REVIEW

Endocrine fibroblast growth factors 15/19 and 21: from feast to famine

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We review the physiology and pharmacology of two atypical fibroblast growth factors (FGFs)—FGF15/19 and FGF21—that can function as hormones. Both FGF15/19 and FGF21 act on multiple tissues to coordinate carbohydrate and lipid metabolism in response to nutritional status. Whereas FGF15/19 is secreted from the small intestine in response to feeding and has insulin-like actions, FGF21 is secreted from the liver in response to extended fasting and has glucagon-like effects. FGF21 also acts in an autocrine fashion in several tissues, including adipose. The pharmacological actions of FGF15/19 and FGF21 make them attractive drug candidates for treating metabolic disease.

The discovery of hormones such as insulin and glucagon that regulate metabolism has a long and storied history. The field of endocrinology continues to be invigorated by the discovery of new metabolic hormones. Among the relatively recent additions are three fibroblast growth factors (FGFs), termed FGF15/19, FGF21, and FGF23. (FGF15 and FGF19 are the mouse and human orthologs, respectively, which we refer to as FGF15/19 unless specifically referring to one species or the other.) In contrast to most FGFs, which act in an autocrine or paracrine fashion, the three "endocrine FGFs" can be released into the bloodstream to act throughout the body. While the fundamental role that FGF23 plays in regulating phosphate metabolism has been known for more than a decade (White et al. 2000; Shimada et al. 2001), the discovery of FGF15/19 and FGF21 and their wide-ranging effects on carbohydrate and lipid metabolism has been more recent. This review focuses on FGF15/19 and FGF21.

Most FGF family members mediate their effects by binding to FGF receptors (FGFRs) on the cell surface (Beenken and Mohammadi 2009). FGFRs are receptor tyrosine kinases encoded by four genes (*FGFR1–4*), with alternate splicing of *FGFR1–3* yielding two isoforms ("b" and "c") that differ in their extracellular domains and ligand-binding profiles. Typically, FGFs require an additional interaction with heparan sulfate glycosaminoglycans in the extracellular matrix to activate their receptors. FGF binding to the FGFR/heparan sulfate complex causes receptor dimerization and autophosphorylation and the subsequent phosphorylation and activation of downstream substrates, including FGFR substrate 2α, and MAP kinases such as ERK1 and ERK2 (Beenken and Mohammadi 2009).

Unlike conventional FGFs, the endocrine FGFs interact only weakly with heparan sulfate. As a consequence, they are able to diffuse away from their cells of origin and enter circulation (Goetz et al. 2007). To compensate for their inability to interact with heparan sulfate, the endocrine FGFs require members of the Klotho family of proteins for high-affinity receptor binding (Kurosu and Kuro 2009). There are three, related Klotho proteins: α-Klotho, β-Klotho, and lactase-like. All three are single-pass transmembrane proteins that interact with FGFRs to enable selective binding of the three endocrine FGFs. α-Klotho serves as the coreceptor for FGF23, and β-Klotho serves as the coreceptor for FGF15/19 and FGF21 (Kurosu et al. 2006, 2007; Urakawa et al. 2006; Ogawa et al. 2007; Wu et al. 2007; Kharitonenkov et al. 2008). FGF15/19 can also signal through lactase-like/FGFR complexes (Fon Tacer et al. 2010). While the FGFRs have very broad tissue distributions, expression of the Klotho proteins is more restricted (Fon Tacer et al. 2010). Thus, the sites of action for the endocrine FGFs are largely dictated by the presence or absence of the Klotho proteins.

FGF15/19

Discovery and role in regulating bile acid homeostasis

FGF19 was originally identified in a screen for novel FGFs in the fetal brain (Nishimura et al. 1999). While most FGFs are highly conserved between rodents and humans, sharing >90% amino acid identity, FGF19 shares only ~50% amino acid identity with its rodent ortholog, FGF15 (McWhirter et al. 1997). However, the *Fgf15* and *FGF19* genes are syntenic (Katoh 2003) and have similar tissue expression profiles (e.g., small intestine and fetal brain) (Nishimura et al. 1999; Xie et al. 1999; Gimeno et al. 2002; Inagaki et al. 2005; Krejci et al. 2007; Fon Tacer et al. 2010), and their protein products elicit similar effects on gene expression and metabolic parameters in mice (Inagaki et al. 2005; Potthoff et al. 2011). During mouse embryo-

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genesis, *Fgf15* has a complex and dynamic pattern of expression in the CNS (McWhirter et al. 1997; Gimeno et al. 2002, 2003; Ishibashi and McMahon 2002). This includes prominent expression in secondary organizers of the brain, which induce and maintain the regional characteristics of the surrounding neuroepithelium. Studies done with FGF15 knockout (KO) embryos show that FGF15 suppresses proliferation and promotes differentiation of neural precursors (Borello et al. 2008; Fischer et al. 2011). *Fgf15* is not detected in the adult CNS (Fon Tacer et al. 2010).

A primary function of FGF15/19 in the adult is to regulate bile acid homeostasis. Bile acids are amphipathic molecules that are released post-prandially from the gallbladder into the small intestine, where they play an essential role in the solubilization of dietary lipids (Russell 2003). After traversing the intestine, ~95% of bile acids are reabsorbed in the ileum and returned to the liver and gallbladder via the portal vein. Because of their intrinsic toxicity, intracellular levels of bile acids must be tightly regulated, which is accomplished by the transcriptional regulation of proteins involved in bile acid biosynthesis, transport, and metabolism. Chief among the transcriptional regulators of bile acid homeostasis is the farnesoid X receptor (FXR), a member of the nuclear receptor family of ligand-activated transcription factors that is activated by the bile acids cholic acid and chenodeoxycholic acid (Kalaany and Mangelsdorf 2006). Among the genes regulated by FXR in the liver is cholesterol 7α-hydroxylase (*CYP7A1*), which encodes the enzyme that catalyzes the first and rate-limiting step in the classical bile acid biosynthetic pathway (Russell and Setchell 1992; Chiang 1998). FXR potently represses *CYP7A1* and thus bile acid synthesis through a two-pronged mechanism (Fig. 1). First, FXR induces hepatic expression of small heterodimer partner (SHP), an orphan nuclear receptor that binds to the *CYP7A1* promoter and represses its transcription (Goodwin et al. 2000; Lu et al. 2000). Unlike most nuclear receptors, SHP lacks a DNA-binding domain and binds indirectly to the *CYP7A1* promoter through interactions with two other orphan nuclear receptors: liver receptor homolog-1 (LRH-1) and hepatocyte nuclear factor 4α (HNF4α) (Goodwin et al. 2000; Lu et al. 2000; De Fabiani et al. 2001). The importance of the FXR–SHP pathway in bile acid homeostasis has been demonstrated in FXR-KO and SHP-KO mice, both of which have increased *Cyp7a1* expression and a corresponding increase in bile acid pool size (Kerr et al. 2002; Wang et al. 2002; Kok et al. 2003).

FGF15/19 is part of a second, interrelated mechanism through which bile acids feed back to repress their own synthesis (Fig. 1). It has been known for more than two decades that intestinal administration of bile acids suppresses *CYP7A1*, suggesting that the intestine produces a secreted factor that acts on the liver to repress bile acid synthesis (Pandak et al. 1991; Nagano et al. 2004). FGF19 was first implicated as this factor when it was shown that FXR agonists induced FGF19 in cultured human hepatocytes and, further, that FGF19 repressed *CYP7A1* expression in both isolated hepatocytes and mice (Holt et al. 2003). Subsequent studies showed that FXR-mediated repression of *Cyp7a1* was lost in FGF15-KO mice, demonstrating the physiological importance of this pathway (Inagaki et al. 2005). While the initial studies suggested that FGF15/19 might be part of an autocrine pathway in liver, more recent studies in mice showed that FGF15/19 is not normally made in hepatocytes, but is instead expressed in ileal enterocytes, where it is induced by bile acids acting on FXR (Inagaki et al. 2005). Consistent with these findings, FXR-mediated repression of *Cyp7a1* requires the presence of FXR in the intestine but not the liver (Kim et al. 2007a). In humans, circulating FGF19 concentrations increase in response to oral administration of bile acids and decrease in response to treatment with bile acid sequestrants, demonstrating that the regulation of FGF15/19 is conserved (Lundasen et al. 2006; Brufau et al. 2010). Thus, FGF15/19 signals from the intestine to the liver to regulate bile acid homeostasis.

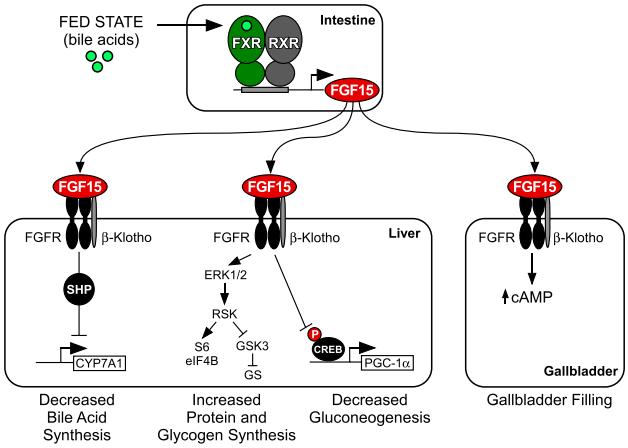

Figure 1. Endocrine actions of FGF15. FGF15 expression is induced in the small intestine by bile acids acting on the FXR/RXR heterodimer. Secreted FGF15 acts on FGFR/β-Klotho receptor complexes in the liver to repress *CYP7A1* and bile acid synthesis through a mechanism that requires SHP, to induce protein synthesis through activation of the ERK1/2–RSK pathway that activates translation factors S6 and eIF4B, to stimulate glycogen synthase (GS) activity and glycogen synthesis through inactivation of glycogen synthase kinase 3 (GSK3), and to repress gluconeogenesis by blocking the phosphorylation and activation of CREB, a transcription factor that induces *peroxisome proliferator-activated receptor γ coactivator 1-α* (*PGC-1α*) and other gluconeogenic genes. FGF15 also acts on the gallbladder to increase cAMP levels, which promotes filling of the gallbladder with bile.

FGF15/19 represses *CYP7A1* in the liver by binding to the FGFR4/β-Klotho receptor complex. As was observed in FGF15-KO mice, FGFR4-KO and β-Klotho-KO mice have increased *Cyp7a1* expression and bile acid synthesis, and administration of exogenous FGF15/19 fails to repress *Cyp7a1* in these animals (Yu et al. 2000; Inagaki et al. 2005; Ito et al. 2005; Tomiyama et al. 2010). Conversely, transgenic overexpression of a constitutively active FGFR4 in either wild-type or FGFR4-KO mice decreased *Cyp7a1* expression and bile acid pool size (Yu et al. 2005). Importantly, FGF15/19-mediated repression of *Cyp7a1* is lost in SHP-KO mice (Inagaki et al. 2005), demonstrating that the

FGF15/19 and SHP pathways converge. The mechanistic basis for this cooperativity is not yet known. One possibility is that FGF15/19 increases the stability of SHP by preventing its ubiquitination (Miao et al. 2009). However, since increased basal SHP expression in the liver has only relatively modest effects on *Cyp7a1* expression compared with FGF19 administration (Boulias et al. 2005; Inagaki et al. 2005; Potthoff et al. 2011), this is unlikely to account for the full effect of FGF19 on *CYP7A1*.

Recent studies indicate that the FGF19–CYP7A1 pathway has medical relevance in humans. Circulating FGF19 concentrations are high in patients with extrahepatic cholestasis, a condition in which bile flow from the liver is impeded (Schaap et al. 2009). Interestingly, FGF19 is expressed in the liver under these disease conditions, suggesting the existence of an adaptive response to reign in bile acid production. Conversely, low serum FGF19 concentrations were found in patients with idiopathic bile acid malabsorption (Walters et al. 2009), a disease associated with excess bile acid production and chronic diarrhea. Based on these findings, it was proposed that low FGF19 serum levels, not defective ileal reuptake of bile acids, are the basis for this disease (Walters et al. 2009). Another study found a single-nucleotide polymorphism in *β-Klotho*, which regulated β-Klotho protein stability and was associated with colonic transit in diarrhea-predominant irritable bowel syndrome (Wong et al. 2011). In a mouse model of bile acid malabsorption, FGF15 administration suppressed *Cyp7a1* and normalized the bile acid pool (Jung et al. 2007). These findings raise the intriguing possibility that FGF15/19 or FXR agonists may have utility in the treatment of diseases associated with excess bile acids.

In addition to its effects on bile acid synthesis, FGF15/19 also regulates gallbladder filling (Fig. 1). FGF15-KO mice have a virtually empty gallbladder even in the fasted state, when the gallbladder is normally full (Choi et al. 2006). Likewise, FGFR4-KO and β-Klotho-KO mice have small gallbladders (Yu et al. 2000; Ito et al. 2005). Injection of recombinant FGF19 into FGF15-KO mice causes a rapid filling of the gallbladder without stimulating bile flow. This effect is mediated in part via relaxation of the gallbladder smooth muscle (Choi et al. 2006). We conclude that FGF15/19 plays an overarching role in regulating bile acid homeostasis, including the post-prandial refilling of the gallbladder. Interestingly, in humans but not mice, FGF19 is abundantly expressed in the gallbladder, where it is secreted into the bile, not the blood (Zweers et al. 2011). The physiological consequences of this exocrine secretion of FGF19 remain to be determined.

FGF15/19 in energy homeostasis

A role for FGF19 in controlling energy homeostasis was first discovered using FGF19 transgenic mice. Mice with constitutively elevated levels of FGF19 have lower body weights due to reduced fat content, despite having elevated food intake (Tomlinson et al. 2002). In this study, reduced adiposity and increased food intake were explained by elevated energy expenditure resulting from increased brown adipose tissue (BAT) mass and enhanced hepatic lipid oxidation. FGF19 transgenic mice were also protected from diet-induced obesity and had lower serum glucose, insulin, cholesterol, and triglyceride levels. Administration of recombinant FGF19 to mice fed a high-fat diet recapitulated most of these metabolic effects (Fu et al. 2004). To date, the molecular basis of these changes has not been elucidated, and this remains an active area of research.

Consistent with the effects of FGF15/19 on metabolism, FGFR4-KO mice show characteristics of metabolic syndrome, including dyslipidemia, hypercholesterolemia, increased adiposity, and glucose intolerance (Huang et al. 2007). Paradoxically, however, obese FGFR4-KO mice have reduced hepatic triglyceride and cholesterol levels due to increased hepatic fatty acid oxidation and hepatic triglyceride secretion (Huang et al. 2007). Interestingly, re-expression of a constitutively active form of FGFR4 in the liver of FGFR4-KO mice decreased plasma lipid and cholesterol levels but failed to improve the glucose tolerance and insulin sensitivity that is seen with FGF19 treatment (Huang et al. 2007). In addition, administering FGF19 to FGFR4-KO mice on a high-fat diet improved glucose homeostasis (Wu et al. 2011). Thus, FGF19 requires FGFR4 in the liver to improve dyslipidema and hypercholesterolemia but not hyperglycemia and insulin resistance.

How does FGF15/19 impact carbohydrate metabolism? While FGF19 acts preferentially through FGFR4 (Xie et al. 1999; Kurosu et al. 2007), it also binds and activates FGFR1c, which is abundantly expressed in white adipose tissue (WAT) but not in hepatocytes (Kurosu et al. 2007). Administration of FGF19 to isolated adipocytes increases FGF signaling and glucose uptake in a β-Klotho-dependent manner, and FGF19 administration induces ERK1/2 signaling in murine WAT (Kurosu et al. 2007; Wu et al. 2009). In addition to WAT, FGF19 may act on other tissues. For example, intracerebroventricular injection of FGF19 increased the metabolic rate, suggesting that FGF19 can act directly on the CNS (Fu et al. 2004). Thus, the pharmacological effects of FGF19 on metabolism may involve signaling through multiple FGFRs in multiple tissues.

Two recent studies demonstrated physiological roles for FGF15/19 in regulating glucose homeostasis. Kir et al. (2011) showed that FGF15-KO mice are glucose-intolerant and store 50% less hepatic glycogen than wild-type mice. Consistent with this finding, pharmacological administration of FGF15/19 stimulated hepatic glycogen synthesis (Fig. 1). FGF19 increased hepatic glycogen synthase activity by inducing the phosphorylation and inactivation of glycogen synthase kinase 3α and 3β (Kir et al. 2011). Administration of FGF19 to mice devoid of insulin restored hepatic glycogen concentrations to normal, demonstrating the insulin-independent effects of this endocrine pathway. These findings may explain why IRS1/IRS2-KO mice, which lack insulin signaling in the liver, are still able to store hepatic glycogen after a meal (Dong et al. 2006). In addition to glycogen synthesis, FGF19 also activated components of the protein translation machinery and increased hepatic protein synthesis in vivo (Kir et al.

2011). Mechanistically, FGF19 uses a RAS–ERK–p90RSK pathway to induce phosphorylation of ribosomal protein S6 and eukaryotic initiation factors eIF4B and eIF4E.

In addition to stimulating glycogen synthesis and protein synthesis in the liver, FGF15/19 also represses gluconeogenesis (Fig. 1; Potthoff et al. 2011). Overexpression of FGF15 in lean mice suppressed hepatic gluconeogenesis without affecting insulin sensitivity. The physiological importance of FGF15 was demonstrated using FGF15-KO and FGFR4-KO mice, which were hyperglycemic and had elevated gluconeogenesis in the fed state (Potthoff et al. 2011). FGF15/19 represses gluconeogenesis by inhibiting the activity of the transcription factor CREB, a key regulator of *peroxisome proliferator-activated receptor γ coactivator-1α* (*Pgc-1α*) and other gluconeogenic genes.

The overlap in the effects of FGF15/19 and insulin on hepatic metabolism is striking: Both stimulate hepatic glycogen and protein synthesis and repress gluconeogenesis. However, there are important differences. First, unlike insulin, FGF15/19 does not stimulate lipogenesis, which is a key advantage in considering the FGF19 pathway as an anti-diabetic therapy. Second, whereas insulin acts through the insulin receptor–PI3K–Akt pathway, FGF15/19 mediates its effects through the FGFR/β–Klotho–ERK–RSK pathway. Finally, there are important temporal differences. Insulin is released within minutes of a meal, and in rodent experiments, serum insulin concentrations and downstream hepatic Akt phosphorylation peak ~15 min after a high-carbohydrate/high-fat meal. In contrast, *Fgf15* mRNA levels in ileum and downstream ERK1/2 phosphorylation in the liver peak ~1 h after feeding (Potthoff et al. 2011). Likewise, in humans, FGF19 serum levels peak ~2 h after a meal (Lundasen et al. 2006), and, accordingly, circulating FGF19 levels in humans negatively correlate with fasting glucose levels and metabolic syndrome (Stejskal et al. 2008; Reiche et al. 2010; Mraz et al. 2011). Thus, FGF15/19 acts after insulin in the transition from the fed to the fasted state. Taken together, these studies establish a post-prandial hormonal program in which insulin and FGF15/19 coordinately govern nutrient metabolism.

FGF15/19 in cancer

Although the studies discussed above point to a novel therapeutic use for FGF19 in treating diabetes, an important caveat is that chronic administration of FGF19 stimulates proliferation. FGF19 transgenic mice develop hepatocellular carcinomas (HCCs) and liver dysplasia by 10–12 mo of age (Nicholes et al. 2002), and an FGF19-neutralizing antibody inhibited tumor formation in this model (Desnoyers et al. 2008). Recently, *FGF19* was shown to be amplified and overexpressed in human HCCs. In mouse xenograft studies, a neutralizing antibody to FGF19 blocked the growth of a human HCC cell line harboring the amplified FGF19 gene (Sawey et al. 2011).

As with the effects of FGF15/19 on metabolism, there is controversy as to whether its proliferative effects are mediated through FGFR4. Although one study showed that FGFR4 is required for FGF19-stimulated growth (Wu et al. 2010b), deletion of FGFR4 accelerates HCC progression in a carcinogen-initiated mouse model of tumorigenesis (Huang et al. 2009). A possible explanation for these seemingly contradictory findings is that the FGFR4–FGF19 pathway has both pro- and anti-carcinogenic actions. For example, while on the one hand FGF19 induces β-catenin and cell proliferation (Nicholes et al. 2002; Desnoyers et al. 2008; Pai et al. 2008), it also suppresses the synthesis of bile acids, which are tumor promoters. Several other mouse KO models that increase bile acid synthesis, including the FXR-KO and SHP-KO mice, also spontaneously develop HCCs (Kim et al. 2007b; Yang et al. 2007; Y Zhang et al. 2008). Thus, FGF19 may alternately suppress or enhance tumorigenesis through different mechanisms. There has been keen pharmacological interest in developing novel FGF19 mimetics that eliminate the growth-promoting effects while preserving the potent metabolic effects of FGF19. To this end, it was recently shown that replacement of two short regions of FGF19 eliminated its proliferative effects without affecting its glucose-lowering actions in ob/ob mice (Wu et al. 2010a).

FGF21

The study of FGF21 exploded with the discovery of its potent insulin-sensitizing actions in rodents (Kharitonenkov et al. 2005). However, despite this plethora of work, much of the biology of FGF21 and its mechanism of action remain controversial and incompletely understood. Part of the problem in developing a coherent understanding of FGF21 has come from trying to compare its systemic pharmacological effects in obese animals with its tissue-specific effects that occur under more physiological conditions. In this next section, we attempt to provide some clarity to this complex picture by considering the pharmacological and physiological actions of FGF21 within the context of the different experimental conditions in which they have been observed.

Discovery and pharmacological actions

Fgf21 was isolated from a mouse embryo cDNA library in a screen for new FGF family members and was shown to be abundantly expressed in the adult mouse liver (Nishimura et al. 2000). A role for FGF21 in regulating metabolism was first reported by Kharitonenkov et al. (2005), who showed that FGF21 induced glucose transporter-1 and promoted glucose uptake in murine 3T3-L1 and human primary adipocytes. In this and subsequent studies done in genetic and diet-induced models of obesity, it was shown that FGF21 administration decreased hepatic triglycerides, decreased plasma triglycerides and glucose levels, and caused weight loss by increasing energy expenditure and reducing fat mass without decreasing food intake (Kharitonenkov et al. 2005; Coskun et al. 2008; Xu et al. 2009a). In similar studies performed with diabetic rhesus monkeys, FGF21 decreased plasma glucose, insulin, and triglyceride concentrations; lowered LDL cholesterol and increased HDL cholesterol levels; and caused modest but significant weight loss (Kharitonenkov et al. 2007). Overall, FGF21 was shown to have remarkable beneficial effects on several metabolic parameters in obese rodents and primates.

Consistent with its effects on glucose and lipid metabolism, a prominent feature of the pharmacological studies with FGF21 is its profound effect on insulin sensitivity. When administered to insulin-resistant rodents, a single injection of FGF21 decreases blood glucose concentrations and improves glucose tolerance and insulin sensitivity in both leptin-deficient ob/ob mice and diet-induced obese mice (Xu et al. 2009b). In diet-induced obese mice, these changes are accompanied by increased whole-body glucose turnover without a decrease in hepatic glucose production. Treatment of diet-induced obese mice with FGF21 for longer periods (3–6 wk) reverses hepatic steatosis, decreases hepatic glucose production, and increases insulin-stimulated glucose uptake in the heart, adipose tissue, and skeletal muscle (Xu et al. 2009a). Likewise, administration of FGF21 to ob/ob mice for 8 d improves hepatic insulin sensitivity and also increases liver glycogen content (Berglund et al. 2009). However, there was no effect of FGF21 on insulin-stimulated glucose uptake in peripheral tissues in ob/ob mice. While the basis for this difference is not clear, there is agreement that FGF21 improves insulin sensitivity at least in part through effects on hepatic metabolism.

On which tissues does FGF21 act directly to regulate metabolism? FGF21 functions through receptors composed of β-Klotho and either FGFR1c, FGFR2c, or FGFR3c (Ogawa et al. 2007; Kharitonenkov et al. 2008; Suzuki et al. 2008). While these FGFRs are ubiquitously expressed, β-Klotho is enriched in several metabolically active tissues, including the liver, WAT, and BAT (Ito et al. 2000; Fon Tacer et al. 2010). Consistent with this profile, FGF21 stimulates ERK1/2 phosphorylation and modulates gene expression in each of these tissues (Kurosu et al. 2007; Fisher et al. 2010). While relatively little is known about how FGF21 regulates transcription, it reduces levels of the lipogenic transcription factor sterol regulatory element-binding protein-1 in the liver (Badman et al. 2009b; Xu et al. 2009a) and induces expression of the metabolic coactivator protein PGC-1α in the liver and WAT (Badman et al. 2009b; Potthoff et al. 2009; Chau et al. 2010; Fisher et al. 2011). β-Klotho is also expressed in both the endocrine and exocrine pancreas (Johnson et al. 2009; J Repa, pers. comm.). In rat pancreatic islets, FGF21 treatment stimulates ERK1/2 and Akt signaling and increases insulin mRNA and protein levels (Wente et al. 2006). Accordingly, FGF21 treatment increases islet number and insulin staining in db/db mice, suggesting that preservation of β-cell function contributes to the beneficial glycemic actions of FGF21 (Wente et al. 2006). Finally, β-Klotho is expressed in the hypothalamus (Coskun et al. 2008), although its precise localization in this tissue has yet to be reported. FGF21 appears able to cross the blood–brain barrier (Hsuchou et al. 2007), and direct intracerebroventricular infusion of FGF21 into the brains of diet-induced obese rats increases food intake and energy expenditure and increases insulin sensitivity due to increased insulin-induced suppression of both hepatic glucose production and gluconeogenic gene expression (Sarruf et al. 2010). In a study in which FGF21 was administered for 2 wk by injection, FGF21 increased expression of the orexigenic hormone neuropeptide Y (NPY) and decreased expression of the anorexigenic hormone pro-opiomelanocortin in the hypothalamus (Coskun et al. 2008). Thus, the pharmacological effects of FGF21 are likely to be a consequence of it acting both centrally and peripherally. While one study reported that FGF21 can function in the absence of β-Klotho to induce an immediate early transcription factor (Tomiyama et al. 2010), no additional parameters were measured in these experiments, and it remains to be determined whether FGF21 has β-Klotho-independent actions.

Physiological actions of FGF21

In contrast to the insulin-sensitizing, pharmacological actions of FGF21 that are observed in obese animals, the physiological actions of FGF21 occur in lean animals. In addition, whereas the pharmacological effects include the systemic action of FGF21 in multiple organ systems and tissues (e.g., liver, pancreas, adipose, and CNS), the physiological actions are likely to occur at lower hormone concentrations and in a more restricted set of tissues. Important clues as to the physiology of FGF21 have come initially from studying its regulation. FGF21 expression is induced in various tissues in response to fasting, feeding, and cold. Summaries of the role of FGF21 in these responses follow.

FGF21 in fasting and starvation In 2007, three groups showed that FGF21 is strongly induced in the mouse liver by fasting ≥12 h (Badman et al. 2007; Inagaki et al. 2007; Lundasen et al. 2007). Optimal induction of FGF21 by fasting requires the glucagon receptor (Berglund et al. 2010), which is consistent with FGF21 acting subsequent to glucagon in the temporal cascade of hormones that regulate the fasting response. There is also evidence that FGF21 feeds back to suppress glucagon concentrations (Kharitonenkov et al. 2005, 2007; Berglund et al. 2009). Fasting-mediated induction of FGF21 requires the peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor activated by fatty acids and the fibrate class of hypolipidemic drugs (Badman et al. 2007; Inagaki et al. 2007; Lundasen et al. 2007). PPARα binds directly to the *Fgf21* gene promoter to induce its transcription (Inagaki et al. 2007). PPARα plays a crucial role in the adaptive starvation response: PPARα-KO mice are unable to adequately catabolize fatty acids in the liver and, as a consequence, become steatotic, hypoketonemic, hypoglycemic, and hypothermic during fasts lasting ≥24 h (Kersten et al. 1999; Leone et al. 1999; Hashimoto et al. 2000). This hypoketonemia and hepatic steatosis can be partially reversed by administration of exogenous FGF21 to the PPARα-KO mice (Inagaki et al. 2007). FGF21 is also strongly induced in the mouse liver by a high-fat, low-carbohydrate "ketogenic" diet (Badman et al. 2007) and by suckling in mouse neonates (Hondares et al. 2010), conditions that mimic starvation in forcing the body to burn fatty acids rather than carbohydrates. FGF21 has effects in lean mice on metabolism, growth, and the phenomenon of torpor that are all consistent with an important role for FGF21 in coordinating the adaptive starvation

response. These effects are summarized in Figure 2 and are discussed below.

Metabolism: In the fed state, FGF21 transgenic mice have increased hepatic tricarboxylic acid cycle flux, gluconeogenesis, fatty acid oxidation, and ketogenesis (Potthoff et al. 2009). FGF21 induces hepatic expression of the transcriptional coactivator protein PGC-1α, which stimulates transcription of genes involved in each of these metabolic pathways. However, the importance of PGC-1α induction for FGF21 action remains in question (Potthoff et al. 2009; Fisher et al. 2011). While the metabolic actions of FGF21 are similar to those of glucagon, FGF21 does not increase glycogenolysis (Potthoff et al. 2009), which is consistent with it being induced 12–24 h into a fast, when glycogen reserves are already depleted. Acute knockdown of FGF21 in the livers of mice fed a ketogenic diet causes hepatic steatosis (Badman et al. 2007), demonstrating that FGF21 is required for the efficient catabolism of fatty acids. Like PPARα-KO mice, FGF21-KO mice become hypoglycemic during a 24-h fast, although the effect is more modest (Potthoff et al. 2009). These data reveal an important role for FGF21 in regulating glucose production and fatty acid catabolism during starvation.

Growth: During starvation, circulating growth hormone (GH) concentrations increase to stimulate lipolysis. However, the anabolic actions of GH, including the induction of its downstream effector, insulin-like growth factor 1 (IGF-1), are lost in starving animals (Thissen et al. 1994). This phenomenon of dissociating the catabolic from the anabolic effects of GH is referred to as "growth hormone resistance." A remarkable phenotype of FGF21 transgenic mice is their diminutive size: FGF21 transgenic mice weigh substantially less than wild-type mice, while retaining their appropriate body proportions (Inagaki et al. 2008). Growth retardation is not due to a decrease in GH concentrations. Rather, basal GH concentrations are modestly increased. Notably, circulating IGF-1 concentrations are reduced in FGF21 transgenic mice, as are hepatic levels of the active, phosphorylated form of the transcription factor STAT5, a major regulator of IGF-1 transcription (Inagaki et al. 2008). These studies reveal a role for FGF21 in inducing growth hormone resistance as part of the adaptive starvation response.

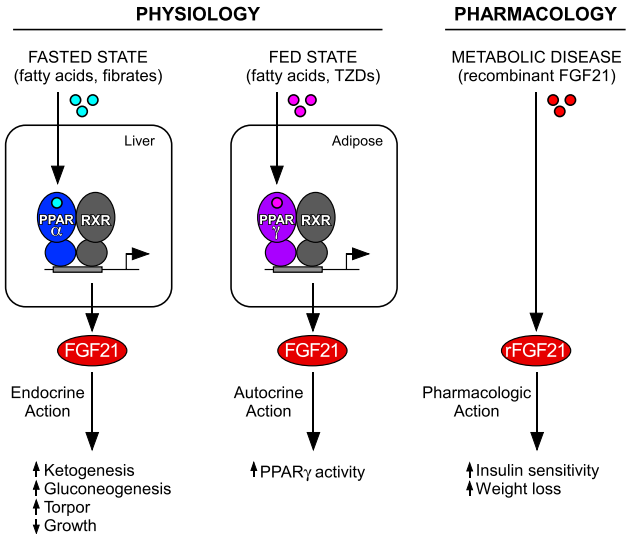

Figure 2. Endocrine, autocrine, and pharmacological actions of FGF21. (*Left* panel) In response to fasting or fibrate drugs, FGF21 expression is induced in the liver by the PPARα/RXR heterodimer. Secreted FGF21 acts as an endocrine hormone to induce ketogenesis, gluconeogenesis, and torpor and to inhibit somatic growth. (*Middle* panel) In response to feeding or thiazolidinedione drugs (TZDs), FGF21 expression is induced by the PPARγ/RXR heterodimer in WAT, where FGF21 acts through an autocrine mechanism to stimulate PPARγ activity. (*Right* panel) Pharmacological administration of recombinant FGF21 (rFGF21) affects multiple tissues and has beneficial effects in metabolic disease.

Torpor: Torpor is a starvation-induced phenomenon in which body temperature and physical activity are reduced to conserve energy. Depending on the species, torpor can reduce energy utilization by as much as 90% (Geiser 2004). A possible link between FGF21 and torpor was first suggested by the induction of pancreatic lipases in the livers of FGF21 transgenic mice, a phenomenon previously observed in torpid mice (Zhang et al. 2006). This extrapancreatic induction of lipases may provide a means for the continuous hydrolysis of fatty acids under conditions of reduced body temperature (Squire et al. 2003). Under fed conditions, FGF21 transgenic mice do not have reductions in body temperature or activity. However, during a 24-h fast, FGF21 transgenic mice exhibit an ~10°C drop in body temperature, accompanied by a marked decrease in physical activity (Inagaki et al. 2007). Importantly, FGF21 is not sufficient to cause torpor. Rather, its overexpression sensitizes lean mice to starvation-induced torpor. This effect is not a function of reduced adiposity, since wild-type and FGF21 transgenic mice have comparable amounts of adipose tissue (Inagaki et al. 2008). Studies with FGF21-KO mice showed that FGF21 is not required for hypothermia or reduced physical activity during a 24-h fast (Oishi et al. 2010). However, ketogenic diet-induced hypothermia was reduced in FGF21-KO mice, suggesting that FGF21 may be important in controlling torpor under conditions of longer-term starvation.

How might FGF21 affect torpor? One possibility is via regulation of hypothalamic NPY, which regulates fasting-induced torpor. FGF21 administration increases NPY mRNA levels in the hypothalamus (Coskun et al. 2008). Similarly, treatment of mice with a fibrate PPAR agonist induces hypothermia, accompanied by increased NPY expression in the hypothalamus, and an NPY-Y1 receptor antagonist prevents this fibrate-mediated hypothermia (Chikahisa et al. 2008). However, neither PPARα nor FGF21 is essential for fasting-mediated induction of NPY (Oishi et al. 2010). These data suggest that there is redundancy in the regulation of NPY and that the effects of FGF21 on body temperature and physical activity are likely to depend on the metabolic status of the animals.

FGF21 in cold adaptation Morphologic alterations in BAT from FGF21 transgenic mice first suggested that FGF21 may regulate thermogenesis (Kharitonenkov et al. 2005). Two groups subsequently showed that FGF21 is induced by cold in BAT (Chartoumpekis et al. 2011; Hondares et al. 2011), although it is unclear whether BAT-derived FGF21 acts hormonally or just locally in BAT itself. Injection of FGF21 into mice stimulated the expression of thermogenic genes such as *uncoupling protein-1* and *deiodinase-2* in BAT and *uncoupling protein-1* in WAT (Coskun et al. 2008; Hondares et al. 2010). Consistent with this activation of BAT and "browning" of WAT, FGF21 administration causes weight loss in obese rodents and monkeys, with more striking weight loss in rodents, which have more BAT (Kharitonenkov et al. 2005, 2007; Coskun et al. 2008; Xu et al. 2009a). An interesting conundrum is how FGF21 contributes to both decreases (i.e., torpor) and increases in body temperature. As suggested above, the response elicited by FGF21 is likely a function of both the physiological context (e.g., cold or starvation) and the tissues that are exposed to FGF21 under these conditions. Interestingly, catecholamines show the same dichotomy in that they are required for both torpor and thermogenesis (Swoap and Weinshenker 2008).

FGF21 in feeding In a surprising twist, FGF21 is induced in the liver by high-carbohydrate diets (Ma et al. 2006; Uebanso et al. 2011) and in WAT by fasting–refeeding regimens (Oishi et al. 2011; Dutchak et al. 2012). These responses in the liver and WAT are likely mediated by carbohydrate response element-binding protein and PPARγ, respectively (Ma et al. 2006; Muise et al. 2008; Wang et al. 2008; Iizuka et al. 2009). Regarding PPARγ, we recently showed that the full insulin-sensitizing effects of the thiazolidinedione drug (TZD) rosiglitazone, a potent PPARγ agonist, require FGF21 (Dutchak et al. 2012). Importantly, diet-induced obese FGF21-KO mice are refractory to both the beneficial, insulin-sensitizing effects of TZDs and TZD side effects such as weight gain and fluid retention. FGF21 stimulates PPARγ at least in part by preventing its post-translational sumoylation and inactivation. These results reveal FGF21 to be part of a feed-forward regulatory pathway that contributes to the fed-state response in WAT (Fig. 2). Notably, unlike the fasting response that elicits FGF21 release from the liver into circulation, feeding and pharmacological induction of FGF21 in WAT do not cause a corresponding increase in circulating levels of FGF21 protein (Dutchak et al. 2012). Thus, FGF21 acts in an autocrine or paracrine fashion in WAT, much like canonical FGFs. In this regard, basal expression of FGF21 is high in the exocrine pancreas under nonfasted conditions when circulating FGF21 is low (Fon Tacer et al. 2010). Thus, the release of FGF21 into the blood by tissues may be the exception rather than the rule.

We suggest two possibilities as to why a hormone that elicits diverse aspects of the starvation response is also induced in WAT by feeding. First, FGF21 may regulate the efficient capture of nutrients when they become available following an extended fast. In support of this idea, FGF21 is very strongly induced in WAT under fasting–refeeding conditions (Oishi et al. 2011). Thus, the actions of FGF21 in WAT may represent an extension of its role in the adaptive starvation response. A second possibility is that FGF21 regulates pathways that are important in both the fed and fasted states. A prominent example of this is triglyceride synthesis. In the fed state, glucose and fatty acids are converted to triglyceride in the liver and WAT, with most triglyceride ultimately stored in WAT. However, triglyceride synthesis also occurs during fasting, when only a small fraction of the free fatty acids released by lipolysis are immediately oxidized. Most free fatty acids are re-esterified to triglyceride by various tissues, including WAT and the liver (Reshef et al. 2003). This so-called "triglyceride/fatty acid cycle" may serve as a mechanism for controlling the delivery of fatty acids and glycerol to the liver and other tissues during starvation. A role for FGF21 in this pathway may explain why FGF21-KO mice have elevated plasma triglyceride and free fatty acid concentrations during fasting (Hotta et al. 2009; Potthoff et al. 2009). Moreover, a role for FGF21 in the triglyceride/fatty acid cycle may also help explain why FGF21 has alternately been reported to stimulate or repress lipolysis (Inagaki et al. 2007; Arner et al. 2008; Li et al. 2009). In experiments in lean, fed FGF21 transgenic mice and in lean, fed wild-type mice injected with recombinant FGF21, circulating free fatty acid concentrations were increased (Inagaki et al. 2007). These studies further showed that FGF21 treatment increased expression of hormone-sensitive lipase and adipose triglyceride lipase in murine adipose and induced glycerol release from differentiated 3T3-L1 adipocytes, a surrogate measure of lipolysis. While there is agreement that FGF21 increases lipase expression in adipose (Inagaki et al. 2007; Coskun et al. 2008), other groups have observed that FGF21 treatment decreases free fatty acid release from 3T3-L1 adipocytes and primary cultures of human adipocytes (Arner et al. 2008; Li et al. 2009), and FGF21 administration lowers circulating free fatty acids in obese mice (Xu et al. 2009a). The pro- and anti-lipolytic effects of FGF21 are reminiscent of those of PPARγ agonists (Oakes et al. 2001; Nagashima et al. 2005; Festuccia et al. 2006; Kershaw et al. 2007). We conclude that the effects of FGF21 on adipose are complex and likely depend on the precise physiological or pathophysiological context.

An adverse consequence of the effect of FGF21 on adipocytes occurs in bone, where pharmacological levels of FGF21 decrease bone mass (Wei et al. 2012). Conversely, FGF21-KO mice have increased bone mass. FGF21 causes bone loss in part by enhancing the differentiation of bone marrow mesenchymal stem cells into adipocytes instead of osteoblasts (Wei et al. 2012). Bone loss is a potential clinical concern as FGF21 is developed as a drug for treating metabolic disease.

FGF21: mice vs. humans

How is FGF21 regulated in humans? As in mice, circulating FGF21 levels are induced by fibrates and other PPARα

agonists in humans (Galman et al. 2008; Christodoulides et al. 2009; Mraz et al. 2011). Circulating FGF21 concentrations are also increased in rheumatoid arthritis patients fasted for 7 d (Galman et al. 2008) and in obese individuals fed a very low-calorie diet for 3 wk (Mraz et al. 2011). While these data suggest similarities in the way that FGF21 is regulated across species, the magnitude of FGF21 induction by PPARα agonists and fasting is modest in humans compared with mice. Notably, circulating FGF21 levels are not increased in humans by either shorter-term fasts or ketogenic diets (Galman et al. 2008; Christodoulides et al. 2009; Dushay et al. 2010) or in subjects with anorexia nervosa (Dostalova et al. 2008; Fazeli et al. 2010), suggesting that there may be important differences in the regulation and function of FGF21 between rodents and humans.

It also remains to be determined whether the function of FGF21 as a fed-state hormone is conserved between mice and humans. While some groups have detected *FGF21* mRNA in human WAT (X Zhang et al. 2008; Mraz et al. 2009), others have not (Dushay et al. 2010). Interestingly, circulating FGF21 concentrations are increased in human subjects who either are overweight or have type 2 diabetes, impaired glucose tolerance, or nonalcoholic fatty liver disease (Chen et al. 2008; Chavez et al. 2009; Mraz et al. 2009; Cuevas-Ramos et al. 2010; Dushay et al. 2010; Li et al. 2010, 2011; Matuszek et al. 2010; Yilmaz et al. 2010). It seems likely that this circulating FGF21 is derived from the liver, perhaps due to the induction of *FGF21* by elevated hepatic lipid and carbohydrate levels. While the human findings appear to be at odds with the insulin-sensitizing actions of FGF21 in rodents and monkeys, hepatic *FGF21* mRNA levels and plasma FGF21 concentrations are similarly increased in diet-induced and genetically obese mice (Muise et al. 2008; X Zhang et al. 2008; Badman et al. 2009a; Fisher et al. 2010). Importantly, these mice still respond to pharmacological doses of FGF21 with improved insulin sensitivity. One possibility is that obesity and insulin resistance cause "FGF21 resistance" in rodents and humans. While a study from one group supports this hypothesis (Fisher et al. 2010), another study does not (Hale et al. 2011). The basis and consequences of increased FGF21 in metabolic disease remain to be determined.

Summary

Insulin and glucagon are the prototypical fed- and fasted-state hormones. In this context, FGF15/19 and FGF21 can be considered "late-acting" fed- and fasted-state hormones, respectively, acting on the heels of insulin and glucagon to regulate metabolism in response to nutritional status (Fig. 3). While FGF15/19 and FGF21 have effects that overlap with those of insulin and glucagon, they also have their own distinct actions. For example, FGF21 sensitizes mice to torpor and suppresses growth, whereas glucagon does not have these effects. A surprising twist to the paradigm of FGF21 serving as a starvation hormone is that it also functions as a fed-state signal in WAT and the liver. Moreover, while both FGF15/19 and FGF21 circulate as hormones, there is emerging evidence that they can also function as autocrine or exocrine factors. While reconciling all of these findings into a simple model is difficult, we conclude that FGF15/19 and FGF21 play important roles in coordinating energy homeostasis under a variety of nutritional conditions.

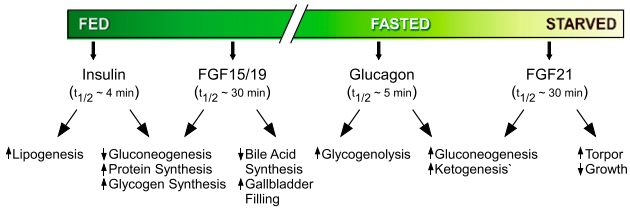

Figure 3. FGF15/19 and FGF21 function in a temporal cascade of hormones to regulate responses to nutritional stress. The temporal relationship among insulin, FGF15/19, glucagon, and FGF21 is shown along with hormone half-lives and biological actions.

Both FGF15/19 and FGF21 have remarkable pharmacological effects on carbohydrate and lipid metabolism, particularly in the context of obese animals. Their similar effects on weight loss and insulin action suggest that they may act through some of the same tissues and pathways, but this remains to be determined. The pharmacological actions of FGF15/19 and FGF21 make them attractive as future drugs for treating metabolic disease. Indeed, FGF21 is already in clinical trials. However, given their pleiotropic effects, additional studies will be required to address the safety of their long-term use. Obvious concerns include the effects of FGF19 and FGF21 on hepatocyte proliferation and bone mass, respectively. For FGF19, there is already evidence that it may be possible to engineer safer versions of this hormone for use in humans (Wu et al. 2010a). In any case, based on their profound pharmacological and physiological effects on metabolism, the endocrine FGFs appear poised to have their own long and storied history.

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