Review

Daniel Cuevas-Ramos* and Carlos A. Aguilar-Salinas

Modulation of energy balance by fibroblast growth factor 21

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Abstract: Fibroblast growth factors (FGFs) are a superfamily of 22 proteins related to cell proliferation and tissue repair after injury. A subgroup of three proteins, FGF19, FGF21, and FGF23, are major endocrine mediators. These three FGFs have low affinity to heparin sulfate during receptor binding; in contrast they have a strong interaction with the cofactor Klotho/β-Klotho. FGF21 has received particular attention because of its key role in carbohydrate, lipids, and energy balance regulation. FGF21 improves glucose and lipids metabolism as well as increasing energy expenditure in animal models and humans. Conditions that induce human physical stress such as exercise, lactation, obesity, insulin resistance, and type 2 diabetes influence FGF21 circulating levels. FGF21 also has an anti-oxidant function in human metabolic diseases which contribute to understanding the FGF21 compensatory increment in obesity, the metabolic syndrome, and type 2 diabetes. Interestingly, energy expenditure and weight loss is induced by FGF21. The mechanism involved is through "browning" of white adipose tissue, increasing brown adipose tissue activity and heat production. Therefore, clinical evaluation of therapeutic action of exogenous FGF21 administration is warranted, particularly to treat diabetes and obesity.

Keywords: browning; exercise; free fatty acids; FGF21; glucose; klotho; lipids; oxidative stress.

Introduction

Fibroblast growth factors (FGFs) are a superfamily of 22 proteins associated with cell proliferation, differentiation, migration, and tissue repair after injury [1–4]. A subgroup of three proteins, FGF19, FGF21, and FGF23, showed endocrine effects [5]. FGF19 (FGF15 in mice) is associated with cholesterol and bile acid synthesis [6], FGF21 regulates glucose and lipid metabolism [7]; and FGF23 control phosphorus circulating levels [8]. They were therefore grouped in an endocrine superfamily with particular features [9]. First, in comparison to the remaining FGFs, this endocrine superfamily showed low affinity to heparin sulfate during receptor binding which conferred their endocrine function [10]. Secondly, reduction in the heparin-binding affinity is compensated with a cofactor interaction named Klotho/β-Klotho (Figure 1). This cofactor binds together with the endocrine FGF and its receptor, achieving proper signal transduction at their target tissues [10, 11]. Finally, such factors have been related to clinical consequences after being overexpressed or knocked out in different artificially created animal models or when evaluated in human disease [7, 12–14]. FGF21 has received particular attention because of its key role in carbohydrates, lipids, and energy balance regulation [12, 15], protection against oxidative stress [16], and "browning" of white adipose tissue (WAT) increasing energy expenditure and weight loss [17–19]. Our group was the first to report increased FGF21 levels after intense physical activity in sedentary young women, which was significantly and independently correlated with noradrenaline levels and FFAs, suggesting that increase sympathetic activity and lipolysis was the mechanism that induce FGF21 augmentation [20]. The effect of exercise in FGF21 levels were consistent with other studies [21, 22], as well as the key role of adrenergic activity to induce FGF21 expression [23]. Conditions with high FFAs mobilization or under increased chronic inflammation such as lactation, patients under growth hormone treatment, insulin resistance, heart ischemic disease, hypothyroidism, and kidney failure associated with preeclampsia have higher circulating FGF21 levels [15, 25–29].

FGF21 has a similar structure to other FGFs. However, currently it is not considered a FGR as it does not have activity in fibroblasts, and does not promote growth in vivo [30]. Nowadays, FGF21 is considered a key hormone for human energy homeostasis. Also, the pathophysiological role of FGF21 in human diseases such as metabolic syndrome,

***Corresponding author: Daniel Cuevas-Ramos,** Department of Endocrinology and Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Sección XVI, Tlalpan 14000, Mexico City, Mexico,
E-mail: ceptamim@gmail.com
Carlos A. Aguilar-Salinas: Department of Endocrinology and Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

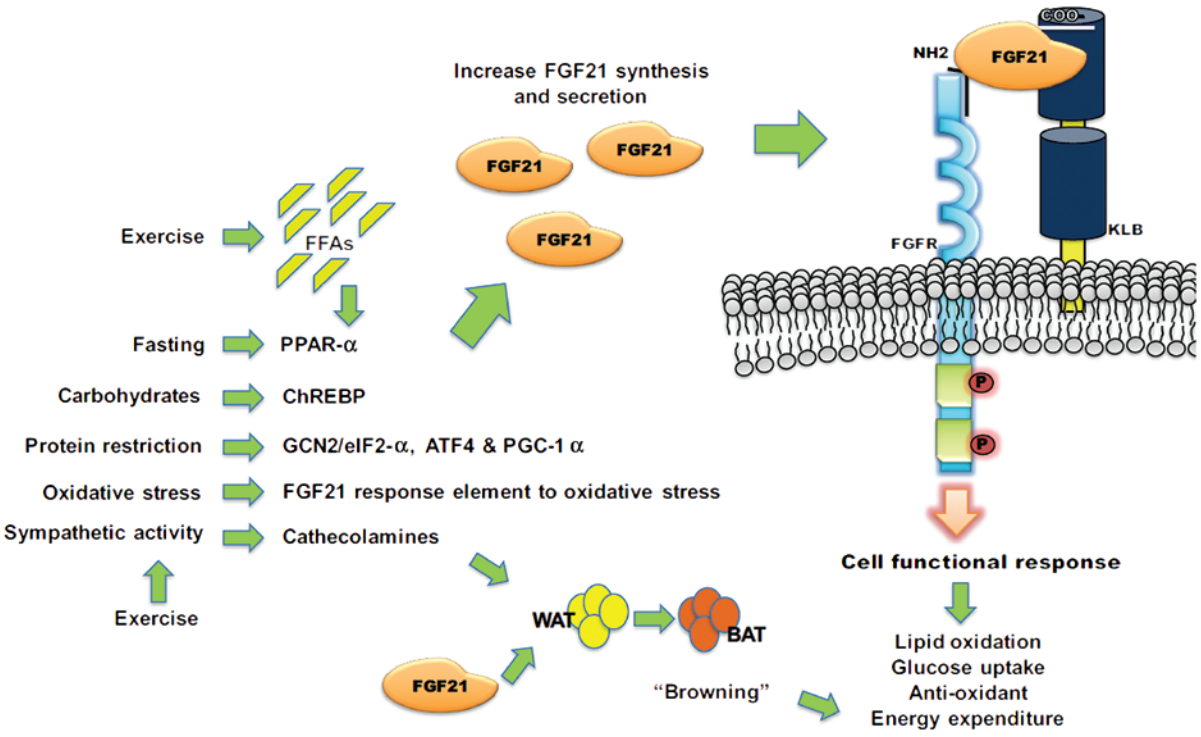

Figure 1: Role of FGF21 on energy balance.
Modulators of FGF21 synthesis are prolonged fasting, carbohydrates, protein restriction, increase in oxidative stress and sympathetic activity. Different stimuli that cause supraphysiologic free fatty acid (FFAs) levels, like exercise, increase FGF21 circulating levels. Peroxisome-proliferator-activated receptor-α (PPAR-α) activity is induced by fasting and FFAs. Carbohydrates increase FGF21 promoter expression through carbohydrate response element-binding protein (ChREBP). Protein restriction increases the activity of general control nonderepressible 2 (GCN2)-eukaryotic initiation factor 2 (eIF2) α pathways activating the transcription factor 4 (ATF4) and the transcriptional coactivator protein of PPAR-γ (PGC)-1 α. Oxidative stress markers interact directly with a FGF21 response element increasing FGF21 synthesis. After FGF21 is released into circulation, interacts with its receptor (FGFR) particularly subtype 1c (FGFR1c). However, FGFR is tissue specific. Cell functional response is induced and FGF21 promotes lipid oxidation and ketogenesis as energy source in low glucose environment because starvation. Also, FGF21 increase glucose uptake in periphery and has an important anti-oxidative effect in multiple organs such as liver and kidney. Finally, FGF21 induces "browning" of white adipose tissue (WAT) to brown adipose tissue (BAT), increasing heat production and energy expenditure. This is also induced by brain sympathetic activity and cathecolamines (adrenaline) release.

diabetes, dyslipidemias, and obesity with a potential role as therapeutic target has been suggested. This review focuses specifically on interventions that modulate FGF21 circulating levels and actions in glucose and lipid metabolism as well as energy expenditure. The consequences of different human metabolic diseases and oxidative stress on FGF21 expression is also reviewed. Finally, an update in novel FGF21-based pharmacotherapy is briefly summarized.

Fibroblast growth factor 21 (FGF21)

FGF21 is a 209 amino acid protein in humans which regulates glucose and lipids metabolism. It is synthetized mainly in liver [31] but also in white [32] and brown [18] adipose tissue, skeletal muscle [33], heart [34], and β cells [12]. Action of FGF21 is through cell membrane receptor FGFR1c and β-Klotho interaction (Figure 1) [35–37]. Intracellular signaling is then activated through phosphorylation of FGFR substrate 2 α (FRS2 α), extracellular response kinase 1/2 (ERK1/2), and Akt (protein kinase B) pathways [38, 39].

Conditions that influence human energy balance and physical stress modify FGF21 synthesis and secretion with measurable changes in circulating FGF21 plasma levels. The response elements involved in the regulation of *FGF21* expression are shown in Table 1. Because they are numerous and highly interrelated, the regulation of FGF21 secretion is complex and critical for the energy balance.

Interventions that modulates FGF21 synthesis and secretion

Fasting

Prolonged fasting was one of the first stimuli to be associated with an important increment on FGF21 liver

Table 1: Relevant transcription factors acting at FGF21 gene promoter.

Transcription factor	Name	Concise function	Clinical significance
PPAR-γ	Peroxisome proliferator-activated receptor γ	Nuclear receptor regulating fatty acid storage, and glucose metabolism	Physiopathology of cancer, obesity, type 2 diabetes, vascular atherosclerosis
PPAR-α	Peroxisome proliferator-activated receptor α	Controls lipid metabolism at liver and is mainly activated under energy deprivation	Metabolic disruption including hypoglycemia, low ketone bodies, and fatty liver
MyoD	Myogenic D factor	Regulates muscle differentiation and p21 expression. Induces fast-twitch muscle fiber phenotype.	No clear clinical association. Probably related to muscle-related diseases (i.e. cachexia).
STAT3	Signal transducer and activator of transcription 3	Transcription activator activated by growth factors and cytokines in response to inflammation or cell proliferation	Hyperimmunoglobulin E syndrome with recurrent infections, bone disorders, auto-inmmune diseases, human cancer, and acromegaly
AP-2 α, β, γ	Transcription factor family activating enhancer binding protein 2	Recruits transcription machinery. It is induced by retinoic acid mainly at liver	Branchio-oculo-facial syndrome (α); Char syndrome (β); early development with fetal death (γ)
AP-4	Activating enhancer binding protein 4	Repressor and an activator of multiple genes	Unknown
CREB	AMPc response element binding protein	Transduction of intracellular signaling associated with AMPc in multiple tissues	Huntington's disease; Rubinstein-Taybi syndrome; insulin resistance physiopathology, cognition, circadian rhythms
Nrf-2	Nuclear factor (erythroid-derived 2)-like 2	Expression of antioxidant proteins such as FGF21.	Protects against oxidative damage.
SREBP	Sterol regulatory element-binding protein	Cholesterol biosynthesis and uptake of fatty acids	Physiopathology of different lipid abnormalities
ChREBP	Carbohydrate-responsive element-binding protein	Binds to DNA in a glucose-dependent manner	Williams-Beuren syndrome
NR3C1	Glucocorticoid nuclear receptor response element	Transactivation and transrepression of target genes	Familial glucocorticoid resistance; neuroendocrine integration; depression; post-traumatic stress disorder; Cushing's disease
ATF4	Activating transcription factor 4	Encodes cAMP-response element binding protein 2 (CREB2)	Bone mineralization; reduces oxidative stress.
AAREs	Amino acid response element	Enhances ATF2 and ATF4 activity to control mammalian gene transcription	Unknown

synthesis in animal models [40, 41]. Prolonged fasting induces lipolysis and free fatty acids (FFAs) release from WAT. Then, FFAs increases peroxisome-proliferator-activated receptor-α (PPAR-α) activity (Figure 1). PPAR-α is a nuclear receptor that response to endogenous signaling ligands such as FFAs, increasing their oxidation and ketone bodies formation as energy source in a carbohydrate deprivation state caused by fasting. The FGF21 promoter has PPAR-α response element which is activated after FFAs/PPAR-α/retinoid X receptor (RXR) interaction (Table 1) [40, 42]. Therefore, increase PPAR-α activity also increases FGF21 liver synthesis and release to circulation in order to improve energy production, increase ketogenesis, gluconeogenesis, appetite, and systemic glucose uptake as adaptive responses to starvation [43, 44].

However, regulation of glucose levels during fasting seems to be different in humans than in animal models. In mice, FGF21 is rapidly induced by fasting whereas in humans, fasting does not consistently increase FGF21 [44–46]. In one recent study, a notable surge in FGF21 occurred after 7–10 days of fasting, and did not drive starvation-mediated ketogenesis like in mice [46]. Also, in humans FGF21 increment was stimulated after decreased thermogenesis, reduction in adiponectin levels, and tissue breakdown markers like transaminases elevation rather than changes in FFAs [46]. Nevertheless, several clinical studies have shown increment in FGF21 levels after other well-known inducers of supraphysiological concentrations of circulating FFAs different than prolonged fasting. Lactation [25], milk ingestion in neonates [17], growth hormone therapy [26, 47], and intensive physical activity

[20] increased FGF21 circulating levels, probably through liver PPAR-α activation as well [43, 44] (Figure 1). Mechanisms associated with higher FGF21 expression are inhibition of histone deacetylase 3 enzyme (HDAC3) by sodium butyrate. HDAC3 usually suppress PPAR-α activity by removing acetyl groups. Inhibition of HDAC3, therefore, permits expression of PPAR-α and in turn higher expression of FGF21 [48]. The thyroid hormone receptor β (TR-β) [49], retinoic acid receptor β (RAR-β) [50], retinoic acid receptor-related orphan receptor α (ROR-α) [51], and the cyclic AMP response element-binding protein H (CREBH) [52], mediate liver responses regulating FGF21 expression. FGF21 is a mechanism involved in the cross talk between liver and brain to maintain glucose homeostasis during prolonged fasting. Liver-derived FG21 exerts its action in the FGFR1 of hypothalamic neurons inducing liver gluconeogenesis, the so-called "liver-brain" axis [53]. FGF21 induces direct activation of hypothalamic mitogen-activated protein kinase extracellular signal-related kinase 1/2 (ERK1/2), thus increasing the expression of corticotropin-releasing hormone (CRH) by activation of the transcription factor cAMP response element binding protein (CREB) [54]. CRH increase corticotropin (ACTH) release from anterior corticotroph pituitary cells, which in turn will increase cortisol release from adrenal cortex. Cortisol stimulates liver gluconeogenesis and glucose release, correcting glucose homeostasis. FGF21 and glucocorticoids regulate each other production in a feed-forward loop. Glucocorticoids induce FGF21 expression in liver through glucocorticoid nuclear receptor response element located in *fgf21* transcription site (Table 1) [54]. FGF21, in turn, induces glucocorticoids synthesis and release in adrenal gland in response to ACTH. This loop bypasses the negative feedback of glucocorticoids in the hypothalamic-pituitary-adrenal axis in order to sustain gluconeogenesis in liver during starvation [54]. Recently, it has been reported that FGF21 expression is regulated by activating transcription factor 4 (ATF4), a transcription factor activated by various stimuli such as endoplasmic reticulum (ER) stress [55]. ATF4 binds to the amino acid response element (AARE), a binding site for ATF4, in the promoter region of the target genes. The two response elements for ATF4 (AARE1 and AARE2) have been reported in the promoter region of FGF21 gene [55].

Carbohydrates

Carbohydrates also influence *FGF21* gene expression. FGF21 increases to overcome the insulin resistance induced by prolonged fasting, but also remains active during early refeeding, therefore maximizing glucose uptake [56]. Then, liver-derived FGF21 expression and release is modulated by carbohydrates through the carbohydrate response element-binding protein (ChREBP) in liver (Figure 1) [57], and ChREBP and PPAR-γ interaction in adipocytes [32, 58]. ChREBP is a central regulator of glycolysis and de novo FFAs synthesis in liver [59]. The beneficial effects on insulin sensitivity observed upon ChREBP overexpression may be due to FGF21 induction [59]. Fructose ingestion also increased circulating FGF21 levels in humans [60]. Recent report also showed metabolic benefit by FGF21 in the liver, modulating the nutrient flux through both carbohydrate [mediated by suppression of a hepatic pyruvate dehydrogenase (PD) complex through PD kinase 4 activity] and fat (mediated by deactivation of acetyl-CoA carboxylase) metabolism [61]. In humans after resting or exercising, FGF21 was regulated depending on splanchnic bed blood flow which in turn modified glucagon-to-insulin ratio stimulating splanchnic FGF21 secretion [22].

Proteins

In addition to starvation, FGF21 expression is regulated by nutritional status such as complete (fasting) or partial deprivation (50% food restriction), when nutrients are over consumed, and also depending on diet amino acid composition [62]. Food restriction causing malnutrition induced FGF21 levels in mice, however, role of the FGF21 increment is not clear since metabolic changes were similar in *Fgf21*$^{-/-}$ and *Fgf21*$^{+/+}$ models under caloric restriction [63], except for the finding that *Fgf21* knockout model were resistant to malnutrition-induced reduction of bone growth [64]. In some studies, when caloric restriction did not caused malnutrition, FGF21 was not induced neither in mouse or human subjects [63, 65]. A potential theory therefore is that FGF21 is regulated by specific macronutrients rather than caloric status. As the effect of fasting and ketogenic diet in mice showed impressive induction of liver FGF21 synthesis and secretion, proteins have been studied as FGF21 regulators. In fact, recent reports suggested that reduced protein intake was an important regulator of liver FGF21 production [66]. FGF21 promoter has amino-acid response elements (AAREs) inducing its expression [55, 67, 68]. After amino acid deprivation the general control non-derepressible 2 (GCN2)-eukaryotic initiation factor 2 (eIF2) α pathway is activated inducing binding of activating transcription factor 4 (ATF4) and the transcriptional coactivator protein of PPAR-γ PGC-1 α [45, 67, 68]. PGC-1 α also is induced with exercising. In both conditions liver FGF21 expression is increased [16]. Amino

acid composition also changes FGF21 expression. Methionine restriction in mice showed higher FGF21 expression in liver, lower fat mass and better insulin sensitivity [69, 70]. The effects of low protein diet on FGF21 induction was not overcome by carbohydrates suggesting the primarily role of protein intake rather than the reduced carbohydrate ingestion. Also, increment of FGF21 may be influenced by subsequent changes in energy expenditure and decrease in fat mass with protein restriction.

Lipids

FGF21 induces fatty acid oxidation to increase energy production by different substrates than carbohydrates, increasing ketogenesis [43, 71]. As further described below, FGF21 exogenous administration consistently causes reduction in plasma triglycerides suggesting increased metabolism induced by treatment [72, 73]. Lipids utilization is induced by FGF21 in liver and adipose tissue [74–76]. The mechanisms involve are the fatty acids production by adipose tissue which in turn are transformed in triglycerides-rich lipoproteins like very low density lipoproteins (VLDL-c) by the liver, and chylomicrons by the intestine. These particles are metabolized by lipoprotein lipase (LPL) in peripheral organs such as skeletal muscle and adipose tissue. Recent information suggests that FGF21 increase LPL activity in brown adipose tissue (BAT) to induce clearance of such high-triglycerides particles. Also, CD36, a fatty acid scavenger, is induce to improve this process. FGF21 reduced VLDL secretion by liver, increase lipid influx to BAT reducing the lipid flux to other organs, and all this through LPL and CD36 overexpression after FGF21 stimulation, lowering serum triglycerides and increasing their catabolism [77].

Oxidative stress

High carbohydrate or fat diet, obesity, and insulin resistance impairs energy balance increasing reactive oxygen species such as peroxides and free radicals that damage proteins, lipids, and DNA. Interestingly, FGF21 expression increase importantly in conditions related to high oxidative stress. FGF21 expression in heart [34, 78], and skeletal muscle [79, 80], occurs mainly and may be only after mitochondrial dysfunction, exerting cardioprotective effects attenuating heart remodeling, inflammation, and oxidative stress [81]. FGF21 has an anti-oxidant function in animal models and human metabolic diseases related to increase pro-inflammatory proteins and oxidative stress. These recent results contribute to understand the FGF21 increment in humans with obesity, the metabolic syndrome and type 2 diabetes. Furthermore, FGF21 seems to play an important role to reduced lipotoxicity and glucotoxicity to reduce cell dysfunction and apoptosis.

FGF21 expression and synthesis is induced with liver [82–84] and kidney disease [24, 85–87]. Higher FGF21 synthesis and release has been reported in patients with liver resection. Also, FGF21 is induced after lipid load and high hepatocyte intracellular lipid content is demonstrated [82, 83, 88, 89]. FGF21 correlates with genetic markers and serum proteins associated with oxidative stress. Serum markers of oxidative stress in humans are serum anti-oxidative activity, isoprostane, reactive oxygen species, malondialdehyde, and oxidized low-density lipoprotein (LDLox) [90]. Treatment with LDLox in endothelial cells caused mRNA FGF21 and protein overexpression up to 20-fold [91]. FGF21 was then correlated with the nuclear factor erythroid 2-related factor (Nrf2). Nrf2 is a transcription factor that has emerged as a key regulator of cell detoxification when oxidative stress is present [92–94]. The Nrf2 system regulates the cell baseline anti-oxidative capacity and cell response under acute oxidative stress challenge. In vitro studies of multiple human and mice cell cultures have shown increase gene expression of several proteins related to cell protection under oxidative stress challenge. Such genes are activated through Nrf2 transcription activity [95]. In contrast, a Nrf2 knock-out mice model (Nrf2-KO) caused increase cell oxidative stress, high cancer incidence and lung disease among other inflammatory conditions [96, 97]. Nrf2 have shown important functions for cell adaptation to metabolic distress too, for example, after caloric restriction or lipid metabolism with high carbohydrates and fat diet [98]. Nrf2-KO mice also have impairment in adipocyte cell differentiation, reduced adipogenesis, and resistance to diet-induced obesity [99]. Interestingly, overexpression of Nrf2 caused FGF21 gene promoter repression with FGF21 protein synthesis suppression [100]. The Nrf2-KO mice are the only animal model, until now, with increase insulin sensitivity but high FGF21 liver expression and serum levels [100]. These findings contrasted with previous results showing a FGF21 serum levels reduction after insulin sensitivity improvement [7]. A possible explanation has been related to the specific FGF21 gene promoter that has specific transcription factor response elements that are activated after oxidative stress [16]. In one side, the Nrf2-KO mice showed better metabolic profile with resistance to diet-induced obesity, in the other, Nrf2 absence eliminates oxidative-stress protection. Therefore, it is hypothesized that in absence of Nrf2, repression of FGF21 is also eliminated

in order to increase FGF21 synthesis and protect against oxidative stress [100]. The Nrf2-FGF21 loop could be a key regulation in human metabolic diseases related to oxidative stress such as insulin resistance, obesity and type 2 diabetes but also in complications such as liver steatosis or diabetic nephropathy. Recent evidence showed, for example, that FGF21 pharmacologic effect corrected diabetic nephropathy in mice, reduced albuminuria, suppress pro-inflammatory proteins with important reduction in oxidative stress markers [85, 101]. Consistently, mitochondrial damage induced by critical illness and inflammatory stress response caused overexpression of *fgf21* gene inducing higher serum FGF21 [102]. Moreover, skeletal muscle-specific deletion of ATG7 (encoding autophagy-related 7) caused mitochondrial dysfunction and increased FGF21 expression through induction of ATF4 [63]. The term "mitokine" has been proposed to refer to proteins like FGF21 that showed important over expression with mitochondrial dysfunction [63].

FGF21 role in energy expenditure

FGF21 improves insulin sensitivity through increasing translocation of glucose transporter 1 (GLUT1) to cell membrane in adipose tissue. The consequence is higher glucose uptake with an additive effect to glucose uptake through GLUT4 induced by insulin [7, 103]. In addition to this increment in insulin sensitivity, such a mechanism has also been associated with higher energy expenditure and weight loss [104]. FGF21 overexpression in transgenic mice model was resistant to weight gain after high-fat and high-carbohydrate diet-induced obesity [7]. Exogenous administration of FGF21 as pharmacological treatment reduced weight and fat mass in animal models with obesity and insulin resistance such as *ob/ob* and *db/db* mice [104]. Moreover, when FGF21 couldn't interact with its receptor in the β-Klotho knock-out mice model, overexpression of FGF21 cause less weight reduction [105]. Increased glucose and lipid metabolism with impressive weight reduction were showed in obese monkeys treated with monoclonal antibody directed to β-Klotho/FGFR1c receptor complex confirming FGF21 metabolic benefits on energy balance [106]. Higher adiponectin levels, improvement of insulin sensitivity and lipid profile with higher high-densitiy lipoproteins (HDL) and lower triglycerides was also previously reported with exogenous administration of FGF21 in diabetic monkeys [7]. The mechanism of weight reduction in these different animal models was higher energy expenditure induced by FGF21 rather than less caloric consumption or higher physical activity [104, 107].

"Browning" of WAT

The next question to be responded was how FGF21 induced increment in energy expenditure. The answer was found in BAT activation. BAT is usually present in small mammals and neonates. Then, a progressive loss of BAT in human adults results in a small proportion of BAT (50–80 g) in the organism in comparison of WAT. Human BAT involution was associated with non-significant role in adult human physiology. However, active BAT plays an important role in energy expenditure in humans burning 100 kcal/day equivalent of 5 kg of fat loss per year [108–110]. Positron emission tomography (PET) assays using [18F]-fluorodeoxyglucose revealed the presence of metabolically active adipose tissue in neck and shoulder areas [108–110]. BAT activity is higher in women, induced with cold temperature, lower in obesity, and decrease with aging [111]. Like in mice, human BAT is able to express FGF21, FGFR1c, and β-Klotho, with good correlation with mitochondrial uncoupling protein-1 (UCP1) expression [23, 112, 113]. UCP1 is a key protein to use lipids and glucose for heat production [113]. Mice models knocked-out for UCP1 expression developed obesity as well as overexpression of UCP1 cause diet-induced obesity resistance [114]. Recent studies showed that FGF21 targets BAT to increase heat production activating the oxidative phosphorylation pathway (respiration) and glucose oxidation [17]. Also, genes of those enzymes needed to improve these pathways were induced by FGF21 administration [17, 19, 107]. FGF21 also induce expression of GLUT1 in BAT [17]. After chronic stimulation with FGF21 or hypothermia, BAT developed hyperplasia and if stimulation persists, transformation of WAT to BAT was activated, the so-called "browning" process (Figure 1). There is evidence that FGF21 promotes the transformation of WAT to "beige" and then BAT, particularly in subcutaneous adipose tissue [115].

UCP1 in BAT is necessary to FGF21 energy expenditure promotion. However, when UCP1 is absent in UCP1-null mice, FGF21 decrease appetite and food intake resulting in weight loss as well [61, 116]. Interestingly, UCP1-null mice showed impressive increment in FGF21 levels with very high expression in BAT and WAT [61, 117]. Therefore, FGF21 is an important compensatory mechanism when UCP1 mechanism is diminished, and anti-obesity effect do not necessarily involve the generation of brown adipocytes in WAT and UCP1 activity in mice [116]. Actually, a diet-induced obesity model treated with FGF21-analog showed

that significant "browning" of WAT by FGF21 is indeed temperature dependent present only in a cold (21 °C) environment [116]. Under 30 °C temperature, mice also reduce weight and improve glucose metabolism after FGF21-analog treatment but without increasing BAT and UCP1 expression, suggesting other mechanisms different to UCP1 for energy expenditure induced by FGF21 [118]. Consistent with this finding, novel mechanisms were described such as, first, expression of the mitochondrial gene, *Ppargc1* that was increased [116], second, increment of the exercise-induced myokines irisin and FGF21 under cold exposure, which in turn increased shivering, thermogenesis, and browning of fat [119]; third, mitochondrial dysfunction because of skeletal muscle–specific deletion of ATG7 that decreased fat mass, accompanied by increased fatty acid oxidation and browning of WAT [63]; fourth, FGF21 induction of PGC-1-α which exerts strong effect on "browning" of WAT [19, 107]. Then, this "new" BAT becomes the target of FGF21 and also a site of FGF21 synthesis and secretion [18, 100]. Further mechanisms proposed are through hypothalamic adrenergic activation by FGF21 to induce BAT thermogenesis activating adenosine monophosphate (AMP) kinase (AMPK) and Sirt-1 (sirtuin protein 1) pathways [58, 120–123]. Patients with pheochromocytoma and very high adrenergic action occasioned by high catecholamine release by tumor showed higher proportion of beige cells in omental WAT with higher expression of FGF21 and UCP1 [23]. Finally, browning of WAT by FGF21 may be related to perilipin 5 (PLIN5). PLIN5 is highly expressed in oxidative tissues and skeletal muscle, in order to increase energy metabolism. Overexpression of PLIN5 increased "browning" factors in adipose tissue through 80-fold higher FGF21 gene expression in muscle with the subsequent increase in serum FGF21 concentration [124].

FGF21 and adiponectin

Initially, mice treated with exogenous FGF21 showed higher adiponectin serum levels [7] and such increment was confirmed in further studies done in monkeys [125]. FGF21 acts in adipose tissue with a key function in glucose and lipid metabolism. First, increase GLUT1 translocation to rise glucose uptake [7]. In addition, FGF21 induces PPAR-γ activation which in turn increased adiponectin expression, particularly after high-fat diet [76, 126]. Adiponectin is a key adipokine consider today the mediator of the favorable metabolic actions of FGF21 [76, 127]. This was confirmed in lipodystrophic mice who showed lack of FGF21 metabolic effects as adiponectin expression is absent [123, 128, 129]. Importantly, FGF21 regulated adiponectin secretion in adipose tissue, but adiponectin does not regulate FGF21 secretion.

FGF21 to combat obesity in humans

Humans with obesity, metabolic syndrome, and type 2 diabetes have higher levels of serum circulating FGF21 levels [12, 112, 130, 131]. This paradox may reflect a FGF21 compensatory response in humans to metabolic disruptions induced mainly by weight increment such as hyperinsulinemia, higher levels of FFAs, increased oxidative stress, and a lower amount of BAT. Unfortunately, this FGF21 elevation in patients with obesity or type 2 diabetes suggests low benefit of exogenous therapeutic FGF21 administration. Nevertheless, FGF21 actions in glucose and lipids metabolism as well as its effects in BAT with potential energy expenditure justifies an opportunity as novel treatment for human metabolic diseases. Multiple different engineering approaches have successfully improved manufacturing of a FGF21 with higher plasma half-life, stability and solubility [132, 133]. PEGylated FGF21 [134, 135], FGF21-antibody conjugates [136], and antibody-based activation of the FGFR/β-Klotho complex [137] are under study. Also, the FGF21 analogs LY2405319 and PF05231023 were tested in two different pilot studies in humans [138, 139]. LY2405319 was administered subcutaneously once daily for 4 weeks in patients with diabetes and obesity. PF-05231023 was administered intravenously in patients with type 2 diabetes. Both drugs showed improvement of glucose levels, lipid profile and weight loss [138, 139]. Administration of PF-05231023 to obese cynomolgus monkeys or in a placebo-controlled, clinical trial in humans with type 2 diabetes showed that ascending doses caused significant decrease in body weight, improved lipoprotein profile, and increased adiponectin level but there were no significant changes in hyperglycemia control [140]. Also, an increased concentrations of bone resorption markers and insulin-like growth factor 1 (IGF-1) was reported. Other FGF21 analogs with potential therapeutic utility are under study, however, development status is unclear [72, 73, 141].

Integrative view of the FGF21 regulation and actions

Initial impression with FGF21 studies in different animal models suggested it main role in glucose uptake at

adipose tissue [7]. Then, important energy balance regulation was shown by FGF21 in mice under different conditions such as hypothermia or starvation [41, 43]. Further actions have emerged to date, associating FGF21 as an integrative hormone for multiple actions in mammals' organism, such as a regulator of insulin, glucagon, leptin, adiponectin, growth hormone, IGF-1, and most recently glucagon-like peptide 1 (GLP1) [142] and their metabolic processes. Recent studies seem to integrate FGF21 actions in the regulation of mitochondrial function which is interesting as it explains multiple if not all the associations between FGF21 and weight, glucose and lipid metabolism, and heat production involving actions in BAT activity as well as human resting or exercising. Also, when these metabolic mechanisms are disrupted by obesity, insulin resistance or type 2 diabetes among others, FGF21 seems to compensate in humans by increasing its circulating levels against oxidative stress. Diseases with higher oxidative stress which are characterized by mitochondrial dysfunction affect FGF21 expression. The clinical translation is that FGF21 is associated with many biochemical, clinical, and tissue parameters associated with energy balance such as the ones summarized here in this review. Today, FGF21 should be seen as a key energy balance regulator acting at a cell mitochondrial function level.

Highlights

- The FGF21-β-Klotho pathway switches to oxidative metabolism during fasting and starvation. Currently FGF21 is considered a link between nutrition, heat production, and energy expenditure with human fat mass, body weight, glucose and lipid metabolism.
- FGF21 improves glucose and lipids metabolism as well as increases energy expenditure in animal models and humans.
- The action of FGF21 is at central and systemic levels, modifying human physiology from brain to periphery at adipose tissue, liver, and skeletal muscle.
- Conditions that induce human physical stress such as exercise, lactation, obesity, insulin resistance, and type 2 diabetes influence in FGF21 circulating levels and action.
- FGF21 has an anti-oxidant function in animal models and human metabolic diseases which contribute to understanding of FGF21 compensatory increment in humans with obesity, the metabolic syndrome, and type 2 diabetes.
- FGF21 has a key role in human metabolism inducing "browning" of WAT, increasing BAT activity and heat production.
- As a FGF21 response to different human stressors such as starvation, nutrient excess, autophagy deficiency, mitochondrial stress, exercise, and cold exposure, it has been proposed as a "mitokine" and as a "stress" hormone.
- Clinical evaluation of the therapeutic action of exogenous FGF21 administration as novel molecules, analogs, or through antibody-based activation of the FGFR/β-Klotho complex is warranted, particularly to treat diabetes and obesity.

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