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Fibroblast Growth Factor 21: A Versatile Regulator of Metabolic Homeostasis

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Abstract

Fibroblast growth factor 21 (FGF21) is an endocrine hormone derived from the liver that exerts pleiotropic effects on the body to maintain overall metabolic homeostasis. During the past decade, there has been an enormous effort made to understand the physiological roles of FGF21 in regulating metabolism and to identify the mechanism for its potent pharmacological effects to reverse diabetes and obesity. Through both human and rodent studies, it is now evident that FGF21 levels are dynamically regulated by nutrient sensing, and consequently FGF21 functions as a critical regulator of nutrient homeostasis. In addition, recent studies using new genetic and molecular tools have provided critical insight into the actions of this endocrine factor. This review examines the numerous functions of FGF21 and highlights the therapeutic potential of FGF21-targeted pathways for treating metabolic disease.

Keywords

FGF21; macronutrient; β -klotho; adipose; thermogenesis; alcohol

1. INTRODUCTION TO FGF21

Fibroblast growth factors (FGFs) have diverse roles in signaling and development across a wide array of tissues. In addition to their vital roles in regulating cell growth, altered FGF function may also contribute to diseases ranging from cancer to bone disorders (8). Mammalian genomes encode 22 FGFs, which are classically considered paracrine, autocrine, or intracrine. The majority of FGFs are secreted and interact with heparan sulfate glycosaminoglycans (HSGAGs), which sequester the proteins to the extracellular matrix and prevent their dispersion from their cell of origin (8). FGFs interact with one of four FGF receptors (FGFR1–4), which are classical tyrosine kinase receptors that dimerize upon interaction with FGFs and initiate intracellular signaling. Unlike traditional FGFs, however, the FGF19 subfamily of FGFs, consisting of FGF19, FGF21, and FGF23, has reduced affinity for HSGAGs and diffuses into circulation to act on distal tissues (9, 106). Thus, these FGFs function as endocrine hormones. Importantly, since FGF receptors are ubiquitously expressed, endocrine FGFs require a coreceptor to provide signaling specificity, which for FGF21 and FGF19 is the coreceptor β -klotho (71, 99, 140). Due to the potent

metabolic actions and therapeutic potential of FGF21, this review focuses on its diverse functions.

FGF21 was first identified as a novel FGF with high expression in the liver and thymus (98). Subsequently, the metabolic role of FGF21 was identified through a screen for factors that induced glucose uptake in 3T3L1 adipocytes (64). A myriad of studies ensued demonstrating that administering FGF21 to obese or diabetic rodents and primates markedly reduced body weight and significantly improved carbohydrate and lipid homeostasis (21, 64, 65, 141). Clinical trials with FGF21-based analogs revealed that many of the metabolic effects of FGF21 observed in rodents also translate to humans (41, 66, 126). In addition to the pharmacological studies, numerous studies have uncovered physiological functions of FGF21, and with these have come the unraveling of unique endocrine pathways regulating nutrient homeostasis.

The expression levels and metabolic functions of FGF21 are dynamically impacted by nutritional status (37, 102). The expression of *Fgf21* messenger RNA (mRNA) is detectable in multiple tissues, including in the liver, thymus, white adipose tissue (WAT), brown adipose tissue (BAT), pancreas, and heart (39, 98). Notably, despite the expression of *Fgf21* mRNA in these tissues, under most physiological conditions, circulating levels of FGF21 are derived primarily from the liver (46, 86). As is discussed in subsequent sections, the induction of FGF21 occurs in specific tissues during diverse physiological states, ranging from fasting to overfeeding (88). FGF21 functions in target tissues that express the traditional FGF receptor FGFR1c and the obligate coreceptor β -klotho (2, 27, 71, 99, 124, 140). While FGFR1c is ubiquitously expressed, β -klotho expression is limited to metabolic tissues and confers specificity for FGF21 signaling. β -klotho is expressed in the liver, WAT, BAT, pancreas (39), and distinct regions of the brain, including the area postrema, nucleus tractus solitarius, suprachiasmatic nucleus (12), and the paraventricular nucleus (PVN) (81). FGF21 initiates signaling to target tissues by binding to the extracellular domain of β -klotho via the C terminus of FGF21, which then facilitates the interaction of FGF21 with FGFR1c (77, 89, 144, 145). This trimeric receptor complex leads to the dimerization of FGFR1c, transphosphorylation of the receptor, and initiation of a signaling cascade (64, 145). Currently, the best readouts for FGF21 signaling in target tissues are general signals of growth factors, including increased phosphorylation of FRS2 α and ERK1/2 (64). FGF21-specific signaling cascades have yet to be identified. The relative contribution of FGF21-mediated signaling in specific tissues to its metabolic effects is examined in later sections.

2. THE PHYSIOLOGICAL EFFECTS OF FGF21

While the metabolic effects of pharmacological FGF21 administration have been clear, the physiological effects of FGF21 have been less straightforward. This is likely due to the complexity of the conditions that regulate FGF21 production, the hormonal milieu that impact FGF21 function, and differences in the experimental design and animal models used in studies that have examined physiological FGF21 function. In the following sections, we examine what we consider to be the major physiological functions of FGF21 and explore the biological networks that contribute to its role in regulating nutrient and energy homeostasis.

2.1. Physiological Role of FGF21 During Fasting and Refeeding

The liver is a critical regulator of energy and nutrient homeostasis. During states of nutrient deprivation, such as fasting, the liver maintains energy availability for peripheral tissues through increased production of hepatic glucose and ketones. As fasting progresses, fuel utilization shifts from carbohydrates to ketone bodies. To accomplish this, WAT undergoes lipolysis to release glycerol and free fatty acids to be used by the liver for gluconeogenesis and ketogenesis, respectively. In the liver, the nuclear hormone receptor peroxisome proliferator-activated receptor- α (PPAR α) becomes activated by fatty acids, leading to a transcriptional orchestration of the hepatic fasting response for glucose and lipid metabolism (62). Multiple groups discovered that PPAR α -mediated induction of FGF21 is important for the adaptive fasting response (7, 54). The *Fgf21* promoter contains PPAR α response elements that lead to the induction of hepatic and circulating levels of FGF21 during prolonged fasting (54). These circulating levels of FGF21 are derived from the liver, as the genetic loss of *Fgf21* specifically from the liver ablates plasma FGF21 levels during fasting (86). Consistent with data from rodents, circulating levels of FGF21 are also induced in humans during prolonged fasting (33, 42). The significance of FGF21 induction during fasting was demonstrated by gain- and loss-of-functions studies. The overexpression of FGF21 was sufficient to increase hepatic gluconeogenesis, β -oxidation, and ketogenesis, whereas the loss of FGF21 prevented fasting-induced hepatic gluconeogenesis, β -oxidation, and ketogenesis in mice (105). While fasting plasma glucose levels were slightly decreased in two different *Fgf21* knockout mouse lines (81, 105), this was not observed in another *Fgf21* knockout line (6). However, liver-specific *Fgf21* knockout mice also exhibited reduced plasma glucose levels during fasting (86).

An important observation made during these studies was that FGF21 does not act directly on the liver to mediate these effects on hepatic metabolism. The administration of FGF21 to primary hepatocytes or infusion into an isolated perfused liver failed to recapitulate the induction of hepatic gluconeogenic gene expression observed in wild-type mice administered FGF21 (105). A subsequent study validated these results and also revealed that FGF21 elicits its effects on hepatic gluconeogenesis through actions in the hypothalamus that activate the hypothalamic-pituitary-adrenal axis to release corticosterone (81). Loss of FGF21 impaired hepatic gluconeogenesis due to blunted corticosterone production (81). Importantly, the FGF21 coreceptor β -klotho is expressed in the hypothalamus (12, 81), and FGF21 crosses the blood-brain barrier (50). Administration of FGF21 to the PVN of the hypothalamus restored corticosterone production and reversed the impaired hepatic gluconeogenesis observed in *Fgf21* knockout mice (81). Therefore, FGF21 coordinates the hepatic fasting response through a liver-to-brain signaling axis (Figure 1).

FGF21 also functions to coordinate the transition from the fasting to the refeed state (Figure 1). FGF21 is well known to be a potent and rapid insulin sensitizer (27, 142), which is not congruent with its actions as simply a fasting factor. During fasting, elevated fatty acid levels in circulation induce physiological peripheral insulin resistance to spare glucose utilization for the brain. However, when nutrients become available following a prolonged fast, FGF21 functions as a metabolic switch to facilitate the transition to the refeed state by enhancing insulin-stimulated glucose uptake while acutely maintaining the actions of the fasting

response (86). Plasma FGF21 levels are still present acutely during refeeding, and liver-specific *Fgf21* knockout mice have elevated plasma glucose levels and impaired insulin sensitivity during refeeding (86). In addition, in humans, plasma FGF21 levels have been shown to be induced by increases in both glucose and insulin levels (31, 84, 110, 133), supporting a role during refeeding. Thus, FGF21 functions as a critical mediator of nutrient homeostasis during both the fasted and refeed states (Figure 1).

2.2. FGF21-Mediated Lipid Metabolism on a Ketogenic Diet

High-fat, low-carbohydrate ketogenic diets lead to a unique metabolic state that promotes lipid oxidation and weight loss (23). Similar to fasting, low-carbohydrate ketogenic diets decrease overall carbohydrate levels, thereby promoting lipid oxidation and increased ketogenesis. FGF21 is markedly induced by ketogenic diets in rodents and has been proposed to be a key mediator of hepatic lipid metabolism during ketotic states (6, 7). Knockdown of hepatic *Fgf21* using adenovirus-mediated expression of short hairpin RNAs directed against *Fgf21* mRNA resulted in significantly elevated hepatic triglyceride and plasma lipid levels, and impaired ketosis (7). Consistent with these data, *Fgf21* total knockout mice fed a ketogenic diet developed hepatosteatosis and exhibited impaired ketogenesis and glucose control (6). However, these conclusions have been controversial because other groups have reported that FGF21 is not required for regulating glucose homeostasis and ketosis induced by ketogenic diets (97, 123). Importantly, it is unclear whether there is any translational relevance of FGF21 to the beneficial effects of ketogenic diets in humans because circulating FGF21 levels fail to increase in humans in response to a ketogenic diet (19, 42, 73).

2.3. FGF21 Signals Protein Restriction

Low-protein diets (73, 85), amino acid starvation (25, 121), and the amino acid-depleting drug asparaginase (138) also induce plasma FGF21 levels. Interestingly, protein restriction promotes systemic energy expenditure through this elevation in plasma FGF21 levels (73, 85). In order to maintain macronutrient balance, animals modify their caloric intake to reach a sufficient amount of protein in the body, a process termed protein leverage (118). That is, to meet daily protein requirements, animals consume more of a diet low in protein, which also results in an increase in calorie consumption. However, to maintain energy balance, a corresponding increase in energy expenditure is required to offset this increase in caloric intake needed to meet protein demands (85, 118). Elevation of circulating FGF21 levels in response to low protein levels increases both the browning of adipose tissues and energy expenditure (73), likely to offset this increased caloric intake (Figure 2). FGF21 is absolutely necessary in mice to mediate the effects of low-protein diets (72, 73, 85). Both *Fgf21* total knockout (73) and *Fgf21* liver-specific knockout mice (85) fail to increase energy expenditure and food intake in response to protein dilution.

FGF21 is markedly induced in response to protein restriction (25, 73, 85) but not caloric restriction (73, 148). Interestingly, the increase in circulating FGF21 levels induced by a ketogenic diet in rodents also appears to be largely due to reduced protein intake rather than to high lipid intake (73). The addition of protein to a ketogenic diet reduces plasma FGF21 levels, although it does not completely return plasma FGF21 levels to baseline (73). Notably

in humans, unlike a ketogenic diet, the administration of a low-protein diet for 28 days significantly increases plasma FGF21 levels (73), demonstrating that in humans amino acid starvation is an important regulator of FGF21 production. Low-protein diets reduce essential amino acid levels, leading to the activation of the amino acid sensor general control nonderepressible 2 (GCN2) (136). GCN2 phosphorylates eukaryotic translation initiation factor 2 α (eIF2 α), which subsequently leads to the activation of activating transcription factor 4 (ATF4) (90). The *Fgf21* promoter has multiple ATF4 binding sites, and GCN2-mediated activation of ATF4 leads to the induction of hepatic and circulating levels of FGF21 (24, 68, 72). Interestingly, the induction of FGF21 during protein restriction requires both the GCN2–ATF4 and PPAR α signaling pathways (73), consistent with the observation that adding protein to a ketogenic diet does not completely restore plasma FGF21 levels to baseline (73). In addition, recent studies have identified the liver-integrated stress response–driven nuclear protein 1 (NUPR1) as being required for normal FGF21 production and associated metabolic remodeling in response to low protein levels (85). Thus, FGF21 is a key mediator of energy homeostasis under conditions of macronutrient challenge, including protein dilution (Figure 2).

2.4. Regulation of Macronutrient Intake and Sweet Taste Preference by FGF21

The regulation of food intake is complex and integrates both peripheral and central cues (92). While the mechanisms controlling total caloric intake have been explored extensively, little is known regarding the regulation of macronutrient intake. Since there is evidence that the consumption of major macronutrients has a genetic component (107), common genetic variants associated with macronutrient intake have been explored through genome-wide association studies of carbohydrate, fat, and protein intake. Two independent studies revealed that single nucleotide polymorphisms (SNPs) at the *FGF21* locus are associated with changes in macronutrient intake (20, 127). Specifically, these studies reported that variants rs838133 and rs838145 were associated with higher carbohydrate intake and that *FGF21* was a candidate gene in the region (20, 127). A third report also identified SNPs at the *FGF21* locus associating with a preference for sweets (120). Although variants around the *FGF21* locus are associated with changes in macronutrient intake, they are not associated with obesity or type 2 diabetes (120). However, while changes in caloric intake can affect body weight and metabolic homeostasis, changes in macronutrient intake alone can affect insulin signaling, cardiometabolic health, and longevity (122). Importantly, an additional study using data from 451,000 individuals in the UK Biobank found that not only is the rs838133 allele associated with higher carbohydrate intake but it is also associated with stronger effects on body fat distribution and higher blood pressure than with effects on body mass (40). Thus, FGF21 may regulate nutrient homeostasis and metabolic health beyond changes in body weight.

Consistent with these data in humans, *Fgf21* total knockout mice exhibit an increased preference for simple sugars, but not lipids or protein (133). In addition, this alteration in macronutrient preference did not result in a change in overall caloric intake (133). Ad libitum feeding of sucrose for 6–24 hours gradually increases hepatic and plasma FGF21 levels in wild-type mice, and this effect is lost in liver-specific *Fgf21* knockout mice (133). Consistent with total *Fgf21* knockout mice, liver-specific *Fgf21* knockout mice exhibited a

preference for a high-carbohydrate diet (133). Simple sugars also increase plasma FGF21 levels in humans. The ingestion of a single bolus of fructose or glucose induces plasma FGF21 levels in both lean healthy participants and patients with metabolic syndrome. While fructose markedly and acutely increased plasma FGF21 levels in humans, glucose caused a delayed and gradual increase in plasma FGF21 levels (31). The difference in the time course for FGF21 induction by these two sugars is likely due to differences in their uptake. The uptake of absorbed glucose and fructose differs between tissues, with glucose largely escaping first-pass removal by the liver, whereas fructose uptake occurs primarily in the liver (114). Thus, a high fructose load results in higher carbohydrate levels in the liver compared with a high glucose load, which may result in a more potent induction of FGF21 by fructose. A separate study examined plasma FGF21 levels in individuals fed a carbohydrate-rich diet without reductions in protein intake (84). Notably, without affecting protein levels, excess carbohydrate led to an approximately eightfold increase in circulating FGF21 levels, an effect not observed with increased fat intake (84). Finally, an infusion of dextrose used to induce hyperglycemia in healthy individuals also significantly increased circulating plasma FGF21 levels (133). Together, these data reveal that FGF21 is induced in response to excess levels of carbohydrate and that the loss of FGF21 signaling increases carbohydrate preference.

The stimulation of FGF21 production by carbohydrates is mediated by the transcription factor carbohydrate-response element-binding protein (ChREBP). In response to increased carbohydrate intake, particularly fructose, increased hepatocyte carbohydrate concentrations drive substrate into the pentose phosphate pathway. An intermediate in this pathway, xylulose-5-phosphate, activates ChREBP (1) and promotes its binding to the carbohydrate response element sites in the promoters of target genes, including *FGF21* (53, 130). Mice that have been fasted and re-fed a high-carbohydrate diet have markedly elevated hepatic and plasma FGF21 levels (112), which can be recapitulated by treating hepatocytes with simple sugars (53, 130, 133). Mice lacking ChREBP fail to show an increase in plasma FGF21 levels in response to simple sugar intake (35, 133). Recent work also shows that the loss of PPAR α disrupts ChREBP-mediated induction of FGF21 and the regulation of sucrose preference (55). This dependence on PPAR α for the full induction of FGF21 in response to carbohydrate is reminiscent of the mechanism for the protein restriction-mediated induction of FGF21 (Figure 2).

Based on the FGF21 loss-of-function data, which showed an increase in carbohydrate preference (133), the administration of FGF21 would be predicted to suppress carbohydrate preference. Indeed, the administration of recombinant FGF21 to mice markedly reduces carbohydrate preference without affecting the preferences for lipid and protein (133). Moreover, the overexpression of FGF21 decreases sucrose preference (125, 133) but not the preference for lipid and protein (133). Interestingly, FGF21 also suppresses the intake of noncaloric sweeteners (125, 133). In fact, FGF21 has a more pronounced effect on reducing the intake of noncaloric sweeteners, such as sucralose, than it does on sucrose intake (133). Due to its energetic value and pre- and postabsorptive mechanisms that drive sucrose intake, these data suggest that FGF21 suppresses some, but not all, of the rewarding effects of sucrose. FGF21 mediates these effects on simple sugar and sweet taste preference not by affecting sweet taste sensing, but rather by affecting taste processing. Chorda tympani nerve

recordings in wild-type mice administered FGF21 were not different from those of vehicle-treated mice in response to various tastants, including simple sugars and noncaloric sweeteners (133). Instead, intracerebroventricular administration of FGF21 was sufficient to suppress sucrose preference (133), and the loss of the obligate FGF21 coreceptor β -klotho in the brain abolished the ability of FGF21 to suppress sweet taste preference (125). Importantly, loss of β -klotho in the PVN of the hypothalamus, but not the suprachiasmatic nucleus, impaired FGF21-mediated suppression of sucrose intake (133). Together, these data identified a novel liver-to-brain negative feedback loop mediated by FGF21 to regulate carbohydrate homeostasis and sweet taste preference, possibly as a mechanism to protect the liver from carbohydrate overload (Figure 2).

FGF21 also regulates sweet taste preference in primates and humans. When an FGF21 analog, PF-05231023, was administered to monkeys, they rapidly and significantly reduced their saccharin intake (125). To explore the relevance of these observations to humans, variants in the *FGF21* locus were examined in participants from the Danish Inter99 cohort to study the relationship of these genotypes to a detailed range of ingestive behaviors. As was observed with increased carbohydrate intake (20, 127), statistically significant associations were identified between the *FGF21* rs838133 allele and increased candy consumption (120).

Multiple conditions, diets, and nutritional states affect circulating levels of FGF21. To examine the nutritional context for the regulation of FGF21 production, the Geometric Framework for Nutrition was utilized to assess the influence of macronutrient and energy intakes on hepatic and plasma FGF21 levels (121). The framework was composed of 25 diets varying in macronutrient (carbohydrate, protein, and lipid) and energy density. This study found that FGF21 was elevated robustly by low protein and that this induction was maximized when low-protein intake was coupled with high-carbohydrate intake (121). While FGF21 may be induced by excess carbohydrate to suppress simple sugar intake and prevent liver injury, it may be maximally induced when carbohydrate consumption progresses to a point of low protein availability. Thus, this FGF21 signaling pathway may be utilized to prevent liver damage as well as to promote the protein leverage effect.

2.5. Alcohol, FGF21, and Liver Function

Similar to excess fructose consumption, binge ethanol intake can be a source of liver damage that may progress to fatty liver disease. Acute binge consumption of ethanol significantly increases plasma FGF21 levels in both rodents and humans (26), and FGF21 protects against alcohol-induced liver injury. Mice that lack FGF21 develop marked liver steatosis and injury in response to chronic alcohol consumption despite being able to clear ethanol as effectively as wild-type controls (26). *Fgf21* knockout mice exhibit increased hepatic lipogenesis and decreased β -oxidation following alcohol exposure (83). In addition, FGF21 regulates alcohol-induced adipose tissue lipolysis (149). Importantly, pharmacological administration of FGF21 for 5 days to wild-type mice that were chronically fed alcohol significantly attenuated hepatic steatosis and liver injury (83).

FGF21 also regulates alcohol intake. The *FGF21* rs838133 variant associates with increased alcohol intake (40), and the rs11940694 SNP in the β -Klotho (*KLB*) locus associates with alcohol consumption (116). Interestingly, administering recombinant FGF21 (116) or the

overexpression of FGF21 (125) significantly reduced alcohol preference but not plasma ethanol concentrations. This effect of FGF21 is lost in mice lacking β -klotho in the central nervous system (CNS; *Klb^{fl/fl};CamK2a-Cre*) (116). FGF21 may mediate these effects by altering dopamine signaling (125), but additional studies are necessary to determine the potential mechanism for these effects. Finally, as would be predicted from the human SNP data, *Klb^{fl/fl};CamK2a-Cre* mice consume more alcohol and prefer alcohol more than wild-type control mice (116).

While FGF21 decreases alcohol intake, it should be noted that the effect on alcohol preference is impacted by the fact that FGF21 administration also increases total fluid intake through increased water intake in the presence of alcohol (125). This effect of FGF21 to increase total fluid intake, and thus preference, was not observed in sucrose preference studies in lean, wild-type mice administered FGF21 for 1–3 days (133; M.J. Potthoff, unpublished data). An increase in water intake in response to FGF21, however, was observed in lean and diet-induced obese wild-type mice administered FGF21 by minipump for 1 week (14). This increase in fluid intake in response to FGF21 may be due to increased energy expenditure. In rodents, pharmacological levels of FGF21 increase energy expenditure (64, 141), which results in increased food intake (54, 64). Rodents and humans tightly control drinking behavior during feeding to ensure an adequate water supply for food ingestion and digestion (151). This regulation of drinking by eating is termed prandial thirst (151). Increases in total fluid intake in response to FGF21 may be secondary to increased energy expenditure as opposed to a direct regulation of thirst. Alternatively, FGF21-induced thermogenesis may affect visceral sensory information and that could influence thirst (91). A possible explanation for the FGF21-stimulated induction of total fluid intake during alcohol consumption, but not sucrose consumption, may be that sucrose is rewarding and itself increases total fluid intake compared with water alone. In contrast, alcohol consumption can lead to dehydration. Thus, FGF21's effect on fluid intake needs to be examined in a model in which FGF21-mediated increases in energy expenditure are abrogated.

2.6. FGF21 and Adaptive Thermogenesis

One of the earliest physiological functions attributed to FGF21 was its ability to regulate adipose tissue thermogenesis. Since FGF21 administration increases energy expenditure and the browning of adipose tissues in vivo (21, 64, 141), it was hypothesized that FGF21 regulates adaptive thermogenesis in response to cold. *Fgf21* mRNA expression is upregulated in both WAT (36, 52) and BAT (15, 36, 49, 52) in response to cold. However, while an increase in circulating levels of FGF21 has been reported in response to cold in mice (49), this has not been observed in other studies (36, 52). In brown adipocytes, norepinephrine activates β -adrenergic receptors and cyclic adenosine monophosphate (cAMP)-dependent, protein kinase A (PKA)- and p38 mitogen-activated protein kinase (MAPK)-mediated mechanisms to regulate ATF2 binding to the *Fgf21* promoter to induce FGF21 production (Figure 3a) (49). Similar mechanisms may upregulate FGF21 expression in white adipocytes during cold, but FGF21 expression is also regulated by both PPAR γ (32, 49, 94) and PPAR α (49) in white adipocytes. Consistent with its role in regulating thermogenesis, FGF21 treatment is sufficient to increase thermogenic gene expression in

mouse primary white adipocytes (36). In addition, treating human primary neck adipocytes with FGF21 increases both thermogenic gene expression and cellular respiration (76). This has led to the hypothesis that adipose FGF21 may act in an autocrine manner to regulate adipose thermogenesis (Figure 3a).

Consistent with a functional role for FGF21 during cold, both *Fgf21* total knockout mice (36) and adipose-specific *Fgf21* knockout mice (52) exhibit reduced body temperature in response to acute cold exposure. During chronic cold exposure, however, FGF21 is dispensable for adaptive thermogenesis (60). During acute cold, FGF21 signals directly to adipose tissue to regulate adipose thermogenesis by inducing the expression and secretion of the chemokine CCL11 (52). CCL11 then functions to recruit eosinophils to subcutaneous adipose tissues, which promotes the beiging of WAT depots (52). These studies provide a novel pathway linking sympathetic nervous system activation to the activation of type 2 immunity and subcutaneous WAT thermogenesis (Figure 3a). In addition, these studies illustrate another role for FGF21 in maintaining energy homeostasis.

In contrast to the studies supporting a role for adipose-derived FGF21 in regulating thermogenesis, recent work from our lab has found that liver-derived, but not adipose-derived, FGF21 regulates core body temperature during cold exposure. Liver-specific, but not adipose-specific, *Fgf21* knockout mice are susceptible to acute cold exposure (lasting 1–3 days) (M. Ameka, K.M. Markan & M.J. Potthoff, unpublished data). Plasma FGF21 levels are induced acutely from the liver in response to cold exposure and liver-derived FGF21 signals to the CNS to increase sympathetic nerve activity to BAT and regulate core body temperature (Figure 3b) (M. Ameka, K.M. Markan & M.J. Potthoff, unpublished data). Therefore, our data suggest that liver-derived FGF21 regulates energy homeostasis during acute cold exposure by signaling to the CNS to, at least in part, regulate sympathetic nerve activity to BAT. Additional studies are needed to evaluate the relative importance of adipose versus liver-derived FGF21 in regulating energy homeostasis.

2.7. Induction of FGF21 by Cellular and Mitochondrial Stress

Cells are naturally exposed to a wide range of environmental stressors and thus have developed responses to specific stimuli to adapt to these situations. These hazardous stimuli can lead to conditions of mitochondrial stress, autophagy, endoplasmic reticulum stress, or activation of the unfolded protein response. Although FGF21 is induced under conditions of metabolic stress (e.g., fasting, cold), it is also induced under many conditions of cellular stress, leading FGF21 to be referred to in some contexts as a stress hormone (69). For example, FGF21 production from skeletal muscle (56, 61, 103, 128), BAT (59), and cardiac tissue (143) is increased in genetic mouse models of cellular or mitochondrial stress. However, while FGF21 can be induced in extrahepatic tissues in these genetic mouse models, the physiological relevance of this induction is unclear. For example, while chronic feeding of a high-fat diet clearly represents a state of cellular and mitochondrial stress for skeletal muscle, skeletal muscle does not contribute to circulating levels of FGF21 during feeding of a high-fat diet; instead, circulating levels of FGF21 are derived exclusively from the liver under these conditions (86). In addition, in humans in response to exercise, FGF21 is produced from the liver not muscle (46). Thus, it is unclear whether there is a

physiological context for the extrahepatic production of FGF21 or whether this is simply a by-product arising from artificial genetic stress. Nevertheless, these studies demonstrate that extrahepatic tissues have the capacity to contribute to circulating levels of FGF21 and that the activation of pathways to induce FGF21 production from muscle may be a beneficial therapeutic strategy.

3. THE PHARMACOLOGICAL EFFECTS OF FGF21

When administered to diabetic and obese animal models, FGF21 provides multiple metabolic benefits and has been shown to decrease blood glucose levels and body weight, improve insulin and leptin sensitivity, improve plasma lipid profiles, and decrease hepatic steatosis (21, 64, 141, 142). Importantly, in animal models FGF21 treatment not only prevents (64) but also reverses diabetes and obesity (21, 141). Pharmacological administration of FGF21 has both acute and chronic effects on metabolism. A single injection of FGF21 to diabetic and obese animal models is sufficient to decrease plasma glucose levels by more than 50% (142). This effect of FGF21 can be recapitulated in wild-type lean mice through coinjection with insulin (11, 27), suggesting that FGF21 functions to directly enhance insulin sensitivity. In contrast to the acute metabolic effects, repeated daily administration of FGF21 increases metabolic rate, promotes weight loss, and thus secondarily improves insulin sensitivity and glucose homeostasis (141). It is clear that FGF21 treatment elicits significant metabolic changes in many tissues, including the liver and adipose tissues (21, 141). However, whether these changes result from the direct actions of FGF21 on these particular tissues has been greatly debated (63). Thus, significant efforts have been made to identify the primary cellular targets and downstream pathways responsible for the metabolic actions of FGF21. While this topic has been contentious in the past, studies within the past couple of years from multiple groups have revealed the direct target tissues that regulate these specific actions of FGF21. In the following sections, we review the cellular targets of FGF21 action and the mechanisms regulating FGF21 activity.

3.1. FGF21 Signals to Adipose Tissues to Acutely Increase Insulin Sensitivity

The acute pharmacological administration of FGF21 increases insulin sensitivity and lowers plasma glucose levels, primarily by stimulating peripheral glucose disposal, while also slightly reducing hepatic glucose production (27, 142). Multiple lines of evidence implicate adipose tissues as important regulators of these acute effects of FGF21 (reviewed in 88). Initial studies exploring the significance of direct FGF21 signaling to adipose tissues used *ap2-Cre* transgenic mice crossed with mice with a conditional *Klb* (27) or *Fgfr1* allele (4). Both *Fgfr1^{fl/fl};ap2-Cre* and *Klb^{fl/fl};ap2-Cre* mice were refractory to the acute insulin-sensitizing effects of FGF21 (4, 27). However, these studies were confounded by the fact that *ap2-Cre* transgenic mice also express Cre recombinase in nonadipose tissues, including in the CNS (47). Thus, to explore the significance of FGF21 signaling directly to adipose tissues, investigators crossed mice with a conditional *Klb* allele with *Adiponectin-Cre* transgenic mice (11), which drive Cre expression specifically in adipose tissues (75). Consistent with the earlier studies, while FGF21 significantly increased insulin sensitivity in both lean and diet-induced obese wild-type mice, this effect was completely lost in *Klb^{fl/fl};Adiponectin-Cre* mice (11). These findings were subsequently confirmed in a separate study using

hyperinsulinemic–euglycemic clamps (74). Thus, FGF21 signals directly to adipose tissue to acutely increase insulin sensitivity (Figure 4).

There are multiple types of adipocytes in the body that differ in function and arise from distinct lineages. Whereas white adipocytes function to store energy, brown and beige adipocytes expend energy through heat dissipation via activation of the mitochondrial uncoupling protein 1 (UCP1) (108). Multiple studies have demonstrated that FGF21 stimulates glucose uptake in BAT in vivo (11, 27, 86, 115). In addition, FGF21 enhances insulin sensitivity in primary brown adipocytes (86) but not in primary white adipocytes (64, 86). Thus, to determine the relative contribution of direct FGF21 signaling to white versus brown adipocytes, investigators crossed mice with a conditional *Klb* allele with *UCP1-Cre* transgenic mice to ablate FGF21 signaling to brown and beige adipocytes. Notably, as observed with *Klb^{fl/fl};Adiponectin-Cre* mice, mice lacking β -klotho in UCP1⁺ adipocytes (*Klb^{fl/fl};UCP1-Cre*) failed to show enhanced insulin sensitivity in response to acute FGF21 administration (11). Positron emission tomography and computed tomography imaging revealed that acute administration of FGF21 with insulin markedly induced glucose uptake in interscapular BAT compared to insulin alone, and this effect is impaired in mice lacking β -klotho in BAT (11). Together, these data demonstrate that FGF21 signals directly to brown adipocytes to acutely enhance insulin sensitivity (Figure 4).

The exact mechanism for the insulin-sensitizing effects of FGF21 has not been elucidated. Two studies reported that the adipokine adiponectin is induced in response to acute FGF21 administration and that adiponectin is essential for the insulin-sensitizing effects of FGF21 (48, 82). However, more recent studies have challenged these findings by failing to observe increases in plasma adiponectin levels in response to acute FGF21 administration (11, 95). Moreover, in contrast to previous studies, both FGF21 (11) and a bispecific activating antibody for the FGFR1– β -klotho receptor complex (70) were found to efficiently retain their acute insulin-sensitizing effects in adiponectin knockout mice (Figure 4). Instead, insulin sensitization specifically in adipose tissues is required for the acute insulin-sensitizing effects of FGF21 (11). Mice lacking the insulin receptor specifically in adipose tissues (*IR^{fl/fl};Adiponectin-Cre*) do not respond to the acute insulin-sensitizing effects of FGF21 (11). Increased insulin sensitization in adipose tissues results in increased glucose uptake in BAT (11) and attenuation of lipolysis in WAT (5). Thus, FGF21 increases insulin sensitivity in adipose tissues to lower plasma glucose levels.

3.2. FGF21 and the Central Nervous System

One of the main areas of controversy regarding FGF21 biology is the site of action responsible for the beneficial metabolic effects of chronic FGF21 administration on body weight and glucose homeostasis. Multiple studies have implicated adipose tissues, liver, and the CNS as direct targets of FGF21 action (reviewed in 88). The following studies have addressed this controversy and have identified the target tissue mediating the beneficial metabolic effects of FGF21. Last year, it was demonstrated that the acute insulin-sensitizing effects of FGF21 can be dissociated from the chronic metabolic effects of FGF21 (Figure 4). Although FGF21 signals directly to adipose tissues to acutely enhance insulin sensitivity, the chronic effects of FGF21 on body weight and improved insulin sensitivity are retained in

adipose-specific *Klb* knockout mice (*Klb^{fl/fl};Adiponectin-Cre*) (11, 74), demonstrating that direct signaling to adipose tissues is not required for the chronic effects of FGF21 (Figure 4). In addition, the potent effects of FGF21 on lipid metabolism (115) are also present in adipose-specific *Klb* knockout mice (11). Consistent with these results, adipose-specific *Fgfr1* knockout mice (*Fgfr1^{fl/fl};Adiponectin-Cre*) were also recently shown to respond to chronic FGF21 administration similarly to control mice (17) in contrast to the results obtained with *Fgfr1^{fl/fl};ap2-Cre* mice (4). In addition to the loss-of-function studies, independent groups also found that the activation of FGFR1-dependent signaling specifically in adipose tissues is not sufficient to induce the beneficial metabolic effects of FGF21 in vivo (11, 17). Neither the inducible expression of a constitutively active FGFR1 protein specifically in adipose tissue (11) nor antibody-mediated activation of a chimeric human FGFR1 receptor in brown adipocytes (17) is sufficient to increase energy expenditure or affect body weight in vivo.

FGF21 crosses the blood–brain barrier and acts centrally to regulate multiple processes (50). It is now clear that the chronic metabolic effects of FGF21 and FGFR1 agonists are mediated through actions in the CNS. The chronic metabolic effects of FGF21 on body weight and glucose homeostasis are lost when β -klotho is deleted from the CNS (*Klb^{fl/fl};Camk2a-Cre*) (101, 126). In contrast, the loss of β -klotho in hepatocytes (74) or adipocytes (11, 74) does not impair FGF21-mediated effects on body weight. Consistent with recombinant FGF21 administration, the administration of a bispecific FGFR1– β -klotho agonist to diet-induced obese wild-type mice promotes weight loss and improves glucose homeostasis, effects that are lost when β -klotho is removed from the CNS (74). FGF21 administration increases sympathetic nerve activity to BAT (101) and WAT (28), and browning of WAT depots (28, 101). Interestingly, FGF21-mediated increases in sympathetic nerve activity to BAT appear to require hypothalamic corticotropin-releasing hormone. The administration of a corticotropin-releasing hormone antagonist blocks FGF21-mediated increases in sympathetic nerve activity to BAT (101). Together, these data demonstrate that the chronic metabolic effects of FGF21 are mediated through effects in the CNS (Figure 4).

Although direct FGF21 signaling to adipose tissues is not required for its chronic effects on metabolism, adipose tissue is still important for mediating the metabolic effects of FGF21 (Figure 4). Lipodystrophic mice, which lack mature adipocytes, are refractory to the metabolic effects of FGF21 (131) and FGFR1-based agonists (38). It is unclear whether this is due to the loss of adipose tissue metabolism or to the loss of an adipose-derived factor. Extended FGF21 administration increases energy expenditure, promotes lipid and glucose uptake in BAT, and increases adipose tissue metabolism (88). However, while adipose tissues are critical for these metabolic effects, UCP1 is dispensable for FGF21-mediated increases in energy expenditure (17, 85, 111, 132). Studies within the past few years have demonstrated that adipose tissue depots possess alternative futile cycles for increasing energy expenditure independent of UCP1 (10, 58). Thus, compensatory adipose tissue thermogenic pathways may be utilized in the absence of UCP1 to maintain energy homeostasis. In addition to centrally activated adipose-tissue thermogenesis, adipose tissues may contribute to FGF21-mediated increases in energy expenditure through the production of adipokines such as leptin. While lipodystrophic mice do not respond to FGF21 administration, the transplantation of WAT from wild-type mice increases leptin levels and

restores FGF21 responsiveness (131). In addition, disrupting leptin signaling using ob/ob or db/db mice abrogates the beneficial effects of FGF21 in lowering body weight in response to extended FGF21 administration (3, 45, 51, 67, 139). Finally, FGF21 administration enhances leptin-mediated decreases in body weight (96, 131). Thus, future studies are needed to elucidate the mechanism of the CNS–adipose circuit (or circuits) targeted by FGF21 that regulates energy homeostasis.

3.3. Mechanisms Regulating FGF21 Activity

Numerous studies have explored the mechanisms regulating FGF21 induction, but much less is known regarding the mechanisms regulating FGF21 activity. Circulating levels of FGF21 are significantly elevated in humans during pathophysiological states, including type 2 diabetes (16, 18), obesity (22, 30, 93), hepatosteatosis (30, 79, 146), pancreatitis (117, 137), and mitochondrial dysfunction (78). These elevations in circulating FGF21 levels are paradoxical, given the potent pharmacological actions of FGF21. However, although levels of FGF21 are increased during these different states, the form of FGF21 in circulation may actually be nonfunctional due to proteolytic processing. Alternatively, FGF21 signaling may be impaired by some pathological stimulus that results in FGF21 resistance, similar to insulin or leptin resistance. Indeed, obesity has been proposed to be a FGF21-resistant state (34). However, the concept of FGF21 resistance is controversial, with another group failing to observe FGF21 resistance in mouse models of obesity and insulin resistance (45). A proposed mechanism for FGF21 resistance is the marked downregulation of β -klotho protein expression observed in WAT during obesity. Although one study found that overexpression of β -klotho in adipose tissues provided protection against diet-induced weight gain via increased energy expenditure (109), another study found that maintaining physiological β -klotho expression levels in adipose tissues during diet-induced obesity failed to prevent weight gain, glucose intolerance, or insulin resistance (87). Since adipose tissues are not the target tissue responsible for chronic FGF21-mediated effects on body weight and glucose homeostasis (11, 17, 74), it seems likely that changes in adipose tissue β -klotho levels would not affect systemic FGF21 sensitivity. Additional studies are needed to examine the existence and etiology of FGF21 resistance.

In addition to regulating FGF21 sensitivity, FGF21 activity could also be regulated by altering its stability or half-life. The half-life of FGF21 is approximately 0.4–2 hours, depending on the species and method of delivery (51, 65, 142). Circulating human FGF21 is composed of 181 amino acids and is proteolytically cleaved, and inactivated, between Pro-171 and Ser-172 by the endopeptidase fibroblast activation protein (FAP) (29, 150). The deletion of these 10 residues on the C terminus of human FGF21 disrupts its ability to interact with β -klotho and signal to target tissues (89, 144). Interestingly, mouse FGF21 is neither cleaved nor inactivated by FAP because mouse FGF21 has a glutamate residue at the corresponding glycine residue at position 170, which disrupts the stringent glycine–proline sequence required for FAP-mediated cleavage (29, 150). In humans, however, it is unclear what percentage of circulating FGF21 is active during disease states (e.g., obesity, diabetes). Since FGF21 is cleaved and inactivated by FAP, the administration of FAP inhibitors may represent a therapeutic strategy to potentiate FGF21 function *in vivo* by prolonging the endogenous half-life (43, 113).

FGF21 levels can also be regulated by adipose-derived exosomes. Although circulating levels of FGF21 are derived from the liver, adipose-derived circulating microRNAs are packaged into exosomes and regulate plasma FGF21 levels (129). Since FGF21 acts directly and indirectly on adipocytes, this may be a potential feedback mechanism for regulating FGF21 signaling in vivo.

4. TRANSLATING THE METABOLIC EFFECTS OF FGF21

Multiple FGF21 analogs have been developed or are in development for treating metabolic disease (44). Clinical trials using FGF21 analogs have demonstrated the therapeutic potential of this pathway in treating obesity and diabetes. A study from Eli Lilly and Company was the first to report the effects of an FGF21 analog, LY2405319, in obese subjects with type 2 diabetes. Administering this analog for 28 days resulted in decreased body weight, improved lipid profiles, lowered fasting insulin levels, and a trend toward reduced plasma glucose levels (41). A subsequent clinical trial, performed by Pfizer Inc., used the FGF21 analog PF-05231023 in subjects with type 2 diabetes (126). PF-05231023 significantly decreased body weight and markedly reduced plasma cholesterol and lipid levels. However, unlike the previous study, PF-05231023 had no significant effect on plasma insulin or glucose levels (126). Notably, this FGF21 analog also significantly reduced body weight and metabolic profiles in nonhuman primates, but did so by reducing their food intake rather than by increasing the browning of subcutaneous WAT (126). Thus, while the effects of FGF21 may translate to humans, the mechanism for the effects may be species specific (104). Since humans possess relatively less BAT than rodents, adipose tissue thermogenesis may contribute less to overall energy homeostasis in humans. Instead, FGF21 may activate a central circuit that regulates energy homeostasis in both species, manifesting functionally as a change in food intake in humans and nonhuman primates, but as an increase in energy expenditure in rodents.

While FGF21-based therapies have beneficial effects, they may also cause adverse effects. One controversial concern is whether FGF21 affects bone loss. In mice, FGF21 has been reported to promote bone loss (134, 135); however, this finding could not be replicated (80). When PF-05231023 was administered to obese cynomolgus monkeys, it decreased body weight and mean abdominal fat content but did not affect bone mineral content (126). In humans, PF-05231023 treatment resulted in changes in markers of bone turnover (126). However, it is unclear whether this is a direct effect of FGF21 or an indirect effect on bone turnover that is known to occur with weight loss. In the same study, PF-05231023 did not affect systolic or diastolic blood pressure, pulse rate, or electrocardiogram readings. Instead, the most frequent adverse effect was diarrhea (126). A subsequent clinical study was conducted with PF-05231023 in obese participants with hypertriglyceridemia who were taking atorvastatin, with or without type 2 diabetes (66). Similar to the previous study (126), once-weekly administration of PF-05231023 markedly improved plasma lipid profiles without significantly affecting fasting plasma glucose levels (66). However, in contrast to the previous study, no significant changes in body weight were observed (66). In addition, the latter study found that PF-05231023 caused multiple adverse effects. The majority of adverse effects were gastrointestinal, but unlike the previous study, systolic and diastolic

blood pressure, and pulse rate, increased in a dose-dependent manner (66). Interestingly, similar effects on blood pressure were observed in rats but not in monkeys (66).

FGF21 has also been shown to affect female reproduction (100, 119). Female mice that over-express FGF21 are infertile, but whether this is a direct effect of FGF21 on fertility or rather a secondary consequence of FGF21-induced energy expenditure and relative caloric insufficiency has been disputed. One study found that FGF21 signals to the brain to suppress vasopressin–kisspeptin signaling, which impairs the proestrus surge in luteinizing hormone (100). However, a separate study suggests that this phenotype is due to caloric insufficiency from chronic energy expenditure (119). While *Fgf21* transgenic mice are infertile on a chow diet, this infertility is reversed by high-fat diet feeding (119). In addition, the direct infusion of FGF21 into the brain at concentrations sufficient to acutely increase energy expenditure failed to alter the estrus cycle (119), suggesting that FGF21 may not function acutely to regulate female fertility. Future studies are necessary to determine whether FGF21-based therapies affect female fertility.

5. CONCLUSIONS

After more than a decade of research, tremendous progress has been made in identifying the physiological actions of FGF21 and assessing its therapeutic potential to treat metabolic disease. Going forward, additional work is necessary to determine the mechanisms for both the beneficial and adverse effects of FGF21. Since FGF21 increases sympathetic nerve activity (28, 101) and affects blood pressure (66), it is unclear whether FGF21 will suffer the same fate as previous sympathomimetics that promoted weight loss at the cost of cardiovascular complications (57). As for the gastrointestinal issues, extended exposure to elevated levels of FGF21 has been shown to increase bile acid biosynthesis by opposing the actions of another endocrine FGF, FGF15/19 (147). Additional studies are needed to determine whether bile acid–induced diarrhea (13) is the cause of the gastrointestinal issues in humans associated with the administration of FGF21 analogs. Finally, it is now evident that the metabolic effects of FGF21 that lower body weight and improve carbohydrate and lipid homeostasis are mediated through actions on the CNS. In addition, FGF21 regulates macronutrient preference, sweet taste preference, and alcohol intake through central mechanisms. Therefore, the future of FGF21 lies in unraveling the complex neural networks mediating the effects of this therapeutically promising endocrine factor.

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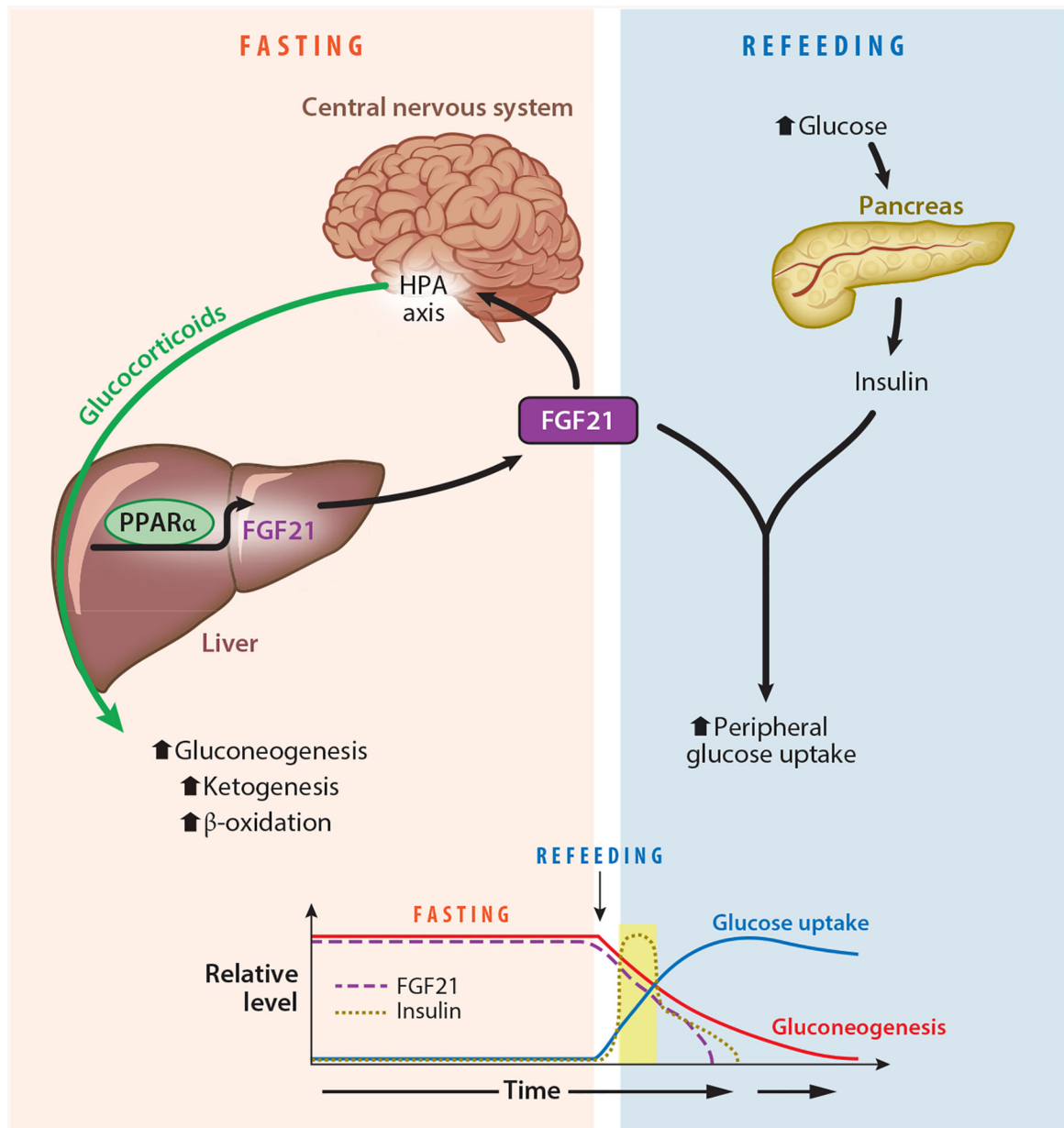


Figure 1. The role of fibroblast growth factor 21 (FGF21) during fasting and refeeding. During fasting, FGF21 expression is induced by peroxisome proliferator-activated receptor- α (PPAR α) in the liver. Circulating FGF21 then activates the hypothalamic-pituitary-adrenal (HPA) axis to increase glucocorticoid release and increase gluconeogenesis. When nutrients become available during refeeding, insulin is released from the pancreas, and while both FGF21 and insulin are in circulation, FGF21 functions to enhance insulin-stimulated glucose uptake in peripheral tissues.

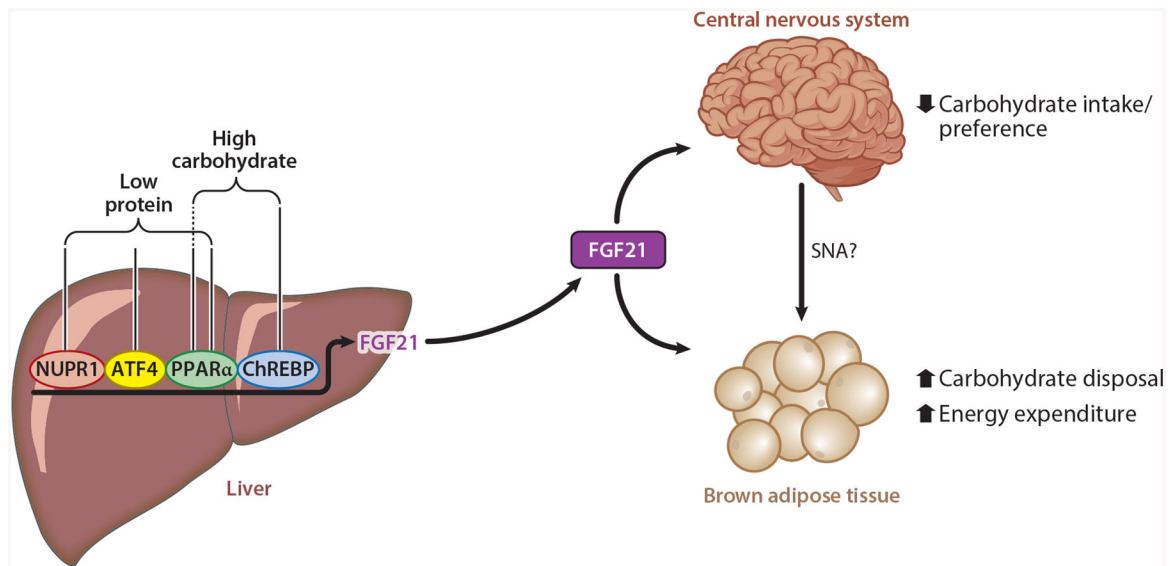


Figure 2.

Nutritional regulation of FGF21 production. The production of circulating levels of FGF21 is regulated by PPAR α and ChREBP in response to high carbohydrate levels and by ATF4 and PPAR α in response to amino acid starvation in the liver. NUPR1 is also critical for protein dilution–mediated increases in hepatic FGF21. In response to these nutritional cues, circulating FGF21 decreases carbohydrate intake through actions on the central nervous system. In addition, FGF21 enhances carbohydrate disposal by direct action on brown adipose tissue, and increases energy expenditure through central nervous system–mediated regulation of adipose tissue function. Abbreviations: ATF4, activating transcription factor 4; ChREBP, carbohydrate-response element-binding protein; FGF21, fibroblast growth factor 21; NUPR1, nuclear protein 1; PPAR α , peroxisome proliferator–activated receptor- α ; SNA, sympathetic nerve activity.

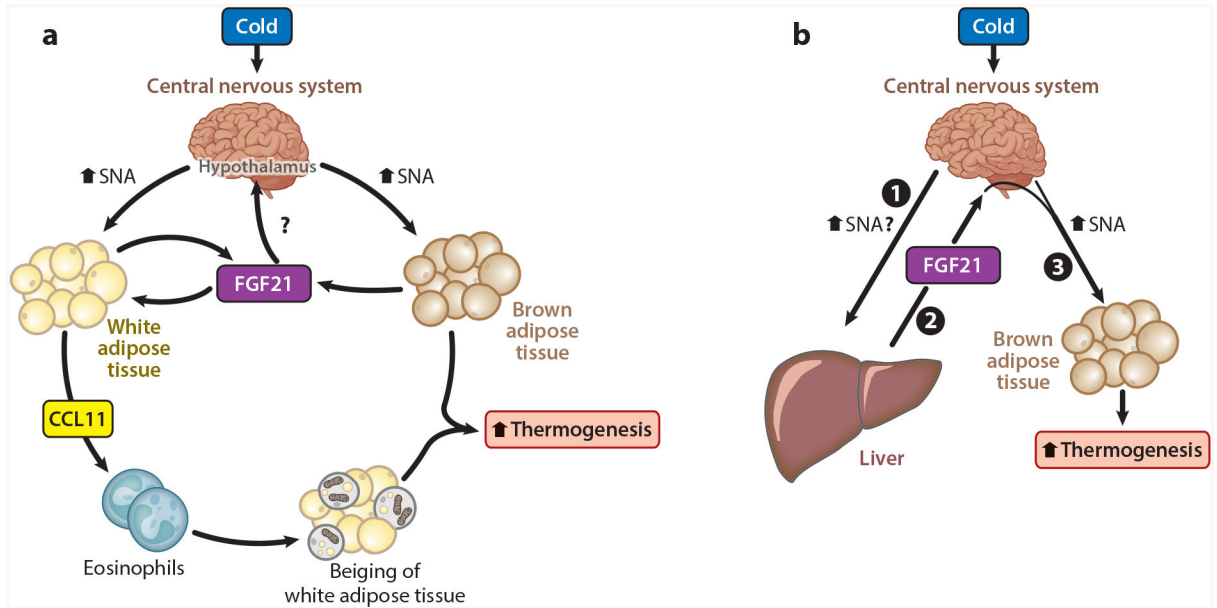


Figure 3.

Fibroblast growth factor 21 (FGF21) coordinates adipose tissue thermogenesis during acute cold. Two models are shown for the physiological regulation of energy homeostasis by FGF21 during acute cold exposure. (a) Exposure to cold stimulates sympathetic nerve activity (SNA) to adipose tissues. Increased SNA to brown adipose tissue increases thermogenesis and the production of FGF21, which enhances thermogenesis. Increased SNA to white adipose tissue stimulates FGF21 to act in an autocrine fashion on adipose tissue to induce the production of the cytokine CCL11. CCL11 recruits eosinophils and macrophages to promote the beiging of subcutaneous white adipose tissue and increase adipose tissue thermogenesis. (b) During acute cold exposure, FGF21 production from the liver is increased through an unknown mechanism, but it may involve central nervous system (CNS)–mediated sympathetic output to the liver (1). Elevated circulating levels of FGF21 (2) then act centrally to increase sympathetic nerve output to drive brown adipose tissue thermogenesis (3).

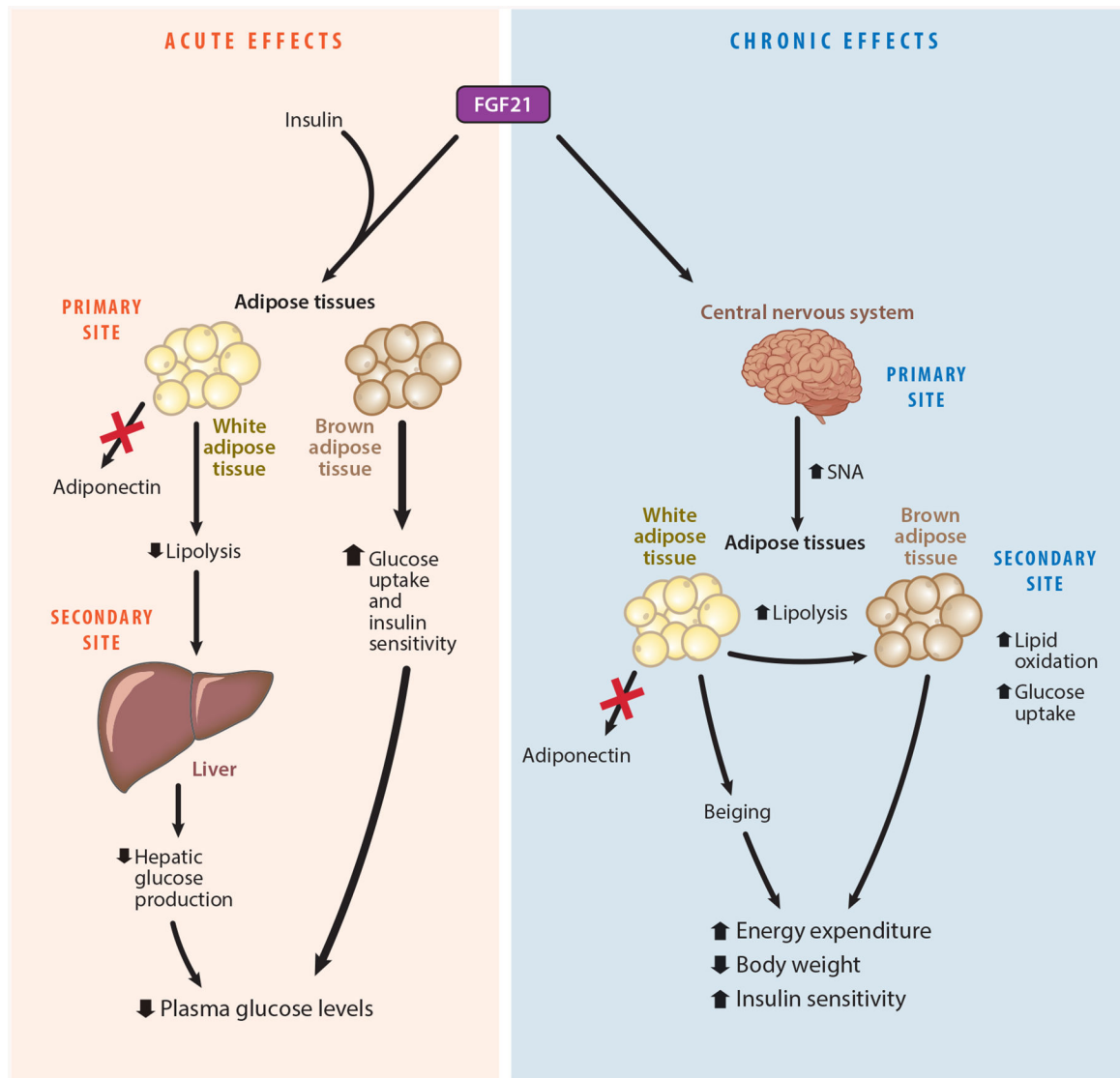


Figure 4.

Dissociation of the pharmacological actions of fibroblast growth factor 21 (FGF21). FGF21 has acute and chronic effects on metabolism. Primary, or direct targets, and secondary sites of FGF21 action are indicated. The acute insulin-sensitizing effects are mediated through direct signaling to adipose tissue in the presence of insulin signaling, but do not require adiponectin (*red X*). FGF21 signaling to brown adipose tissue is primarily responsible for the acute glucose-lowering effect of FGF21. The chronic effects of FGF21 are mediated through direct signaling in the central nervous system (CNS). The activation of specific FGFR1 and β -klotho neurons by FGF21 leads to increased energy expenditure through increased sympathetic nerve activity (SNA) to brown adipose tissue and the beiging of white adipose tissue. These effects result in decreased body weight and a secondary improvement in insulin sensitivity. figure adapted from Reference 11.