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Review Article

Exploring endocrine FGFs - structures, functions and biomedical applications

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Abstract: The family of fibroblast growth factors (FGFs) consists of 22 members with diverse biological functions in cells, from cellular development to metabolism. The family can be further categorized into three subgroups based on their three modes of action. FGF19, FGF21, and FGF23 are endocrine FGFs that act in a hormone-like/endocrine manner to regulate various metabolic activities. However, all three members of the endocrine family require both FGF receptors (FGFRs) and klotho co-receptors to elicit their functions. α-klotho and β-klotho act as scaffolds to bring endocrine FGFs closer to their receptors (FGFRs) to form active complexes. Numerous novel studies about metabolic FGFs' structures, mechanisms, and physiological insights have been published to further understand the complex molecular interactions and physiological activities of endocrine FGFs. Herein, we aim to review the structures, physiological functions, binding mechanisms to cognate receptors, and novel biomedical applications of endocrine FGFs in recent years.

Keywords: Fibroblast growth factors, FGF19, FGF21, FGF23, metabolic FGFs, endocrine FGFs, metabolic diseases, diabetes, obesity, biomedical applications

Introduction

Fibroblast growth factors (FGFs) are a family of proteins responsible for important functions in the body, such as angiogenesis, cell proliferation, migration, and metabolism [1]. Their sizes range from 17 to 34 kDa, with a core region composed of β-strands arranged in a trefoil manner [2]. There are 22 FGF members divided into seven subfamilies based on their functions and structural similarities (**Figure 1**). The FGF1 subfamily comprises FGF1 and FGF2 [3, 4]. Both these FGF isoforms are first isolated from bovine brains and are found to have high proliferation activities [5]. Based on their isoelectric points, FGF1 and FGF2 are often called acidic and basic growth factors, respectively. The FGF4 subfamily, consisting of FGF4, FGF5, and FGF6, is also involved in cell proliferation and migration [1, 6]. FGF3, FGF7, FGF10, and FGF22 are members of the FGF7 subfamily with cell migration and differentiation activities [1, 6]. The members of the FGF8 subfamily (FGF8, FGF17, and FGF18) play important roles in ear, eye, and brain development [1]. The FGF9 subfamily comprises FGF9, FGF16, and FGF20, which are involved in cell proliferation and differentiation [7]. Due to their modes of action, the 5 FGF subfamilies mentioned above are further classified as paracrine FGFs [8]. They can signal nearby cells through their binding with heparin sulfate/heparan sulfate proteoglycans (HS/HSPG) and their specific FGF receptors (FGFRs) [3, 9-11].

In comparison, the subfamily FGF11 has an intracrine function composed of FGF11, FGF12, FGF13, and FGF14. This group of FGFs is not considered a canonical FGF subfamily but is classified as FGF homologous factors (FHF) [12, 13]. These proteins share high sequence identity with other FGFs, assume similar β-trefoil core structures, and can bind to heparin but do not activate FGFRs [13]. Olsen et al. (2003) identified two surface residues at Arg52 and Val95 that may be responsible for FHFs' inabili-

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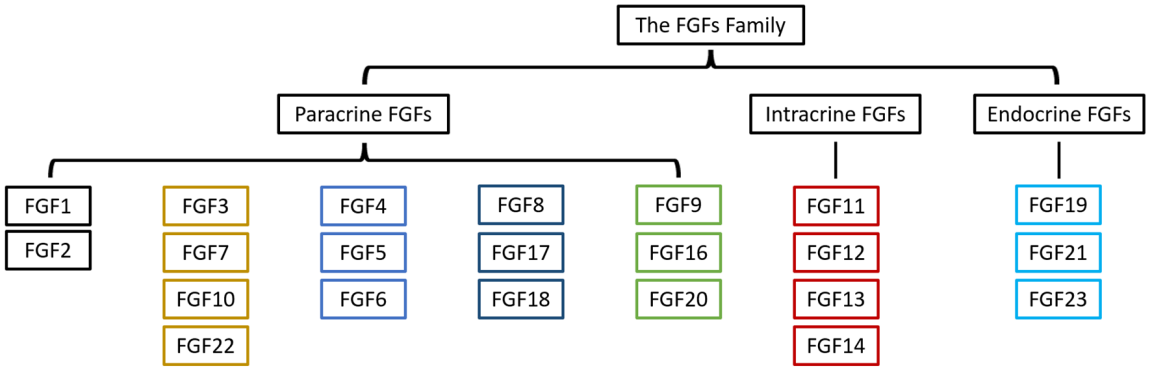

Figure 1. FGF subfamilies within the FGF family. Representation of all FGF members in the FGF family and their subgroups based on evolutionary relationships.

ty to activate FGFRs [13]. Instead of binding to FGFRs, they regulate voltage-gated sodium channels [14] and interact with intracellular kinase scaffold proteins and islet brain-2 [13]. Despite sharing notable similarities with canonical FGFs, FHFs have evolved to function differently than their FGF counterparts.

The last subfamily, FGF19, is composed of FGF19, FGF21, and FGF23. There is no known human FGF15. The murine ortholog of FGF15 is FGF19 [15]. This pair is referred to as FGF15/19. The members of the FGF19 subfamily are well known for their structural flexibility and unique signaling processes. Compared to canonical paracrine FGFs, this subfamily has a reduced affinity for HS, allowing these proteins to act in an endocrine manner [16]. They also require a co-receptor, called klotho protein, to form stable FGFR-FGFs-klotho complexes to elicit their functions [17]. Although they possess proliferative activities, they are also highly metabolic. FGF19, FGF21, and FGF23 are mainly involved in, but not limited to, bile acid synthesis, glucose and lipid homeostasis, and phosphate regulation [17]. Since their discoveries in the early 2000s, the FGF19 subfamily has attracted the interest of several researchers due to its potential applications in phosphate loss therapy, diabetes, and obesity treatments [18, 19]. This review aims to provide background information on endocrine FGFs' structures, physiological functions, and binding mechanisms and to discuss some novel biomedical applications of this FGF subfamily in recent years.

Brief discussion of FGF and receptor structures

Endocrine FGFs

Review of FGF19 structure: FGF19 is secreted in the ileum as a 216 amino acid protein from which the 24 residues at the N-terminus constitute the signal peptide [15]. The recombinant FGF19 obtained from a baculovirus-infected insect cell started with the 25th residue, suggesting that the first 24 amino acids only play a role in signaling the protein's secretion process [20] (**Figure 2A**).

Typically, the members of the FGF family are characterized by their β-trefoil structure composed of 12 β-sheets arranged in a triangular figure. The β1-β2 and β10-β12 regions are the HS binding sites (HBS), ensuring the functions of the proteins [11, 21]. HS is a glycosaminoglycan and a co-receptor to FGFs and allows for downstream signaling of the proteins [21]. However, FGF19 deviates from the typical FGF core structure. The crystal structure of the protein shows the β1-β2 and β10-β12 regions stretching out of the core. The β11 strand is not identified in FGF19 [22]. Instead, the β-strand is replaced by an α-helix (α-11) [23]. Similarly, the secondary HBS in β1-β2 also seems to extend out of the core [22, 23]. This variation in its structure is responsible for the decreased affinity of FGF19 for HS, thus allowing the protein to act in an endocrine manner. Among the endocrine FGFs, FGF19 has been reported to have the longest β1-β2 region and the strongest affinity for HS among the subfamily members.

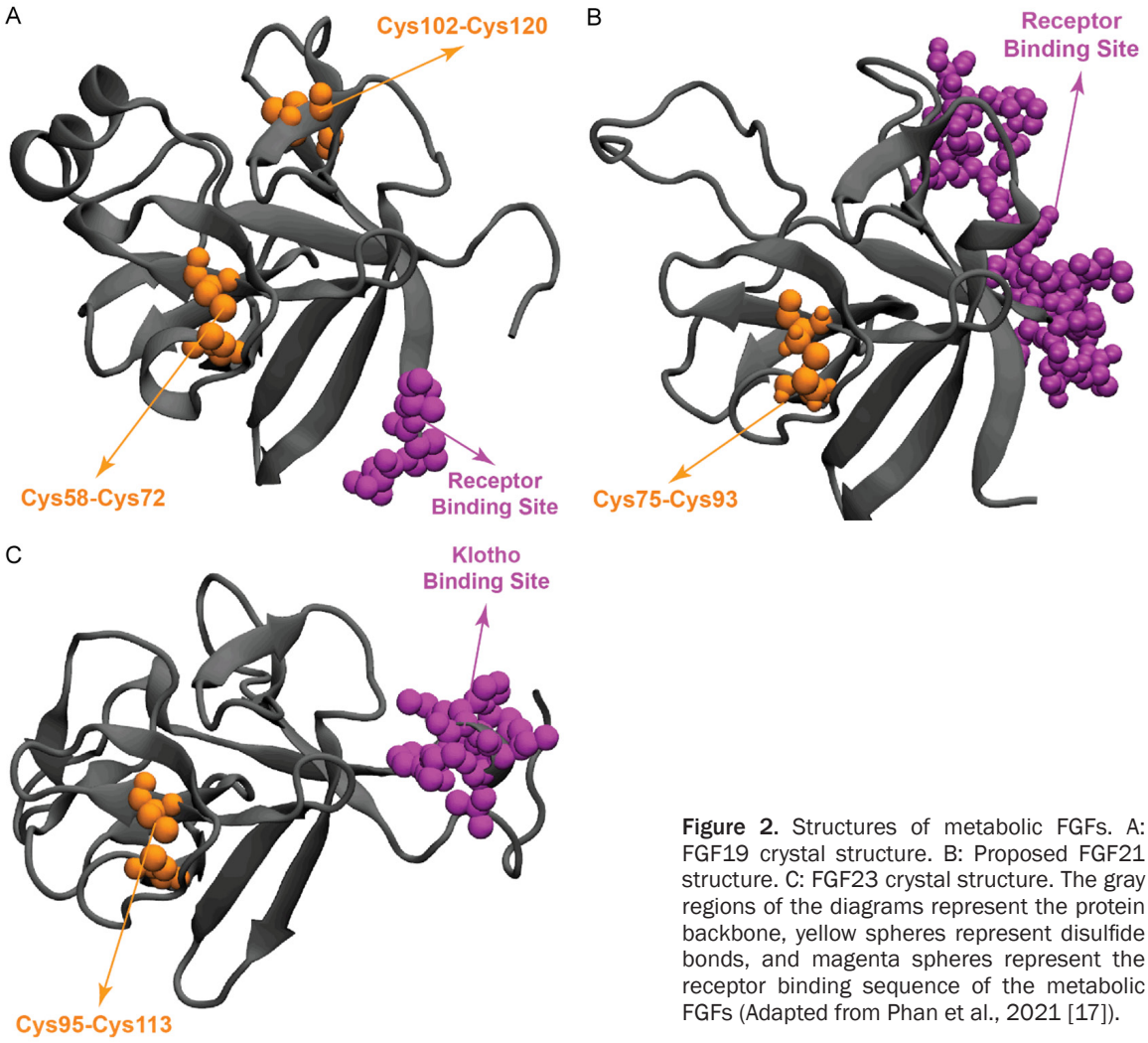

Figure 2. Structures of metabolic FGFs. A: FGF19 crystal structure. B: Proposed FGF21 structure. C: FGF23 crystal structure. The gray regions of the diagrams represent the protein backbone, yellow spheres represent disulfide bonds, and magenta spheres represent the receptor binding sequence of the metabolic FGFs (Adapted from Phan et al., 2021 [17]).

Another essential feature in the FGF19's structure is the presence of four cysteine residues involved in disulfide bridge formation. The first one is between C58-C70, and the second is C102-C120. C58-C70 connects β1 to β2, while C102-C120 connects β6 to a small α-helix located between β7 and β8, stabilizing their β-hairpin structures [17, 22, 23]. It is also important to note that C70 is specific to FGF19, as other paracrine-acting FGF molecules, such as FGF2 or FGF10, usually have a conserved glycine residue instead of the cysteine residue [17, 22, 23]. These disulfide bonds help stabilize the two nearby β-hairpin structures, further increasing the structural stability of FGF19 [22]. Despite these unique features to increase FGF19's structural stability, this protein is well known for its instability and short half-life like other endocrine FGFs [24], with a documented half-life of approximately 30 minutes [25, 26].

The dynamic structures of FGF21: Premature human FGF21 comprises 210 amino acids and weighs 22.3 kDa. Like FGF19, the mature molecule loses its signaling peptide at the N-terminus to generate an 181-amino-acid long protein [27]. These cleavages usually occur between Ala29 and Tyr30 [28]. The mature FGF21 amino acid sequence starts with a 13-residues N-terminus, a β-trefoil core, and ends with a 40-residues C-terminus [29, 30]. Only one disulfide bond is observed in FGF21 at position Cys75-Cys93 [31] (**Figure 2B**).

Attempts to crystallize FGF21 have yet to be successful, but the structure of FGF21 core has been elucidated using nuclear magnetic resonance spectroscopy [32]. Although the sequence identity is only 38% between FGF19 and FGF21, the two molecules present some structural similarities, especially in the HBS

region. FGF21 also comprises 11 strands (β1-β10, β12) for its 120-amino-acid β-trefoil core [30]. The typical β11 region, present in paracrine FGFs, is replaced by a proline-rich region extending out of the core, similar to FGF19 [30]. As a result, the HBS structure in FGF21 is distorted compared to typical HBS in paracrine FGFs, further decreasing FGF21's affinity to HS. The altered HBS structure reveals that the nearby β2-β3 hairpin structure and the neighboring areas are also flexible, contributing to FGF21's unstable core and decreased affinity to FGFR and HS complex [30]. Furthermore, the HBS region in FGF21 is more negatively charged than what is usually observed in other paracrine-acting FGFs, like FGF2 and FGF10, creating electrostatic repulsion with HS and further decreasing FGF21's affinity to HS [30]. Such alterations in FGF21's core and their negative impacts on FGF21's affinity to FGFRs and HS complex allow FGF21 to escape from the local extracellular matrix and act in an endocrine manner [30].

FGF21's N-terminus and C-terminus function differently during binding events with its cognate receptors. Specifically, the N-terminus is involved in receptor activation. From serial truncation experiments of the N-terminus, FGF21's ability to bind to FGFRs is not affected but decreased activation efficiency is observed [29, 33]. On the other hand, the C-terminus of FGF21 is crucial for binding to β-klotho (KLB). Serial truncation experiments with the C-terminus have been shown to decrease the binding affinity of FGF21 to KLB [29, 33]. Additionally, this C-terminus is believed to be flexible and prone to proteolysis. It is cleaved by a specific protease, called fibroblast activation protein, at position proline 171 to inactivate FGF21's biological activities [34, 35].

Features of the FGF23 structure: FGF23 is the latest member of the FGF19 subfamily. It is also the largest of the three proteins. The protein is secreted as a 251-residues protein that loses its signal sequence, similar to FGF19 and FGF21 and matures into the 227-residue-long protein [36]. The mature FGF23, which weighs around 32 kDa, also possesses a disulfide bridge at position C95-C113 [37] (**Figure 2C**).

Consistent with the other members of the family, FGF23 lacks the β11 strand, which is replaced by an α11-helix, but the β10-β12 region and the β1-β2 do not extend out of the protein's core [23]. Additionally, FGF23 possesses a shorter β9-β10 loop that may influence the β10-β12 loop, contributing to the non-canonical core structure [23]. With 72 residues, FGF23 has a longer C-terminus than both FGF19 and FGF21, which have 40 and 34 amino acids, respectively [38]. The C-tail comprises a tandem repeat called R1 and R2 and plays a vital role in signaling functions [38, 39]. Both repeats are observed to bind to α-klotho (KLA) with similar binding affinity [38]. Interestingly, the C-terminus of FGF23 is innately flexible and interferes with the crystallization of FGF23 [23].

Fragments of the FGF23 C-terminus are also found in organisms due to posttranslational cleavage of the peptide chain as a part of FGF23 regulation [40-42]. Specifically, two forms of FGF23 can be seen in the bloodstream [42-45]: the wild-type physiologically active FGF23 with a full-length C-terminus and the truncated form with only residues from Tyr25-Arg179 without the 72-residue C-terminus [42-45]. Such cleavage occurred at the conserved site in FGF23 called the R176-X-X-R179 site to inactivate the protein [46, 47]. O-glycosylation prevents cleavage of Arg176-Arg179 [47]. However, phosphorylation at Ser180 allows for proteolytic cleavage [37, 40].

Brief overview of endocrine FGF receptors and co-receptors

FGF receptors: The FGFR family has four members, namely FGFR1-FGFR4. Each member is encoded in distinct genes but shares a high sequence similarity of 56 to 71%. They are single-pass transmembrane proteins with an extracellular-binding region at the N-terminus and a cytoplasmic tyrosine kinase domain in the C-terminus, resembling the well-known receptor tyrosine kinases. The transmembrane domain helps anchor the receptor and facilitates its dimerization [48]. The intracellular tyrosine kinase domain participates in both dimerization and transduction of signaling [48]. The extracellular domain plays a crucial part in ligand-substrate binding. FGFRs' extracellular domains commonly have three immunoglobulin regions, often called D1-D3. D1 is known as the autoinhibitory region and is connected to D2 via an acid box, a sequence rich in aspartate residues. D1 and this linker region are often

dispensable in paracrine FGF binding but have been discovered to heavily regulate the interaction of endocrine FGFs with their cognate receptors. Briefly, the D1-linker region suppresses both direct interactions of FGF21 with FGFR1c and FGFR1c with KLB, signifying its role as an autoinhibitory region [49]. D2 and D3 are needed for ligand-receptor binding and specificity. FGFR1-3 often undergoes alternate splicing events, specifically in D3 regions, to create other splicing variants (b and c). The variants have unique binding specificities and create seven variants (FGFR1-3a, b, c, and FGFR4) [49].

Among the seven FGFR variants, endocrine FGFs can and prefer to interact with FGFR1c, 2c, 3c, and 4 [49, 50]. However, they may have different specificities for each protein and may be distributed differently in different body regions. For example, FGF21 has been reported to bind to FGFR4:KLB with less affinity than FGFR1:KLB [50]. Additionally, FGFRs distributions are varied in different tissues, such as FGFR1 is more abundant in adipose tissues, while FGFR2 is more expressed in the liver [51]. Combining with the fact that endocrine FGF21 functions in the liver differ drastically from those in adipose tissues [12], one can see that the tissue-specific expressions of klothos and FGFR subtypes determine the tissue selectivity and activity of endocrine FGFs [49].

Klotho co-receptors: A unifying trait of all endocrine FGFs is their requirement for co-receptors klotho to enact their physiological effects through FGFRs [52]. Superficial direct interactions have been reported between FGF19 and FGF21 toward FGFRs, but no definitive physiological effects are observed [49, 53]. Therefore, the presence of the co-receptor klotho is crucial for their direct assertion of biological activities through FGFRs [17].

Klothos are initially studied due to their roles in lifespan elongation in mice when overexpressed [30]. Conversely, premature aging is observed if the *klotho* gene is defective [54, 55]. However, with the initial findings of metabolic endocrine FGFs, the klotho family is found to be involved in many more diseases. Based on primary sequences, there are three subgroups in the klotho family: α-, β-, and γ-klotho [56, 57]. They are transmembrane proteins with a single pass [55]. They comprise a short intracellular domain of 11 amino acids, a transmembrane domain of 21 amino acids, and a long extracellular domain of 980 amino acids [58]. Klothos' extracellular regions have two homologous regions called KL1 and KL2 (also known as D1 and D2), connected by a proline-rich segment [59]. Despite not exhibiting glucosidase activities, both subunits resemble the plant β-glucosidase enzyme [56, 57, 59, 60]. Between these two domains, a cleft is formed to hold the substrates. Thus, the central β-barrel structure and the cleft between the two subunits form the binding pocket for endocrine FGFs [56, 61, 62]. Detailed information regarding binding modes between klothos and FGFs is discussed in the modes of binding section below. Among the three members, only KLA and KLB participate in endocrine FGF binding to FGFRs [17]. KLA is often expressed in kidneys and parathyroid glands where FGF23 asserts functions, while KLB is found in the liver and adipocytes where FGF19 and FGF21 are often found [52]. Interestingly, only KLA interacts with FGF23, while KLB prefers FGF21 and FGF19 [25]. Many works have supported the idea that klotho proteins increase the proximity of FGFs and FGFRs, thereby increasing the affinity between the protein and FGFRs [38, 63].

Modes of binding

The dependence on klotho proteins and the lack of affinity toward HPSGs are key differences that distinguish metabolic FGFs from other subfamilies [49]. Indeed, all metabolic FGFs preferentially signal through the FGFR-klotho complexes [64]. Thus, metabolic FGFs require the participation of klotho proteins to interact with their cognate receptors [65-67] (**Figure 3**). Mutations in endocrine FGFs suggest that the binding sites of the proteins with their co-receptors, KLA and KLB, are located at their C-terminus [23]. This ability contrasts with paracrine FGFs, which have a high affinity for HPSG but require neither HPSG nor the co-receptor klotho to induce their functions through FGFRs [30, 49]. Additionally, endocrine FGFs are believed to form a stable ternary complex with their FGFRs and klothos in a 1:1:1 ratio [49]. Despite the shared key characteristics among metabolic FGFs, the nuances of each protein's interactions with FGFRs and co-receptors are individually distinct and are highlighted in this section.

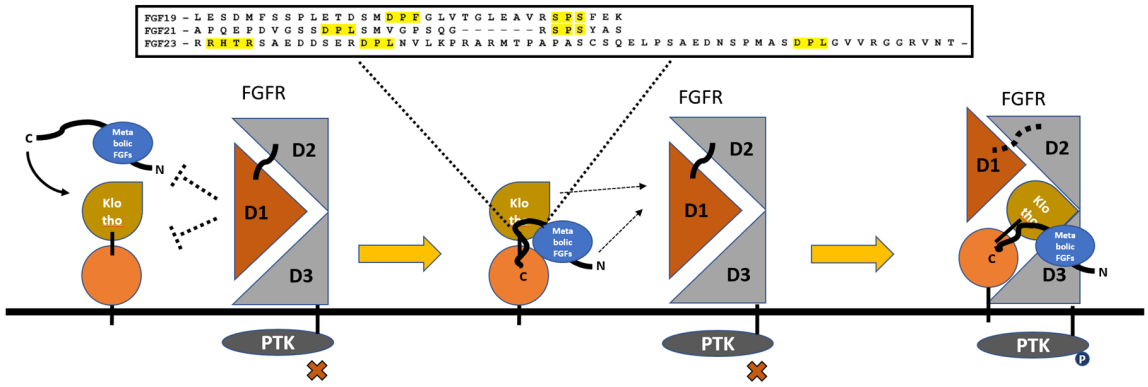

Figure 3. Diagrams of specific interactions of endocrine FGFs with their cognate receptors. FGF19, FGF21, and FGF23 C-terminus alignment are depicted at the top of the figure, showing the important binding motifs. FGF19 and FGF21 bind to KLB with two binding motifs in their C-termini: S-P-S and D-P-L/F. However, FGF23 only binds to KLA and possesses only one motif in its C-terminus: D-P-L/F.

The interaction between FGF19 and its receptors

The FGF19 subfamily requires co-receptor klotho proteins for their signaling through FGFRs. Interestingly, there is no common klotho protein to which all metabolic FGFs bind. Indeed, FGF19 and FGF21 prefer KLB, while FGF23 binds to KLA. Notably, FGF19 can bind and induce signaling through 4 FGFRs: 1c, 2c, 3c, and 4 [68]. However, FGF19's interactions with FGFR1c, 2c, and 3c are KLB-dependent, while those with FGFR4 are KLB-independent [68]. Moreover, its interactions with FGFR4 may contribute to its bile acid regulation but fail to show any improvement in glucose regulatory activities that FGF19 has [68].

Recent crystal structure reports of soluble KLB (sKLB) complex to metabolic FGFs have shown that FGF19 and FGF21 share many similar mechanistic interactions with KLB to assert their functions through FGFR1c [57]. Specifically, FGF19 binds to the co-receptor KLB through the C-terminus between the two domains of KLB. The FGF19 C-terminus (FGF19CT) binds to two sites on KLB: Site 1 and Site 2 on domains D1 and D2 on sKLB, respectively. These two sites are approximately 30 Å apart. Interestingly, Site 1 of the binding sites is maintained by internal hydrophobic interactions and forms a compact and rigid structure with multiple β-turns from P191-V203 of FGF19 [56]. This site contains the D-P-L/F motif in FGF19CT that helps maintain intramolecular hydrogen bonds for the multiple β-turns within the region [57]. Such tight and rigid structures of FGF19 also interact with many residues in KLB, spanning a large surface area [56].

On the other hand, Site 2 is said to mimic the binding site of glycoside hydrolase 1 (GH1) enzymes with many hydrophilic residues [56, 57]. However, site 2 has limited contact with FGF19CT and is distinct from the FGF21 C-terminus-sKLB interaction. This difference is attributed to the differences in the binding affinities of FGF19 and FGF21 toward sKLB. Despite the restrained contacts, Site 2-FGF19CT still includes residues V209-S213 of FGF19. Within this region of FGF19CT, the sugar-mimicking motif S-P-S strongly binds to the E693 residue of KLB, effectively resembling the enzyme-substrate interactions in GH1 [56]. Indeed, this E693 residue in KLB corresponds to one of two "conserved catalytic" glutamates that serve as general acid/base catalysts when interacting with FGF19 residues S211 and S213 in a double-displacement reaction model in glycoside hydrolases, such as the GH1 enzyme [56]. Further support for S-P-S can be seen in sKLB's F826 and F931 through hydrophobic interactions with the motif [57]. As expected, D-P-L/F and S-P-S motifs are also observed in FGF21 across many species [57] (**Figure 3**). The D-P-L/F motif is also found in FGF23 [38, 57].

The FGF19 N-terminus is believed to be essential in determining FGFR-binding specificity, especially FGFR4 [20]. Replacing FGF19's N-terminus with corresponding FGF21 amino acids seems to reduce the protein's ability to activate FGFR4 drastically but did not signifi-

cantly compromise the protein's activity with FGFR1 [69]. Replacing the FGF21 N-terminus with the corresponding FGF19 did not affect the activation of FGFR4, indicating that the N-terminus of FGF19 must play a crucial role in determining the FGFR-binding specificity [69, 70]. Indeed, residues 38-42 at the FGF19 N-terminus (^{38}WGDPI^{42}) are crucial in FGFR4 activation [70, 71]. The FGF19 N-terminus is also involved in mitogenic and tumorigenic activities [70, 71]. Specifically, the recent development of anti-FGF19 antibodies targeting the FGF19 N-terminus can suppress tumor growth [72]. Since FGF19 can follow klotho-independent induction with FGFR4 and HPSGs, its N-terminus is believed to contribute to HPSGs and FGFR interactions [73]. More specifically, it has been suggested that this N-terminus region plays a crucial role in FGFR interactions and downstream events [72, 73]. However, the precise binding events of the FGF19 N-terminus and FGFRs and their tumorigenic activities are still underdeveloped and require future investigations.

Conserved mode of binding in FGF21

Similar to FGF19, FGF21 requires KLB to function through FGFRs. While FGFRs, especially FGFR1, are expressed in a broad array of tissues, KLB is expressed in specific locations, notably the adipose tissue, liver, pancreas [74], and various areas of the brain [75, 76]. Like FGF19, FGF21 is believed to bind to the needed KLB through the protein's C-terminus and interact with the FGFRs through its N-terminus (**Figure 3**). Without FGF21, KLB and FGFRs may not be able to bind. Likewise, there is no evidence that FGF21 and FGFRs can form stable binding without the co-receptor KLB [49]. Unlike FGF19, FGF21 is reported to bind to only FGFR1c, 2c, and 3c but cannot induce signals through FGFR4 efficiently [77].

The underlying binding mechanism between FGF21-KLB-FGFR1c is reported in detail in recent works [33, 56]. Like FGF19, the FGF21 C-terminus (FGF21CT) interacts at the interface of domains D1 and D2 in KLB at Site 1 and 2. FGF21CT's residues P186-V197 bind to Site 1 through hydrophobic interactions, residues S200-S209 bind to Site 2 through S-P-S motif mimicking glycoside hydrolases GH1 enzyme, and residues 198 to 200 do not interact with KLB [57]. One can observe that the D-P-L/F and S-P-S sugar-mimicking motifs are shared between FGF19 (D198-P199-L200, S211-P212-S213) and FGF21 (D192-P193-F194, S204-P205-S206) despite the low identity (approximately 40%) shared between FGF19 and FGF21 C-terminus [56, 78]. These motifs are also conserved within FGF19 and FGF21 variants among different species, indicating their importance in KLB interactions [57].

Despite the highly similar KLB-binding mechanism, FGF21 and FGF19 interactions with KLB produce observable variances in the complexes' structures [57]. An observable 17° change between the D1 and D2 domains of KLB is present in FGF19CT-sKLB. On the other hand, FGF21CT-KLB only produces a change of 6°. Additional changes in distances are also reported in FGF19CT-sKLB and FGF21CT-sKLB, suggesting that FGF19 and FGF21 occupy the same sites within KLB but may utilize different crystal packing interfaces [56, 57]. The differences in degrees of contact between FGF21 and FGF19 within Site 2 binding sites hint at potential differences in sKLB binding affinity between the two proteins. Indeed, the binding kinetics study using biolayer interferometry portrayed dissociation constants (K_D) of 210 ± 13 and 23.9 ± 0.7 nM for FGF19 and FGF21, respectively. Additionally, FGF21 also has a slower dissociation rate (k_{off}) (6.7×10^{-3} s^{-1}) than FGF19 (6.1×10^{-2} s^{-1}). A notable 10-fold increase in K_D of FGF21 binding affinity is driven by a commensurate 10-fold decrease in k_{off} [57]. However, mitogen-activated protein kinases (MAPKs) activation between the two proteins is measured to be the same despite the 10-fold magnitude of binding affinity [57].

On the one hand, the FGF21 N-terminus helps the protein interact with FGFRs [49] at its D2-D3 extracellular segments [33] (**Figure 3**). Deletions of the FGF21 N-terminus did not affect its ability to bind to KLB or drastically decrease the protein's potency in the luciferase assay. However, such deletion affects the efficacy (maximal response) of FGFRs and drastically reduces the receptor's efficacy (6 amino acid deletion) or abolishes its response (8 amino acid deletion) [33]. Additionally, the FGFR D1/linker region, the autoinhibitory region of FGFRs, may regulate the interactions of the FGF21 N-terminus with the FGFR D2-D3

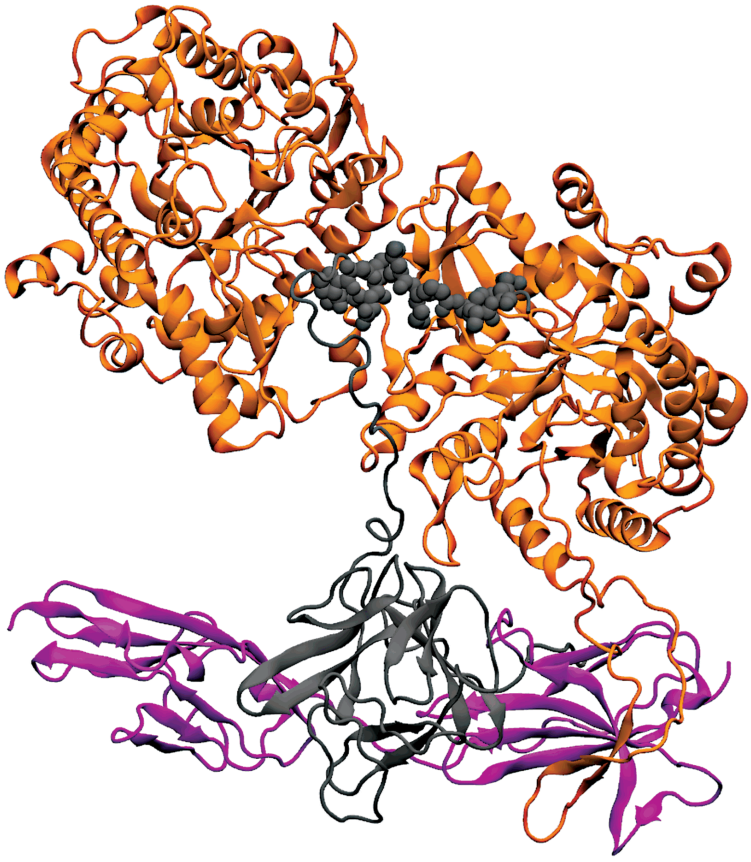

Figure 4. Crystal structure of FGF23-FGFR1c-KLA. Both the KL1 and KL2 domains of the KLA backbone are represented in orange, FGF23 is in gray, and receptor FGFR1c is in magenta (Figure from Phan et al., 2021 [17]).

regions of the receptors [49]. Specifically, FGFRs may lock into an unfavorable conformation by the D1/linker region for neither FGF21 nor KLB to interact directly [49]. On the other hand, a weak and transient but direct FGF21-FGFR interaction is possible and implicated the function of autoinhibitory portions of FGFRs as the determination of the endocrine FGFs' functions. This notion challenges the common understanding that the autoinhibitory region functions to prevent receptor automerizations [49]. Unfortunately, the exact mechanisms between the FGF21 N-terminus and FGFRs have not been reported [33, 49]. However, FGFR1c and FGF21 are suspected to have a 2:2 complex similar to paracrine FGFs and FGFRs [49]. Another study indicated the binding ratio differently, with FGF21:FGFR1:KLB as a 1:2:1 ratio instead of the often proposed 1:1:1 or 2:2:2 ratio [79]. Altogether, the data suggested that KLB is crucial in FGFR-FGF21-KLB binding by bringing FGF21 into proximity of FGFRs for binding, and may also work with FGF21 to open up D2-D3 regions of FGFRs for binding [49].

Multiple binding sites in FGF23

Unlike FGF19 and FGF21, FGF23 depends upon interactions with the co-receptor KLA. In the presence of KLA, FGF23 binding affinity to FGFR is 20 times stronger, implicating KLA's critical roles in FGF23-FGFR activation [39]. FGF23 has high preferences for FGFR1c, 2c, 3c and FGFR4 [80]. Recent works demonstrated that FGF23 signaling is negatively affected if FGFR1c is mutated to become FGFR1b by replacing 1c's serine residue with 1b's tyrosine residue [80].

The detailed mechanical behaviors of FGF23 toward KLA have been well studied. Through the FGF23 C-terminus, FGF23 can bind to KLA [66]. There are two repeated tandem segments in the FGF23 C-terminus, R1 and R2. Both sections lack the S-P-S motif. However, both sections have the D-P-L/F motif conserved in all metabolic FGFs [38, 80]. Such motifs, especially in R1 (D188-P189-L190-N191-V192-L193), form a compact, rigid cage-like structure upon binding with KLA [38]. Moreover, FGF23 variants containing only one section, R1 or R2, show similar binding to KLA compared to native FGF23 and, thus, act as FGF23 antagonists [38, 56]. The data suggested that FGF23 has two binding sites for KLA. However, only one is sufficient for the signaling process [38, 56]. The C-terminus of FGF23 is also believed to be essential for forming the ternary FGF23-FGFR-KLA stable complex [39] (**Figure 4**).

On the other hand, the FGF23 N-terminus consists of residues 25-179. Most residues are the core of the protein and share some homology with other FGF members [81]. This section binds FGFR1c between the D2 and D3 subunits

of the receptor. However, such interactions are weaker when compared to paracrine FGF9-FGFR1c interactions [80]. Without forming a stable ternary complex, FGF23 showed poor binding to either klotho or FGFRs alone, like other metabolic FGFs [39].

Physiological activity and pathways

Since the initial reports of the energy homeostasis regulation activities of FGF19 and FGF21 in the early 2000s [82, 83], an explosion of investigations have delineated the physiological functions and pathways of metabolic FGFs in the human body as detailed and intricate networks of many players with multiple downstream pathways in various tissues. To better understand this complex relationship and unravel FGF19 subfamily functions, the fundamental elements of these pathways are reviewed and highlighted in the following section.

Discussion of FGF19 physiological roles

FGF19 is mainly produced by enterocytes of the ileum due to farnesoid X receptor (FXR) activation upon binding of bile acids [84]. In addition to bile acids, Diet1 (a 236-kDa protein expressed in small intestine enterocytes) levels are involved in regulating FGF19 [85]. Furthermore, endoplasmic reticulum stress molecules, vitamin D receptors, and FXR are documented in the literature as modulators of FGF19 levels in the body [25, 84, 86]. FGF19 mainly interacts with the liver, gallbladder, adipose tissue, and brain and regulates bile acid, lipid, glucose, and protein production by interacting with FGFR4 and KLB [84].

One of the main functions of FGF19 is its involvement in negative feedback control of bile acid synthesis and secretion via hydroxylation of cholesterol by cytochrome P450 7α-hydroxylase (CYP7A1), leading to decreased hydroxylation of bile acids and ultimately resulting in a reduction in bile acid synthesis [86]. It is suggested that this function of FGF19 is partly dependent on small heterodimer partner-1 (SHP1), although the exact molecular mechanisms of such cooperation remain unclear. High bile acid levels stimulate FXR activation in the liver, thus forming a complex with the retinoid x receptor (RXR) in the nucleus of hepatocytes. FXR-RXR will activate SPH1 expression, which is vital in the inhibition of key receptors (LRH1 and HNF4α) responsible for the transactivation of the *CYP7A1* gene, subsequently decreasing bile acid levels [25, 86, 87]. Elevated levels of bile acids will stimulate FXR in enterocytes and induce FGF19 production from enterocytes in the terminal ileum, and then FGF19 will travel through the portal and systemic circulation. Since the liver expresses FGFR4 and KLB, FGF19 will form a ternary complex in the liver, KLB-FGF19-FGFR4, which then activates extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase signaling pathways, further downregulates *CYP7A1* gene expression and bile acid production [88].

Bile acid metabolism is linked with triglyceride and cholesterol homeostasis. Hence, inhibiting bile acid synthesis will lead to elevated plasma triglyceride and cholesterol levels. However, increasing fatty acid oxidation and energy metabolism by FGF19 will lessen this risk. There are records of FGF19 involvement in increasing the abundance of the essential sterol transporters in the liver, namely the ATP-binding cassette sub-family G member 5 and member 8, hence lowering plasma lipid content [89] and inhibiting sterol regulatory element binding protein 1c, which is a major transcription factor of lipid-encoding genes [90]. Thus, elevated levels of FGF19 exert a protective effect on preventing lipid assimilation and hyperlipidemia [91].

Gerhard et al. (2013) first described the association of decreased FGF19 levels in type 2 diabetes mellitus (T2DM) [92]. Elevated gluconeogenesis in the liver of T2DM patients is the leading cause of hyperglycemia and organ damage. Glycogen synthases a and b are crucial enzymes in regulating hepatic glycogen synthesis. FGF19 can stimulate the phosphorylation of hepatic glycogen synthase kinase (GSK) 3α and β isoforms and subsequently inactivate both isoforms. Since GSK-mediated phosphorylation and inactivation of glycogen synthase are inhibited, hepatic glycogen synthesis will be elevated [93]. This FGF19-mediated blood glucose homeostasis is postulated to be regulated by ERK1/2 pathway simulation and the inhibition of AGRP/NPY neurons [94]. However, the exact molecular pathways still need to be fully understood.

FGF19 helps maintain optimal metabolic health and energy consumption by interacting with the intestinal tract and adipose tissue. Many studies have documented that FGF19-mediated brown adipose tissue activation and browning of white adipose tissue are key mechanisms associated with higher energy consumption, protection against obesity, hyperlipidemia, hyperglycemia, and various types of metabolic disorders [93, 95]. The browning of white adipose tissue is proposed to be associated with *uncoupling protein 1* (*UCP1)* gene regulation, a critical gene for thermogenesis. FGF19 and *UCP1* gene expression have a significant correlation, and may indicate that FGF19 is involved with *UCP1* gene expression in subcutaneous adipose tissue [96].

Cancer patients with elevated levels of FGF19 are found to have an elevated risk for cancer progression and metastasis through Ras-Raf-MAPK, phosphoinositide 3-kinases (PI3K)-protein kinase B (AKT), epithelial-mesenchymal transition, single transducer and activator of transcription pathways, which are all stimulated via the FGF19-FGFR4-KLB interaction. Aberrant cell signaling alters cell proliferation, migration, survival, and apoptosis, leading to cancer [97]. Recent findings show that FGF19-FGFR4 axis alterations are involved in hepatocellular carcinoma (HCC), promoting cancer initiation and progression [98]. Furthermore, FGF19 expression is significantly elevated in breast cancer and associated with upregulation of the AKT pathway [99, 100].

Additionally, the FGF19-FGFR4 interaction stimulates lipogenesis upon food intake, whereas during starvation, FGF19 promotes lipolysis through FGFR1c [95]. In addition to the effects above, the association of FGF19 with glucose homeostasis through FGFR1c in the brain has been documented, highlighting the glucose suppression effect of systemic FGF19 in an insulin-independent manner [101]. Zhao et al. (2020) found that FGF19 is significantly decreased in postmenopausal women with osteoporosis compared with healthy controls. Moreover, serum bile acid levels are also found to be lower than those in controls. Collectively, the data infer that FGF19-mediated bile acid metabolism is pivotal in bone metabolism [102].

The roles of FGF21 in metabolism

The main sites of FGF21 production in humans are the liver, adipose tissue (white and brown adipose tissues), and pancreas via various stimuli [103]. However, a minute level of FGF21 production has been recorded in the kidney, heart, thymus, testes, and skeletal and cardiac muscles [84, 104]. Once expressed, FGF21 can affect a selection of tissues and organs determined by a combination of tissue-specific expressions of KLB and specific FGFR isoforms on said tissues [77]. An example of FGF21's effector organs is the brain due to its ability to cross the blood-brain barrier [104-106]. In addition to the brain, white and brown adipose tissue, heart, and skeletal muscles are target sites for FGF21 as well [104]. Upon arrival at target sites, FGF21 mediates lipid and glucose metabolisms, decreases lipogenesis, and increases insulin sensitivity, amongst other activities [107]. Such a wide array of expression locations and target sites for FGF21 creates an intertwined cross-talking system between seven different organs and tissues that can be triggered by many stimuli, rendering FGF21 physiological activities complicated and wildly debated [104]. These complex relationships between different stimuli, site-specific expressions of FGF21, and physiological effects of FGF21 at target sites have been reported in great detail [104].

Indeed, hepatic FGF21 expressions and physiological effects in human and animal models are diverse, complex, and nuanced. Firstly, different stimuli may lead to different site-specific expressions of FGF21 [104]. Excessive oxidative stress, nutritional and cellular stress, cold exposure, and exercise are principal stimuli for FGF21 secretion [103, 107]. Nutritional stress can originate from extended fasting, a ketogenic diet, or amino acid scarcity [103, 107]. However, site-specific FGF21 expressions and effects have been detected depending on the type of nutritional stress stimulus. For example, nutritional stress due to a ketogenic diet and fasting induces FGF21 expression in the liver, conferring FGF21's protective functions in liver through hepatic fatty acid oxidation and reduction of lipid flux into the liver from metabolic stress due to lipid overload. In contrast, cold exposure and feeding signals lead to FGF21 expression in brown and white adipose tissue, stimulating thermogenesis [104, 108-113].

Secondly, the same nutritional stimuli may cause different FGF21 expressions in different experimental models. Specifically, hepatic FGF21 levels increase with fasting and inges-

tion of ketogenic diets in rodents and human subjects with non-alcoholic fatty liver diseases (NAFLD) but not in healthy adult humans [114]. On the other hand, amino acid/protein restriction may cause an increase in FGF21 expression in mice, rats, and humans [115]. However, such regulation of hepatic FGF21 stimulation due to protein restriction is not entirely understood since human results are inconsistent [104].

Thirdly, FGF21 expressions are paradoxically influenced by feeding and fasting signals [116]. As mentioned earlier, liver-derived FGF21 expression is stimulated mainly by nutritional stress, i.e., extended fasting, a high ketogenic diet, protein restriction, and alcohol consumption [108, 109, 114, 115, 117]. Conversely, feeding with simple sugar, specifically fructose consumption, is also a significant stimulant for hepatic FGF21 secretion in humans, especially in subjects with metabolic disorders [118]. Indeed, the production and secretion of FGF21 in the liver due to fasting are found to be regulated by peroxisome proliferator-activated receptor α (PPARα). In contrast, elevated hepatic FGF21 levels due to excess glucose and fructose intake are regulated by carbohydrate response element binding protein (ChREBP) [116]. Thus, FGF21 expression may be linked to an adaptive response to fasting or starvation in rodents, but the same FGF21 may play a different role in fructose metabolism in humans [118]. Liver-derived FGF21 can also be elevated through acute and chronic alcohol consumption in mice [119, 120] and humans [120, 121]. An increase in FGF21 levels after chronic alcohol consumption in mice is shown to lessen alcohol-induced hepatic damage and reduce mortality, indicating that FGF21 may have some protective characteristics against alcoholic liver diseases such as alcoholic steatohepatitis [119, 120]. PPARα and/or ChREBP are believed to combine to fine-tune alcohol-mediated FGF21 elevation in the liver [104]. However, this regulation of hepatic FGF21 expression stimulation caused by alcohol consumption is not yet fully understood. Due to this notable activity of FGF21, this protein is often deemed as an alcohol appetite inhibitor in humans [121] and could be potential treatment for alcoholic liver disease [119].

Once expressed, liver-derived FGF21 finds its way to adipose tissue, one of its primary effector tissues where FGF21 co-receptor KLB is highly expressed [104]. The functions of FGF21 in adipocytes are lipid and glucose metabolism and regulation of energy balance through interactions of the KLB-FGF21-FGFR1 ternary complex [122]. The downstream effects of such interaction are improved insulin sensitivity, reduced lipolysis and glucose homeostasis, and enhanced mitochondrial oxidative capacity [104]. Notably, as a metabolic regulator, FGF21 is involved in glucose uptake via induced glucose transporter (GLUT)-1 expression in 3T3-L1 adipocytes in an insulin-independent manner [82]. In response to FGF21 early treatments (24 hours), adipocytes (3T3-L1) activate the phosphorylation of FGFR1, resulting in acute activation of *Akt, GSK-3, SHP-2, MEK1/2, p70^{S6K}, Stat, Raf, FRS-2*, and *Ras/MAPK*; along with the influx of calcium [82, 123]. Specifically, in response to FGF21 stimulation, primarily ERK1/2 pathways and a low level of protein kinase *Akt* pathways are activated [82, 123, 124]. Afterward, two ERK1/2-responsive transcription factors called E26 Transformation-Specific Like-1 protein (ELK-1) and Serum Response Factor (SRF) are phosphorylated to bind to the *GLUT1* gene promoter [124]. SRF binds to factor called Serum Response Element (SRE) to form a binary SRF-SRE complex within the promoter region. Activated Elk-1 binds to this SRF-SRE complex, leading to the transactivation of its target gene *GLUT1* through a conserved cis-element within its promoter [124], and increasing glucose uptake through this glucose channel. Alongside the ERK1/2-GLUT1 pathways, FGF21 also activates the AMP-activated protein kinase pathway and the silent mating type information regulation two homolog 1 pathway, which leads to the activation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and histone 3 [125]. These pathways promote glucose uptake via both transcription and plasma membrane translocation of GLUT1 in an insulin-independent manner, enhance mitochondrial oxidative capacity via increasing oxygen consumption and citrate synthase activity, and induce expression of other metabolic genes [126]. It has been demonstrated that FGF21 induces a much slower but sustained increase in glucose uptake compared to insulin-stimulated glucose uptake, which is fast and transient [82, 127]. Thus, these two components may work together complementarily to regulate glucose levels.

On the other hand, chronic treatment of FGF21 for 72 hours increases PPARγ protein expression, GLUT1 mRNA, and protein production [123]. When treated with a PPARγ agonist, rosiglitazone, further potentiated FGF21 induced-GLUT1 upregulation effects are observed and induced activation of FGFR2 [123]. Additionally, FGF21 with a low level of rosiglitazone can also aid in the differentiation process of adipocytes, specifically 3T3-L1, with modest effects [123]. Despite the observed decrease in serum glucose levels in animal models through FGF21, clinical studies in humans have failed to demonstrate similar effects [128, 129], suggesting that FGF21 may be targeting the lipid pathways rather than improving hyperglycemia in humans [130].

In addition to regulation of glucose levels through adipocytes, FGF21 also induces lipolysis in adipocytes in both cell culture and animal models [82, 109], releasing the stored lipids for downstream usages by regulating lipase genes during feeding but inhibiting it during fasting [109] and increases lipid oxidation in obese patients [131]. It is believed that FGF21-treated subjects have increased energy expenditure and decreased fat mass from *in vivo* studies [132, 133]. However, conflicting results about FGF21 activities from *in vitro* studies, such as suppressing lipolysis and reducing no esterified free fatty acid levels, are also reported [134, 135]. In obese human subjects, at insulin-stimulated conditions, increased serum FGF21 levels are also noted with increased lipid oxidation rates [131]. As noted previously, FGF21 levels are positively associated with insulin-resistance states (i.e., obesity, T2DM, and NAFLD) and specifically with adiposity, fasting insulin, and triglycerides [131, 136], which is postulated to be a protective mechanism against lipotoxicity induced by obesity or a compensatory upregulation due to obesity-induced FGF21-resistance [52, 136]. Combined with the conflicting FGF21 lipolysis activities, one can see the importance of additional investigation to elucidate further the exact mechanisms and pathways of FGF21 on lipolysis.

The brain and heart are other effector organs of liver-derived FGF21 [104, 133]. Despite not being expressed in the central nervous system, FGF21 can cross the blood-brain barrier in humans and is involved in the regulation/modulation of functions of the central nervous system at the hypothalamus, an area rich in KLB expressions, the co-receptor of FGF21 [106, 133]. Research studies have demonstrated the effect of FGF21 in the brain as an upregulation of the expression of thermogenic genes, weight loss, and elevated energy expenditure via the sympathetic nervous system in white adipose tissue [133, 137, 138]. The proposed model of FGF21 acting on the central nervous system is first through FGF21 action on the hypothalamus, which induces corticotropin-releasing factors and stimulates sympathetic nerve activity to produce sympathetic outflow in adipose tissues, which upregulates Uncoupling Protein 1 and finally induces lipolysis in said adipose tissues [133]. Combined with FGF21's glucose uptake activities and direct substrate mobilization on adipose tissues, FGF21 can efficiently exert the energy expenditure increases observed in mouse models [133]. The effects of hepatic FGF21 on the heart have yet to be thoroughly investigated. However, FGF21 elicited a protective role against oxidative stress in cardiomyocytes in culture through interactions of FGFR1 and KLB [139].

Furthermore, the liver is another FGF21-targeted organ in an autocrine manner. FGF21 attenuates lipogenesis, increases hepatic fatty acid oxidation, ketogenesis, and gluconeogenesis, and stimulates insulin sensitivity in the liver [107, 140, 141]. Additionally, there are records of the involvement of FGF21 in oxidative and endoplasmic reticulum stress [107], downregulation of fat synthesis, and proinflammatory cytokines [142, 143]. Specifically, FGF21 treatment in bovine hepatocytes is shown to affect two transcription factors, SREBF1 and PPARA, through the AMPK pathway [144]. PPARA is translocated in the nucleus, increasing lipid oxidation, lipid transport genes, and protein expressions (such as PPARGC1a, ACOX1, CPT1a, LDLR, and CD36) [144]. On the other hand, FGF21 treatment inhibits SREBF1 translocation into the nucleus, thus inhibiting several other lipogenic genes and protein expressions like ACACA and ACLY, which help heighten lipid oxidation rates while inhibiting lipid transportation and decrease lipogenesis via the AMPK pathways [144, 145]. Similarly, in mouse models, FGF21 treatment also showed to induce PGC-1α expressions in the liver, a transcriptional coactivator that in-

duces lipid oxidation, mitochondrial oxidative phosphorylation, and gluconeogenesis [145] and increases the oxidative capacities of adipocytes [126]. It is believed that FGF21 full expression is required for normal hepatic fatty oxidation activation and is a lipid homeostasis regulator [108]. Thus, FGF21 is effectively involved in lipid profile improvement, and it has been utilized in recent clinical trials to treat metabolic diseases such as NAFLD and non-alcoholic steatohepatitis (NASH) with positive results [142, 146-148]. However, due to the high expression of FGFR4, which has a low affinity for FGF21, and low expression of FGFR1 in the liver, the primary receptor of FGF21 [50, 149], some believe that FGF21 does not contribute directly to the improvement on liver physiology [104] but instead indirectly through the central nervous system [145]. Therefore, one can summarize that although the autocrine effect of hepatic FGF21 on the liver has been reported, its exact effects still need further research [149].

New evidence confirms that FGF21 can also be expressed from adipocytes [104, 136]. It is regulated via PPARγ instead of PPARα, which regulates hepatic FGF21 [104, 125, 150]. Such expression of adipocyte-derived is noted in 3T3-L1 differentiated adipocytes and human adipocytes, as well as in obese mice adipose tissues [136]. Overfeeding, obesity, thermogenic activation, and cold exposure are found to be the main stimuli for adipocyte-derived FGF21 expression [104]. Adipocyte-derived FGF21 effects are assumed to be on adipose tissue in an autocrine manner [151] to increase glucose uptake through the upregulation of *GLUT1* genes [123] but do not contribute to the circulating FGF21 pool within the body, which is primarily hepatic FGF21 [152]. However, under specific circumstances (i.e., severe cold exposure), adipocyte-derived FGF21 (specifically from brown adipose tissues) may join the circulating FGF21 pool [104, 153]. Thus, more investigations are needed to determine whether adipocyte-derived FGF21 can also act in an endocrine fashion like liver-derived FGF21 [104].

It has been found that impaired FGF21-mediated signaling can lead to insulin resistance in the liver [93]. In FGF21-deficient mice, liver insulin resistance and glucose production increased [154]. Similarly, FGF21 is believed to be related to liver insulin resistance in T2DM patients and is involved in insulin regulation [93]. Indeed, FGF21 enhances insulin sensitivity through direct adipose tissue signaling, specifically brown adipocytes [155]. UCP1 is also believed to be involved in FGF21-mediated insulin sensitivity enhancement [155]. UCP1, located in brown adipocytes' mitochondrial inner membrane, dissipates energy as heat and maintains body temperature during cold exposure [156]. Impaired FGF21-insulin sensitization activities are observed in UCP1-deficient mice, thus concluding that UCP1 is essential in FGF21-mediated insulin regulation [157]. Indeed, direct FGF21 signaling to UCP1 in brown adipocytes is critical to enhance insulin sensitivity [155]. Adiponectin (a cytokine secreted by adipocytes and involved in regulating glucose levels, lipid metabolism, and insulin sensitivity through anti-inflammatory, antifibrotic, and antioxidant effects) was initially presumed to be essential in FGF21-mediated insulin sensitivity, relieving obesity-related hyperglycemia [93, 103], and inducing FGF21 expression in liver [158]. However, FGF21 can currently elicit these effects without adiponectin [155]. Thus, in adiponectin knockout mice, adipocyte FGF21-associated activity is mediated via the ERK1/2 pathway [93, 103], rendering adiponectin dispensable in FGF21-mediated insulin sensitivity enhancement [155]. Such intertwined crosstalk between multiple tissues and organs described here demonstrated the notable complexity of FGF21 in humans. Further, it highlighted the need for additional investigations to understand this web of physiological activities of FGF21.

Metabolic activity of FGF23

The primary cellular sources of FGF23 in human adults are osteocytes, osteoblasts, and bone marrow [159]. However, low FGF23 mRNA levels have been detected in renal tubular epithelial cells, cardiac myocytes, and the spleen in various diseases and conditions [40, 159, 160]. Classical stimuli for FGF23 expressions are elevated levels of calcium, parathyroid hormone (PTH), 1,25-dihydroxyvitamin D, and phosphate [40].

Other factors may also influence FGF23 expressions. Recent findings reveal the influence

of iron deficiency, inflammation, hypoxia, and erythropoiesis on FGF23 expressions [40]. Molecular studies have demonstrated that low serum iron levels can stimulate FGF23 transcription and cleavage of a full-length intact FGF23 (iFGF23) into C-terminus cleavage products of FGF23 (cFGF23) simultaneously [161-163]. Furthermore, the levels of serum inflammatory markers such as interleukin 6, tumor necrosis factor α, C-reactive protein, and fibrinogen correlate with FGF23 levels [164], and this association exhibits trends of iFGF23 and cFGF23 levels similar to those in iron deficiency. Hence, chronic inflammation might contribute to disproportionate FGF23 levels and cleavage, which increase the risk of specific pathologies, i.e., chronic kidney disease (CKD) or autosomal dominant hypo-phosphatemic rickets (ADHR) [164]. EPO is a hormone released from the kidneys during hypoxia and hypoxemia to increase the rate of red blood cell production [40]. FGF23 production and secretion from bone marrow are significantly elevated with increased EPO [165]. Rabadi et al. (2017) revealed that acute loss of 10% of the total blood in wild-type C57BL/6 mice indicated increased plasma cFGF23 fragments, suggesting possible existence of EPO-mediated FGF23 production [166]. Agoro et al. (2018) showed a significant reduction in apoptosis of erythroid cells and hematopoietic stem cells committing toward the erythroid lineage when FGF23 secretion is inhibited in mice (male C57BL/6J) with renal failure. Furthermore, they observed reduced inflammation and increased serum iron and ferritin levels, confirming the therapeutic attenuation of FGF23 as a potential treatment for renal anemia and iron deficiency [167]. Moreover, there are records of regulation of FGF23 transcription by calcium through L-type voltage-sensitive calcium channels [168] and evidence of direct regulation of FGF23 by energy intake via mechanistic target of rapamycin [169].

The primary physiological function of FGF23 is phosphate and vitamin D homeostasis in humans [170-172]. Phosphate level is central to cellular energy metabolism, cell growth, signal transduction, skeletal development, and mineralization and must be carefully maintained. Phosphate homeostasis by FGF23 is achieved via harmonious modulation of kidney phosphate and vitamin D regulation, and parathyroid hormone secretion [173]. FGF23 secretion from osteoblasts is stimulated by phosphate loading (accumulation/elevation of phosphate availability) due to increased dietary phosphate uptake and/or reduced phosphate excretion in the kidney. FGF23 has prominent effects in the renal distal convoluted tubule and parathyroid gland [40, 174]. FGF23 regulates phosphate homeostasis directly by regulating the expression of sodium-dependent phosphate cotransporters in proximal tubules, NPT2A and NPT2C, which leads to controlled phosphate reabsorption. FGF23 levels induce decreased membrane expression of NPT2A and NPT2C transporters, thus limiting phosphate reabsorption capacity in the kidney and promoting phosphate excretion and restoring the optimal phosphate balance. However, the molecular mechanism of this process is still not fully understood, and there is evidence of paracrine cross-talk between proximal and distal tubules in nephrons [172]. It has been found that the formation of the KLA-FGF23-FGFR1 complex on the surface of renal tubular cells activates ERK1/2 and serum/glucocorticoid-regulated kinase 1 (SGK1) downstream signaling pathways. SGK1 then phosphorylates sodium-hydrogen exchanger regulatory factor 1 (NHERF1), facilitating the translocation of NPT2A and NPT2C from the apical membrane [174, 175].

Moreover, FGF23 is associated with phosphate homeostasis indirectly by regulating vitamin D metabolism. FGF23-mediated down-regulation of the 1α-hydroxylase enzyme is responsible for the conversion of 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol (1,25(OH)2D), which is the biologically active form of vitamin D. FGF23 upregulates 24-hydroxylase, which leads to the degradation of active 1,25(OH)2D. The subsequent net decline in hormonally active vitamin D in plasma attenuated gastrointestinal phosphate uptake, and thus, systemic phosphate loading is attenuated. The interaction of the KLA-FGF23-FGFR1 complex is postulated to be responsible for the stimulation of *CYP24A1* and inhibition of *CYP27B1* gene expression, which encode 24-hydroxylase and 1α-hydroxylase enzymes, respectively [174, 176]. Furthermore, parathyroid hormone (PTH) regulates phosphate release from bone. The literature documents that PTH secretion is under the strict

control of FGF23. However, the exact molecular mechanisms are still not fully elucidated. It is assumed that such an effect of PTH is based on the upregulation of 1α-hydroxylase production by stimulating *CYP27B1* gene expression and its regulation of NPT2A and NPT2C transporters on the tubular surface through interacting with the KLA-FGF23-FGFR1 complex [172, 174]. Hence, PTH and vitamin D play critical roles in phosphate homeostasis, and FGF23 tightly controls the aforementioned molecular mechanisms via a negative feedback loop [174].

Biomedical applications of metabolic FGFs

Due to their notable biological activities, FGF family members, especially paracrine FGFs, have been studied intensively to develop promising therapeutic applications [4, 177]. Likewise, metabolic FGFs have been used in many drug development projects in recent years for various metabolic diseases [17]. Herein, we discuss some notable clinical applications of endocrine FGFs to highlight their potentially crucial roles in future drug development.

FGF19 recent applications

Changes from optimal FGF19 levels lead to several pathological conditions, primarily in lipid and carbohydrate metabolism, such as NAFLD, alcoholic hepatitis, T2DM, and CKD [17]. Decreased levels of FGF19 have been found in obesity, T2DM, and dyslipidemia [84]. Specifically, in diabetic patients, lower levels of FGF19, triglycerides, glycated hemoglobin, and body mass index exhibited a significant association [84] (**Table 1**).

NAFLD is a medical condition characterized by liver swelling when more than 5% of the organ's weight is fat. Although the exact causes are not known, individuals with T2DM have a higher risk of developing NAFLD. Low fasting FGF19 levels are associated with the development of NAFLD [178]. Impaired FGF19 production is assumed to be associated with increased bile acid production and subsequent higher NAFLD fibrosis scores [84]. If not treated at early stages, NAFLD can progress into NASH and cirrhosis and/or HCC [179, 180], which is expected to be the primary cause of liver transplantation by 2030 [181]. Zhou and colleagues (2017) reported on the efficacy and safety of an FGF19 analog, M70, which could reduce bile acid toxicity and lipotoxicity to reinstate hepatic health. They observed a significant decrease in toxic lipid species (e.g., diacylglycerols, ceramides, and free cholesterol) and an increase in unoxidized cardiolipin levels, which confirms mitochondrial health. Furthermore, M70 significantly restored liver histology and increased insulin sensitivity, energy homeostasis, and optimal lipid metabolism [19]. Harrison et al. (2020) reported that 12 weeks of treatment of patients with histologically proven NASH with an engineered FGF19 analog, NGM282 (previously known as M70), improved histological outcomes with decreased liver fibrosis [148]. On the other hand, the DePaoli group (2019) reported that NGM282 failed to correct hyperglycemia in T2DM [182]. Since insufficient FGF19 levels are presumed to play significant roles in NAFLD, NASH, and cirrhosis, these FGF19 analogs compensate for the needed FGF19 level for optimal liver function.

High FGF19 and FGFR4 levels are detected in hepatocellular carcinoma (HCC) patients [183]. FGF19 also induces hepatocellular carcinoma at high concentrations through its proliferative activities via FGFR4 [184]. Altogether, an inability to bind to FGFR4 would eliminate the protein's ability to cause liver cancer and bile acid dysregulations. As such, Lenvatinib, a new pharmaceutical intervention that treats unresectable HCC, is created. It is a multi-kinase inhibitor that acts on vascular endothelial growth factor receptors 1-3, fibroblast growth factor receptors (FGFR) 1-4, platelet-derived growth factor receptor α, rearranged during transfection oncogene and KIT [185]. Due to treatment with Lenvatinib, serum FGF19 levels are elevated, emphasizing its usefulness as a predictive biomarker of HCC progression [185].

While FGF19 level is monitored in different studies, especially related to liver dysfunction, it is also directly administered to patients to treat health concerns such as NASH. Aldafermin (NGM282 or M70) is an example of an FGF19 analog that treats NASH and bile acids-related disorders such as diarrhea [148, 186]. This analog possesses some substitutions to the original FGF19 sequence, namely the A30S, G31S, and H33L substitutions, and a deletion of the first five residues P2 - S6 at FGF19 N-terminus, not counting the signal sequence [71].

Table 1. Notable analogs of metabolic FGFs in clinical trials

Trial identifier	Disease/Condition	Intervention	Phase	Outcomes
FGF19				
NCT01943045	NASH*	NGM282	Phase 2	Rapid, robust, and sustained reduction of lipid content in the liver and significant improvements in liver histology [182].
NCT01585025	Bile acid diarrhea	Obeticholic acid	Phase 2	Obeticholic acid stimulated the FGF19 synthesis, thus, decreasing bile acid synthesis [219].
NCT05130047	Bile acid malabsorption	NGM282	Phase 2	Stool consistency is improved within 14 to 28 days [220].
NCT04828486	Advanced HCC*	Futibatinib	Phase 2	Ongoing [221].
NCT05441475	HCC*	ABSK-011	Phase 2	Ongoing [222].
FGF21				
NCT05039450	NASH*	AKR-001 (Efruxifermin) and GLP-1 receptor agonists	Phase 2b	Safe profile, with a 65% reduction in liver fat at 12 weeks [201].
NCT04767529	NASH*	AKR-001	Phase 2b	Improved liver fibrosis and resolved NASH over 24 weeks in F2 or F3 fibrosis patients. Ongoing [202].
NCT03486912	NASH*	BMS-986036	Phase 2b	Significantly reduced hepatic fat fractions in NASH* patients [194].
NCT04048135	NASH* and NAFLD*	Pegozafermin (BIO89-100)	Phase 1, 2	Improved lipid metabolism, glucose metabolism, weight, and liver transaminases in diabetic monkeys and healthy humans [198].
NCT03466203	Obesity	LLF580	Phase 4	Lowered serum triglycerides, liver fat, and positive trends in lipoprotein profiles. Fasting glucose level remained unchanged [197].
FGF23				
NCT02915705	XLH*	Burosumab,	Phase 3	Significant clinical improvements in XLH* patients [215].
NCT00830674	XLH*	KRN23	Phase 1	Increased serum phosphorous levels and improved XLH* [223].
NCT02163577	XLH*	Burosumab	Phase 2	Reabsorption of phosphate in kidney tubules increased [224].
NCT05509595	Fibrous Dysplasia of the bone	Burosumab	Phase 2	Ongoing.
NCT03565913	CKD* stage 5	EffCaMgCit	Phase 2	Ongoing.

*Note: NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; HCC = hepatocellular carcinoma; T2DM = type 2 diabetes mellitus; XLH = X-linked hypophosphataemia; CKD = chronic kidney disease.

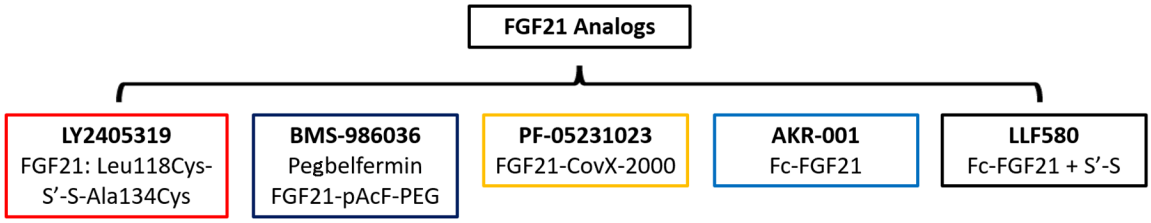

Figure 5. Notable FGF21 analogs in clinical trials. Recent FGF21-based therapeutic applications are listed with the associated names and brief descriptions of the FGF21 analogs.

Following the same concept, FGF19ΔNT is also engineered. FGF19ΔNT is an FGF19 variant with an N-terminus deletion that is effective against hepatocellular carcinoma [72]. Other FGF19 analogs are not yet undergoing clinical trials, but their stability and mitogenicity are being studied for potential improvements and pharmaceutical applications [187].

Other than these effects, patients with end-stage renal disease who undergo hemodialysis also show elevated levels of FGF19. The literature has documented the association of FGF19 with atherosclerosis in T2DM diabetes pathophysiology [107, 188].

FGF21 and its analogs in clinical trials

Due to its notable ability to regulate glucose uptake, FGF21 is an attractive candidate for treating several metabolic diseases. However, FGF21 is unstable and has a short half-life (0.5-2 hours) [189]. Thus, many attempts have been reported to create stable FGF21-based therapeutic agents over the years. Herein, we focus on the development of FGF21 variants as therapeutic agents for metabolic diseases and their results in recent clinical trials. These inventions are typically categorized into three broad groups: FGFR1/KLB agonists, FGF21/FGF21 dual agonists, and FGF21 analogs [189]. Here, we highlighted the notable results of FGF21 analogs in recent clinical trials (**Figure 5** and **Table 1**).

Several randomized clinical trials have been reported to treat human subjects with five FGF21 analogs for obesity or T2DM. The first is LY2405319, a variant of FGF21 that was created by Lilly Laboratory in 2013 [31, 129]. This variant has comparable bioactivity to native FGF21 with improved biophysical characteristics. In the first proof-of-concept study of LY2405319 in diabetic subjects, the variant's effects on lipid, insulin, and body weight measures are positive [129, 190]. However, its glucose uptake effect is less robust in humans than in monkey subjects [31, 129, 190]. The second is PF-05231023, a long-lasting FGF21 analog that combines the protein with an antibody scaffold, CovX-2000. This analog also reduced mouse models' body weights, blood glucose, and lipid levels [128, 191]. In a phase 1 clinical trial, PF-05231023 reduces triglyceride (TG) levels in obese hyper-triglyceridemic adult subjects [128]. The third is Pegbelfermin, also known as BMS-986036 [192]. This agent results from PEGylating a recombinant human FGF21 [192, 193]. Pegbelfermin's phase 2a clinical trial reported that the drug is safe and well tolerated [147, 193]. Furthermore, it could significantly reduce the hepatic fat fraction in patients with NASH or even in obese or diabetic patients who are at risk for developing NASH [147, 193, 194]. However, this agent has limited activity on adipose tissue-FGFR1c in humans and may only work on FGFR2c and 3c in the liver [195]. Additionally, in clinical trials phase 2b (FALCON program), Pegbelfermin is not able to meet its primary endpoint of fibrosis, despite its ability to improve biomarker endpoints and non-invasive measures of liver steatosis, as well as reduce the disease activities of NASH and advanced fibrosis patients [194, 196]. Another variant of FGF21 is called LLF580, a fusion protein between FGF21 and the Fc domain of human immunoglobulin G-1 (IgG1). However, this new variant also introduces a new disulfide bond that increases the thermodynamic stability and decreases the proteolytic proneness of FGF21 [197]. Thus, LLF580 has a half-life of 4 weeks. Like other analogs, LLF580 is safe and well-tolerated [197]. The agent also improves insulin resistance and lowers patients' triglycerides, liver fat, and other lipid levels [197]. It did not lower body weight or

have any glycemic reduction effects, similar to many FGF21 analogs [197]. The most recent development of FGF21-based design is the novel glycoPEGylated FGF21 analog Pegozafermin, also known as BIO98-100 [198]. Such an analog is made to tackle NASH and severe hypertriglyceridemia. The design is shown to interact with FGFR1c, similar to recombinant FGF21, with approximately eightfold higher potency. In diabetic monkeys, Pegozafermin rapidly improved several metabolic markers like triglycerides, fasting glucose, cholesterol, food intake, body weight, etc. [198]. In healthy humans, it is shown to have a higher half-life, around 55 to 100 hours, and improve triglycerides, low and high-density lipoprotein-cholesterol, insulin-sensitizing, adiponectin, etc. [198]. Thus, Pegozafermin is set to proceed to phase 3 clinical trials to treat metabolic diseases.

The most promising current project is the development of FGF21-based therapeutics called AKR-001, also known as AMG 876 or Efruxifermin, a fusion protein between FGF21 and human immunoglobulin 1 Fc with a half-life of 3.0-3.5 days, and increased affinity for KLB [199, 200]. A phase 1 clinical trial for AKR-001 has shown some positive signs of improving lipoprotein and insulin sensitivity while maintaining positive body weight in T2DM patients [199]. In a recent phase 2a trial, Efruxifermin is well tolerated with dose-dependent improvement in liver health by significantly reducing liver fat in NASH and F1-F3 fibrosis patients [195]. Interestingly, this agent is said to have the potential to inhibit adipose tissue lipolysis and liver de novo lipogenesis while improving insulin sensitivity and glycemic control [195]. In clinical trials phase 2b of Efruxifermin (SYMMETRY trials), the drug is safe and reduces 65% of liver fat after 12-week treatments with GLP-1 receptor agonists. It is reported to be much higher than the 10% liver fat reduction for patients treated with GLP-1 receptor agonist alone [201]. Furthermore, in a different clinical trial phase 2b, to treat NASH patients with F2 or F3 fibrosis (HARMONY trials), Efruxifermin alone is capable of improving liver fibrosis and resolving NASH over 24 weeks with acceptable tolerability and awaiting phase 3 trials [202]. Such design showed promising therapeutical activities of FGF21-based design to treat metabolic diseases like NASH and T2DM.

From these observations, one can conclude that most of the proposed models of FGF21-based therapeutics showed positive effects in human subjects, especially in improving lipids and hepatic fat [197]. However, there are limited to no observations of improvement of glycemic changes by these agents in humans, in stark contrast to the results of animal trials of similar agents [128, 129, 197]. Therefore, further development of novel models for FGF21-based therapeutics with glycemic reduction effects in human subjects is warranted.

Clinical reports of FGF23

FGF23 is a phosphaturic hormone involved in mineral metabolism in the body. Thus, alterations in FGF23 levels lead to bone diseases. In the case of rickets, abnormal calcium and phosphate serum levels have been observed. Genetic mutations involved in vitamin D metabolism, FGF23 expression, renal phosphate homeostasis, and bone mineralization have been identified as significant contributors to different forms of rickets. In calciopenic rickets (calcium-related rickets) and phosphophenic rickets (phosphate-related rickets), defects in renal tubules and elevated FGF23 levels have been observed, owing to FGF23-mediated low dietary intake of calcium and phosphate. The other FGF23-dependent types of rickets are X-linked hypo-phosphataemia (XLH), autosomal recessive hypo-phosphataemic rickets (ARHR), autosomal dominant hypo-phosphataemic rickets (ADHR), Raine syndrome, and tumor-induced osteomalacia (TIO) or polyostotic fibrous dysplasia [173, 174]. In all of these conditions, FGF23 levels are significantly elevated. Thus, preventing FGF23 overexpression through clinical modulation of serum FGF23 at optimal levels could be an effective therapeutic strategy to treat hypo-phosphatemic disorders [203] (**Table 1**).

Identifying the FGF23 molecular mechanism and its regulation may be critical to maintaining FGF23 levels in an optimal range. Hence, using FGF23-blocking antibodies to prevent excess FGF23 or screening small molecule drugs that disrupt interactions in the KLA-FGF23-FGFR1 ternary complex will offer successful therapeutic options [204]. A human monoclonal antibody (FGF23 blocking antibody), Burosumab, which inhibits excessive FGF23 signaling, has been granted the U.S. Food and Drug Ad-

ministration (FDA) approval to treat XLH and TIO [205]. It targets the N-terminus of FGF23, abrogating the interactions with FGFRs. More specifically, Burosumab can recognize three epitopes on FGF23, β1-β2 hairpin loop, β8-β9 hairpin loop, and β10 strand [206]. Burosumab improved phosphate metabolism in patients with TIO with an acceptable safety profile [205]. Since complete surgical resection of tumors is not a feasible option for some TIO patients, Burosumab will provide an enormous clinical benefit [204, 207].

Another avenue for inhibiting aberrant FGF23 signaling is using small molecule inhibitors. ZINC12409120, a potent small molecule inhibitor, downregulates the ERK pathway by disrupting the KLA-FGF23-interaction, and could be a potential therapeutic target for the clinical management of hereditary and acquired hypophosphataemic diseases such as XLH and TIO [204, 205]. However, the safety of this approach is questionable due to potential off-target effects since FGFRs are ubiquitously expressed. To mitigate such off-target effects, researchers have developed another small molecule inhibitors, ZINC13407541 (N-[[2-(2-phenylethenyl)cyclopenten-1-yl]methylidene]hydroxylamine) and its analogous forms 8n ((E)-2-(4-(tert-butyl)cyclopent-1-ene-1carbaldehyde oxime) and 13a (E)-2-((E)-4-methylstyryl)benzaldehyde oxime), which specifically interact with Q156 residue at the N-terminus of FGF23, leading to the obstruction of FGF23-mediated MAPK signaling and elevated serum phosphate levels, 1,25D levels, *Npt2a*, *Npt2c* and *Cyp27b1* mRNA levels in mouse models. However, further studies need to be performed to confirm human safety [207, 208].

A key difference in FGF23 structure compared with FGF19 and FGF21 is the presence of 2 tandem repeats at the C-terminus, which can interact with KLA. Site-directed mutagenesis studies confirmed that both KLA binding sites are crucial for FGF23-mediated signaling and bioactivity [208]. Hence, FGF23 C-terminus peptide fragments can compete with intact full-length FGF23 to interact with KLA, forming biologically inactive complexes that cannot initiate cell signaling. Thus, such short peptides can act as internal competitive agonists [63]. Goetz et al. (2010) demonstrated that 72-residues long C-terminus fragment of FGF23 can improve hypophosphataemia by abrogating FGF23-FGFR-KLA complex formation. The proteolytic cleavage of FGF23 at ^{176}RXXR^{179} motif produces a 72-residues long peptide (S180 to I251) that can compete with full-length FGF23 protein for binding for KLA and interrupt the formation of functional signaling complex, thus antagonizing the harmful effects of excessive FGF23 signaling due to elevated full-length FGF23 peptide [39]. Moreover, Zhang et al. (2018) reported that administration of 72-residues C-terminus peptides of FGF23 is effective against diabetic nephropathy in db/db mice. Intraperitoneal injection of the 72-residues peptide improved fibrosis and inflammation conditions in mice kidneys [209]. Hence, the administration of C-terminus fragments of FGF23 could be a viable option for kidney diseases that worth further investigation [39, 209].

Furthermore, FGF23 deficiency can result in hyperphosphatemia and ectopic calcifications in hyperphosphatemic familial tumoral calcinosis and CKD [210]. Clerin et al. (2020) documented that selective pharmacological inhibition of NPT2A via a small-molecule inhibitor, PF-06869206, decreases phosphate uptake in proximal tubules and promotes phosphate excretion in mice [211]. The efficacy and safety of PF-06869206 have been tested both *in vitro* (HEK293 cells) and *in vivo* (C57BL/6 mice), strengthening its applicability in treating hyperphosphatemic disorders [212].

Moreover, an association of FGF23 in acute kidney injury (AKI) and chronic kidney disease (CKD) has also been identified [212]. In CKD patients, serum FGF23 levels are significantly elevated, causing phosphate wasting and renal failure. The relationship between FGF23 and CKD is complex since CKD pathology is multifactorial with many confounding variables. However, current treatment strategies for CKD involve the management of phosphate intake through dietary restrictions and addressing dysregulation of mineral metabolism. David et al. (2016) proposed a putative mechanism explaining the involvement of FGF23 in CKD. This study speculated that, owing to a high pro-inflammatory and iron-deficient environment, FGF23 is overexpressed, and its cleavage is dysregulated [213]. As previously mentioned, biologically active full-length FGF23 peptide

can be cleaved at ^{176}RXXR^{179}, giving rise to C-terminus fragments that can act as internal agonists of FGF23 to maintain biologically active full-length FGF23 levels in an optimal range. Hence, the accumulation of full-length FGF23 protein contributes to detrimental outcomes associated with CKD [213]. Moreover, they noticed that chronic inflammation and iron deficiency, common in many CKD patients, lead to overexpression of FGF23 transcription through HIFI α signaling [213]. Further, FGF23 is associated with renal fibrosis characterized by elevated accumulation of extracellular matrix in the kidney due to tissue injury due to FGF23-mediated activation of tissue growth factor β [214]. Thus, FGF23 can be adopted as a valuable early biomarker of CKD.

In AKI, a rapid surge of FGF23 is observed, which underscores the importance of FGF23 as a biomarker for detecting AKI. Furthermore, other stimulatory factors of FGF23 production, such as a proinflammatory and iron-deficient environment and anemia, can further increase FGF23 expression, leading to aggravation of AKI. Hence, elevated FGF23 levels are usually associated with poor prognosis [84].

Furthermore, FGF23 is associated with the pathogenesis of cardiovascular diseases. In patients with left ventricular hypertrophy, FGF23 levels are found to be significantly elevated. Such effects are postulated to be derived from interactions of FGF23 with FGFR4 in a KLA-independent manner. Elevated FGF23 levels reduced calcium levels in cardiomyocytes, impairing their contraction. Furthermore, FGF23 activates the renin-angiotensin-aldosterone system, leading to renal fibrosis and cardiac hypertrophy [84].

Conclusions

Since their discovery in early 2000, metabolic FGFs have been known to regulate many critical metabolic pathways and to enact their functions differently than other FGFs [17]. However, with continuous development in recent years, endocrine FGFs have emerged as desirable therapeutic agents for many metabolic diseases, including NASH, CKD, obesity, and T2DM, as shown in **Table 1** and discussed in Section 5. For example, LLF580 (based on FGF21) has been used to treat obesity with promising outcomes and has reached clinical trial phase 4 [197]. Likewise, NGM282 (based on FGF19) has been able to treat NASH patients' conditions rapidly [182]. Finally, Burosumab (based on FGF23) has positive results in treating XLH and has undergone clinical trial phase 3 [215]. Such designs show intensive efforts to expand and transition FGF-based therapies from bench studies into bedside applications with positive outcomes.

Along with the notable expansion of endocrine FGFs' biomedical applications, the ongoing discoveries of endocrine FGFs' novel physiological functions and structural features solidify the FGF19 subfamily as exciting fields with many unknowns that warrant further investigations. These promising opportunities can be seen with new findings, such as the dynamic folding NMR structures of FGF21 [30] and the discovery of multiple binding sites of the FGF23 C-terminus [38]. These works provide new insights into metabolic FGFs and may provide new strategies for novel studies of the FGF19 subfamily.

Despite these ongoing studies that enhance our current knowledge of endocrine FGFs, there are still existing challenges within the field of metabolic FGFs. One such problem is the inherent instability of the FGF19 subfamily, which has impeded their structural studies and clinical applications. The tendency of these proteins, specifically FGF19 and FGF21, to form inclusion bodies has rendered large-scale production significantly challenging [22, 38] and warrants innovative methods for consistent production. Additionally, the members of the FGF19 subfamily have unstable C-termini that affect their development as therapeutical drugs, with FGF21's half-life being only around 30 minutes to 2 hours [129], FGF19 at around 30 minutes [216, 217], and FGF23 at around 46-58 minutes [218]. Furthermore, FGF21's nuanced functions and complex cross-talking activities may require further investigations to elucidate fully its biological impacts on the human body [104]. Due to these disadvantageous characteristics, their clinical applications are often accompanied by concerns regarding their stability and biological activities. Nonetheless, significant, and promising progress is being made in this area through many novel drug designs based on metabolic FGFs. With the robust development in both basic

research and biomedical applications, as well as the existence of unsolved problems, there are still plenty of opportunities for breakthroughs in exploring endocrine FGFs.

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Disclosure of conflict of interest

None.

Abbreviations

1,25(OH)2D, 1,25-hydroxycholecalciferol (25-(OH)D) into 1,25-dihydroxycholecalciferol; ADHR, Autosomal dominant hypo-phosphatemic rickets; AKI, Acute kidney injury; AKT, Protein kinase B; ARHR, Autosomal recessive hypophosphataemic rickets; cFGF23, FGF23 C-terminus; ChREBP, Carbohydrate response element binding protein; CKD, Chronic kidney disease; CYP7A1, Cytochrome P450 7α-hydroxylase; ELK-1, E26 transformation-specific like-1 protein; EPO, Erythropoietin; ERK, Extracellular signal-regulated kinase; FDA, U.S. Food and Drug Administration; FGF19CT, FGF19 C-terminus; FGF21CT, FGF21 C-terminus; FGFRs, Fibroblast growth factors receptors; FGFs, Fibroblast growth factors; FXR, Farnesoid X receptor; GLUT, Glucose transporter; GSK, Glycogen synthase kinase; HBS, Heparin binding sites; HCC, Hepatocellular carcinoma; HS, Heparin sulfate; HSPG, Heparan sulfate proteoglycan; iFGF23, Intact FGF23; IgG1, Immunoglobulin G-1; KLA, α-klotho; KLB, β-klotho; MAPKs, Mitogen-activated protein kinases; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; NHERF1, Sodium-hydrogen exchanger regulatory factor 1; PI3K, Phosphoinositide 3-kinases; PPAR, Peroxisome proliferator-activated receptor; PGC-1α, Peroxisome proliferator-activated receptor-γ coactivator-1α; PTH, Parathyroid hormone; RXR, Retinoid x receptor; SGK1, Serum/glucocorticoid-regulated kinase 1; SHP1, Small heterodimer partner 1; sKLB, Soluble KLB; SRE, Serum Response Element; SRF, Serum Response Factor; T2DM, Type-2 diabetes mellitus; TGF-β, Tissue growth factor β; TIO, Tumor-induced osteomalacia; UCP1, Uncoupling protein 1; XLH, X-linked hypo-phosphataemia.

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