Understanding the Physiology of FGF21

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Abstract

Fibroblast growth factor 21 (FGF21) is a peptide hormone that is synthesized by several organs and regulates energy homeostasis. Excitement surrounding this relatively recently identified hormone is based on the documented metabolic beneficial effects of FGF21, which include weight loss and improved glycemia. The biology of FGF21 is intrinsically complicated owing to its diverse metabolic functions in multiple target organs and its ability to act as an autocrine, paracrine, and endocrine factor. In the liver, FGF21 plays an important role in the regulation of fatty acid oxidation both in the fasted state and in mice consuming a high-fat, low-carbohydrate ketogenic diet. FGF21 also regulates fatty acid metabolism in mice consuming a diet that promotes hepatic lipotoxicity. In white adipose tissue (WAT), FGF21 regulates aspects of glucose metabolism, and in susceptible WAT depots, it can cause browning. This peptide is highly expressed in the pancreas, where it appears to play an anti-inflammatory role in experimental pancreatitis. It also has an anti-inflammatory role in cardiac muscle. Although typically not expressed in skeletal muscle, FGF21 is induced in situations of muscle stress, particularly mitochondrial myopathies. FGF21 has been proposed as a novel therapeutic for metabolic complications such as diabetes and fatty liver disease. This review aims to interpret and delineate the ever-expanding complexity of FGF21 physiology.

INTRODUCTION

Energy homeostasis in mammals is a regulated physiological process involving input of calories and expenditure of energy (1). A mismatch of these two processes in favor of excess net calories results in obesity, which is now a major health problem. In the United States, 30% of the population is obese, with a body mass index (BMI) of greater than 30, while 30% is overweight, with BMI ranging between 25 and 29.9. Excess weight is a problem because of the associated comorbidities, such as type 2 diabetes, cardiovascular disease, nonalcoholic fatty liver disease (NAFLD), and increased risk of multiple cancers such as breast and colon cancer. Multiple factors are needed to maintain energy homeostasis, including factors from the periphery as well as leptin and ghrelin from the CNS. Factors include neuropeptides such as agouti-related peptide, melanocortin-stimulating hormone, neuropeptide Y, and melanocortin-concentrating hormone and monoamine neurotransmitters such as serotonin. Fibroblast growth factor 21 (FGF21) is a fairly recent entry to the list of factors controlling energy homeostasis. Although FGF21 does not affect food intake, it significantly increases energy expenditure under specific stimuli. As is outlined in more detail below, the physiology of FGF21 is quite complex largely because it is synthesized in multiple organs and can act on multiple target tissues (**Table 1**) in either a paracrine or an endocrine fashion (**Figure 1**). Furthermore, the molecular mechanism of FGF21 signaling is complex and involves several FGF receptors (FGFRs) as well as an obligate coreceptor, β-klotho (KLB). Tissue specificity of FGF signaling is conferred by the coexpression of a given FGF receptor and KLB. To better understand the biology of FGF21, investigators have leveraged murine models. Several studies have administered FGF21 to both normal and obese animals and have manipulated both diet and environment to increase FGF21 expression. Genetic loss- and gain-of-function studies in mice using global or conditional gene deletion and transgenic overexpression have also been instrumental for understanding FGF21 biology. Collectively, several studies have established that exogenous FGF21 has beneficial metabolic effects, including weight

Table 1 Overview of FGF21 in animal models

Tissue	Stimulus	Transcriptional elements	Signal	Target tissue	Effect
Liver	Fasting, ketogenic diet, MCD diet, sucrose, amino acid deficiency	PPARα, ChREBP, FXR, ATF4	Endocrine, paracrine?	Liver, adipose tissue, heart, brain	↑FFA oxidation, ↓inflammation, ↑uncoupling, ↓cardiac hypertrophy, ↑glucose tolerance, ↑energy expenditure, ↓fertility
BAT	Cold exposure	ATF2	Autocrine, paracrine	BAT	↑Uncoupling
IWAT	Cold exposure	ATF2	Autocrine, paracrine	IWAT	↑Uncoupling
Gonadal WAT	TZD, chow refeed	PPARγ	Autocrine, paracrine	Gonadal WAT	↑Adipocyte differentiation and expansion
Muscle	Myopathy, oxidative stress	ATF4	Endocrine	Adipose tissues	↑Uncoupling
Pancreas	Pancreatitis	MIST1	Autocrine, paracrine, endocrine	Pancreatic acinar tissues	↓Inflammation, ↑islet survival
Heart	Cardiomyopathy	MYoD/ATF2	Autocrine, endocrine	Heart	↓Cardiac hypertrophy ↓Oxidative stress

Abbreviations: BAT, brown adipose tissue; FFA, free fatty acid; IWAT, inguinal white adipose tissue; TZD, thiazolidinedione; WAT, white adipose tissue.

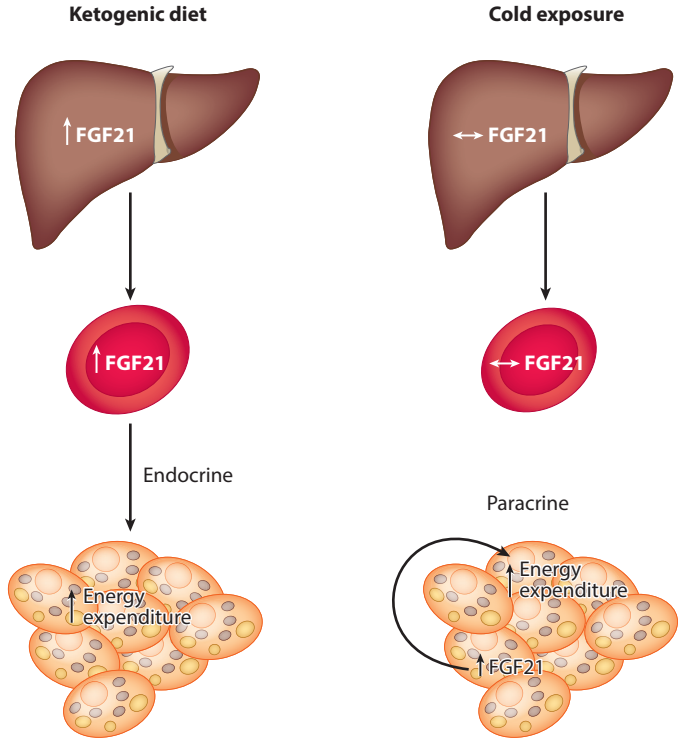

Figure 1

Two example physiological states in which FGF21 exerts its effects through either endocrine or paracrine regulation. In the liver, consumption of a ketogenic diet increases Fgf21 expression, leading to substantial increases in serum levels. This circulating Fgf21 increases energy expenditure through thermogenic activation of adipose tissue. In contrast, cold exposure leads to localized increases of Fgf21 in adipose tissue, resulting in autocrine activation without any changes in systemic Fgf21 levels.

loss and resolution of hepatic triglyceride accumulation. Consistent with these findings, hepatic Fgf21 expression and serum levels increase with either fasting, consumption of a ketogenic diet, or protein and amino acid restriction in mice. In humans, neither a ketogenic diet nor fasting affects FGF21, whereas fructose ingestion leads to a rapid and robust increase in circulating FGF21. Paradoxically, obesity also increases serum FGF21 in both mice and humans, most likely as a result of increased fatty liver. This apparent increase in circulating levels of FGF21 indicates that obesity leads to an FGF21-resistant state. In the last 10 years, the potential of FGF21 signaling as an antiobesity target has prompted new strategies to develop a broad range of therapeutic agents that include protein analogs, pegylated derivatives, and fused bimolecular constructs of FGF21.

GROWTH FACTORS

The FGF family encompasses a large number of factors that are involved in diverse actions such as cell growth, cell differentiation, and embryonic development (2). Most FGFs are small, with a molecular weight range of 17 to 34 kDa. Signaling is propagated after FGF peptides bind to one of several tyrosine kinase receptors (FGFRs) that are expressed on the plasma membrane. Heparin sulfate proteoglycan is required for receptor-ligand interaction, with all except hormonal

FGFs possessing a heparin-binding domain. Ligand binding induces several known signaling cascades, including Ras/MAPK (mitogen-activated protein kinase), phosphatidylinositol 3-kinase (PI3K)/Akt, and protein kinase C (PKC). Phylogenetically, FGF genes are found in a wide variety of organisms (3). For example, four FGF-like genes have been described in beetles (coleopterans) and *Drosophila*, and ancestral genes have been described in *Caenorhabditis elegans*. Genomic analyses indicate that many FGFs arose from gene duplications prior to the appearance of tetrapods (3). FGFs have been divided into seven subfamilies, although some researchers partition FGF3 into its own unique subfamily (2). Hormonal FGFs compose one of these subfamilies and include three FGFs: FGF19 (and the mouse ortholog Fgf15), FGF21, and FGF23. As mentioned above, members of this subfamily are distinct, as they lack a heparin-binding domain and require an obligate coreceptor for signaling: KLB in the case of FGF19 and -21 and klotho for FGF23. Fgf15/FGF19 is produced in the ileum and regulates bile acid synthesis and secretion, whereas FGF23 is made predominantly in bone and regulates phosphate metabolism. FGF21 is more broadly expressed and has diverse metabolic actions, contributing to its complexity, which is discussed below.

GENETICS AND BIOCHEMISTRY

In humans, *FGF21* (gene ID 26291) is on chromosome 19 (19q13.33) and contains three exons that encode a protein of 208 amino acids. For humans, more than 40 single-nucleotide polymorphisms (SNPs), most of which are synonymous, have been reported. A few notable exceptions of potential functional significance include SNP rs838145, which is associated with higher serum FGF21 levels (4), and SNP rs838133, which is associated with increased protein consumption (5). In mice, *Fgf21* (gene ID 56636) is located on chromosome 7.

The mammalian *FGF21* gene is highly conserved. Indeed, there is a difference of only one or two amino acids between humans and gorillas or humans and chimpanzees, respectively, and there is nearly 80% homology between human and rodent FGF21s. FGF21 is also found in other vertebrates, including fish and reptiles.

FGF21 AS A METABOLIC REGULATOR

In 2000, the gene encoding Fgf21 was first identified in embryonic mouse embryo by using RT-qPCR and degenerate primers from human *FGF19* (6). The highest level of *Fgf21* expression in mouse adult tissues was in the liver. Subsequent homology searches using mouse *Fgf21* revealed a highly conserved human ortholog. Possible roles for this new FGF remained undefined until 2005, when FGF21 exhibited bioactivity and stimulated Glut1-mediated glucose uptake in 3T3L1 adipocytes (7). FGF21 infusions were also found to be effective in lowering serum glucose and increasing insulin sensitivity in two genetic murine models of obesity: the *ob/ob*, *db/db* mouse and the Zucker diabetic fatty rat. At the same time, overexpressing human FGF21 in transgenic mice decreased body weight and increased resistance to diet-induced obesity (DIO) on a high-fat, high-sucrose diet. Pharmacological treatment with human FGF21 also led to weight loss in mice with DIO. In contrast, food intake in FGF21-treated animals did not change compared with saline-treated controls. Thus, the FGF21-induced weight loss in DIO mice resulted from increased energy expenditure (8, 9). Consistent with these findings in mice, FGF21 treatment in diabetic rhesus monkeys also led to improved metabolic parameters, including decreased serum lipid and glucose levels (10). Collectively, these studies established that increasing FGF21 levels improves metabolic parameters in obese and diabetic animal models.

Two important subsequent studies increased our understanding as to how FGF21 is regulated. These findings established that hepatic Fgf21 expression is upregulated after fasting or

consumption of a ketogenic diet and by the transcription factor PPARα. Hence, treatment with PPARα agonists—including fenofibrate, Wy-14643, and GW7647—substantially increases hepatic FGF21 expression (11, 12). PPARα is also essential for the fasting response, as mice lacking Pparα showed no increase in Fgf21 expression (12). Nevertheless, these mice had an attenuated response to consumption of a ketogenic diet, suggesting that other transcription factors might also regulate hepatic Fgf21 (11). Adenovirus-mediated knockdown of Fgf21 in livers of mice consuming a ketogenic diet led to significant pathology, including fatty liver, and severe serum hypertriglyceridemia led to reduced hepatic fatty acid oxidation. Liver-specific Fgf21 knockdown also led to impaired assembly and export of VLDL particles, a major pathway by which triglycerides are cleared from the liver. Consistent with a role of Fgf21 in the adaptive response of mice to a ketogenic diet, which includes weight loss and increased energy expenditure despite normal caloric intake, *Fgf21*$^{-/-}$ mice have an atypical response, gaining rather than losing weight (13).

FGF21 SIGNALING

Like FGF19 and -23, FGF21 binds to FGFRs with extremely low affinity, and effective binding and signal transduction require interaction with a coreceptor, KLB. KLB binds FGF21 at the C terminus, resulting in an FGFR/KLB complex (14, 15). Interaction with the FGFR occurs through the N terminus of FGF21 (16). Thus, both termini of FGF21 must be intact for FGFR activation to occur (17, 18). KLB expression is essential for FGF21 signaling and is the primary determinant of tissue specificity. Mice engineered with genetic deletion of *Klb* are refractive to the pharmacological actions of FGF21 in both transgenic overexpression (19) and exogenous treatment paradigms (20). Expression of Klb and specific FGFRs in mice is tissue specific and regulated (21, 22). In mice, Klb is expressed in all adipose tissue depots (21), the liver (9), the exocrine pancreas (23) and endocrine pancreas (24), and the suprachiasmatic nucleus (SCN) (25) and the paraventricular nucleus (26) of the hypothalamus. As such, all these known Klb expression sites are implicated as sites of Fgf21 action. In humans, KLB is only minimally characterized, although SNPs in KLB are associated with higher BMI (27) and accelerated colon transit (28).

FGF21 activates multiple FGFRs, including serotypes FGFR1c, -2c, -3c, and -4 (14, 15, 29). Of these, FGFR1 has the highest affinity for FGF21. The C terminus of FGF21 interacts with KLB and the N terminus of FGF21 interacts with the D2 and D3 immunoglobulin-like domains of the FGFR1 receptor (16). In addition, photobleaching experiments have found that KLB and FGFR1 aggregate to form heterodimeric complexes on the cell membrane at a 1:1 ratio. In the presence of FGF21, the FGFR1 receptor dimerizes and binds at a ligand:receptor ratio of 1:2 (30). Deletion of *Fgfr1* in the adipose tissue of mice attenuates many, but not all, of the metabolic pharmacological actions of FGF21, suggesting that Fgfr1 is the preferential FGFR for FGF21 in vivo (31). However, studies examining the importance of FGFRs in mediating the native physiological effects of FGF21 or the hepatic effects of FGF21 on lipid metabolism have not been performed.

Analyzing FGF21 signaling in the liver is complicated, as KLB is also critical to the action of human FGF19 (mouse ortholog Fgf15), which binds and activates FGFR4 to regulate bile acid metabolism (15, 32). Indeed, the preferential binding of FGF19 to FGFR4 and that of FGF21 to FGFR1 and other receptors are believed to tailor the specific functional response of the liver to these hormones. Thus, FGF19 acting through FGFR4 leads to proliferative cell growth. Indeed, FGFR4 has been positively associated with hepatoma; however, driving increased expression of KLB seems to overcome this association and is protective of proliferation (33), possibly by promoting FGF21 signaling. On the basis of these studies, FGFR4-inhibitory antibodies are being investigated for treatment of hepatocellular carcinoma (34). Similar strategies are being undertaken to target the KLB/FGFR1 complex in metabolic disorders, which would presumably bypass the less

desirable proliferative effects of FGFR1 by selectively targeting FGFR1 only when complexed with KLB. In mice, this approach has been successful, with at least one monoclonal antibody replicating the pharmacological effects of FGF21 in vivo (35).

As with most other FGFs, FGF21 activates signaling through the Ras/Raf MAPK signaling pathway. Upon ligand-receptor interaction, the FGFR dimerizes and autophosphorylates receptor tyrosine residues. This action facilitates binding and activation of the docking protein FGFR substrate 2 alpha (FRS2α). FRS2α transduces FGF signals to MAPK signaling cascades via the recruitment of several adaptor molecules. Extracellular signal–related kinase 1 (ERK1) and ERK2 are critical for FGF signaling, initiating the transcription of immediate early genes. In vitro studies using 3T3L1 adipocytes determined that within minutes FGF21 administration leads to the recruitment and activation of MAPK and PI3K signaling pathways through FRS2α (36). Indeed, inactivating ERK signaling attenuates the ability of FGF21 to acutely induce glucose uptake in adipocytes. However, inhibiting ERK signaling does not always attenuate the biological effects of FGF21, and studies are ongoing to delineate other potential novel signaling pathways recruited by FGF21 (37).

Even though the actions of FGF21 lead to improved metabolic profiles, FGF21 levels are greatly increased in obese humans (38) and mice (39), leading to the suggestion that obesity may give rise to an FGF21-resistance state (40). Consistent with this hypothesis, significant reductions in KLB expression have been found in the adipose tissue of obese mice, impairing FGF21 signaling (37, 40). In adipocytes, TNF-α-induced inflammation reduces KLB expression and diminishes the response of the cells to FGF21 (41). Hypoglycemia similarly reduces KLB levels in pancreatic islets in mice (42). The altered expression of KLB in different metabolic states may explain some of the differential responses observed in obese mice. However, in the studies described above, relatively low pharmacological doses of FGF21 were used to assess resistance to endogenous FGF21. At higher doses, FGF21 overcomes this apparent resistance and is effective in signaling (8, 43). If lower KLB expression contributes to resistance, targeting KLB and increasing its expression are intriguing therapeutic options. In fact, both glucagon-like peptide 1 (44) and thiazolidinediones (TZDs) (45) enhance KLB expression in vivo and may enhance FGF21 sensitivity. This strategy is especially relevant in the adipose tissue of obese humans, where FGF21 receptor expression is reduced (46).

LIVER

The liver emerged early on as a potential site of FGF21 action, after hepatic FGF21 was identified as a downstream target of PPARα. PPARα is activated by fatty acids during both fasting and consumption of a ketogenic diet (11, 12, 47) and regulates the transcription of many enzymes mediating fatty acid oxidation and ketosis (11). Mice lacking Fgf21 have an atypical response to a ketogenic diet: They gain, rather than lose, weight; they fail to increase energy expenditure; and importantly they develop substantial fatty liver, consistent with impaired fatty acid oxidation (13). Subsequently, fatty liver resulting from either genetic obesity or DIO is associated with increased hepatic expression and serum levels of Fgf21, presumably as a result of Pparα induction by fatty acids (40). In the liver, Fgf21 expression is also induced by protein insufficiency, secondary to amino acid deprivation, an effect that is downstream of activating transcription factor 4 (ATF4) (48). Extremely high levels of circulating Fgf21 have been reported in mice consuming diets deficient in methionine (49, 50) or leucine (51), and increased circulating Fgf21 has been found in mice with depletion of plasma alanine levels (52). In the case of ketogenic diets, methionine deficiency appears to be the specific contributor to the rise in FGF21.

Fgf21 also plays a significant role in the hepatic response to the methionine/choline-deficient (MCD) diet. In wild-type mice, consumption of this diet normally leads to fatty liver and eventually

to a histological phenotype consistent with nonalcoholic steatohepatitis (NASH). Consumption is also associated with significant increases in hepatic expression of Fgf21; such increased expression leads to elevated serum levels. *Fgf21*$^{-/-}$ mice have markedly exacerbated accumulation of liver triglycerides (53, 54), which are associated with increased inflammation and fibrosis. This phenotype in *Fgf21*$^{-/-}$ mice is in part due to impaired activation of fatty acids and reduced oxidation (53) as well as increased expression of genes involved in fatty acid uptake and diacylglycerol synthesis (54). Interestingly, a milder phenotype of inflammation and fibrosis associated with fatty liver is seen in *Fgf21*$^{-/-}$ mice consuming a standard obesogenic or high-fat diet. Furthermore, pharmacological treatment of *Fgf21*$^{-/-}$ mice that are consuming an MCD diet with FGF21 leads to resolution of the phenotype, reducing fatty liver and normalizing markers of fibrosis and inflammation (53).

Despite studies showing that hepatic Fgf21 rises in response to different dietary manipulations and that exogenous Fgf21 elicits an anti-inflammatory response in the liver in the context of lipotoxic diets, the precise mechanistic effects of Fgf21 on the liver have been the subject of debate. Under dietary challenges, Fgf21 expression appears to change only in the liver. The hepatocyte is believed to be the major source of circulating FGF21, as deletion in this cell using an albumin Cre results in dramatic reductions in circulating levels of FGF21, and increases in FGF21 serum levels are absent during the fasting response (55). The beneficial effects on the liver may be mediated directly by FGF21 on hepatocytes or, alternatively, through secondary mediators responding to hepatic FGF21 via either adipose tissue or the CNS. The liver as a site of FGF21 action came into question when two papers failed to demonstrate that FGF21 induced hepatic immediate early gene expression through the Erk1/2 pathways. In contrast, signaling responses were readily observed in adipose tissue (15). However, others reported that FGF21 induces Akt phosphorylation in the liver in both lean and *ob/ob* animals, in which FGF21 suppresses glucose production and increases insulin sensitivity (56). In addition, FGF21 is able to induce phosphorylation of Frs2 and Erk1/2 in the liver in both lean and obese animals (21, 40) with a time course and dose response similar to those seen in adipose tissue. In human hepatoma HepG2 cells, FGF21 induces ERK1/2 phosphorylation and suppresses apolipoprotein(a) expression (57). Furthermore, FGF21 is downstream of SIRT in HepG2 cells. SIRT1 is a histone deacetylase that, in part, regulates the adaptive response to caloric restriction in mice. Treatment of HepG2 cells with the SIRT1 agonist resveratrol enhances fatty acid oxidation through induction of FGF21 expression and secretion. This autocrine response occurs in an enclosed in vitro system, further providing evidence that FGF21 may act directly on the hepatocyte (58).

Events downstream of acute signaling that might mediate the FGF21 response are largely unknown. One potential candidate is PGC-1α, a transcriptional coactivator that regulates fatty acid oxidation by enhancing mitochondrial function and biogenesis. Several studies have found that Fgf21 regulates both Pgc-1α mRNA and protein levels in the liver of mice (21, 59). However, this link between Fgf21 and hepatic Pgc-1α remains controversial, as mice with liver-specific deletion of *Pgc-1α* appear to respond normally to exogenous FGF21 (21), whereas an attenuated phenotype is seen in mice with whole-body *Pgc-1α* deletion (59). PGC-1α may regulate processes downstream of FGF21. However, this coactivator is not critical to FGF21 action in the liver, perhaps because of the redundancy of the functions of PGC-1α and PGC-1β in the liver.

Whereas the initial view of FGF21 was that of a key regulator of nutrient deficiency, recent studies note that hepatic FGF21 levels are also increased during states of nutrient excess. This paradoxical increase is regulated by carbohydrate response element–binding protein, a transcription factor that regulates de novo lipogenesis in adipose tissue and the liver in response to carbohydrate load. Several studies have described this process in human (60) and rodent (61, 62) models and further highlight FGF21 as a key regulator of the interface between the nutritional state of

an organism and the required adaptive metabolic response. Although a mechanism for FGF21 downstream of nutrient load in the liver has not been proposed, recent work has found a role in adipose tissue; this role is discussed in the following section.

ADIPOSE TISSUE

The first identified action of FGF21 in adipocytes was induction of glucose uptake in 3T3L1 adipocytes independent of insulin (7). In this case, FGF21 induction of glucose uptake was dependent on the recruitment of Erk1/2 signaling and activation of the serum response factor Ets-like protein-1, leading to the increased expression of the facilitated glucose transporter Glut1 (37). However, the response of Glut1 to FGF21 is not sustained through chronic treatment. Subsequent studies failed to find evidence that FGF21 induced adipose tissue glucose uptake in vivo, even though acute doses of FGF21 potently reduced circulating glucose levels in mice (9, 56). Furthermore, this FGF21-dependent reduction in glucose levels also apparently results from the suppression of hepatic glucose production. These studies using hyperinsulinemic-euglycemic clamps were performed in obese mice. Subsequently, FGF21 was found to increase glucose uptake in both white adipose tissue (WAT) and brown adipose tissue (BAT) in chow-fed nonobese mice. This finding suggests a differential response to FGF21 in adipose tissue depots that depends on nutritional status (63).

The role of FGF21 in regulating the physiological response to fasting led to the hypothesis that FGF21 induces lipolysis in adipose tissue, releasing lipid stores for subsequent utilization (12, 13). However, although this hypothesis emerged from these earlier in vivo studies, FGF21 was also reported to be a potent suppressor of lipolysis in mouse (64) and human (65) adipocytes in vitro. Additionally, acute doses of FGF21 potently reduce circulating nonesterified free fatty acids (NEFAs) (40, 64), whereas chronic administration is associated with low NEFAs (22). These findings are consistent with findings that adipose tissue lipolytic activity is higher in 24-h-fasted Fgf21 knockout mice (66). However, as multiple reports on both the physiological and pharmacological actions of FGF21 show enhanced tissue energy expenditure and loss of fat mass, lipolysis must apparently be activated to release fatty acids for oxidative metabolism. Thus, further work is required to resolve these different findings and to delineate the exact physiological role of FGF21 in regulating adipose tissue lipolysis.

An important role for FGF21 in adipose tissue is regulation of interscapular BAT to increase thermogenesis through induction of uncoupling protein 1 (Ucp1) and to induce expression of genes involved in thermogenesis in susceptible depots, including inguinal WAT. This role was first suggested in an elegant study using neonatal mouse pups in which consumption of maternal milk increased hepatic Fgf21 expression (67). Such increased expression, in turn, increased thermogenic gene expression in neonatal BAT, indicating that Fgf21 may act as a signal to defend core body temperature. Subsequent studies found that cold exposure rapidly induced Fgf21 expression in BAT in adult mice (68, 69), a response that was downstream of beta-adrenergic signaling that induced activating transcription factor 2 transcriptional activity (68). The role of Fgf21 in regulating nonshivering thermogenesis is not limited to BAT, as chronic cold exposure also increases Fgf21 expression in other cold-sensitive fat depots, including subcutaneous and perirenal fat (70), where it plays a role in adaptive thermogenesis. This adaptive response is characterized by the appearance of Ucp1-expressing, multilocular brown-like adipocytes in specific WAT depots. Mice lacking Fgf21 display an impaired response to cold, and pharmacological doses of FGF21 are able to induce chronic adaptive thermogenesis. Small changes in circulating FGF21 levels have also been reported in cold-exposed humans (71).

Although the molecular mechanisms regulating these processes are not well defined, Pgc-1α has been implicated in this process, as FGF21 dramatically increases Pgc-1α protein levels independently of mRNA levels in vivo and in vitro (70). Indeed, fat-specific Pgc-1α knockout mice show an impaired response to FGF21. FGF21 also increases mitochondrial activity in 3T3L1 adipocytes (72), possibly through an Ampk-Sirt1-mediated pathway that leads to deacetylation and activation of Pgc-1α, subsequently leading to enhanced mitochondrial function and to an increased rate of oxygen consumption.

Given the role of FGF21 in regulating nonshivering thermogenesis, it is interesting to speculate that this physiological response may mediate many metabolic effects of FGF21, as increased thermogenesis would contribute to increase energy expenditure, weight loss, and glucose tolerance (8, 22). In fact, pharmacological doses of FGF21 induce weight loss and improve glucose tolerance in DIO mice while concomitantly increasing both glucose uptake in BAT and browning of WAT (73). These effects are independent of hepatic insulin action and occur after the removal of the predominant interscapular brown fat pad, suggesting that many of the pharmacological actions of FGF21 are mediated through the browning of WAT. Recent work has found that Ucp1 knockout mice have improved glucose tolerance when treated with human FGF21, despite no increase in energy expenditure (74), suggesting that there are mechanisms by which FGF21 improves adipose glucose disposal without increasing thermogenesis. However, the role of beige cells downstream of FGF21 signaling remains controversial (75).

FGF21 has also been implicated in the browning of WAT seen with tissue and metabolic stress. Indeed, both essential amino acid restriction (48, 51) and impaired muscular and hepatic autophagy (76) are unexpectedly beneficial to metabolic health in mice. In both models, enhanced tissue expression leads to substantial increases in circulating Fgf21 and to elevated Ucp1. In the absence of Fgf21, mice are heavier and less glucose tolerant. These studies suggest that the role of Fgf21 in browning WAT is an adaptive response to physiological and metabolic manifestations of stress as well as a thermogenic response to cold. Further work is required to understand the mechanistic reasons behind this adaptive response. However, it is clear that both responses represent extremely interesting targets for metabolic pharmacotherapy.

If FGF21 mediates many of its metabolic actions through activating uncoupling in BAT, then successful pharmacotherapy requires that this pathway be preserved in humans. Fortunately, several studies have found evidence to support this fact. Mild cold exposure increased circulating FGF21 levels in humans, correlating with increased energy expenditure and lipolysis (71). Further analyses found that brown-like adipocytes differentiated from the stromal vascular fraction of human neck fat were responsive to FGF21, increasing the rate of oxygen consumption and temperature (77, 78). Additionally, FGF21 expression was observed in browned visceral fat from human neonates and patients with pheochromocytoma, a condition marked by adrenal tumors secreting excessive amounts of catecholamines (79).

Finally, as discussed above, several studies have found that hepatic FGF21 expression is, counterintuitively, increased during high-carbohydrate feeding. Recent data found that this increase also occurs in WAT in response to refeeding a chow diet. This increase in adipose Fgf21 mRNA expression was not observed in the liver, and serum levels of Fgf21 did not increase, suggesting a paracrine or autocrine role within adipose tissue, as observed with cold exposure. Indeed, Fgf21 was enhanced by the Pparγ agonist TZD, and in turn, increased Fgf21 was proposed to stabilize and activate Pparγ by reducing receptor sumoylation (80). These data imply that FGF21 responds to carbohydrate intake by expanding the storage capacity of adipose tissue acting as a sink for excess calories. However, a more recent study found that *Fgf21*$^{-/-}$ mice on another genetic background respond normally to TZD treatment, suggesting that Fgf21 is dispensable in Pparγ signaling. In this case, TZDs robustly enhanced Klb expression in WAT and therefore suggest a different

mechanism whereby the two pathways may interact in vivo through alterations in signaling sensitivity (45). One such example is in 3T3L1 cells, where rosiglitazone and FGF21 synergistically enhanced glucose uptake and improved insulin sensitivity (36). However, more work is required to understand this complex pathway.

The dramatic effect of FGF21 on adipose tissue in enhancing substrate utilization makes it tempting to speculate that fat depots mediate most of the metabolic actions of FGF21. Several labs have tested this hypothesis by investigating FGF21's primary action on adipose tissue (discussed above) and FGF's secondary action on the liver, which is mediated by adiponectin secretion from fat. Adiponectin is one of the most abundantly secreted proteins from fat and is associated with increased liver and muscle insulin sensitivity in mice and humans. FGF21 increases serum adiponectin levels, especially in higher-molecular-weight species, which are considered to be more bioactive (81, 82). Interestingly, adiponectin knockout mice (*Adpoq*$^{-/-}$) fail to show the insulin-sensitizing and glucose-lowering effects of FGF21, although some of the weight loss properties remain. This resistance to FGF21 in *Adpoq*$^{-/-}$ mice is thought to arise from the accumulation of the sphingolipid ceramide and lipotoxicity that impairs insulin action in the livers of obese mice. FGF21 significantly reduced ceramide levels in wild-type mice, but not in *Adpoq*$^{-/-}$ mice, suggesting that the FGF21/adiponectin/ceramide axis is critical to the insulin-sensitizing effects of FGF21 (81). Nevertheless, many of the physiological conditions associated with native FGF21 action, such as thermogenesis, high fat intake, high carbohydrate intake, and starvation, are not associated with adiponectin action, suggesting that such action may be a pharmacological phenomenon restricted to high doses of FGF21 administered in vivo. Additionally, it is hard to explain why *Adpoq*$^{-/-}$ mice are partially refractive to the weight loss effects of FGF21, as this result cannot easily be explained by adiponectin action alone. Furthermore, loss of hepatic insulin action did not impair the effects of FGF21 on weight loss and glucose metabolism, suggesting that sensitizing the liver to insulin is not a major component of whole-body FGF21 function (73).

Despite the sometimes discrepant results, in general adipose tissue appears to be extremely important to the native physiological and pharmacological functions of FGF21. Indeed, inactivating Fgf21 signaling in fat seems to attenuate many of these pharmacological functions. Not only actions of FGF21 on adipose tissue, but also downstream pathways that FGF21 acts through, represent promising targets for metabolic pharmacotherapy.

CENTRAL NERVOUS SYSTEM

In mice, systemically administered Fgf21 crosses the blood-brain barrier in a nonsaturable fashion and remains intact (83). In humans, FGF21 is found in cerebrospinal fluid (CSF), and there is a linear relationship between serum levels and CSF levels (84). FGFR1, FGFR2, and FGFR3 are expressed in the brain, as is KLB, although at fairly low levels. Thus, FGF21 has the potential to act in the brain. Indeed, Fgf21 administered peripherally to obese rats leads to increased energy expenditure, although weight loss is not observed due to a coincident increase in food intake (85). Fgf21 is also reported to affect circadian periodicity and fertility, suggesting direct action of Fgf21 in the CNS. This was characterized in an *Fgf21*-transgenic mouse model that expresses Fgf21 at extremely high concentrations, resulting in smaller and leaner mice with significant increases in energy expenditure throughout the light-and-dark cycle. The abnormal circadian periodicity in these mice can be normalized by genetically deleting central *Klb* by using *Camk2a-Cre* mice and the floxed *Klb* allele (25). Similarly, female mice that overexpress Fgf21 are infertile, secondary to suppressed vasopressin and kisspeptin signaling; as with circadian behavior, the fertility defect in *Fgf21*-transgenic mice is corrected when Klb is deleted from the brain (86). Although these findings demonstrate the importance of a CNS relay in mediating FGF21 action, the precise

site of action remains undefined. Although the SCN has been proposed to mediate the effects of Fgf21 in the brain, its obligate coreceptor Klb is not restricted to the SCN. Importantly, *Camk2a* is expressed in multiple murine brain regions such as the cortex, olfactory structures, the hippocampal formation, the thalamus, the striatum, and several nuclei of the hypothalamus (87).

Two recent reports demonstrate that the central actions of FGF21 can increase energy expenditure by increasing sympathetic activity. One report utilized the Fgf21 transgenic mouse *Klb* floxed/*Camk2a-Cre* model and found that deleting Klb in the brain decreases energy expenditure and increases weight gain on standard chow. Furthermore, intracerebroventricular injection of Fgf21 also increases sympathetic nerve activity in BAT, an effect that is markedly reduced in mice lacking brain Klb (88). Another report demonstrated that FGF21 administered centrally at low doses both activates BAT and leads to browning of inguinal fat through sympathetic activation, as measured directly by evaluating norepinephrine turnover (89). In inguinal fat, browning was confirmed by the induction of genes involved in thermogenesis, including *Ucp1* and *Dio2*. Furthermore, treatment of mice with the β-blocker propranolol attenuated the response to central but not peripherally delivered FGF21. This report also suggests that the paraventricular nucleus may mediate FGF21 actions on sympathetic activity.

The data on central FGF21 action are intriguing and have led to the suggestion that many actions of FGF21 on the liver, particularly those in gluconeogenesis, may be mediated through the brain, possibly through regulation of corticotropin-releasing factor in the paraventricular nucleus, stimulating glucocorticoid (corticosterone) production in the adrenal cortex (26). However, interpretation of these data is potentially confusing, as the particular Fgf21 knockout mouse used displayed fasting hypoglycemia, which also elevates corticosterone. Furthermore, two other strains of mice lacking Fgf21 do not show hypoglycemia (13, 66). The contributions of central FGF21 signaling to reducing hepatic fibrosis and regulating lipid metabolism in NAFLD and NASH models have not been investigated.

PANCREAS

FGF21 mRNA and protein are expressed at high levels in the pancreas, although the function of FGF21 in this tissue remains obscure. FGF21 appears to have a role in modulating inflammation and damage induced by experimental pancreatitis. FGF21-null mice develop more damage than do wild-type mice, whereas mice overexpressing human FGF21 show an attenuated phenotype (23). Further studies identified the transcription factor MIST1 as an upstream regulator of FGF21 and showed that deletion of the MIST1 gene leads to a marked reduction in pancreatic FGF21 levels by epigenetic silencing, resulting in increased susceptibility to pancreatitis (90). FGF21 may also play a role in enhanced islet transplant survival in a model of streptozotocin-induced diabetes (91) and may promote β-cell survival and protect isolated rat islets and insulin-producing INS cells from glucolipotoxicity and cytokine-induced apoptosis (24). However, human FGF21 fails to alter insulin and glucagon secretion from islets isolated from healthy mice (9), although FGF21 stimulates insulin secretion in ex vivo islets isolated from diabetic mice (24). Islets from the obese diabetic *db/db* mouse fail to respond to FGF21, possibly as a consequence of reduced KLB expression (42).

MUSCLE

In the basal state, FGF21 is expressed at low levels in skeletal muscle and is regulated by insulin in mice (92) and humans (93). However, the exact role of muscle-derived FGF21 is not clear. Several studies found that FGF21 can regulate glucose uptake in primary myotubes and in mouse myoblast C2C12 cells, but there is no in vivo evidence for such regulation. Additionally, skeletal muscle

does not express sufficient levels of KLB to respond to FGF21. One study showed that exercise is able to induce serum FGF21 in both mice and humans. In mice, the increase was due to enhanced expression in the liver, as opposed to muscle, and was associated with increased circulating free fatty acids and ketones (94). The sole contribution of the liver to serum levels is consistent with the finding that, under most physiological manipulations, circulating Fgf21 is from the liver (55).

FGF21 is induced in and secreted from muscle in mitochondrial myopathies. Such release is seen in both humans (below) and mice (95), suggesting that a stress response is involved. In mice, the increase in Fgf21 leads to expected changes in metabolism, including low hepatic fat content and resistance to a high-fat diet. Another instance in which Fgf21 is overexpressed in muscle with metabolic consequences is observed with the muscle-specific deletion of autophagy factor 7. In this mouse model, a mild muscle phenotype is accompanied by resistance to DIO and browning of WAT; all these phenotypes depend on Fgf21 (76).

In the heart, FGF21 plays a paracrine role. *Fgf21*$^{-/-}$ mice show increased cardiac hypertrophy and proinflammation when challenged with isoproterenol and also have increased end-systolic and end-diastolic volume. Fgf21 knockout mice also show decreased fatty acid oxidation. In these mutant mice, some changes were apparent before β-agonist challenge, and treatment with Fgf21 ameliorated the effects (96). Similar effects were seen in a diabetic model of cardiomyopathy (97).

FGF21 IN HUMANS

Shortly after the reports that rodent Fgf21 was regulated by fasting and ketosis, studies on FGF21 in humans emerged (**Table 2**). The first report in 2008 (98) measured circulating FGF21 levels in both normal subjects and patients with newly diagnosed and as-yet-untreated type 2 diabetes. FGF21 was measured using an early immunoassay after an overnight fast. Fasting FGF21 levels in study subjects with type 2 diabetes were 20% higher than in nondiabetic individuals. However, a correlation with BMI was not noted. Investigators subsequently reported that lipid infusions in humans over a 6-h period led to a modest, approximately 20% increase in serum FGF21 levels (99). An association between plasma free fatty acid levels and FGF21 levels in subjects during both lipid infusion and saline infusions was also noted. In contrast to the situation for mice,

Table 2 Regulation of FGF21 in humans

Perturbation	Serum levels	Proposed source
Obesity	↑↑	Liver and fat
NAFLD	↑↑	Liver
Mitochondrial myopathies	↑↑↑↑	Muscle
Exercise	↑	Liver
Fructose	↑↑↑	Liver
Ketogenic diet	↔	NA
Lipid infusion	↑	Liver
Overnight fast	↔	NA
Prolonged fast (72 h)	↓	NA
Glucagon	↑	Liver
Insulin	↑	Muscle
Cold exposure	↑	Fat

Abbreviation: NAFLD, nonalcoholic fatty liver disease. NA denotes not applicable.

FGF21 levels in humans did not change either with 48-h fasting or with refeeding. Furthermore, consumption of a ketogenic diet for 4 months led to a substantial reduction in circulating FGF21 levels (100). Two consistent themes emerged from these collective early studies. Multiple groups demonstrated that serum FGF21 levels correlate with BMI (101, 102) and correlate with the risk of metabolic syndrome and diabetes (98). These correlations persist regardless of race or gender. One 2010 study prospectively evaluated multiple dietary manipulations in humans (38). As had been previously reported, there was no effect of ketogenic diet, acute fasting, or acute refeeding on circulating serum FGF21 levels, and 72 h of fasting led to decreased circulating levels of FGF21 (38). In addition, an association of FGF21 in subjects with clinically proven NAFLD was reported. Samples from subjects with NAFLD and with clinically driven liver biopsies showed increased FGF21 expression. Other groups reported similar results (103, 104).

More recently, a large cross-sectional study (105), which included more than 800 subjects, comprehensively analyzed FGF21 and showed positive correlations with multiple parameters, including age, blood pressure, BMI, fat mass, waist/hip circumference, and insulin and glucose metabolism. This study also showed that serum FGF21 levels correlated with liver transaminase levels. Intriguingly, there was a negative association between FGF21 and the insulin-like growth factor IGF1.

Although many groups have reported the positive relationship of FGF21 with BMI and with hepatic fat accumulation, the relationship between serum FGF21 and obesity in children is uncertain. At least one study failed to find such a correlation in children before and around the time of puberty (106), whereas another study found that FGF21 levels were increased in obese children and correlated with levels of free fatty acids and leptin (107).

Thus, early on investigators knew that, in humans, serum FGF21 levels were not induced by fasting, ketosis, glucose, or mixed meals. Many investigators confirmed the correlation with obesity, BMI, and NAFLD. Modest increases with both PPARα and PPARγ agonists were also demonstrated. In addition, researchers found that glucagon treatment increased circulating FGF21 levels in humans and that Fgf21 mediated glucagon's actions in mice (108). However, definition of an acute metabolic response proved elusive. One study demonstrated a 2–3-fold increase in serum FGF21 levels after exercise in healthy subjects; this increase was typically seen 1 h after 30 min of treadmill running (94). A similar effect of increased serum Fgf21 levels with exercise was noted in mice. Analysis of expression in different organs revealed that, at least in rodents, the source of circulating Fgf21 with exercise was the liver. In humans, 28 days of protein restriction led to an average 1.5-fold increase in serum FGF21 levels, suggesting that FGF21 may be a signal mediating protein restriction. However, this interpretation is slightly complicated, as individuals were overfed by 40% and gained weight during the course of the study.

Recently, an acute hormone-like response to FGF21 has been described in humans ingesting a fructose load (109). When humans were challenged with a 75-g oral load of fructose, a robust increase in serum FGF21 levels was observed within 2 h, after which levels returned to baseline. The increase in normal-weight humans ranged from 2 to 10 fold. In subjects with metabolic syndrome, baseline levels of FGF21 were 4-fold higher, as had been previously reported. This group also showed a 4-fold increase in stimulated FGF21 at 2 h. Thus, subjects with metabolic syndrome had a significantly higher area under the FGF21-response-to-fructose curve than did normal-weight subjects, similar to the case seen in the insulin response to glucose in insulin-resistant patients. Glucose ingestion did not immediately stimulate FGF21; indeed, at 60 min, levels were reduced. Interestingly, an increase in circulating FGF21 levels was seen at 4 h, possibly reflecting downstream events secondary to changes in either insulin or glucagon following a glucose load. The acute response to fructose suggests that FGF21, in humans, can be an acute metabolic regulator.

In humans, very high levels of FGF21 have been reported in association with mitochondrial myopathies. The highest levels, in the thousands of pictograms, are seen in patients with mitochondrial severe neurogastrointestinal encephalomyopathy. However, on the spectrum of myopathies, FGF21 levels tend to vary significantly, and although FGF21 may be a helpful predictor of mitochondrial myopathies in association with other indicators (110), the added value for either diagnosis or monitoring diseases is still unclear (111).

FGF21 AND PHARMACOTHERAPY

Pharmacological dosing of FGF21 improves the metabolic profile of mice and nonhuman primates, making FGF21 an extremely attractive candidate for the treatment of obesity and diabetes. Several approaches have been undertaken to target FGF21 signaling in humans. Several companies have generated FGF21 variants or analogs that have increased stability and efficacy over native FGF21 protein. Of these, LY2405319 has been tested in a randomized, placebo-controlled, double-blind proof-of-concept trial in patients with obesity and type 2 diabetes (112). After 28 days of treatment, subjects receiving LY2405319 lost weight and exhibited improved serum lipid profiles, indicating the value of targeting FGF21 signaling. Other companies are stabilizing FGF21 through conjugation to other moieties such as PEG or immunoglobulins, which have proven efficacy in animal models by improving glucose tolerance and reducing body weight, but no data have been published from clinical studies. As discussed above, a few companies are designing antibodies that target FGFR1 and KLB to synthetically activate an FGF21 response. For further reading, please see two recently published in-depth reviews of FGF21 pharmacology (113, 114).

CONCLUSIONS

FGF21 is a systemic peptide hormone, regulating whole-organism adaptation to multiple physiological challenges, including, but not limited to, nutrient restriction, cold exposure, carbohydrate intake, and stress. Unlike other hormones, FGF21 signaling is not confined to a single tissue and does not regulate a single physiological process. In fact, depending on the environmental stimulus and the site of production, FGF21 can function as a classical endocrine hormone and as an autocrine factor; this latter mode of action represents at least one method by which the physiological actions of FGF21 are compartmentalized. Hence FGF21 appears to regulate seemingly opposable physiological states such as the nutritionally limited state of fasting and the energy-depleting processes of adipose tissue thermogenesis. Furthermore, data gathered from pharmacological dosing of FGF21 may not necessarily reflect the native functions of this novel hormone. However, it is clear that FGF21 represents an extremely promising targetable system for therapeutic intervention of the multiple metabolic complications associated with obesity. Future studies concentrating on the native physiological processes regulated by FGF21 will aid in the development and understanding of therapeutics targeting FGF21 signaling.

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