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Mini-Review: Endocrine Actions of Fibroblast Growth Factor 19

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Abstract: Fibroblast growth factor (FGF) 19 is an atypical member of the fibroblast growth factor family of signaling molecules. FGF19, FGF21, and FGF23 comprise a phylogenetic subfamily with attributes that distinguish them from typical FGFs. The FGF19 subfamily has reduced heparin binding resulting from a disrupted β-trefoil domain. Reduced heparin binding allows these FGFs to diffuse beyond their site of origin and act as endocrine hormones. This family of FGFs is regulated, at least in part, by nuclear hormone receptors. FGF19 expression is regulated by the farnesoid X receptor, a nuclear hormone receptor that is a key regulator of bile acid biosynthesis and transport. In line with its regulation by a bile acid receptor, FGF19 is involved in the regulation of bile acid biosynthesis and gallbladder filling. FGF19 originates from intestine and signals to liver via the portal circulation with a pronounced diurnal pattern. FGF19 is the only FGF to not have a closely related mouse homologue. The mouse homologue of FGF19, called FGF15, is only 53% identical to the human FGF19. FGF19 transgenic mice and mice administered exogenous FGF19 are resistant to the effects of a high fat diet, suggesting FGF19 may play a role in metabolic signaling pathways. Hepatocellular carcinoma is seen in mice, predominantly female mice, exposed to FGF19. Further investigation into the cellular mechanisms involved in these activities will allow better understanding of FGF19 biology in the context of human physiology.

Keywords: Fibroblast growth factor 19; FGF19; bile acid; cholesterol 7-alpha hydroxylase; CYP7A1; obesity; diabetes; gallbladder

Introduction

The study of fibroblast growth factors (FGFs) has been an active field of research for over 30 years. The 22 human FGFs play key roles in diverse areas of biology.[1–3] The FGFs bind and signal through plasma membrane tyrosine receptor kinases termed fibroblast growth factor receptors (FGFRs). FGFs also bind to heparin or heparin sulfate proteoglycans which act to increase the affinity and stability of the FGF-FGFR complexes.[4] FGFs typically signal in a paracrine manner across the mesenchymal epithelial interface. Epithelial FGFs bind to mesenchymal FGFRs or vice versa.[1,5] The binding of heparin or heparin sulfate proteoglycans also

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provides a mechanism for limiting the diffusion of the FGF ligands beyond their paracrine targets.

Of the four FGFRs, FGFR1, FGFR2, and FGFR3 exist as splice variants that display differential FGF binding selectivity.[6] There are no known splice variants of the FGFR4. The expression patterns of the FGFs and their receptors provide an additional level of discrimination, since FGFs and FGFRs are differentially expressed. Temporal selectivity further regulates these signaling pathways: expression of FGF3, -4, -8, and -17 is restricted to embryonic tissues, whereas other FGFs play a role in developmental as well as adult biology. For many years, FGF research focused on the differentiation, migration, and cellular proliferation activities of FGF family members, although it is now clear that FGF signaling affects many diverse biological processes in adult as well as developing tissues. This review will focus on a subset of atypical FGFs and on FGF19 in particular.

Atypical FGF Family Members

Within the family of human FGFs, there are seven phylogenetic subfamilies based on amino acid sequence identity.[1] In general, FGFs within the same subfamily tend to share sequence as well as functional similarity. This trend does not hold true for the subfamily consisting of FGF19, -21, and -23. The core sequences of these three FGFs are quite diverse, being only slightly more related to each other than to FGFs outside the subfamily.[1,5] Though diverse in structure and function, there are several properties that FGF19, -21, and -23 have in common that are atypical for the FGF family. First, this subfamily of FGFs has evolved with a common regulatory mechanism; they are transcriptionally regulated by members of the nuclear receptor class of ligand activated transcription factors. FGF19 expression is regulated by Farnesoid X Receptor (FXR, NR1H4) a bile acid receptor.[7] The fatty acid receptor PPARα regulates FGF21 expression and FGF23 levels are regulated, at least in part, by the vitamin D receptor.[8–12]

Another distinguishing feature of this subfamily is their atypical heparin binding properties. FGFs typically have a β-trefoil structural motif which is a positively charged heparin binding region consisting of 12 antiparallel β-strands. Crystallographic analysis of FGF19 and -23 indicate a disrupted β-trefoil motif compared to other FGFs.[5,13] Consequently, this subfamily binds poorly to heparin and heparin sulfate proteoglycans. Reduced heparin binding enables increased diffusion of these FGFs beyond the interstitial spaces. Indeed, these three FGFs have been shown to possess activities analogous to the classical endocrine hormones, whereby the FGFs are secreted in one tissue or organ system but bind and signal in a distant target tissue or organ. For example, FGF23 originates primarily in bone and regulates vitamin D metabolism and phosphate homeostasis in kidney.[14] FGF21 has recently been shown to be a key liver hormone that regulates the response to fasting by signaling to adipose and brain.[8,9] The metabolic and biliary activities of FGF19 will be the focus of this review.

Another common feature shared by this atypical FGF family is the connection with Klotho/βKlotho proteins. Klotho and βKlotho are single pass transmembrane proteins with restricted tissue distribution.[15] It has recently been shown that the FGF19 subfamily members, with their reduced heparin binding, rely instead on the Klotho/βKlotho proteins to stabilize the FGF−FGFR interaction, creating a complex of sufficient affinity to activate downstream signaling pathways.[5,16–18] Prior to the identification of the Klotho/βKlotho binding partners for this subfamily, it was reported

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that FGF19 displayed binding specificity for FGFR4;[19] however, in the presence of βKlotho, FGF19 binds and signals through FGFR1c, FGFR2c, and FGFR3c in addition to FGFR4.[18] Reliance on Klotho/βKlotho expression adds another level of selectivity to the signaling of these FGFs. Klotho, which is required for FGF23 signaling, is expressed primarily in kidney, brain, and reproductive tissues.[15–17] FGF19 and 21 interact with βKlotho, which is expressed in adipose, liver, and pancreas.[5,18] Thus, the requirement for Klotho/βKlotho binding partners strictly focuses the target tissues of this endocrine FGF subfamily.

Mouse FGF15 Is the Orthologue of Human FGF19

Highly conserved mouse FGF orthologues have been identified that map 1:1 and share >90% amino acid identity to all human FGFs with the exception of human FGF19. Conversely, the mouse FGF15 has no highly conserved orthologous human FGF. Mouse FGF15 and human FGF19 share 53% total amino acid identity, which is more than FGF19 shares with its human subfamily members FGF21 and FGF23.[1,20] There is evidence to suggest that FGF19 and mouse FGF15 are orthologues, including the fact that the genes encoding these peptides reside on syntenic regions of the zebrafish, mouse, and human genomes.[20] There is also conservation of the sequence and position of the FXR binding element which regulates expression of these two genes.[7,21] Recent elucidation of the biological activities of FGF19 and mouse FGF15 have provided additional evidence that these peptides have similar, though not always identical, function. Differences between FGF19 and mouse FGF15 function have been most clearly elucidated in the field of vertebrate embryology, where distribution of mouse FGF15 differs from chick FGF19 during otic and retinal development.[22,23]

Biliary Functions of FGF19 and Mouse FGF15

Regulation of Bile Acid Homeostasis. The first evidence that FGF19 plays a role in the regulation of bile acid biosynthesis came from *in vitro* studies aimed at identifying genes regulated by FXR, a bile acid responsive receptor and master regulator of bile acid homeostasis. Treatment of primary human hepatocytes with the FXR agonist GW4064 elicited transcription of FXR target genes.[7] Analysis of these transcripts identified FGF19 as the most strongly induced gene following GW4064 treatment. Induction of FGF19 transcripts, following treatment with the bile acid chenodeoxycholic acid, a natural FXR ligand, and isolation of the FXR binding site in the FGF19 gene, confirmed FGF19 as an FXR target gene.[7] Studies to determine how FGF19 contributed to FXR's effects on bile acid homeostasis built upon the earlier work in *fgfr4*$^{-/-}$ mice.

FGFR4 is the predominant FGFR in mature hepatocytes, and *fgfr4*$^{-/-}$ mice have increased bile acid pool size as well as increased bile acid excretion and a small, depleted gallbladder.[24] Further analysis of livers from these animals revealed elevated expression of cholesterol 7α-hydroxylase (Cyp7a) mRNA and protein. Cyp7a is the first and rate-limiting enzyme in the classical bile acid biosynthetic pathway and is responsible for converting cholesterol to the bile acid precursor cholesterol 7α-hydroxylase. Cyp7a is exquisitely regulated at the transcriptional level by a variety of pathways, including a network of nuclear receptors. Of the nuclear receptor transcription modulators, Cyp7a is positively regulated in mice by oxysterols, derivatives of its cholesterol substrate, via the liver X receptor (LXR; NR1H3) and negatively regulated by its bile acid products via FXR in conjunction with small heterodimer partner (SHP, NR0B2) and liver receptor homologue 1 (LRH-1, NR5A2).[25]

Elevation of both bile acid pool size and bile acid excretion in *fgfr4*$^{-/-}$ mice suggested either a surplus of cholesterol substrate driving bile acid biosynthesis or the loss of a key pathway regulating Cyp7a directly.[24] HMG-Co-A reductase

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transcript levels were used to address this question since it is transcriptionally induced by low sterol levels and repressed by high levels. FGFR4$^{-/-}$ mice had elevated HMG-CoA reductase mRNA levels, suggesting there was not a cholesterol surplus driving Cyp7a activity.[24] This was confirmed by feeding the mice a high cholesterol diet which gave the expected repression of HMG-CoA reductase message.[24] These studies indicate a role for FGFR4 as a negative regulator of Cyp7a expression and bile acid biosynthesis.

These data from *fgfr4*$^{-/-}$ mice combined with the knowledge that FGF19 is a target gene of FXR prompted an analysis of the effects of FGF19 on CYP7A expression. Treatment of primary human hepatocytes with recombinant human FGF19 decreased CYP7A1 transcript levels in a dose-dependent manner.[7] These results were further extended by injecting mice with an FGF19 expressing adenovirus which also caused a reduction in Cyp7a message.[7] Further investigation into the mechanism of Cyp7a repression by FGF19 utilized the selective pan c-Jun N-terminal kinase (JNK) inhibitor SP600125 to demonstrate the necessity of this phosphoprotein signaling cascade for FGF19-mediated repression of Cyp7a message.[7] Additional data supporting involvement of the JNK cascade in FGF19 signaling to Cyp7a came from studies using CahR4 mice that are *fgfr4*$^{-/-}$ and overexpress a constitutively active form of human FGFR4. While *fgfr4*$^{-/-}$ mice display reduced liver levels of phosphorylated, activated JNK than wild-type mice, the CahR4 mice have increased liver levels of phosphorylated JNK.[26] Interestingly, bile acid-mediated repression of Cyp7a and JNK activation were retained in the *fgfr4*$^{-/-}$ mice fed high levels of bile acid, indicating additional JNK-mediated bile acid feedback pathways.[26]

Enterohepatic Signaling. Neither human FGF19 nor murine FGF15 mRNA has been detected in liver,[21,27,28] so it is unclear why cultured primary human hepatocytes express FGF19 in response to FXR agonists. This puzzle was addressed by Inagaki et al., who demonstrated induction of mouse FGF15 message in small intestine, but not liver, following administration of GW4064 or cholic acid to mice.[21] FGFR4 had the reverse expression pattern, with abundant expression in liver and little or no expression in small intestine.[21] Administration of recombinant mouse FGF15 or an FGF15-expressing adenovirus to mice decreased the expression of CYP7A1 message in liver, similar to what was seen previously with FGF19.[21] CYP7A1 repression was lost in *fgfr4*$^{-/-}$ mice injected with the FGF15-expressing adenovirus.[21] Similarly, CYP7A1 repression was lost in SHP$^{-/-}$ mice injected with the FGF15-expressing adenovirus.[21] These data indicate that FGF15, normally produced in the small intestine, signals in an endocrine fashion through liver FGFR4 to repress CYP7A1 and bile acid biosynthesis in a SHP dependent manner. Further support for this model was seen in FGF15$^{-/-}$ mice which had elevated liver CYP7A1 expression, cholesterol 7α-hydroxylase protein levels, and fecal bile acid excretion.[21] Furthermore, repression of CYP7A1 was not seen in GW4064-treated FGF15$^{-/-}$ mice, suggesting FGF15 is required for FXR-mediated bile acid feedback repression of CYP7A1.[21] This new model of negative feedback of bile acid biosynthesis addresses the perplexing mystery of bile duct-ligation in rodents resulting in upregulation of CYP7A1 protein, despite bile acid overload in the liver. It had been proposed that an unknown "intestinal factor" was required for CYP7A1 repression, with bile duct ligation somehow removing the activity of this signaling agent.[21,29,30] FGF15 may be this "intestinal factor". Restoration of FGF15 in the intestine, via GW4064 treatment, enables repression of CYP7A1 in the bile duct-ligated animal.

Recently, evidence of analogous endocrine actions of FGF19 was published. Serum FGF19 levels were measured in human volunteers. Fasting FGF19 levels varied from 49 to 590 pg/mL and were increased 250% in volunteers treated with chenodeoxycholic acid.[28] Chenodeoxycholic acid administration produced a corresponding 26% decrease in serum 7α-hydroxy-4-cholesten-3-one (C4), a marker of Cyp7a1 enzymatic activity.[28] Conversely, volunteers treated with the bile acid binding resin cholestyramine for 2–3 weeks had an 87% decrease in serum FGF19 and an 18-fold increase in C4.[28] An important lesson from this study was that serum FGF19 levels fluctuate when monitored over a 25.5 h period in the absence of pharmacological intervention. It has been known for many years that bile acid biosynthesis follows a diurnal rhythm and serum FGF19 follows a similar pattern. Serum FGF19 levels peak twice during the day, at 3 and 9 pm, approximately 1.5–3 h after the peak in serum bile acids that occurs postprandially as bile acids are reabsorbed by the intestine and transported back to the liver via the portal vein.[28] C4 levels, on the other hand, were suppressed ~2 h after serum FGF19 levels peaked.[28] Under fasting conditions, serum bile acid levels remained low, serum FGF19 remained low, and bile acid biosynthesis in the liver increased 100%.[28] This striking pattern supports the model of postprandial bile acid induction of intestinal FGF19 (via FXR) which then signals to the liver, repressing CYP7A1 and bile acid biosynthesis (Figure 1).

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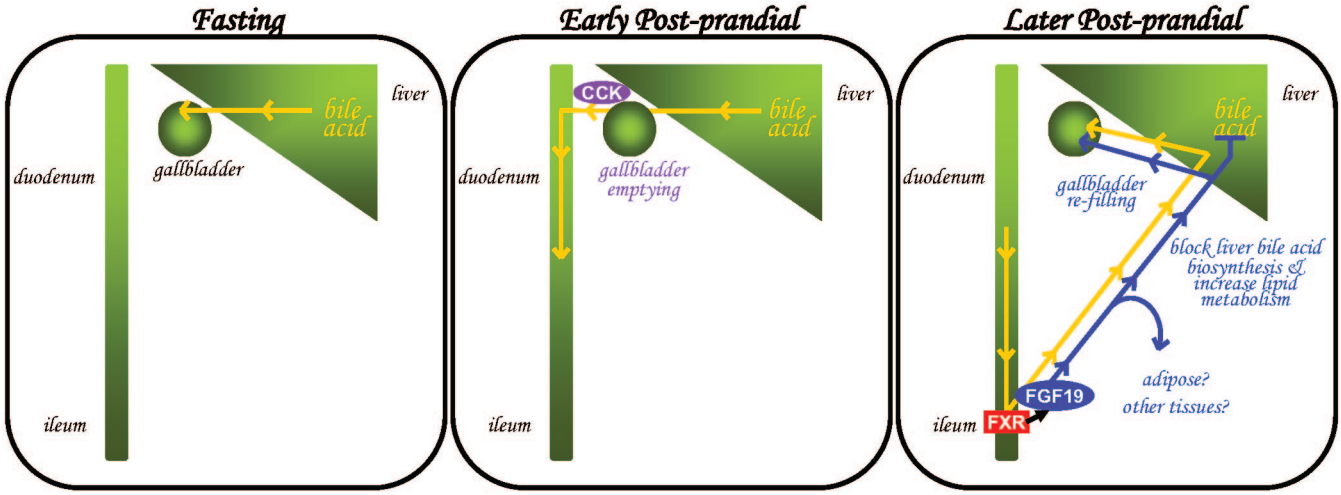

Figure 1. Induction of the gut hormone FGF19 following feeding, its target tissues and activities. In the fasted state, bile acids are synthesized in the liver and transported to the gallbladder for storage. Following feeding, the gut hormone cholecystokinin (CCK) signals to the gallbladder to contract, releasing bile into the duodenum. As the bile acids reach the ileum they enter enterocytes and activate the nuclear receptor FXR causing FGF19 transcription. FGF19 is released from enterocytes and signals to the liver to block bile acid biosynthesis and stimulate lipid metabolism as well as to the gallbladder to stimulate gallbladder refilling.

Regulation of Gallbladder Filling. In addition to hormonal control of liver bile acid biosynthesis, intestinal FGF15 and FGF19 have recently been identified as regulators of gallbladder filling. Gallbladder emptying is stimulated by the hormone cholecystokinin (CCK) which is released from the proximal duodenum in response to feeding. The regulatory mechanisms of gallbladder filling are less well understood. Both *fgf15*$^{-/-}$ and *fgfr4*$^{-/-}$ mice have small, depleted gallbladders.[24,31] Administration of recombinant mouse FGF15 or human FGF19 to *fgf15*$^{-/-}$ mice resulted in a >10-fold increase in gallbladder volume within 15 min.[31] Because there is no FGF15 expressed in gallbladder, common bile duct, or the sphincter of Oddi (site of CCK regulation of gallbladder emptying), it is suggested that this is another endocrine action of intestinal FGF15 and FGF19.[31] The precise site of action and the downstream signaling pathways are still unclear but likely involve receptors other than FGFR4, since FGF19 was able to induce gallbladder filling in *fgfr4*$^{-/-}$ mice.[31]

Metabolic Effects of FGF19

Other interesting functions of FGF19 have been identified in FGF19 transgenic mice. FVB mice, constitutively expressing FGF19 under the control of myosin light chain promoter, weigh less that their wild-type littermates, primarily due to a decrease in white adipose tissue.[32] Although FGF19 transgenic mice have increased food intake, they also have a higher metabolic rate that is independent of increases in leptin, IGF-1, growth hormone, or thyroid hormone levels.[32] When challenged with a high fat diet for 12 weeks, FGF19 mice retain a strikingly lean phenotype and have decreased muscle and liver triglyceride levels.[32] These mice also have lower serum glucose and insulin levels, improved glucose tolerance, and improved insulin sensitivity compared to wild-type littermates.[32]

Similar effects (except for changes in food intake) were also seen upon administration of recombinant human FGF19 for 7 days to FVB mice maintained on a high fat diet for 4–6 weeks.[33] In comparison, vehicle-treated animals gained weight over the 7-day treatment period. Strikingly, mice treated with recombinant FGF19 lost weight.[33] Mice treated with FGF19 also had a further reduction in respiratory quotient over that seen with the high fat diet, suggesting increased lipid oxidation.[33] FGF19 administration improved glucose tolerance and decreased serum insulin, leptin, cholesterol and triglycerides.[33] Importantly, administration of recombinant FGF19 to *ob*/*ob* mice or crossing the FGF19 transgenic mice onto the *ob*/*ob* background resulted in mice

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that weighed less and had lower serum glucose levels and improved glucose sensitivity compared to *ob*/*ob* mice.[33] These data suggest FGF19 acts as an insulin sensitizer independent of leptin signaling.

Research aimed at understanding the mechanism of the metabolic effects of FGF19 has focused on liver and brown adipose tissue (BAT). Interscapular brown adipose depots were enlarged in the FGF19 transgenic mice and showed gene expression changes consistent with increased BAT activity; however, when FGF19 transgenic mice were backcrossed to UCP-DTA mice that have reduced BAT, the FGF19 expressing mice were protected from obesity and hyperglycemia, suggesting the actions of FGF19 are independent of its activity in BAT.[33]

In both FGF19 transgenic mice and mice treated with recombinant FGF19, several genes regulating lipid metabolism were altered in liver. These changes include repression of steroyl CoA desaturase-1 (SCD1) and mitochondrial acetyl CoA carboxylase-2 (ACC2) message.[32,33] SCD1 is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acid required for cholesterol ester, wax ester, triglyceride, and phospholipid biosynthesis. ACC2 converts acetyl CoA to malonyl CoA, which negatively regulates carnitine palmitoyltransferase 1 (CPT1) transport of fatty acids into the mitochondria for β-oxidation; thus, a decrease in ACC2 activity would translate into increased fatty acid oxidation. Similar to FGF19 transgenic mice, $SCD^{-/-}$ and $ACC2^{-/-}$ mice are lean and resistant to the effects of a high fat diet.[34,35] In mice treated with recombinant FGF19, repression of SCD1 occurs over several days, whereas ACC2 expression is decreased within 6 h.[33] Consistent with decreases in SCD1 and ACC2, FGF19 administration decreases respiratory quotient and decreases liver triglyceride levels in fat fed mice;[33] however, direct evidence of increased fatty acid oxidation is lacking at this time.

Mitogenic Signaling

FGFs play a prominent role in cell proliferation, and some FGFs and FGFRs have been linked to the development and progression of tumors. Of the FGFRs, FGFR4 has low mitogenic potential,[19] but it is now clear that FGF19 can signal through FGFR1c, -2c, and -3c as well.[18] FGF19 has been reported to have weak mitogenic signaling through FGFR1c, -2c, -3,c and FGFR4; however, these studies did not include the βklotho signaling partner.[6]

FGF19 transgenic mice, predominantly the female mice, develop hepatocellular carcinoma (HCC) in a relatively short time (12 months).[36] Whether this is a consequence of normal FGF19 biology is not clear; constitutive expression of human FGF19 protein in mouse may not reflect normal FGF19 activity in human. The human FGF19 protein and the mouse FGF15 protein are 53% identical.[1,20] While there are clearly systems where human FGF19 and mouse FGF15 share similar biology, there are other areas where there are differences such as during otic and retinal development. It may be that expression of human FGF19 in the developing transgenic mouse elicits signals in liver that are unrelated to FGF19 signaling in its natural human context. The diurnal rhythm of FGF19 signaling is certainly a key aspect of its biology that is not reflected in transgenic mice expressing constitutively high levels of human FGF19 continuously throughout their lifespan.

Hepatocellular proliferation was seen in nontransgenic mice following six daily injections of 30 μg of recombinant human FGF19.[36] Normal circulating levels of FGF19 varied between ~50 and 600 pg/mL in human subjects.[28] This is many orders of magnitude lower than the dose administered to mice. Similarly, the transgenic mice that develop HCC express FGF19 at 18–78 ng/mL[36] in addition to the normal expression of FGF15, again, more than 2 orders of magnitude higher than the normal range in human subjects. Typical circulating levels of mouse FGF15 are unknown, so it is difficult to draw parallels between species and models. There have been no reports of hepatocellular proliferation in the constitutively active human FGFR4 mice; such systems may help clarify the signaling pathways involved.

Future Directions

It is unclear at this point whether the metabolic effects of FGF19 seen in transgenic mice and mice administered exogenous FGF19 represent physiological properties of FGF19. Although high circulating levels of FGF19 seen in these models may activate FGFR1-βKotho signaling pathways in adipocytes,[18] it is unclear if normal FGF19 signaling from intestine to liver via the portal vein would result in similar effects. Although FGF19 has reduced heparin binding affinity compared to most FGFs, it has been suggested that the first pass through the liver may trap some fraction of the FGF19 via heparin binding.[18] Further investigation into the relative concentrations of FGF19 in portal and systemic circulation and its effects on metabolic signaling pathways in vivo is needed.

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It is also possible that the metabolic and biliary effects of FGF19 are intertwined. If repression of CYP7A1 expression by FGF19 translates into contraction of the bile acid pool size, it would likely decrease dietary lipid absorption. This could lead to decreased fat mass and tissue triglycerides, resulting in improved insulin sensitivity. Again, however, the high circulating levels seen in the mouse models do not appear to reflect the physiological diurnal pattern of FGF19 seen in humans.[28] Further investigation into the cellular mechanisms involved will allow better understanding of FGF19 biology in the context of human physiology.

Elucidation of the endocrine actions of the atypical FGFs is evolving at a rapid pace. Further investigation into the pathways involved in the metabolic, biliary, and putative proliferative effects of FGF19 will determine whether one or more of these pathways can be selectively targeted to provide therapeutic benefit to patients. Based on our current understanding, it has been proposed that the FGF19 pathways could impact human diseases including metabolic disorders such as obesity, diabetes, and hypercholesterolemia as well as gallstone disease and diseases of bile acid metabolism.[18,5,31,28,24] Further elucidation of the pathways involved in FGF19 biology may also uncover new therapeutic targets for these diseases.

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