. matthew-potthoff@uiowa.edu.

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still have important autocrine or paracrine roles[8–10]. Beyond its physiological production, FGF21 is also produced by non-hepatic tissues in non-physiological genetic models of altered mitochondrial function[11–16], and circulating levels of FGF21 have been proposed to be a biomarker of mitochondrial disease in humans[17–19]. However, the relevance of elevated FGF21 to this disease state is unclear.

In many tissues, including the liver, FGF21 levels are basally low and are markedly induced by a myriad of nutritional and cellular stress signals. For example, FGF21 was initially proposed to be a late-fasting/starvation factor, because it is transcriptionally induced during extended fasting, through a mechanism dependent on the transcription factor PPARα, and it regulates the adaptive fasting response[20,21]. However, FGF21 is also subject to transcriptional regulation mediated by nutritionally sensitive transcription factors. Hepatic mRNA and plasma protein levels of FGF21 are induced by high sugar intake through a mechanism dependent on the transcription factor ChREBP[22–25] and protein restriction through pathways dependent on the transcription factors ATF4 and NRF[26,27]. FGF21 is maximally induced under a nutritional state of a combined high-sugar/low-protein condition[28]. Interestingly, the induction of FGF21 by both carbohydrate[25] and protein[26] requires PPARα in addition to the transcriptional mechanisms described above. However, although lipid consumption has been proposed to increase plasma FGF21 levels[21,29], a thorough analysis of macronutrient intake has revealed that fat intake does not significantly alter FGF21 expression in rodents[28]. The importance of these transcriptional mechanisms in the induction of FGF21 is exemplified by their conservation in humans. Starvation[30,31], high sugar intake[32,33] and dietary protein restriction[26] all induce circulating FGF21 levels in humans. In contrast, ketogenic diets do not induce plasma FGF21 levels in humans[26,30,34], in agreement with the findings of more comprehensive macronutrient studies in mice[28]. Obesogenic diets, which are termed high-fat diets but are actually high-fat/high-carbohydrate diets, also significantly elevate plasma FGF21 levels[35]. The induction of FGF21 by obesogenic diets may be caused by numerous factors, including macronutrient levels, organelle stress including endoplasmic reticulum stress and mitochondrial stress[36], and/or FGF21 resistance[37,38]. The diversity in the signals regulating FGF21 expression has made generating a unifying physiological model for FGF21 function complicated.

Although transcriptional regulation of FGF21 expression is the primary mode of FGF21 production, plasma FGF21 levels may also be regulated at the level of secretion. YIPF6 is a membrane receptor that is associated with secretory vesicles and has been shown to interact with FGF21 in the endoplasmic reticulum, thus limiting its secretion[39]. YIPF6 also specifies packaging of FGF21 into coat-protein complex II vesicles[39]. Once secreted, FGF21 has a short half-life of approximately 0.5–1.5 hours (refs. [40,41]). FGF21 is also subject to proteolytic cleavage by the serine proteases fibroblast activation protein (FAP) and dipeptidyl peptidase IV (DPP-IV)[42,43]. DPP-IV is responsible for the cleavage of human FGF21 on the amino terminus at residues 2 and 4, and FAP is responsible for the cleavage of FGF21 at residue 171 (refs. [42,43]). While cleavage at residues 2 and 4 does not significantly impair FGF21 function, cleavage at residue 171 inactivates FGF21, because the last ten residues of FGF21 are critical for its interaction with the obligate FGF21 co-receptor β-klotho (KLB)[44,45]. Notably, human but not mouse FGF21 is cleaved and

processed by FAP, because mouse FGF21 possesses a single–amino acid difference in the stringent FAP-recognition motif[42,43].

FGF21 signals through a FGFR1-KLB receptor complex

Once secreted, FGF21 elicits its biological effects by binding and activating a receptor complex composed of the co-receptor KLB and a traditional FGF receptor, fibroblast growth factor receptor 1c (FGFR1c)[46,47]. Whereas FGFR1c exhibits a ubiquitous tissue expression pattern[5], KLB expression is primarily restricted to specific metabolic tissues (for example, the liver, pancreas and adipose tissue)[5], and lower expression is observed in the brain[22,48–50]. Despite some reports to the contrary[51,52], KLB is not expressed in muscle[5,41]. Thus, KLB is believed to confer specificity for FGF21 signalling. Indeed, loss of KLB abolishes all effects of FGF21 in vitro and in vivo[46,53–56]. KLB is thought to function as a targeting receptor for FGF21, thus promoting association with FGFR1c, which functions as the effector receptor[57]. FGF21 binding promotes the formation of a dimeric FGFR1c–KLB signalling complex, which activates the intracellular tyrosine kinase domains of FGFR1c and propagates signalling through phosphorylation by the kinase ERK[46,53,58,59] (Fig. 1). Although FGF21 signalling can promote post-translational protein modifications[60,61] and alter gene expression[62,63], little is known regarding the intracellular signalling pathways and mechanism of FGF21 action within cells.

Effects of FGF21 on metabolism

Through the years, FGF21 has been proposed to have multiple functions and has been given many designations, including a starvation hormone[64] or stress hormone[36]. However, we propose that FGF21, rather than functioning as a master regulator of metabolism, instead functions as a ‘master sensitizer’ of specific hormonal signals regulating metabolism. Depending on the metabolic state, induction of FGF21 instructs the system to reestablish homeostasis through actions on multiple tissues. Although not meant to be exhaustive, this review highlights the main functions of FGF21 in regulating nutrient and energy homeostasis and describes the key target tissues mediating these effects. Specifically, we explore the effects of FGF21 on (1) enhancing insulin sensitivity, (2) increasing energy expenditure and weight loss, (3) decreasing hepatic triglycerides and (4) regulating macronutrient preference.

FGF21 enhances insulin sensitivity.

An interesting aspect in the discovery of FGF21’s metabolic actions is that FGF21 was identified in a screen for factors that increased white adipocyte glucose uptake independently of insulin[58]. Remarkably, however, FGF21 was subsequently discovered to possess potent insulin-sensitizing actions in vivo[41]. In fact, FGF21 is one of the most potent acute insulin sensitizers ever identified. A single injection of FGF21 can decrease plasma glucose levels by more than 50% in animal models with genetically induced and diet-induced obesity[41,65]. This decrease in plasma glucose levels occurs primarily through increased peripheral glucose disposal[41,56,65], although decreases in hepatic glucose production have also been observed[66]. FGF21 acutely enhances insulin sensitivity through direct actions on adipose tissues[65,67]. While induction of adiponectin, an insulin-sensitizing

hepatoprotective adipokine[68], has been proposed to mediate these insulin-sensitizing effects of FGF21 (refs. [69,70]), other studies have found that adiponectin is dispensable for the metabolic effects of FGF21 (refs. [65,71]). Indeed, more recent studies have confirmed that adiponectin is not required for FGF21 signalling but instead have suggested that adiponectin may induce physiological FGF21 production from the liver[10].

Interestingly, in lean, ad libitum–fed wild-type mice, a single intraperitoneal injection of FGF21 has no effect on plasma glucose levels[65]. However, coadministration of FGF21 with insulin synergistically increases plasma glucose disposal many fold beyond that induced by insulin administration alone[65]. This insulin-sensitizing effect of FGF21 may function physiologically during the fasted-to-refed state, thus maximizing nutrient uptake after the depletion of energy stores during a prolonged fast[6]. Therefore, FGF21 may function by regulating glucose and lipid homeostasis during late fasting[62], then function acutely during refeeding through enhancing insulin-stimulated glucose uptake[6] and suppressing lipolysis[72]. FGF21 may consequently serve as a metabolic switch that maintains nutrient homeostasis by acting as a master sensitizer of endocrine signals during fasting (for example, glucagon) and different endocrine signals during refeeding (for example, insulin), thereby facilitating the transition from the fasted to the refed state[3].

Given the extent of the decrease in plasma glucose, a tissue such as the liver might logically be assumed to be involved in FGF21's metabolic actions. However, loss of KLB from hepatocytes is dispensable for the insulin-sensitizing effects of FGF21 (ref. [67]). Instead, loss of FGF21 signalling to adipose tissues, as accomplished by crossing *Klb*$^{fl/fl}$ mice with adiponectin-Cre mice[65,67], abolishes the insulin-sensitizing effects of FGF21 in both lean and diet-induced obese (DIO) mice, thus demonstrating the importance of direct FGF21 signalling to adipose tissues in mediating this insulin-sensitizing effect. Thermogenic (UCP1$^+$) adipocytes are particularly important, because the insulin-sensitizing effects of FGF21 are also impaired in mice lacking KLB in UCP1$^+$ adipocytes (*Klb*$^{fl/fl}$;*Ucp1-Cre*)[65]. Importantly, positron emission tomography/computed tomography studies using radiolabelled [^{18}F]fluorodeoxyglucose in mice have found that coadministration of FGF21 and insulin markedly increases glucose uptake in brown adipose tissue, but not muscle or white adipose tissue[65]. The capacity of brown adipose tissue, when activated, to dispose of glucose is substantial, accounting for a large fraction of ingested glucose in rodents[73,74]. In agreement with the in vivo fluorodeoxyglucose data[65] and previous in vitro results in white adipocytes[58], FGF21 treatment of isolated primary brown adipocytes, but not white adipocytes, enhances insulin-stimulated glucose uptake[6]. Finally, mice with acute ablation of UCP1$^+$ adipocytes[75] or those lacking UCP1 exhibit impaired FGF21-mediated regulation of glucose homeostasis[76]. Together, these data reveal that FGF21 enhances insulin sensitivity through direct actions on adipose tissues (Fig. 2).

FGF21 regulates energy homeostasis.

In contrast to its acute actions, prolonged administration of FGF21 or FGF21 analogues has important metabolic effects, including a marked decrease in body weight in rodents and non-human primates, and more modest effects on body weight in humans[3,40,58,77,78]. Pharmacological administration of FGF21 to obese rodents reverses diabetes and obesity

through increasing energy expenditure[77,78]. Prolonged FGF21 administration to DIO mice significantly decreases body weight, adiposity, and hepatic triglycerides and cholesterol; reverses plasma hyperglycaemia and hypertriglyceridaemia; and secondarily increases insulin sensitivity[78]. In addition, prolonged overexpression of FGF21 in mice increases insulin sensitivity and extends lifespan[64,79]. These effects of extended FGF21 signalling in promoting weight loss through increased energy expenditure are mediated through direct actions on the central nervous system[67,80–84], not adipose tissues[65,67,85] (Fig. 2). In DIO non-human primates, similarly to rodents, FGF21 or FGF21-analog administration also promotes weight loss[40,81,86]. Notably, however, whereas some studies have reported FGF21-mediated weight loss without effects on food intake[40,86], another study using an FGF21 analogue (PF-05231023) in non-human primates has reported that the weight loss is attributable to significant decreases in food intake[81]. In agreement with the differences observed in non-human primates between administration of FGF21 and an FGF21 analogue, administration of various FGF21 analogues to humans also has been reported to result in differences in body weight and insulin sensitivity. Independent administration of two different FGF21 analogues, PF-05231023 (ref. [81]) and LY2405319 (ref. [87]), to people with obesity and type 2 diabetes significantly decreases body weight and improves lipid profiles. In contrast, two other FGF21 analogues, AKR-001 (also known as efruxifermin)[88] and pegbelfermin[89,90], have not been found to significantly decrease body weight in people with obesity or type 2 diabetes. Beyond the differing body-weight regulation among FGF21 analogues, the effects of different compounds on glucose homeostasis and insulin sensitivity also vary: some compounds do not significantly affect insulin sensitivity[81], while others significantly increase insulin sensitivity in people with type 2 diabetes[87,88]. Nevertheless, the magnitude of the glucose-lowering effects of FGF21 analogues is clearly lower in humans than rodents[81,87–91], perhaps because of differences in brown adipose tissue metabolism between species[65]. However, all analogues consistently have a pronounced ability to decrease plasma triglyceride levels, in many cases by as much as ~70% (refs. [81,87–91]). The differences in reported outcomes with the various FGF21 analogues may reflect differences in analogue development and/or study design. Thus, until studies are conducted with recombinant FGF21 protein in humans, the analogue-specific versus FGF21-specific effects on metabolism may not be known with certainty.

The same holds true for differences observed in the potential adverse effects observed with different FGF21 analogues versus FGF21 administration: while most FGF21 analogues are generally well tolerated, one discontinued FGF21 analogue increases heart rate and blood pressure[92]. In contrast, these effects have not been observed with other analogues[88,89], and administration of FGF21 is actually cardioprotective in numerous rodent models[93–95]. Another potential but controversial adverse effect of FGF21 is bone loss. In mice, FGF21 has been reported to promote bone loss[96,97], but this effect has not been independently validated in rodents[98] or obese non-human primates[99]. In humans, administration of FGF21 analogues has conflicting effects on bone turnover: PF-05231023 has been found to increase markers of bone turnover in humans[81], whereas AKR-001 (ref. [88]) and pegbelfermin[89] have not. Because PF-05231023 affects body weight, and AKR-001 and pegbelfermin do not, changes in markers of bone turnover might possibly be an indirect consequence of weight loss. Finally, most adverse effects associated with FGF21-analogue administration

are gastrointestinal[81,87,88]. Although the exact cause of these gastrointestinal effects is unclear, it may be a consequence of FGF21-analogue interference with FGF19-mediated regulation of bile acid metabolism[100].

The mechanism underlying FGF21-mediated weight loss through actions in the central nervous system (CNS) is incompletely understood. While increases in sympathetic-nerve activity and adipose tissue thermogenesis have commonly been proposed to mediate FGF21's effect in promoting weight loss[80,101], several lines of evidence suggest that FGF21-mediated control of energy homeostasis is not one dimensional. An effective example has recently been demonstrated in an exploration of the metabolic effects of FGF21 in mice in which thermogenic ($UCP1^+$) adipocytes were acutely ablated[75]. Notably, in these studies, FGF21 significantly decreased body weight to the same extent in wild-type mice and mice acutely lacking thermogenic adipose tissues. A particularly interesting finding was the mechanism underlying the weight loss. Whereas FGF21 increased energy expenditure and caused significant weight loss in wild-type mice, as expected, mice with acute ablation of thermogenic adipose tissues exhibited no increase in energy expenditure but still exhibited increased weight loss through increased physical activity and a trend towards decreased food intake[75]. Relatedly, two independent studies have revealed that FGF21 significantly decreases body weight in the absence of UCP1 (refs. [102,103]). Notably, while one study found that deletion of UCP1 strongly blunts the effects of FGF21 on energy expenditure and appears to mediate its effects on body weight through decreased food intake[103], the other study observed a strong trend in increased physical activity and the maintenance of increased energy expenditure after FGF21-analogue administration[102]. Indeed, administration of high doses of FGF21 has been shown to increase both energy expenditure and physical activity[78]. Thus, we speculate that FGF21 may act on higher-order neural circuits in the brain, thereby coordinating a resetting of body-weight homeostasis by effectively modulating energetic balance in a species-specific and context-specific manner. For example, although a common neural circuit may exist between species, rodents possess more brown adipose tissue than humans relative to total body weight, and increasing energy expenditure may provide a more effective way to achieve a negative energy balance and return to metabolic homeostasis. If this primary mode is disrupted, alternative mechanisms (such as food intake or physical activity) may be used by these circuits (Fig. 3a). Conversely, because non-human primates and humans have relatively less thermogenic adipose tissue than rodents, body-weight regulation may be mediated by a coordinated effect on caloric intake and/or physical activity, thus promoting a negative energy balance.

Therefore, determining the central targets of FGF21 action is critical. Attempts to address this question have been hampered by the very low level of KLB expression in different cells in the CNS. Recently, however, studies that have crossed mice expressing Cre recombinase under the control of the endogenous *Klb* locus (*Klb*-Cre) with mice expressing the tdTomato reporter (*Klb*-Cre;tdTomato) have revealed critical sites of KLB expression in the hypothalamus[48]. Moreover, through single-cell RNA sequencing of cells from the hypothalamus in *Klb*-Cre;tdTomato mice, FGF21's hypothalamic target cells have been identified. In contrast to the reported restriction of KLB expression to the suprachiasmatic nucleus[49], KLB is expressed in numerous cell types and regions throughout the hypothalamus[48] (and CNS). Future work is needed to identify which neural circuits

coordinate FGF21-mediated regulation of energy homeostasis and how FGF21 sensitizes these neurons to respond to metabolic cues.

FGF21 reverses fatty liver.

Physiological circulating levels of FGF21 are produced primarily by the liver in response to homeostatic imbalance, perhaps partly to protect the liver itself from metabolic or cellular stress[3,104]. As mentioned in the previous section, FGF21 has potent lowering effects on hepatic and plasma triglycerides in obese rodents and humans, and FGF21 mimetics are actively being pursued as therapeutic agents for the treatment of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH)[95,105]. An important contributor to the progression from non-alcoholic fatty liver disease to NASH is altered lipid metabolism[106,107]. Administration of the FGF21 analogues AKR-001 (ref. [88]) and pegbelfermin[89] in humans with NASH results in marked absolute decreases in liver fat (up to 70%) and significant amelioration of liver fibrosis[88,89]. FGF21's potent triglyceride-lowering effects are mediated by altered adipose and liver metabolism. Pharmacological administration of FGF21 or FGF21 analogues exerts lipid-lowering effects through accelerated lipoprotein catabolism[108], suppression of postprandial lipolysis in adipose tissues[88], decreased hepatic endoplasmic reticulum and oxidative stress[109,110], and increased beta-oxidation of lipids in the liver[62] (Figs. 2 and 3a). The mechanisms underlying these effects have been controversial, and both the liver and CNS have been proposed as the responsible target tissue in which direct signalling occurs. Whereas some studies have shown that FGF21 signals directly to the hepatocytes or liver[111,112], others have found that FGF21 does not signal directly to hepatocytes[50,62,113]. Importantly, FGFR1c, FGF21's primary target, is not expressed in primary hepatocytes[46,114]. However, FGFR1 is expressed in immortalized hepatocyte cell lines (HepG2 cells)[115], thus raising doubts regarding the relevance of these models in the assessment of FGF21 function[112]. Although FGF21 may be capable of binding and signalling through other FGF receptors (FGFR2 and FGFR3)[46] in hepatocytes, KLB is absolutely required for signaling[46,53–56]. Notably, loss of KLB in hepatocytes does not alter the FGF21-mediated decreases in body weight, and the improvements in both insulin sensitivity and glucose and lipid homeostasis in DIO mice[67]. Instead, FGF21 signals directly to the CNS (Fig. 3a), at least in rodents, and consequently regulates energy expenditure and improves glucose and lipid homeostasis[80,82].

Importantly, although FGF21's effects on hepatic lipid metabolism appear to require FGF21 signalling in the brain, no studies have directly assessed whether FGF21 signalling to the brain is required for FGF21's pharmacological ability to reverse NASH. FGF21 or FGF21 analogues could possibly act on the liver in mediating the beneficial effects on NASH. Although hepatocytes do not express FGFR1 under normal conditions[46,114], FGFR1 expression in hepatocytes could potentially be induced during NASH, similarly to its induction during hepatocarcinogenesis[115], thus making it a target for FGF21 signalling. Alternatively, FGF21 analogues, whose receptor binding profiles may differ from those of recombinant FGF21 protein[95], might possibly mediate their effects through signalling to the liver. Finally, clinical studies in patients with obesity and type 2 diabetes have found that FGF21-analogue administration markedly induces plasma adiponectin levels[81,87–89], and this induction has be proposed to contribute to FGF21-mediated effects in

ameliorating hepatic steatosis and inflammation during NASH[116]. The potential contribution of adiponectin to mediating the beneficial effects of FGF21 against NASH in vivo warrants further investigation. These studies highlight the clinically meaningful therapeutic potential of targeting FGF21 signalling in the treatment of fatty liver disease and reveal important unanswered questions surrounding the mechanisms of these pharmacological agents.

Macronutrient imbalance and FGF21.

A major physiological role of FGF21 is the regulation of nutrient homeostasis. Genome-wide association studies correlating changes in macronutrient intake with genetic variants have identified single-nucleotide polymorphisms at the *FGF21* locus that significantly correlate with changes in carbohydrate intake[117,118]. These single-nucleotide polymorphisms are also associated with increases in sweet-taste preference[119] and greater waist-to-hip ratios[120]. In agreement with these human data, mice lacking FGF21 also exhibit a greater preference for sucrose[22]. Subsequent work has revealed that FGF21 is induced by simple-sugar intake in both mice and humans[22,32,33], and that sugar-induced production of FGF21 from the liver occurs in a ChREBP-dependent manner[22,121]. Once in circulation, FGF21 signals to the CNS and consequently suppresses simple-sugar intake and sweet-taste preference[22,122], but not protein or lipid intake[22,84]. In agreement with the findings in rodents, administration of an FGF21 analogue to people with obesity decreases the preference for sweet-tasting food and carbohydrate intake[123]. Thus, increased FGF21 production from the liver in response to carbohydrate intake is a negative feedback loop regulating carbohydrate intake[22].

Recent work has demonstrated that FGF21 signals directly to glutamatergic, but not GABAergic or dopaminergic, neurons in regulating simple-sugar intake and sweet-taste preference[48]. While physiological FGF21 signalling to glutamatergic neurons in the paraventricular nucleus regulates basal sucrose intake[22,48], FGF21 signalling to glutamatergic neurons in the ventromedial hypothalamus (VMH) is required for FGF21-mediated suppression of simple-sugar intake[48]. In agreement with our proposed role of FGF21 as a master sensitizer, FGF21 increases the sensitivity of both glucose-excited and glucose-inhibited neurons in the VMH in response to increased, but not decreased, glucose concentrations[48]. FGF21 accomplishes this action by increasing the excitability of KLB^+ neurons in the VMH[48]. By enhancing the excitability of KLB^+ glucose-responsive neurons in response to high, but not low, glucose concentrations, FGF21 achieves specificity in suppressing simple-sugar intake under conditions of elevated plasma glucose and not during fasting. Importantly, however, these same neurons are dispensable in FGF21-mediated increases in energy expenditure. These findings indicate that FGF21 signals through distinct populations of neurons, thereby mediating effects on macronutrient intake and energy expenditure[48]. The production of FGF21 in the liver in response to excess carbohydrate intake functions in a feedback loop by signalling to the hypothalamus to suppress further carbohydrate intake[22]. In addition, FGF21 signalling to adipose tissues promotes the disposal of excess carbohydrates[41,65]. Thus, FGF21 production from the liver, in combination with elevated glucose levels, protects the liver from metabolic stress through a coordinated regulation of carbohydrate intake and disposal (Fig. 3b).

Beyond the response to high carbohydrate, FGF21 is induced in response to protein imbalance, specifically dietary-protein dilution[26,27,124]. During amino acid restriction, mammals increase their consumption of diets low in protein to acquire sufficient protein to satisfy organismal requirements, despite increasing their overall caloric intake[125–129]. Physiological induction of FGF21 during dietary-protein dilution, which by definition involves higher carbohydrate or lipid consumption, is likely to coordinate an increase in en energy expenditure to offset the increased caloric intake and maintain body-weight homeostasis. FGF21 is required for the increases in energy expenditure and the prevention of weight gain associated with low-protein diets[26,27]. In addition, as expected according to the mechanism of FGF21's action in increasing energy expenditure, FGF21 signalling to the CNS is required for the weight loss associated with a low-protein diet[83,84]. Interestingly, similarly to its effects in suppressing simple-sugar intake and sweet-taste preference, FGF21 signalling to glutamatergic, but not GABAergic, neurons is required for the weight loss associated with dietary-protein dilution[84]. Therefore, induction of FGF21 from the liver, in combination with low amino acid levels, signals the CNS to increase energy expenditure and protect against weight gain from excess caloric intake. In addition, FGF21 may promote foraging for other macronutrients (that is, protein and lipids) by suppressing carbohydrate intake[22,84] (Fig. 3c).

Critical questions for future research

Great strides have been made in deciphering the mechanisms underlying FGF21's metabolic effects. Although specific sites of action that coordinate FGF21's effects have broadly been identified, the mechanisms of action within these targets remain unclear. For example, how FGF21 signals within target cells and what pathways are required for FGF21 signalling are unknown. In addition, how FGF21 alters the sensitivity, or impinges on the signalling, of a diverse set of metabolic cues is an important unanswered question. Interestingly, both FGF21 and another endocrine FGF, FGF15/19, share a requirement for KLB for signaling[46,54], but what cross-talk, if any, exists between these signalling pathways in both physiological and pharmacological settings is unclear. Beyond the identification of FGF21 targets and signalling cross-talk, whether FGF21 signalling pathways are impaired during obesity and diabetes remains unresolved[38,61,130]. Because circulating levels of FGF21 are significantly elevated during obesity and diabetes[3], and given the known metabolic effects of FGF21, FGF21's action might logically be reasoned to somehow be impaired, or 'FGF21 resistant', during obesity[37]. Importantly, in contrast to their leptin resistance[131], obese rodents, primates and humans are responsive to pharmacological levels of exogenous FGF21 or FGF21 analogues in this potentially FGF21-resistant state.

Similarly to its regulation of nutrient homeostasis, FGF21 production by the liver is also increased in response to alcohol intake[132,133], and administration of FGF21 suppresses alcohol intake[122,134]. In agreement with findings in other models described above, FGF21 signalling in response to alcohol consumption may function as a feedback loop that protects the liver from damage. Whether these effects on alcohol intake are mediated through the same central targets that exert FGF21's effect on suppressing sugar intake remains to be determined. Although many of FGF21's functions require KLB expression in the brain, the cellular targets of FGF21 signalling in the CNS have only recently been discovered.

Because distinct cellular targets and brain regions coordinate different effects of FGF21, identification of the neural substrates and circuits regulating these processes is likely to provide novel insights into FGF21's function and reveal previously unrecognized circuits controlling higher-order regulation of energy homeostasis and reward-seeking pathways. The ability to define how central and peripheral FGF21 target tissues coordinate FGF21-mediated responses in a context specific manner, perhaps through communication via both the afferent and efferent nervous system, will be a critical research area in the future.

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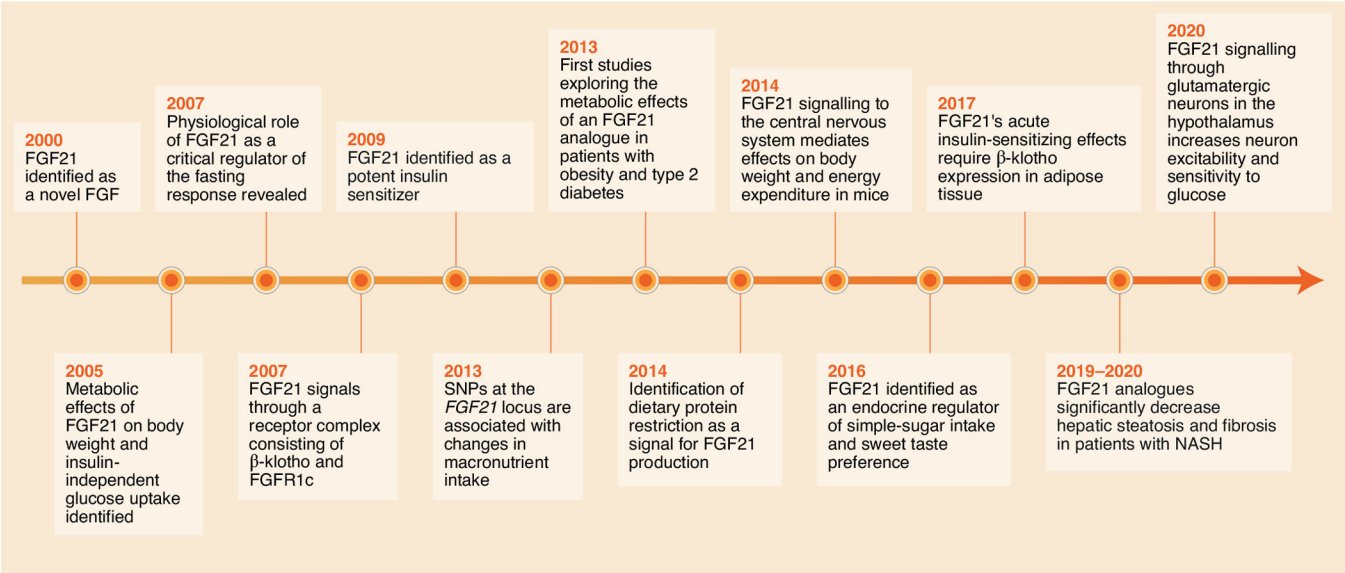

Fig. 1. Discovery of FGF21 as a metabolic messenger.

After the discovery of FGF21 in the year 2000, insight into FGF21's function has advanced rapidly. FGF21 was first identified as a regulator of body weight and an insulin-independent modulator of glucose uptake (2005). Subsequent work revealed that FGF21 signals through a unique receptor complex (KLB–FGFR) (2007) and has potent insulin-sensitizing effects in obese rodents (2009). Physiological roles in which FGF21 regulates fasting (2007) and macronutrient intake (2013 and 2016) were later revealed, thus underscoring its function in energy and nutrient homeostasis. Important cellular targets, specifically the CNS (2014) and adipose tissues (2017), were then identified as direct targets mediating distinct aspects of FGF21's metabolic effects. Clinical studies have revealed the therapeutic potential of FGF21 analogues in the treatment of diabetes and obesity (2013) and NASH (2019–2020). Most recently, direct central targets of FGF21's actions have been shown to underlie its effects on neuronal function to suppress simple-sugar intake (2020).

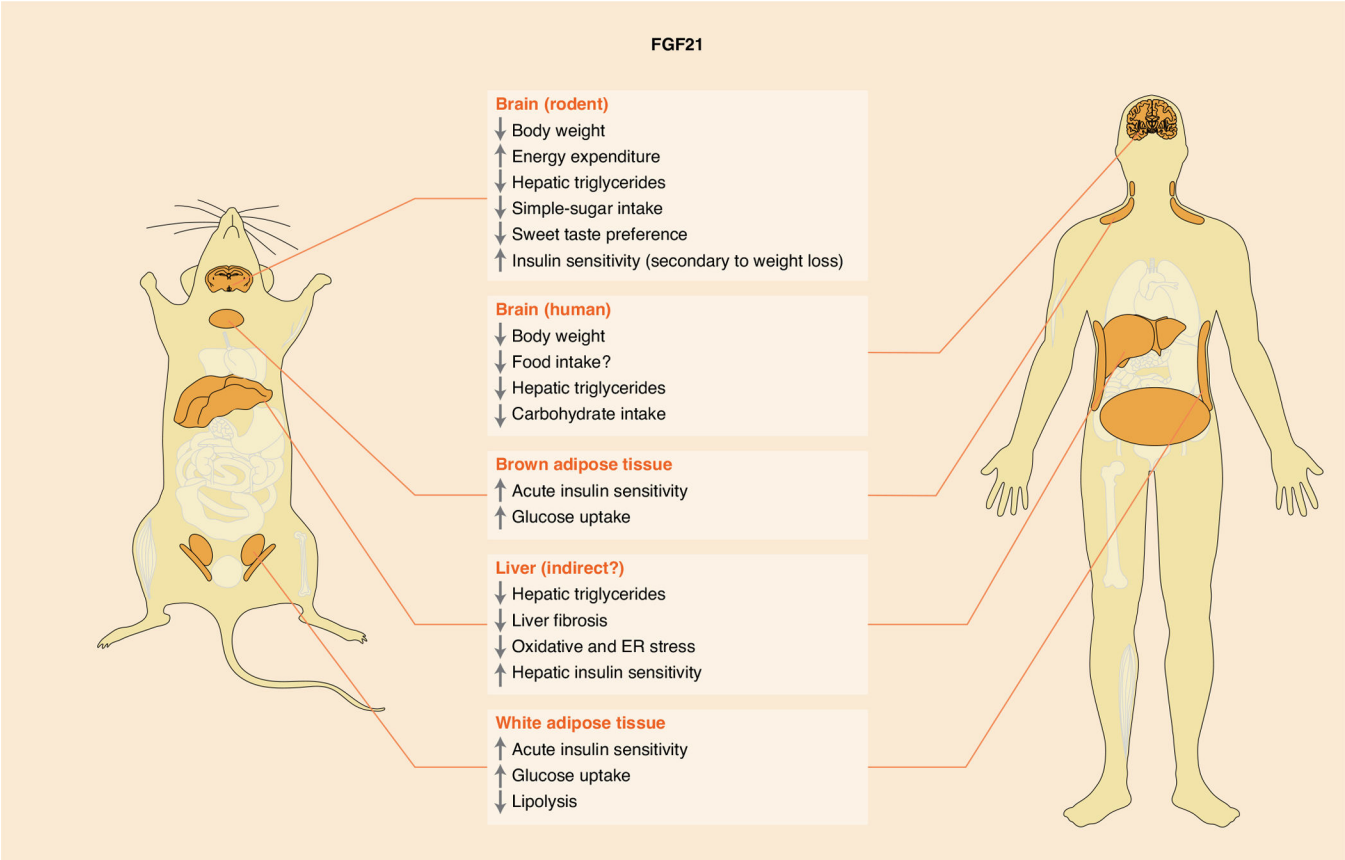

Fig. 2. Target tissues and metabolic activities of FGF21.

The metabolic effects of FGF21 in regulating body weight, hepatic triglycerides and macronutrient preference are conserved. However, species-specific differences in FGF21's glucose-lowering effects have been observed. Most of FGF21's metabolic effects are mediated through direct signalling to the brain and adipose tissues. Although FGF21 signalling to other tissues indirectly affects hepatic metabolism, whether FGF21 analogues signal directly to the liver is unclear. Evidence in rodents suggests FGF21's abilities to regulate macronutrient preference, decrease body weight and hepatic triglycerides, and secondarily increase insulin sensitivity (associated with weight loss) are mediated through direct FGF21 signalling to the central nervous system. FGF21's acute insulin-sensitizing effects are mediated through direct signalling to adipose tissues. ER, endoplasmic reticulum.

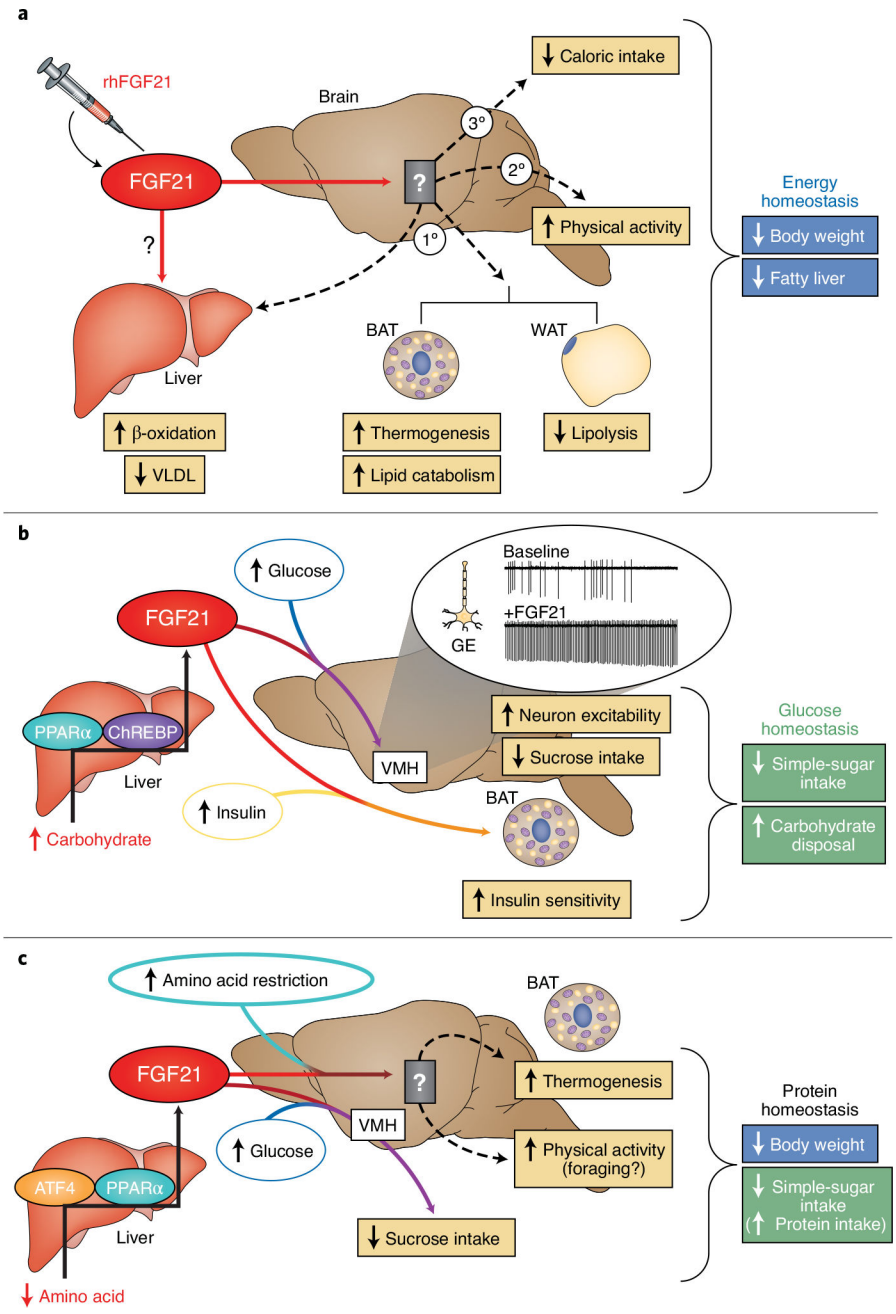

Fig. 3. FGF21 modulates signals, thus regulating energy and nutrient homeostasis.

a, In rodents, pharmacological administration of recombinant human FGF21 (rhFGF21) signals to unknown regions in the brain and invokes the primary (1°) energy-expending effects of FGF21 in decreasing body weight and liver triglycerides. However, in rodents lacking thermogenic adipose tissues, or in primates and humans, which have lower thermogenic adipose capacity than rodents, secondary (2°) and tertiary (3°) mechanisms increase physical activity and decrease caloric intake, thereby decreasing body weight. Direct actions of FGF21 on the liver in humans are unclear. **b**, FGF21's ability to regulate glucose homeostasis is bimodal. FGF21 production from the liver is increased in a ChREBP-

and PPARα-dependent transcriptional response to excess hepatic carbohydrate levels. Once secreted, FGF21 signals to the CNS and consequently enhances the excitability of glucose-excited (GE) neurons in the VMH in response to glucose. This increase in glucose sensitivity signals the suppression of simple-sugar intake. To complement FGF21's effects on simple-sugar consumption, FGF21 also promotes carbohydrate disposal by enhancing insulin sensitivity in adipose tissues. **c**, FGF21 is produced by the liver in response to amino acid restriction through transcriptional regulation mediated by ATF4 and PPARα. Liver-derived FGF21 then enters the circulation and signals the brain to modulate macronutrient preference and energy expenditure. FGF21 signalling to the brain suppresses sugar intake, which secondarily promotes the intake of other macronutrients (such as protein). The brain regions mediating FGF21's effects on thermogenesis and physical activity during amino acid dilution remain to be identified. BAT, brown adipose tissue; WAT, white adipose tissue; VLDL, very low-density lipoprotein.