Biological role, clinical significance, and therapeutic possibilities of the recently discovered metabolic hormone fibroblastic growth factor 21

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Abstract
Fibroblast growth factor 21 (FGF21), a 181 amino acid circulating protein, is a member of the FGF superfamily, with relevant metabolic actions. It acts through the interaction with specific FGF receptors and a cofactor called β-Klotho, whose expression is predominantly detected in metabolically active organs. FGF21 stimulates glucose uptake in adipocytes via the induction of glucose transporter-1. This action is additive and independent of insulin. β-Cell function and survival are preserved, and glucagon secretion is reduced by this protein, thus decreasing hepatic glucose production and improving insulin sensitivity. Lipid profile has been shown to be improved by FGF21 in several animal models. FGF21 increases energy expenditure in rodents and induces weight loss in diabetic nonhuman primates. It also exerts favorable effects on hepatic steatosis and reduces tissue lipid content in rodents. Adaptive metabolic responses to fasting, including stimulation of ketogenesis and fatty acid oxidation, seem to be partially mediated by FGF21. In humans, serum FGF21 concentrations have been found elevated in insulin-resistant states, such as impaired glucose tolerance and type 2 diabetes. FGF21 levels are correlated with hepatic insulin resistance index, fasting blood glucose, HbA1c, and blood glucose after an oral glucose tolerance test. A relationship between FGF21 levels and long-term diabetic complications, such as nephropathy and carotid atheromatosis, has been reported. FGF21 levels decreased in diabetic patients after starting therapy with insulin or oral agents. Increased FGF21 serum levels have also been found to be associated with obesity. In children, it is correlated with BMI and leptin levels, whereas in adults, FGF21 levels are mainly related to several components of the metabolic syndrome. Serum FGF21 levels have been found to be elevated in patients with ischemic heart disease. In patients with renal disease, FGF21 levels exhibited a progressive increase as renal function deteriorates. Circulating FGF21 levels seem to be related to insulin resistance and inflammation in dialysis patients. In summary, FGF21 is a recently identified hormone with antihyperglycemic, antihyperlipidemic, and thermogenic properties. Direct or indirect potentiation of its effects might be a potential therapeutic target in insulin-resistant states.

Introduction
Human fibroblast growth factor 21 (FGF21) is a 181 amino acid (~20 kDa) circulating protein derived from a 209-amino acid mature protein encoded by the FGF21 gene located in chromosome 19 (1, 2). FGF21 belongs to the human FGF superfamily, initially named by its ability to stimulate fibroblast proliferation, which contains 22 proteins (3). The FGF gene family can be divided into three subfamilies: the intracellular FGFs (FGF11/12/13/14), the endocrine FGFs (FGF15/19/21/23), and the paracrine FGFs (the rest) (3, 4, 5). FGFs bind extracellularly to four cell surface tyrosine kinase FGF receptors (FGFRs 1–4) (6, 7, 8, 9). The amino acid sequence of human FGF21 is highly identical (~75% amino acid identity) to that of mouse FGF21. FGF21 mRNA is preferentially expressed not only in the liver but also in other tissues, such as white adipose tissue (WAT), skeletal muscle, and pancreas (1, 10, 11, 12). FGF21 is detected in plasma, suggesting that it is secreted into circulation acting as a true hormone. FGF21 activity depends on its binding to FGFRs and a cofactor called β-Klotho, a single-pass transmembrane protein whose expression is induced during differentiation from preadipocytes to adipocytes. The cofactor β-Klotho is predominantly expressed in metabolic organs including liver, WAT, and pancreas (13). This cofactor is crucial for the FGF21 specificity of...
the target cells increasing the ability of FGFRs to bind FGF21 (14, 15). The FGF21–β-Klotho–FGFR complex acts by inducing MAP kinase phosphorylation in WAT (14). FGF21 expression is controlled by different transcriptional factors such as peroxisome proliferator-activated receptor α (PPARα (PPARA)) in the liver (16, 17) and PPARγ (PPARG) in adipocytes (18).

The purpose of this review has been to update the most relevant aspects of FGF21 focusing on its biological role and clinical significance, as well as its possible role as a potential therapeutic agent in human disease.

**Biological roles of FGF21: animal studies**

FGF21 has been recently considered as a metabolic hormone regulated by nutritional status with multiple beneficial effects on glucose homeostasis and lipid metabolism in animal models. Indeed, FGF21 improves insulin sensitivity, glucose, and lipid homeostasis and preserves β-cell functions in diabetic animal models (11, 19, 20, 21, 22, 23, 24) (Table 1). Furthermore, unlike the majority of the members of the FGF family, FGF21 is free of the proliferative and tumorigenic effects (11, 19, 25).

**Glucose and insulin metabolism**

FGF21, through its binding to β-Klotho–FGFR complex, stimulates glucose uptake in differentiated adipocytes via the induction of glucose transporter-1 (GLUT1) through sequential activation of transcription factors, requiring several hours for this activity (19, 26, 27) (Fig. 1). Glucose uptake induced by FGF21 is additive and independent of insulin. This glucose entry into adipocytes results in its storage as triglyceride (TG). Moreover, the ability of FGF21 for increasing the thermogenic capacity of WAT could, at least in part, lead to a greater clearance of glucose (17, 28).

FGF21 might also act on glucagon metabolism and vice versa. FGF21 suppresses hepatic glucose production, increases liver glycogen, and lowers glucagon in mice (29). On the other hand, hepatic expression of PPARα and FGF21 is stimulated by hepatic glucagon receptor activation in a manner that is further augmented by fatty acids (30). It has also been reported that FGF21 preserves β-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways (11).

All these actions would be associated with a reduction of blood glucose. Indeed, systemic administration of FGF21 has shown to reduce plasma glucose to near normal levels in genetically compromised diabetic rodents (19) and fasting plasma glucose, fructosamine, insulin, and glucagon without developing hypoglycemia in diabetic rhesus monkeys (20, 31). Lastly, continuous i.c.v. infusion of FGF21 in high-fat diet-induced obese (DIO) male Wistar rats increased hepatic insulin sensitivity due to increased insulin-induced suppression of both hepatic glucose production and gluconeogenic gene expression indicating the central nervous system (CNS) as a potentially important target for the beneficial effects of FGF21 (23). In fact, FGF21 has shown to have a significant, nonsaturable, unidirectional influx across the blood–brain barrier (32).

**Lipid metabolism**

FGF21 has shown beneficial effects on lipid profile in animal models. Systemic administration of FGF21 was also followed by a decrease in plasma TG, free fatty acids (FFA), and cholesterol in genetically compromised diabetic and obese rodents (19, 31). Moreover, FGF21 administration also induces changes in the mRNA profiles of several genes involved in lipid metabolism in DIO mice (21). In WAT, FGF21 increased mRNA levels of uncoupling protein 1 (UCP1), PPARγ coactivator 1α (PGC1α), hormone-sensitive lipase (HSL), and adipose TG lipase (ATGL), whereas in brown adipose tissue (BAT), FGF21 administration led to an elevation of

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**Table 1 Metabolic effects of FGF21 in animal models.**

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<tr>
<th>Carbohydrate metabolism</th>
<th>Glucose uptake in differentiated adipocytes</th>
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<tr>
<td>GLUT1 induction in differentiated adipocytes</td>
<td>Stimulation of glucose uptake in differentiated adipocytes</td>
<td>Decreases glucagon secretion</td>
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<tr>
<td>Improves insulin sensitivity and glucose clearance</td>
<td>Promotes lipolysis in WAT in response to fasting</td>
<td>Increases ketogenesis in liver in response to fasting</td>
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<td>Lipid metabolism</td>
<td>Increases energy expenditure</td>
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<td>Increases ketogenesis in liver in response to fasting</td>
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<td>Reduces plasma triglyceride levels</td>
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<td>Decreases GH-stimulated lipolysis in adipocytes</td>
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UCP1 and acetyl-coenzyme A (CoA) carboxylases 2 (ACC2) transcripts (21). In diabetic rhesus monkeys, chronic FGF21 therapy significantly improved lipid profile, including a decrease in TG and LDL and an increase in HDL (20).

**Obesity**

FGF21 also seems to be involved in the regulation of body fat mass. Circulating FGF21 concentrations are significantly elevated in obesity (33) in rodents responding poorly to exogenous FGF21, indicating that obesity could be an FGF21-resistant state (34). FGF21 increases energy expenditure in mice with free access to food, thereby correcting obesity (21, 31), and transgenic mice overexpressing FGF21 have shown to be resistant to diet-induced metabolic abnormalities and obesity (19). A small but significant weight loss was also reported after chronic FGF21 therapy in diabetic nonhuman primates (20). These antiobesity actions of FGF21 appear to be mediated by an increase in energy expenditure and preferential fat utilization because weight loss is accompanied by elevated oxygen consumption, increased core body temperature in the absence of decrease in total caloric intake, or effect on physical activity (21, 31). Moreover, FGF21 enhances the expression of genes involved in thermogenesis within brown fat increasing body temperature (17).

**Hepatic steatosis**

In addition to correcting multiple metabolic alterations, FGF21 reverses hepatic steatosis and decreases tissue (muscle and BAT) lipid contents in DIO mice (31). This favorable effect on hepatic steatosis might be related to the inhibition of the maturation of sterol regulatory element-binding protein-1c, a transcription nuclear factor that activates all genes required for lipogenesis (31, 35).

**Adaptive response to fasting**

A link between FGF21 and fasting in animals has been reported. The adaptive response to fasting seems to be mediated by the activation of PPARα, a nuclear receptor activated by fatty acids, which leads to stimulation of gluconeogenesis, ketogenesis, and fatty acid oxidation (36, 37). These metabolic responses of PPARα to fasting are mainly mediated by FGF21 (19, 37, 38, 39). Several observations support this statement: i) PPARα agonist therapy, low-carbohydrate ketogenic diet and fasting induce hepatic expression, and circulating levels of FGF21 that are rapidly suppressed by refeeding (16); ii) FGF21 regulates fasting promoting lipolysis in WAT from murine adipocytes and ketogenesis in liver in response to fasting directly induced by PPARα (22, 36, 37), although this FGF21-induced lipolysis has not been demonstrated by others (40, 41); iii) knockdown of FGF21 in the liver results in impaired ketogenesis and fatty acid oxidation in mice (16); iv) FGF21 reduces physical activity and promotes torpor in mice, a short-term hibernation state with the aim of saving energy by reducing physical activity and body temperature energy (37); and v) FGF21-overexpressing transgenic mice show reduced fat mass and resistance to obesity (19, 38, 39). These findings would demonstrate a role for the PPARα-FGF21 endocrine signaling pathway in regulating diverse metabolic and behavioral aspects of the adaptive response to starvation (Fig. 1).

The opposite effects of FGF21 on glucose metabolism might depend on several factors such as nutritional status, fasting or feeding, and normal or altered glucose metabolism.

**Other effects**

It is known that GH and FGF21 are metabolic hormones involved in the regulation of glucose and lipid metabolism. Both hormones are induced in response to fasting and act on adipocytes promoting lipolysis. The existence of a possible feedback loop between GH and FGF21 has recently been documented (42, 43). GH induces hepatic production of FGF21 by activation of the transcription factor PPARα through FFA released from adipose tissue by GH-induced lipolysis, and elevated FGF21, in turn, acts as a negative feedback signal to terminate GH-stimulated lipolysis in adipocytes (42). These effects suggest that FGF21 and GH might act in a coordinated way to control the amplitude and duration of lipolysis during adaptive response to fasting (42).

FGF21 has proved capable of inhibiting apoptosis in cultured cardiac endothelial cells from male adult Wistar rats and might play physiological roles in improving the endothelial function at an early stage of atherosclerosis and in stopping the development of coronary heart disease (CHD) (44).

**FGF21 in health and human disease**

In humans, FGF21 is mainly produced not only in liver and adipose tissue but also in skeletal muscle and thymus (1, 45, 46). The contribution from each tissue to plasma is not known. Several recent studies have analyzed circulating FGF21 levels in community-dwelling adults (47), as well as its role related to diabetes, insulin resistance, metabolic syndrome (MetS), nonalcoholic fatty liver disease (NAFLD), and weight status in humans (45, 47, 48, 49, 50, 51, 52, 53, 54, 55) (Table 2).

The normal reference range for FGF21 varies considerably among different studies. For example, the median (interquartile range) morning fasting serum FGF21 concentration was 468 (295–520) pg/ml in a group of 50 healthy subjects (50), whereas it was
FGF21 was also an independent predictor of DM2 in diabetes and latent autoimmune diabetes in adults (61).

have been found elevated in insulin-resistant states, and insulin resistance in community-dwelling adults (47). On the other hand, 24-h profiles of serum FGF21 concentration have shown various oscillation patterns ranging from 6 to 12 times per day, with an average oscillation duration of about 2.5 h and without circadian rhythm, suggesting that FGF21 may be secreted into systemic circulation in a pulsatile manner (56). However, other authors have documented a circadian rhythm of FGF21 during fasting in both obese and lean individuals (57, 58, 59), and it has been suggested that it could be caused by the oscillation of FFA (59). Lastly, FGF21 increases in healthy subjects only after a 7-day fast, supporting the hypothesis that FGF21 is induced by prolonged fasting in humans as also occurs in mice (57).

FGF21, insulin resistance, and type 2 diabetes
Increased serum FGF21 concentrations have been recently associated with abnormal glucose metabolism and insulin resistance in community-dwelling adults (47). In this regard, circulating FGF21 concentrations have been found elevated in insulin-resistant states, such as impaired glucose tolerance and type 2 diabetes (DM2) (45, 48, 49, 50, 51, 60) but decreased in type 1 diabetes and latent autoimmune diabetes in adults (61). FGF21 was also an independent predictor of DM2 in humans and its elevation is independent of the DM2 duration (62). Lastly, FGF21 seems to be also independently associated with markers of insulin resistance and an adverse lipid profile in polycystic ovary syndrome (63) and gestational diabetes (64).

FGF21 correlates inversely with insulin sensitivity at muscle level and directly with the hepatic insulin resistance index, fasting plasma glucose, and 2-h plasma glucose after an oral glucose tolerance test and HbA1c, indicating a clear relationship with hepatic and whole-body insulin resistance in DM2 (48, 49, 62, 65). In addition, FGF21 is expressed in human skeletal muscle in response to insulin stimulation, suggesting that FGF21 is an insulin-regulated myokine (46).

The role of insulin on FGF21 is currently not well understood. Some studies have shown that artificial hyperinsulinemia performed in healthy subjects is accompanied by an increase in FGF21 levels (66). However, it has also been reported that FGF21 also increases in hypoinsulinemic states (67). This discrepancy might be in relation to an elevation of stimulators of FGF21 secretion such as FFAs, resulting from complete insulin deficiency (66). Therefore, FGF21 responses to insulin might be affected by several confounders, such as obesity, endogenous circulating FFAs, insulin levels, and insulin resistance.

FGF21 has direct effects in enhancing skeletal muscle glucose uptake, providing additional points of regulation that may contribute to the beneficial effects of FGF21 on glucose homeostasis (60). On the other hand, several other studies on humans have reported that FGF21 inhibits lipolysis in adipocytes, indicating that the antilipolytic effect could be a mechanism through which FGF21 promotes insulin sensitivity in man (40).

Several studies have linked high levels of FGF21 with the presence of chronic complications associated with diabetes (50). In fact, serum FGF21 level was independently correlated with urinary albumin excretion in a group of DM2 patients, indicating that circulating FGF21 may be involved in diabetic nephropathy. Moreover, serum FGF21 levels were significantly higher in the DM2 subjects with carotid artery plaques compared with those without plaque (51).

In relation to therapy of diabetes, the addition of rosiglitazone (68) or pioglitazone and exenatide (69) on ongoing metformin therapy in DM2 patients as well as the use of mitiglinide (70) or short-term continuous s.c. insulin infusion (71) in patients with newly diagnosed DM2 was followed by a significant reduction in circulating FGF21 levels suggesting that FGF21 decreases as insulin sensitivity improves. Based on all these observations, FGF21 should be considered as a new hormone with a significant role in insulin-resistant states and complications associated with DM2, possibly promoting insulin sensitivity in man as a compensatory mechanism (Fig. 2).
FGF21 and obesity

It has recently been reported that human FGF21 gene expression is paradoxically and independently regulated by both fasting and feeding signals, suggesting that human FGF21 is increased with nutritional crisis, including starvation and overfeeding (72). Increased FGF21 serum levels have been found to be associated with obesity in both children (52) and adults (45, 53, 54), indicating a connection between FGF21 and body fat mass.

Obesity in childhood not only has shown to be associated with increased FGF21 serum levels compared with normal-weight children but also a positive correlation between FGF21 and body fat mass. Although it has been reported that FGF21 is significantly correlated to BMI and leptin as markers of VAT in children (52), this fact does not always occur in adults. In this case, several metabolic alterations such as high liver fat, TG, insulin, homeostasis model assessment (HOMA) index, and area under the curve of glucose and lower HDL rather than overall adiposity have been associated with high FGF21 levels (73).

Therefore, FGF21 seems to be an independent marker for the presence of MetS in obesity (53, 73, 74). Indeed, serum FGF21 level has also been reported to be significantly increased and positively correlated with intrahepatic TG content in NAFLD, representing an independent predictor of liver steatosis (54, 75, 76, 77).

FGF21 and cardiovascular disease

A relationship between FGF21 and CHD has recently been reported. In a clinic-based study, median serum FGF21 levels were significantly higher in CHD patients than in control subjects. Moreover, CHD patients with diabetes, hypertension, or both showed higher FGF21 levels than those of patients without these comorbidities (55). In this study, FGF21 levels correlated positively with TG, fasting blood glucose, apolipoprotein B100, insulin, and HOMA index of insulin resistance (HOMA-IR) but negatively with HDL and apolipoprotein A1, indicating a positive association between FGF21 and adverse lipid profile in CHD patients and a possible compensatory response or resistance to FGF21 (55).

FGF21 and renal disease

Circulating FGF21 levels are independently associated with renal function and progressively increased from early- to end-stage renal disease (ESRD) (81, 82, 83, 84). Compared with healthy subjects, serum FGF21 levels have been found to be ~8–15 times higher in long-term dialysis patients (81, 82). It has been suggested that FGF21 might play a role in insulin resistance in patients with ESRD (82). Indeed, FGF21 was positively correlated with inflammatory markers (interleukin-6, fibrinogen, and high-sensitivity C-reactive protein) and HOMA-IR and negatively with residual renal function in a group of 72 nondiabetic peritoneal dialysis patients (82).
Impaired renal excretion combined with compensatory mechanisms to counteract metabolic stress and/or insulin resistance and FGF21 resistance in peripheral tissues might explain the marked elevation of serum FGF21 concentration in dialysis patients (81, 82).

Potential therapeutic applications

Evidence reported so far suggests that FGF21 possesses favorable metabolic effects not only on carbohydrate but also on lipid metabolism showing antihyperglycemic and antihyperlipidemic properties promoting insulin sensitivity and thermogenesis. In humans, high FGF21 levels have been invariably linked to insulin-resistant states, such as glucose intolerance, DM2, MetS, and obesity, as well as some of their complications such as CHD, indicating a possible compensatory elevation of FGF21 to overcome insulin resistance. Based on the previous studies, as it has been suggested by some authors, FGF21 represents a novel and attractive therapeutic agent.

In this regard, the pharmacological use of recombinant human FGF21, FGF21 analogs or agonists, and drugs that increase circulation levels of endogenous FGF21 might be of interest in the therapeutic armamentarium against disease states associated with insulin resistance, mainly DM2, obesity, polycystic ovary syndrome, and hepatic steatosis (21, 85, 86, 87, 88, 89, 90). Further investigations in patients with the above-mentioned states and other metabolic disorders associated with insulin resistance are, therefore, required to clarify the true role of the potential therapeutic applications of this new metabolic hormone.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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