

Albert Pérez-Martí, Viviana Sandoval, Pedro F. Marrero, Diego Haro* and Joana Relat*

Nutritional regulation of fibroblast growth factor 21: from macronutrients to bioactive dietary compounds

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Abstract: Obesity is a worldwide health problem mainly due to its associated comorbidities. Fibroblast growth factor 21 (FGF21) is a peptide hormone involved in metabolic homeostasis in healthy individuals and considered a promising therapeutic candidate for the treatment of obesity. FGF21 is predominantly produced by the liver but also by other tissues, such as white adipose tissue (WAT), brown adipose tissue (BAT), skeletal muscle, and pancreas in response to different stimuli such as cold and different nutritional challenges that include fasting, high-fat diets (HFDs), ketogenic diets, some amino acid-deficient diets, low protein diets, high carbohydrate diets or specific dietary bioactive compounds. Its target tissues are essentially WAT, BAT, skeletal muscle, heart and brain. The effects of FGF21 in extra hepatic tissues occur through the fibroblast growth factor receptor (FGFR)-1c together with the co-receptor β -klotho (KLB). Mechanistically, FGF21 interacts directly with the extracellular domain of the membrane bound cofactor KLB in the FGF21- KLB-FGFR complex to activate FGFR substrate 2 α and ERK1/2 phosphorylation. Mice lacking KLB are resistant to both acute and chronic effects of FGF21. Moreover, the acute insulin sensitizing effects of FGF21 are also absent in mice with specific deletion of adipose KLB or FGFR1. Most of the data show that pharmacological administration of FGF21 has metabolic beneficial effects. The objective of this review is to compile existing

information about the mechanisms that could allow the control of endogenous FGF21 levels in order to obtain the beneficial metabolic effects of FGF21 by inducing its production instead of doing it by pharmacological administration.

Keywords: beta-klotho; diet; energy metabolism; fibroblast growth factor 21; obesity.

Introduction

Fibroblast growth factor 21 (FGF21) increases energy expenditure. It thus has beneficial effects on glucose/lipid homeostasis and on body weight control and emerges as a novel therapeutic agent for the treatment of metabolic diseases such as obesity, type 2 diabetes and metabolic syndrome. In rodent and primate models of the aforementioned conditions, FGF21 has the capacity to restore glycemia and lipid profile, and to improve insulin resistance [1, 2].

It is widely accepted that FGF21 participates in metabolic homeostasis in health but its action takes on greater relevance in diseases.

To date, the pharmacological use of FGF21 is limited due to its half-life of around 1–2 h. In order to improve the pharmacokinetics, selectivity, and potency of FGF21, several laboratories have focused on designing FGF21 analogs. Two such analogs (LY2405319 and PF05231023) are currently being tested in clinical trials and have yielded similar results: benign toxicology, decreased plasma TGs and low-density lipoprotein cholesterol, increased high-density lipoprotein cholesterol, modest weight loss, elevated adiponectin, reduced insulin levels, and elevated plasma ketones. Surprisingly, no glucose-lowering effect was registered in any trial. This is a major setback as both compounds were assessed mainly as anti-diabetic drugs. However, the results of these two trials highlight the capacity of FGF21 to ameliorate lipid and cholesterol metabolism [3–5].

Nutritional signals play an important role in controlling gene expression in mammals. Macronutrients

*Corresponding authors: **Diego Haro and Joana Relat**, Department of Nutrition, Food Sciences and Gastronomy, Food Campus (Torribera), School of Pharmacy, University of Barcelona, Av Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Barcelona, Spain; and Institute of Biomedicine from University of Barcelona (IBUB), Barcelona, Spain, E-mail: dharo@ub.edu (D. Haro), jrelat@ub.edu (J. Relat)

Albert Pérez-Martí, Viviana Sandoval and Pedro F. Marrero: Department of Nutrition, Food Sciences and Gastronomy, Food Campus (Torribera), School of Pharmacy, University of Barcelona, Santa Coloma de Gramenet, Barcelona, Spain; and Institute of Biomedicine from University of Barcelona (IBUB), Barcelona, Spain

[carbohydrates, fatty acids (FAs), proteins], micronutrients (minerals and vitamins), and some bioactive dietary compounds have the capacity to regulate gene expression and thus metabolic homeostasis. In this context, it has been described that endogenous FGF21 levels are regulated by various nutritional challenges such as high-fat diets (HFDs), low-protein diets (LPDs), amino acid-deficient diets, fasting, and polyphenols [6, 7] (Figure 1). However, the levels of this molecule are also determined by metabolic stress, including obesity, type 2 diabetes (T2DM), and non-alcoholic fatty liver disease (NAFLD) [8]. In this regard, this review summarizes how various nutrient stimuli and diet components regulate the expression of FGF21, and it also seeks to shed light on the molecular mechanisms and the clinical implications of the crosstalk between diet composition FGF21 levels and signaling.

FGF21 signal transduction

FGF21, together with FGF15/19 and FGF23, is an atypical member of the fibroblast growth factor (FGF) family. With

endocrine, paracrine and autocrine properties, FGF21 lacks the heparin domain present in the rest of the family members, thus allowing it to be secreted. Defined as a hepatokine, myokine, and adipokine, FGF21 is expressed in several tissues, including the liver, pancreas, thymus, heart, testis, skeletal muscle, white adipose tissue (WAT), brown adipose tissue (BAT), heart, and brain. It also exerts action on multiple target tissues, ranging from peripheral to central [9].

FGF21 acts as a hormone-like peptide and its signaling pathway requires FGF21 binding to a fibroblast growth factor receptor (FGFR). FGFRs are tyrosine kinase receptors, and seven isoforms have been described (1b, 1c, 2b, 2c, 3b, 3c and 4). FGFR1c has been defined as the main mediator of FGF21 response in vivo [10] through an obligate dimerization with the co-receptor β -klotho (KLB) [11]. The co-expression of these two receptors determines the sensitivity of a tissue or organ to FGF21 signaling.

Regarding the signal transduction pathway, the binding of FGF21 to the FGFR-KLB dimer stimulates the phosphorylation of FGFR substrate 2 α (FRS2 α) and the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt [12].

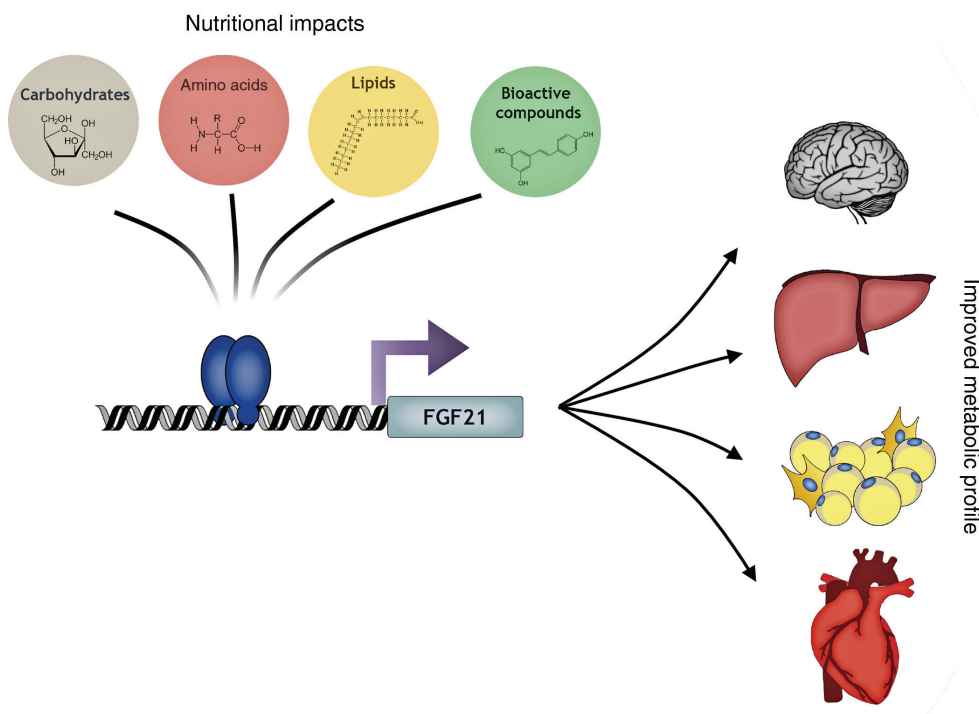


Figure 1: FGF21 expression is regulated by diet and its effects are widely distributed. Endogenous levels of FGF21 are regulated by different macronutrients and bioactive dietary compounds. Acting as a hormone, FGF21 impacts on several tissues where regulates mainly lipid and glucose metabolism.

FGF21 as a hepatokine, myokine, adipokine and other kines

Liver production and secretion

The main source of FGF21 is the liver, where its expression is induced in response to stress. FGF21 was initially described as a fasting-adaptation hormone, as its hepatic production coupled to plasma levels is dramatically increased during prolonged fasting [13, 14]. It is now known that hepatic FGF21 expression is also induced in other liver-stress circumstances such as in obesity, specific nutritional conditions, liver injury, viral infection, chemical insult, hepatosteatosis, steatohepatitis, NAFLD, cirrhosis, and liver cancer [8, 15–18].

Hepatic overexpression of FGF21 triggers ketogenesis, gluconeogenesis, and FA oxidation (FAO) and suppresses lipogenesis in the liver [15, 19]. However, the autocrine effects of FGF21 on this organ are still under debate. FGFR4 is the predominant isoform in the liver, but the FGFR4-KLB complex cannot activate the FGF21 transduction pathway [10]. In contrast, hepatic FGFR1 levels are low, and it is unclear whether they are enough to ensure FGF21 signaling. Later studies reported contradictory results regarding the role of FGF21 in ketogenesis, thereby suggesting that the physiological effects of this molecule may differ from the pharmacological ones.

As a hepatokine, FGF21 affects WAT and BAT, tissues in which it regulates lipid metabolism – mainly by inducing lipolysis and browning and by increasing thermogenic capacity [20, 21]. It also exerts action in the brain, where it is able to reduce physical activity, induce torpor, and regulate circadian behavior [22, 23]. In conclusion, all data indicate that FGF21 is produced and secreted by the liver when the function of this organ is compromised by stress and that it is responsible for restoring and maintaining metabolic homeostasis. Hepatic FGF21 expression is highly sensitive to nutritional status, and the molecular mechanisms that modulate its expression in the liver will be reviewed in the following chapter.

White adipose tissue: autocrine and paracrine effects

Adipose tissue is the main target tissue of FGF21 and the major mediator of its beneficial effects. While the physiological effects of FGF21 during fasting remain elusive, most data on its signaling in WAT derive from studies in which it was pharmacologically administered or overexpressed in obese mice. Nevertheless, FGF21 shows paradoxical actions on WAT depending on its source.

Fgf21-overexpressing mice show induced lipolysis [13, 24]. In contrast, *Fgf21*-knockout mice present enhanced lipolysis in late fasting [21]. In addition, while FGF21 suppresses lipolysis in mouse and human adipocytes [25], it is induced by peroxisome proliferator activating receptor α (PPAR α) in WAT upon feeding, thus stimulating adipogenesis [26, 27].

Regarding glucose metabolism, FGF21 induces glucose uptake in 3T3L1 adipocytes by increasing glucose transporter 1 (GLUT1) independently of insulin action [2, 28]. Moreover, later studies showed increased glucose uptake in both WAT and BAT of lean mice infused with FGF21 and fed a chow diet [29].

In summary, in WAT, FGF21 induces genes involved in glucose uptake, lipogenesis and lipolysis, depending on the metabolic state of the adipocytes. These apparently contradictory effects may be due to compensatory effects of genetic modifications in mice, different nutritional status and different FGF21 concentrations reached between pharmacological administration and physiological secretion.

WAT is not only a FGF21 target tissue but also a mediator of the effects of this growth factor. In this regard, the glucose- and insulin-sensitizing effects of FGF21 require the production and secretion of adiponectin from WAT. Accordingly, FGF21 stimulates this mechanism in rodents, and adiponectin-knockout mice fail to reproduce the sensitizing effects of FGF21 [30]. Similarly, FGF21 also reduces the levels of the sphingolipid ceramide. Sphingolipid ceramides have been associated with insulin resistance caused by lipotoxicity. By inducing adiponectin secretion, FGF21 diminishes the accumulation of ceramides in obese animals [31]. Overall, despite some contradictory effects of FGF21 in adipose fat depots, adipose tissue is considered indispensable for the physiological and pharmacological effects of FGF21.

Finally, FGF21 induces the expression of uncoupling protein 1 (Ucp1), thus producing the so-called browning process of WAT in an autocrine, paracrine or endocrine fashion [20, 32]. Browning occurs in multilocular beige adipocytes in specific susceptible WAT depots, such as inguinal and perirenal tissue, through an increase in the expression of genes involved in thermogenesis and confers a brown fat-like phenotype to white adipocytes.

Brown adipose tissue: FGF21 induces thermogenic capacity

BAT is a FGF21 target tissue since it expresses FGFR1 and KLB; however, it is also a source of FGF21. In BAT, FGF21

stimulates glucose uptake and thermogenesis through the induction of UCP1 in the interscapular depot in an autocrine and paracrine fashion [33]. Upon cold exposure, FGF21 expression is increased in BAT and other cold-sensitive fat depots in the β -adrenergic/ATF2-dependent pathway [32–34]. In this regard, *Fgf21*-deficient mice respond poorly to cold exposure and show greater shivering [32]. The mechanisms underlying the action of FGF21 on BAT/WAT are still not well understood. Part of the FGF21-induced activation of the thermogenic program is driven by PGC1 α , as FGF21 increases the protein levels of this molecule. Similarly, *Pgc1a*-knockout mice show an impaired response to FGF21 [32].

Furthermore, hepatic FGF21-mediated thermogenesis has also been described in response to maternal milk consumption in neonatal pups [35], and also in situations of metabolic stress, for example upon amino acid restriction [20]. These observations suggest that the hepatic FGF21-mediated increase in thermogenic capacity is an adaptive response to metabolic stress.

It has been proposed that the effect of FGF21 on energy expenditure and weight loss may be due to an increased thermogenic capacity of BAT and WAT (browning). However, recent experiments in *Ucp1*-null mice and interscapular BAT-excised mice show that when FGF21 is administered pharmacologically, UCP1 is not required for the improvement of the glucose, cholesterol, and free FA profile. Nonetheless, the increment in metabolic rate associated with the administration of FGF21 is diminished in these mice [36–38]. These data suggest that the metabolic benefits of FGF21 are partly UCP1-independent.

Skeletal muscle: FGF21 is produced in response to mitochondrial dysfunction

FGF21 expression in muscle was first described in Akt1 transgenic mice in which the mRNA and serum levels of FGF21 were induced [39]. In normal conditions, basal expression of FGF21 in skeletal muscle is low but its expression is increased by insulin [39, 40], exercise [41], mitochondrial myopathies [42], impaired mitochondrial FAO [43], muscle-specific autophagy deficiency [44] and transgenic overexpression of Akt1, perilipin-5 [45] or *Ucp1* [46] in skeletal muscle. The induction of FGF21 in muscle due to a metabolic dysfunction is driven by the transcription factor ATF4, which is activated by endoplasmic reticulum (ER) stress. The AMPK and PI3K/Akt1 signaling pathways are also able to increase FGF21 expression [43].

The impact of FGF21 on skeletal muscle is not clear, as the expression levels of KLB do not seem to be sufficient

to respond to FGF21. Several studies suggest that FGF21 administration can improve glucose uptake in vitro [47, 48], and a model of impaired mitochondrial FAO has recently shown the same in vivo [43].

In contrast, the role of FGF21 as a myokine is more evident. In the abovementioned conditions where FGF21 is overexpressed in muscle, the plasma levels of this growth factor also increase. The metabolic effects of this increase include the reduction of fat content in liver, increased FAO, resistance to a HFD and browning of WAT. These results show that FGF21 can act as a myokine when secreted in response to muscle stress and that it exerts its effects on metabolism in an endocrine fashion.

Heart: FGF21 exerts cardioprotective actions

Initially, the heart was discarded as a target tissue or source of FGF21 due to the low levels of FGF21 and KLB mRNA detected in this organ. However, later studies showed that FGF21 is expressed and secreted by cardiac cells in response to various stress conditions, including obesity, type 1 diabetes, fasting, ER stress, inflammation, infarct or hypertrophy, and some cardiovascular diseases [49, 50]. In the heart, FGFR1 and KLB have been detected in cardiac cells, where FGF21 exerts protective effects in an autocrine and endocrine fashion. The mRNA expression of FGF21 in these cells is driven by the transcriptional activation of Sirtuin1 – peroxisome proliferator-activated receptor α (Sirt1-PPAR α) [50]; however, it can also be regulated by ATF4, especially when FGF21 induction is caused by ER or oxidative stress [49, 51].

It is now well established that FGF21 plays a key role in cardiac remodeling and pathophysiology. FGF21 protects cardiomyocytes from hypertrophy through a mechanism that involves the activation of the cAMP responsive element binding protein (CREB), the induction of PGC1 α expression, and the reduction of the NF- κ B pro-inflammatory pathway [50]. Cardiac FGF21 also induces the expression of anti-oxidant genes such as *Ucp3* and superoxide dismutase (*Sod2*), thus preventing the production of reactive oxygen species (ROS) in cardiac cells and oxidative stress in the heart [52]. In an autocrine fashion, FGF21 modulates cardiac lipid homeostasis [49] and protects against diabetes-induced cardiomyopathy by activating the ERK-p38MAPK-AMPK pathway [50]. Finally, as an endocrine peptide, FGF21 can also inhibit cardiomyocyte apoptosis, thus reducing damage to the heart [53, 54].

Central nervous system: target tissue of FGF21

FGF21 has the potential to act in the central nervous system (CNS) since FGFRs are widely expressed in this tissue and KLB is specifically expressed in the suprachiasmatic nucleus, the dorsal vagal complex of the hindbrain, the area postrema, the nucleus tractus solitari, the nodose ganglia, and the paraventricular nucleus [22, 55, 56]. Immunoblotting experiments have revealed that FGF21 is expressed in several regions of the brain, such as the substantia nigra, striatum, hippocampus, and cortex [57]; however, this growth factor also crosses the blood brain barrier and is present in the cerebrospinal fluid in a linear relationship with serum levels [58]. FGF21 modulates circadian rhythm and fertility [22, 59], but current data point to the CNS as a mediator of the effects of FGF21 on energy expenditure and browning [60]. However, in all cases, the presence of KLB appears to be required for FGF21 to exert its effects. This observation thus indicates that this growth factor is an endocrine signal. Regarding the effects of FGF21 on energy expenditure and browning, experiments with diet-induced obese (DIO) mice show that the lack of KLB in the CNS abrogates all the effects of FGF21 on body weight, insulin sensitivity, metabolic regulation in liver, WAT, and BAT. These data suggest that direct signaling in the CNS causes an increase in the sympathetic outflow.

In the hypothalamus, FGF21 affects the expression of corticotropin-releasing factor. Intracerebroventricular injection of FGF21 in *Fgf21*-KO mice restores the metabolic effects of FGF21 essentially through the corticotropin-releasing hormone (CRH) and the activation of CREB, which finally enhances hepatic gluconeogenesis and sympathetic nerve activity in BAT [56, 61, 62]. According to that, lack of KLB in the brain blunts these effects [56, 60, 61]. Finally, treatment with the β -blocker propranolol diminishes the effects of FGF21 when it is centrally administered but not when delivered peripherally [60].

Given the wide range of biological processes regulated by the CNS and the capacity of FGF21 to act in the brain, it is likely that future studies will reveal new actions of FGF21 signaling through the CNS.

Pancreas: FGF21 preserves β -cell function

FGF21 is highly expressed in the pancreas and has protective effects against cerulein-induced pancreatitis [63]. Supporting data show that FGF21-deficient mice are more susceptible to damage, and FGF21-overexpressing mice are partly protected. In addition, FGF21 may be involved in enhancing islet engraftment [64] and in the

preservation of β -cell function and survival [65]. Another point is that KLB expression is critical for these beneficial effects, but the expression of KLB is reduced when islets are treated with high glucose concentrations [66]. In the pancreas, FGF21 increases insulin content and glucose-dependent secretion and inhibits glucagon release in isolated islets [65].

Other effects of FGF21

In addition, and consistent with its metabolic benefits, FGF21 transgenic overexpression extends lifespan in mice. The authors of that study proposed that inhibition of the GH/IGF-1 signaling pathway would explain life extension in *Fgf21* transgenic mice. Furthermore, microarray analysis showed that FGF21 modulates gene expression in the liver in a similar manner to caloric restriction (which is known to extend lifespan in mammals). These data suggest that FGF21 extends lifespan by acting as a selective caloric restriction mimetic in the liver [67].

Interestingly, FGF21 appears to be not only a metabolic regulator but also a nutrient intake and taste regulator. Both pharmacologic administration and hepatic secretion of FGF21 produce a satiety signal that suppresses the intake of “sweets” [68, 69]. In addition, two genome-wide meta-analyses associated genetic variations in a locus including *Fgf21* with significant differences in macronutrient intake [6, 70].

While FGF21 boasts numerous beneficial effects, a major adverse effect is a decrease in bone mass. Both genetic overexpression of *Fgf21* and pharmacological administration of this molecule lead to the inhibition of osteoblastogenesis and the stimulation of adipogenesis. In contrast, the absence of *Fgf21* leads to a high bone mass phenotype. The mechanism underlying bone mass loss is the potentiation of PPAR γ activity [71]. Although this non-desirable effect has been described only in rodents, it has to be taken into account when considering FGF21 as a potential drug candidate.

FGF21 expression is regulated by nutrition

Fasting and feeding

FGF21 is expressed and produced by multiple tissues. However, under normal physiological conditions, all circulating protein appears to derive from the liver [72]. FGF21 was initially described as a protein induced in the liver to control metabolic adaptation to long periods of

starvation. This mRNA induction goes through the PPAR α [13, 73, 74] and CREBH [75, 76]. Later, several studies showed that FGF21 expression is regulated not only by fasting but also by other nutritional states [77]. In addition to PPAR α and CREBH FGF21 expression responds to the retinoic acid (RA) receptor β (RAR β) [78], the RA receptor-related orphan receptor α (ROR α) [79], the thyroid hormone receptor β (TR β) [80], the activating transcription factor-4 (ATF4) [81], the farnesoid X receptor (FXR) [82], and the carbohydrate responsive element binding protein (ChREBP) [83]. The following chapter will summarize the effects of various macronutrients and bioactive dietary components on FGF21 expression, the metabolic consequences and the putative signaling transduction pathways involved.

Carbohydrates upregulate FGF21 expression

Hepatic FGF21 is induced by prolonged fasting and also by refeeding with a high carbohydrate diet (HCD) (mixed sugar and starch) [84]. Rats starved for 24 h and refed with a HCD for 12 h show an increase in hepatic mRNA and serum levels of FGF21 and metabolic adaptations in the liver and WAT. Specifically in liver, there is an induction of lipogenesis, glucose uptake and metabolism, and a reduction of FA uptake and FAO. Similarly, in WAT, refeeding with a HCD induces lipogenesis, glucose uptake and metabolism, and lipolysis [84]. These results support the hypothesis that FGF21 is produced to compensate any imbalanced nutritional states.

Hepatic FGF21 mRNA expression and plasma levels are also induced in male C57BL/6 mice fed a HC diet containing 77% of energy as dextrose, 0.5% as fat and 22.5% as protein [85]. These results agree with previous studies performed in isolated rat hepatocytes cultured with high glucose [86]. Under this dextrose-rich diet, the liver induction of FGF21 increases *de novo* lipogenesis, probably as a result of excess carbohydrate intake. In contrast, the same diet supplemented with exogenous lipids [a soybean oil-based emulsion that is rich in C-18:1 and C-18:2 (n-6) unsaturated FAs] reduces FGF21 mRNA levels and *de novo* lipogenesis.

In rodents and humans, glucose is not the only molecule able to induce FGF21 expression *in vitro*. The human promoter of FGF21 responds to xylitol [77] and fructose [87] through the transcription factor ChREBP, which binds to a carbohydrate response element (ChoRE) present in human and mouse promoters [77, 83]. These data again reveal the independent mechanisms that regulate the expression of FGF21 downstream of fasting and feeding signals. *In vivo*,

both in humans and rodents, fructose ingestion, but not glucose, leads to an increase of FGF21 serum levels, which peak 2 h after an acute load [87]. In this case, the remaining question is how and why FGF21, which is considered a metabolic positive hormone, is induced by a metabolically pernicious sugar such as fructose.

Finally, it has also been demonstrated that FGF21 expression is regulated by dietary fiber. The intake of such fiber facilitates weight loss and improves lipid and glucose profiles. Sugarcane fiber (SCF: 85% insoluble fiber, 70% particles <1 μ M) administered to mice fed a HFD for 12 weeks enhances insulin sensitivity, diminishes fasting plasma glucose and TGs, and attenuates weight [88]. Without altering caloric intake, SCF regulates leptin and glucagon-like peptide 1 (GLP-1) in these mice. In the liver, SCF decreases TGs, cholesterol content, and FGF21 expression, but induces mRNA levels of KLB, FGFR1, FGFR3 and PPAR α , thus enhancing FGF21 signaling and ameliorating the obesity observed in the FGF21-resistant state. In parallel SCF also increases AMPK signaling [88].

High-fat diets induce FGF21 resistance

The notion of crosstalk between HFDs and FGF21 expression is controversial probably because of the variety of FAs included in diets. For example, mice fed a corn-oil based HFD (cHFD) for 5 weeks express more FGF21 in liver than those fed a HFD in which corn-oil is replaced by a fish-derived long-chain polyunsaturated n-3 FA (PUFA) [89]. Moreover, another study reported that mice fed a HFD for 16 weeks show no differences in FGF21 mRNA levels versus those fed a low-fat diet [85].

In HepG2 cells, oleate, linoleate and trans-10, cis-12 conjugated linoleic acid (t-10, c-12-CLA) induce FGF21 expression and secretion while palmitate has no effect [90, 91]. Lipid infusion in humans increases the circulating levels of FGF21 [90]. Similarly, in neonatal mice, hepatic FGF21 expression is induced at the initiation of suckling, mainly due to the high FA content of milk [35]. In this case, the FGF21 secreted induces the thermogenic program in BAT.

In the abovementioned situations, mRNA induction occurs through the activation of PPAR α and causes an increase in serum levels of FGF21.

In addition to long-chain FAs, butyrate and α -lipoic acid also modulate FGF21 expression in the liver. The former is produced mainly by bacterial fermentation of dietary fiber in the large intestine, and its effect on FGF21 expression seems to be due to its capacity to inhibit histone deacetylase-3 (HDAC3) [92]. Another short FA,

α -lipoic acid, is also involved in the regulation of FGF21. α -lipoic acid can be obtained from the diet (leafy green vegetables and red meats), and its dietary supplementation induces hepatic and plasma levels of FGF21 in vivo and in vitro [93, 94]. The effect of α -lipoic acid on FGF21 expression depends on a CREBH-dependent mechanism that includes the induction of its expression and an increase in its binding to the FGF21 promoter [95].

Finally, several models of obesity in rodents, primates and humans showed that FGF21 levels are higher than in normal weight littermates [17, 96, 97]. In this context, obesity can be defined as an “FGF21-resistant state” [98]. The hepatic induction of FGF21 mRNA in DIO mice positively correlates with an attenuated responsiveness in liver and WAT as a result of a reduction in FGFR1, FGFR4 and KLB levels.

Amino acid-deficient and low-protein diets increase energy expenditure

Several studies have described that hepatic FGF21 is regulated by protein intake. In general, LPDs or diets deficient in a specific amino acid (i.e. leucine or methionine) cause an increase in the hepatic expression and serum levels of FGF21. In this regard, diets with a relatively low content of essential amino acids, such as many vegan diets, are known to be protective against cancer, autoimmunity, obesity, and diabetes. These benefits could be partly due to increased circulating levels of FGF21 [99]. Several studies have demonstrated that FGF21 is the link between imbalanced amino acid intake and adaptive metabolic response and that it serves to restore metabolic homeostasis.

In liver – but not in WAT or BAT–, FGF21 is induced by leucine deprivation. In wild-type mice, the metabolic response to leucine deprivation includes dramatic changes in lipid metabolism. In liver, such deprivation inhibits FA synthase (FAS) activity, decreases the expression of lipogenic genes, and increases the mobilization of lipid stores. In WAT, it decreases FAS activity and the expression of lipogenic genes and increases the expression of FAO genes. Finally, there is an induction of UCP1 expression in BAT [20, 100, 101]. In contrast, in *Fgf21*-deficient mice this metabolic response to leucine is impaired, thus indicating that FGF21 is a key hormone in the regulation of lipid metabolism during leucine deprivation [20, 81].

In the same way, methionine-deprived mice show a comparable phenotype to that of leucine deprivation. The metabolic response to methionine deficiency includes resistance to diet-induced obesity, improved glucose

homeostasis, increased FA activation and oxidation in liver, increased lipolysis in WAT, and increased Ucp1 expression in BAT [102–104]. All these effects are coupled to an increase in FGF21 levels.

The metabolic response to protein restriction is similar to that observed under leucine or methionine restriction [7]. Serum levels of FGF21 increase in both rodents and humans upon exposure to LPDs, regardless of overall caloric intake [105, 106]. Protein restriction is accompanied by weight loss and an increase in both food intake and energy expenditure. Remarkably, neither food intake nor energy expenditure of *Fgf21*-deficient mice are altered by the administration of LPDs [7]. Moreover, ketogenic diets (KDs), which are widely known to induce FGF21 expression, are usually low in carbohydrates and proteins but rich in fat. The protein content of KDs underlies the increased levels of circulating FGF21, since protein supplementation but not carbohydrate supplementation blunts the induction [107]. This effect could also explain the induction of FGF21 observed in HCDs, which are characterized by a low protein content.

Protein undernutrition caused by LPDs or imbalanced diets (which are common in mammals confronted with deficient sources of certain amino acids like legumes, grains or corn) strongly affects aminoacidemia. Additionally, pathological situations caused by various forms of stress, such as trauma, thermal burning, sepsis and fever, can lead to a negative nitrogen balance. In this context, several scenarios that alter aminoacidemia also lead to an increase in FGF21 expression. The absence of *slc6a19* (neutral amino acid transporter) causes a lack of systemic neutral amino acids, resulting in an increase in FGF21 transcription [108]. The treatment with the antileukemic agent asparaginase depletes circulating asparagine and glutamine levels, promoting FGF21 expression [109], and the skeletal muscle-specific knockout mice for glucocorticoid receptor (GR) show reduced alanine flux from skeletal muscle during fasting, resulting in an increase in FGF21 plasma levels [110]. Hence, it is likely that many other situations that reduce amino acid availability also lead to an induction of FGF21 expression.

GCN2, a kinase that acts as a sensor of amino acid supply [111], and PPAR α are indispensable for the induction of FGF21 in response to protein restriction. In this regard, the respective knockout mice present blunted induction of FGF21 when fed a LPD. Nevertheless, it is worth mentioning that in both *Ppara*-KO and *Gcn2*-KO mice, the LPD still induces FGF21 expression [7]. This observation suggests that additional signaling pathways are involved in triggering the increase in FGF21 expression in response to protein restriction.

To date, there is no evidence of PPAR α activation in response to a LPD, thus suggesting that PPAR α plays a role in the constitutive expression of FGF21. In contrast, the LPD increases GCN2-dependent phosphorylation of eIF2 α , resulting in greater ATF4 protein levels [100, 112]. Therefore, the GCN2/eIF2 α /ATF4 cascade emerges as the main signaling pathway in the induction of FGF21 by protein restriction. ATF4 directly or indirectly induces the transcription of a subset of specific target genes, including FGF21, to modulate many cellular processes to adapt to amino acid deficiency [81, 113, 114]. The 5' regulatory region of the human FGF21 gene contains two evolutionarily conserved functional ATF4-binding sequences (AARE), which are responsible for ATF4-dependent transcriptional activation in response to ER stress or amino acid restriction [81, 115].

Hepatic mTORC1 activity is also related to FGF21 expression. The mTOR signaling pathway monitors amino acid sufficiency and promotes protein translation and cell growth, among other processes [116]. In this case, L-Tsc1 KO mice, which present mTORC1 hyperactivity in the liver, show increased expression of FGF21 and depleted levels of glutamine [117]. Moreover, when these animals are treated with rapamycin (mTORC1 inhibitor) or glutamine, the increase in FGF21 is blunted. Finally, in human hepatic tumors, mTORC1 activation also correlates with FGF21 levels [117]. It has been proposed that the mechanism underlying the increase in FGF21 expression occurs through PGC1 α ; however, additional mechanisms could be involved and it is feasible that depleted glutamine levels trigger an amino acid response (AAR).

In summary, amino acid-deficient diets diminish aminoacidemia and trigger AAR, thereby resulting in elevated FGF21 levels. The contribution of each single amino acid to the modulation of FGF21 and how a deficiency in specific types of dietary protein alters FGF21 expression require further study.

Bioactive dietary compounds affect FGF21 expression and signaling

Polyphenols are the most abundant phytochemicals in nature. These bioactive compounds are synthesized as secondary metabolites by plants and are thus abundant in fruits, vegetables, legumes, cocoa and some beverages, such as tea, coffee and wine. Polyphenols comprise a large heterogeneous group of chemical structures, all with a phenolic ring with one or more hydroxyl groups, and they are classified mainly into two families, namely flavonoids and non-flavonoids, and also into many subfamilies.

Flavonoids are the most abundant polyphenols and consequently those most greatly ingested in human diets.

Due to the diversity of polyphenols, their absorption in the body is dose- and type-dependent and their effects are related to their bioavailability and pharmacokinetics. It is estimated that most of the polyphenols ingested go directly to the colon and only between 5 and 10% are absorbed in small intestine. The effects of polyphenols in the colon have been related to a prebiotic capacity, as they are believed to induce the growth and activity of some bacteria, such as *Bifidobacterium*, *Enterococcus* and *Prevotella*. Furthermore, once absorbed, polyphenols enter portal circulation and are metabolized in the liver. Finally, the conjugate metabolites reach the bloodstream and the target tissues [118, 119].

Various epidemiological studies have reported that the regular consumption of polyphenols has beneficial effects in obesity, insulin resistance, cardiovascular diseases, and cancer. Several lines of evidence support the notion that polyphenol-rich diets play a key role in regulating lipid and glucose metabolism and are thus pivotal in the prevention and treatment of pathologies related to energy homeostasis [120–123].

It has been described that resveratrol has a protective effect on cardiovascular risk associated to ROS overproduction but also on the prevention of hepatic steatosis and the improvement of insulin resistance [124]. Green tea polyphenols, mainly epigallocatechin-3-gallate (EGCG), reduce the LDL and increase HDL levels in humans and improve insulin sensitivity in genetic models of insulin resistance, thus exerting a beneficial effect on body weight and lipid profile [125, 126]. Isoflavones and polyphenol-rich grape extract can partially prevent hepatic steatosis associated with obesity by restoring the correct secretion of adipokines – mainly leptin and adiponectin, by up-regulating FAO, and by down-regulating lipogenesis in adipose tissue [127, 128].

For many years, the health benefits of polyphenols were attributed to their anti-oxidant capacity as free radical scavengers. It has recently been described that polyphenols activate cell-signaling pathways that are not related to ROS production but rather those involved in metabolic regulation. It is remarkable that most of these pathways are downstream of FGF21. It has been reported that, in vitro, polyphenols downregulate SREBP-1c and its main target genes in lipogenesis, namely FAS and acetyl-CoA carboxylase (ACC) [129]. Stilbens, mainly resveratrol, exert their effects by promoting the phosphorylation and activation of the AMP-activated protein kinase (AMPK) and the activity of SIRT and PGC1 α , thus inducing FA catabolism through the AMPK/SIRT1/PGC1 α axis [130, 131]. On

the other hand, an anthocyanin-rich juice extract up-regulates PPAR α activity in mice on a HFD and down-regulates lipogenic gene expression in liver by promoting FA consumption. Coffee polyphenols and resveratrol also induce FAO in rats through PPAR α and PGC1 α -dependent mechanisms [132]. Chalcones and flavokawain cardamomin type B inhibit lipid accumulation and adipocyte differentiation by increasing the phosphorylation of the ERK in the early phase of adipogenesis [133].

In summary, polyphenols exert beneficial metabolic effects especially in obese and in insulin-resistant animal models, and FGF21 restores homeostasis in scenarios of metabolic stress. The crosstalk between the two signals remains unclear, but a positive correlation between the beneficial effects of polyphenol-rich fruit extracts and FGF21 activity has been described in various rodent models of diet-induced obesity. In some cases, the effects of polyphenols were due to an induction of FGF21 levels in liver [134, 135] but also to an enhancement of FGF21 signaling by increasing the expression of FGF21 receptors and KLB, [136, 137]. Moreover, flavokawain B and cardamomin also modulate the secretion of FGF21 in mature adipocytes [133].

FGF21 in humans

After the identification of rodent FGF21 as an endocrine regulator induced downstream of PPAR α and its agonists by both fasting and a KD [13, 73], it was shown that fasting FGF21 levels are significantly increased in patients with T2DM without a correlation with BMI, thereby pointing to a potential role of FGF21 in the pathogenesis of insulin resistance and T2DM [138].

Later, in contrast to previously published data, it was described that serum FGF21 levels in overweight and in obese subjects are significantly higher than in lean individuals and that they correlate positively with adiposity and the metabolic syndrome [17, 139]. Also, an increase in plasma levels of FGF21 in hepatic- and muscle-insulin resistant states and a correlation with BMI have been reported [140].

Accordingly, a study performed in 2010 again showed a positive correlation between FGF21 expression and BMI; however, the authors did not find that fasting and refeeding or 12 days of a KD regulated the hormone in humans [8]. Moreover, FGF21 concentrations are reversibly increased and are related to leptin and free FAs in obese children. However, the results of that study do not support a significant relationship between FGF21, insulin resistance, and features of metabolic syndrome or NAFLD

in this age group [141]. The same pattern of FGF21 expression was described in obese adolescents with T2DM compared with obese adolescents without. As in rodents, this increment in FGF21 levels in obesity and T2DM points to a FGF21-resistant state and an impaired capacity of FGF21 to improve insulin sensitivity [142]. No significant association between FGF21 and growth or IGF-1 was found in either cross-sectional or longitudinal analyses; these findings do not support a relationship between FGF21 and growth in obese children [143].

In contrast, in the pubertal transition, it was found that FGF21 concentrations do not differ by obesity status or by sex. An inverse association between FGF21 and bone mineral content (BMC) among non-obese individuals and an inverse association between FGF21 and lean mass among females were observed, which were both independent of fat mass. FGF21 was inversely associated with HOMA-IR in males but not in females. The existence of relationships between FGF21, musculoskeletal parameters, and insulin resistance raises the possibility of crosstalk between these systems. These data suggest that circulating FGF21 differs in its association with bone, lean mass and insulin resistance depending on the sex and weight of the individual [144].

Globally, several studies have proposed FGF21 as a biomarker for metabolic pathologies such as cardiovascular diseases, NAFLD, and mitochondrial disease [8, 145]. Serum FGF21 concentrations are significantly elevated in patients with mitochondrial disease. This prospective study established serum FGF21 levels as a sensitive biomarker of mitochondrial disease and demonstrated that they are the best predictor of this disorder when compared to serum levels of other classical indicators [146]. In the same way, a cross-sectional study with a large well-characterized sample (913 subjects) assessed the interaction between clinical parameters (renal function, metabolic, hepatic and vascular risk markers), as well as growth hormone (GH) status, with FGF21 levels. Those authors concluded that FGF21 serum concentrations are associated with several aspects of the metabolic syndrome, hepatocellular function, as well as with GH status [147].

In a healthy population, there is also an age-related increase in serum FGF21 levels. This observation highlights a potential age effect in response to metabolic demands during the lifespan. FGF21 levels increase with age independently of body composition. At lower levels of FGF21, bone mineral density (BMD), but not other body composition parameters, attenuates the association between FGF21 levels and age, thereby suggesting that the metabolic demands of the skeleton serve to link FGF21 and energy metabolism [148].

Regarding the nutritional regulation of FGF21 in humans, the data are less clear than in rodents. Increased levels of FGF21 in human serum after extreme fasting (7 days) and PPAR α activation have been described in healthy non-diabetic individuals but with a wide inter-individual variation. The induction of ketogenesis independently of FGF21 levels suggests that the physiological role of FGF21 in humans differs from that in mice [149]. Although FGF21 is elevated in response to pharmacological activation of PPAR α and PPAR δ , the absence of variation in human plasma during fasting and refeeding and the decrease after a 3-month KD confirm that FGF21 does not play a major role in regulating fasting response or ketosis in humans [150].

Unlike mice, which showed an increase in circulating FGF21 after only 6 h of fasting, human subjects did not have a notable surge in FGF21 until 7–10 days of fasting. Moreover, FGF21 induction was associated with decreased thermogenesis and adiponectin, an observation that contrasts with previous reports based on supraphysiological dosing. In addition, FGF21 levels increased after ketone induction, thereby demonstrating that endogenous FGF21 does not drive starvation-mediated ketogenesis in humans. Instead, a longitudinal analysis of biologically relevant variables identified serum transaminase markers of tissue breakdown as predictors of FGF21. These data establish FGF21 as a fasting-induced hormone in humans and indicate that it contributes to the late stages of adaptation to starvation, when it may regulate the utilization of fuel derived from tissue breakdown [151].

A large number of studies have shown that dietary protein content markedly influences food intake and metabolism. In this context, in humans, circulating FGF21 levels increase dramatically after 28 days on a LPD, thereby suggesting that FGF21 is a signal of protein restriction and providing an explanation of the effect of dietary protein deficiency on metabolism [7].

As mentioned earlier, FGF21 levels increase rapidly following fructose ingestion and return to baseline within 5 h. Moreover, both baseline and fructose-stimulated FGF21 levels are 2–3 fold higher in subjects with metabolic syndrome compared to those in healthy subjects. In contrast, FGF21 does not increase in the first 2 h after the ingestion of a glucose load, although a modest increase is observed after 3–4 h. These data suggest that FGF21 plays a key role in fructose metabolism in humans [87].

Other nutritional challenges that affect FGF21 levels are fish oil supplements and betaine. The inclusion of the former in a diet for 3 months markedly reduces the circulating levels of FGF21 and other biomarkers, combined with decreases in serum lipids, glucose, liver enzymes,

and other NAFLD risk factors. These results suggest that fish oil might contribute to reversing FGF21 resistance [152]. Regarding betaine, it is known that plasma betaine levels are reduced in insulin-resistant humans and that they correlate closely with insulin sensitivity. In this context, in addition to the beneficial metabolic effects of betaine, supplementation with this compound robustly increases hepatic and circulating FGF21 levels in mice. On the basis of these observations, betaine supplementation merits further investigation for the treatment or prevention of T2DM in humans [153].

Circulating levels of FGF21 can also be boosted by exercise. Increased levels of the protein were observed in the serum of healthy male volunteers performing a treadmill run at 50 or 80% VO_{2max} . These results suggest that FGF21 is also associated with exercise-induced lipolysis [41].

Finally, in healthy human volunteers, the injection of natural glucagon increased plasma FGF21 within hours, showing for the first time that glucagon regulates glucose, energy, and lipid metabolism, at least in part via FGF21-dependent pathways [154].

Expert opinion

FGF21 is a peptide hormone involved in metabolic homeostasis and considered a promising therapeutic candidate for the treatment of obesity and associated comorbidities such as insulin resistance, T2DM, and cardiovascular diseases. Although the effects of FGF21 in humans seems to be weaker than in mice and interindividual variability in the levels of this growth factor are observed, it is obvious that FGF21 exerts beneficial effects on metabolic homeostasis in both species. To date, the therapies related to FGF21 overexpression have involved pharmacological administration; however, this approach has considerable limitations due to the kinetics and bioavailability of the compound. Recently, several studies have demonstrated that specific macronutrients and bioactive dietary compounds can modulate endogenous FGF21 expression and signaling, thereby opening up the possibility to induce FGF21 production or increase activity through dietary intervention. The most feasible approaches to define a nutritional intervention able to induce FGF21 activity and reduce obesity and associated-comorbidities are probably through protein restriction and polyphenol-enriched diets. However, more research is needed in order to establish the role of FGF21 as the link between the dietary components. It is also important to highlight that all these nutritional interventions must be done in compliance

with a balanced diet in order to prevent the loss of muscle mass or BMD and essential nutrient deficiencies.

Outlook

To design therapeutic approaches based on a nutritional intervention it is essential to determine the molecular mechanisms through which macronutrients, micronutrients, and bioactive dietary compounds affect FGF21 expression and signaling. It is likely that the metabolic effects of certain diets such as vegan or the beneficial effects of established healthy diets, such as the Mediterranean diet, occur at least in part through FGF21. Further studies in humans will be required to define the effect of a nutritional profile on FGF21 levels and signaling in healthy, obese and T2DM patients. Also it will be important to define if there are any differential effects between the pharmacological administration of FGF21 and its endogenous overproduction. Moreover, obesity that is one of the possible diseases treatable with FGF21 is also a FGF21-resistant state. In this context it will be strictly necessary to study the way to overcome this resistance, thus increasing the levels of the FGF21 receptors in its target tissues. Some studies have revealed that some nutritional interventions are able to induce the expression of FGFR and KLB.

In summary, FGF21 is a promising therapeutic candidate against metabolic pathologies such as obesity or T2DM and its expression and signaling are highly regulated by different nutritional states. The remaining question is how we can increase the responsiveness to FGF21 in obese or T2DM patient just through a dietary intervention.

Highlights

In animal models has been is well described that:

- The liver is the main organ in contributing to serum levels of FGF21.
- The transcription factors network responsible for the control of FGF21 gene expression is highly complicated. Both, positive and negative factors have been described.
- The expression of hepatic FGF21 responds to several stimuli, mainly metabolic stress signals.
- The hepatic expression of FGF21 can be modified by dietary components. Mainly high fat diet, high carbohydrate diet, low protein diet or amino acid restricted diets.

In order to confirm FGF21 as a therapeutic candidate against metabolic pathologies such as obesity or T2DM:

- The role of FGF21 as the link between the dietary components and its metabolic effects has to be set precisely.
- The effect of dietary bioactive compounds on the expression of hepatic FGF21 needs further studies.
- The mechanisms responsible for the establishment of the FGF21-resistant state have to be proven.
- The effect of different dietary interventions on the expression of FGF21 receptors in target tissues needs future evaluation.

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