Fibroblast Growth Factor 21—Metabolic Role in Mice and Men

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ABSTRACT Since its identification in 2000, the interest of scientists in the hepatokine fibroblast growth factor (FGF) 21 has tremendously grown, and still remains high, due to a wealth of very robust data documenting this factor's favorable effects on glucose and lipid metabolism in mice. For more than ten years now, intense *in vivo* and *ex vivo* experimentation addressed the physiological functions of FGF21 in humans as well as its pathophysiological role and pharmacological effects in human metabolic disease. This work produced a comprehensive collection of data revealing overlaps in FGF21 expression and function but also significant differences between mice and humans that have to be considered before translation from bench to bedside can be successful. This review summarizes what is known about FGF21 in mice and humans with a special focus on this factor's role in glucose and lipid metabolism and in metabolic diseases, such as obesity and type 2 diabetes mellitus. We highlight the discrepancies between mice and humans and try to decipher their underlying reasons. ***(Endocrine Reviews 38: 468 – 488, 2017)***

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With a prevalence of ~8% in adults, type 2 diabetes mellitus (T2D) is the most prominent metabolic disease worldwide (World Health Organization fact sheet on diabetes at http://www.who.int/mediacentre/factsheets/fs312/en). The hallmark of the disease, that is, hyperglycemia, results from insulin resistance of liver, skeletal muscle, adipose tissue, and brain and a concomitant failure of pancreatic β-cells to compensate for this resistance by increased insulin secretion (1). Current therapeutic options aim at reducing insulin resistance (metformin, thiazolidinediones), enhancing insulin secretion (sulfonylureas, meglitinides, dipeptidyl peptidase IV inhibitors, incretin mimetics), or excreting glucose via the urine (sodium/glucose cotransporter 2 inhibitors), but a progressive loss of β-cell function and mass is often observed (2). Eventually, the *ultima ratio* to normalize blood glucose is the replacement of endogenous insulin by injection of human recombinant insulin or insulin analogs. New insights into the molecular pathomechanisms behind insulin resistance and β-cell failure point toward a crucial role for a well-balanced humoral crosstalk between metabolically relevant tissues (*e.g.*, adipose, muscle, liver). Highly relevant players and promising targets for novel therapeutic strategies in this crosstalk were recently identified, among them fibroblast growth factor (FGF)21 (3–5). FGF21 is an endocrine factor secreted by liver acting as a metabolic regulator. Interest in the metabolic effects of FGF21 was aroused by the emergence of FGF21 as hit in an *in vitro* assay testing secreted proteins of unknown function for stimulation of glucose uptake in murine 3T3-L1 adipocytes in an insulin-independent manner (6). Based mainly on preclinical studies in mice showing improvements in glucose and lipid metabolism, FGF21 emerged as an interesting new candidate for T2D treatment,

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ESSENTIAL POINTS

- Fibroblast growth factor (FGF)21 is a liver-derived circulating hormone (hepatokine) with very robust favorable effects on glucose and lipid metabolism in mice
- First clinical trials with FGF21 analogs in type 2 diabetic patients revealed improvements in plasma lipids, but also an unexpected lack of glucose-lowering efficacy
- Differences between mice and humans in the tissue expression and physiological functions of FGF21 may underlie unexpected clinical findings
- A better understanding of the role of FGF21 in human physiology and pathophysiology will facilitate the translation of experimental findings from bench to bedside
- In both mice and humans, FGF21 exerts adverse effects on bone mass and density, and this has to be taken into account in the development of FGF21-based therapeutics

and several pharmaceutical companies made huge efforts to put this idea into practice. However, the glucose-lowering potential of FGF21 in humans has been questioned.

Molecular Biology of FGF21 in Mice and Humans

FGF21 gene and gene products

In 2000, murine and human FGF21 were identified and characterized at the cDNA level by Nishimura *et al.* (7). The murine gene is located on chromosome 7, consists of three coding exons, and gives rise to a single transcript that encodes a preprotein of 210 amino acids (aa), including an N-terminal signal peptide of 28 aa. The secreted form has an apparent molecular mass of ~23 kDa (182 aa). The human orthologous gene resides on chromosome 19, likewise consists of three coding exons (and one noncoding 5′-flanking exon), and gives rise to two transcripts due to usage of alternative promoters. Both transcripts encode the same preprotein of 209 aa, including a signal peptide of 28 aa. Human FGF21 shares 146 aa with the murine ortholog (79% identity) and the secreted form has an apparent molecular mass of ~23 kDa (181 aa).

FGF21 and the FGF superfamily

Depending on the species, between 19 and 27 FGF genes were identified in vertebrates (8). In mice and humans 22 genes (FGF1 to FGF23) exist, which can be phylogenetically grouped into eight subfamilies (8). Functionally, FGFs may be grouped into three subfamilies: intracellular FGFs (FGF11 to FGF14) that lack a signal peptide, FGFs [FGF19 (murine ortholog: FGF15) and FGF21] with low heparin-/heparan sulfate–binding capacities indicating release into circulation and systemic action, and the remaining FGFs with high heparin-/heparan sulfate–binding capacities, thus potentially mainly acting in an autocrine/paracrine manner (9).

FGF21 receptors and their signaling

At target sites, FGF21 binds and activates members of the FGF receptor (FGFR) family of receptor tyrosine kinases. Mammals have seven primary FGFR isoforms (1b, 1c, 2b, 2c, 3b, 3c, 4) (10). FGFR activation by FGF21 *in vitro* and *in vivo* is crucially dependent on β-Klotho (KLB), an FGFR-binding single-pass transmembrane protein (11–15). Accordingly, a clear preference of FGF21 for FGFR1c/KLB and FGFR3c/KLB complexes has been demonstrated (13, 16). Activation of these complexes by FGF21 leads to a plethora of rapid signaling events [for a review of general FGF signaling, see Ornitz and Itoh (17)]. Among these, the hitherto best described events are phosphorylation of FGFR substrate 2α and subsequent activation of the mitogen-activated protein kinase cascade, including Raf-1 and extracellular signal–regulated kinases (ERKs) 1 and 2 (6, 11, 18–21). Interaction of FGF21 with FGFR4/KLB is very weak and does not induce ERK phosphorylation (10, 22).

Tissue specificity of FGF21 and FGF21 receptor gene expression

In the initial study discovering FGF21, Nishimura *et al.* (7) reported predominant expression of the murine FGF21 gene in liver and lower messenger RNA (mRNA) levels also in thymus. This preliminary view has been modified and several studies have reported, although too a much lower extent, expression of FGF21 mRNA also in pancreas, testes, gastrointestinal tract, brain, skeletal muscle, brown adipose tissue (BAT), and white adipose tissue (WAT) (23, 24). In humans, the FGF21 gene under basal conditions is considered to be nearly exclusively expressed in liver, and weaker signals were shown in the brain (24) and, according to unpublished results, in the pancreas (25) (Fig. 1).

Because FGFR1 and FGFR3 are rather ubiquitously expressed in mice and humans, the target organ selectivity of FGF21 is probably determined by the restricted tissue expression of KLB: in keeping with a previous report by Ito *et al.* (26), large-scale RNA sequencing data reveal that the murine KLB gene is predominantly expressed in liver, pancreas, and

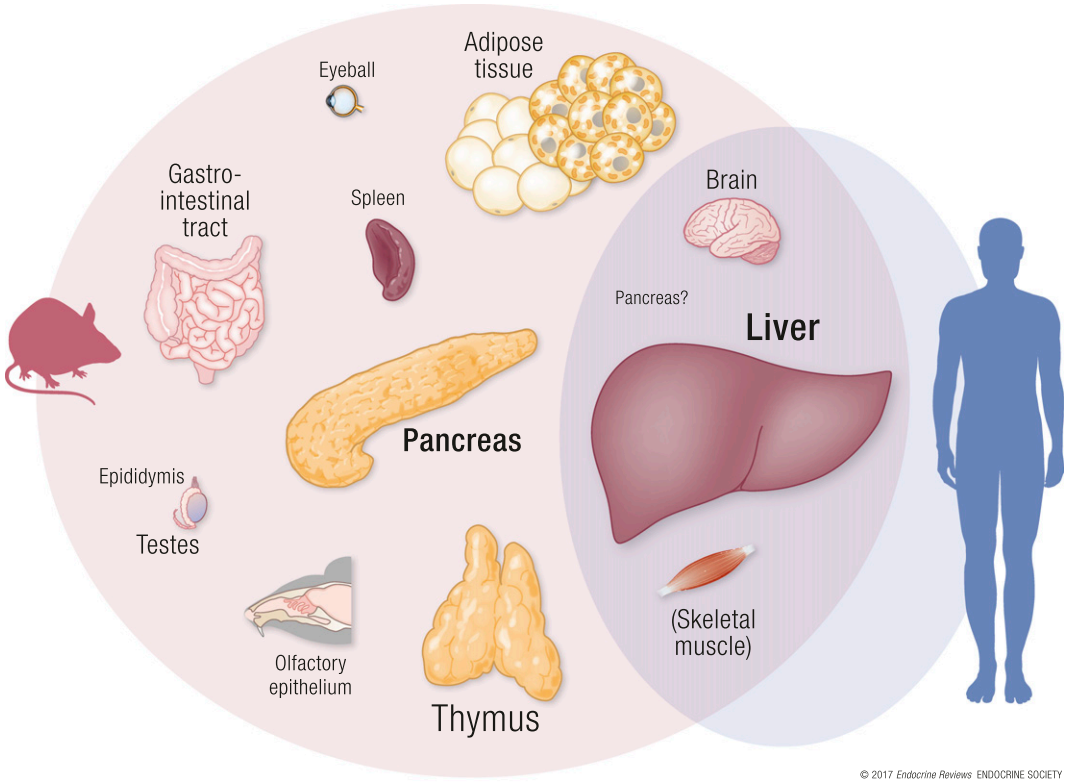

Figure 1. Comparison of major anatomical expression sites of FGF21 in mice and humans. The most prominent expression sites are highlighted by using bold fonts. The intensity of expression is indicated by font/image size. Note that skeletal muscle FGF21 expression signals were found in mice already in the basal state, in humans only upon hyperinsulinemia. Figure produced with graphics from Servier Medical Art (smart.servier.com) under Creative Commons BY 3.0 license.

adipose tissue (no KLB expression in muscle) (24). In humans, major KLB expression sites are, beyond liver and adipose tissue, breast and bone marrow. Only weak expression signals are observed in human pancreas (24). It is conceivable that differences at the KLB expression level between mice and humans may contribute to some of the divergent findings regarding the metabolic actions of FGF21 described for the two species.

FGF21 in the circulation

Serum FGF21 concentrations measured in chow-fed mice range from 0.1 to 1 ng/mL (19, 27–29) depending on the strain tested, the age of the mice, and the assay used. Serum concentrations of healthy humans exhibit marked interindividual variation ranging from 5 pg/mL to 5 ng/mL (30–34).

As to the stability of FGF21 in blood, only limited information is available for the half-life of human recombinant (nonglycosylated) FGF21, with slightly deviating data ranging from 20 min to 2 hours in different mice strains, rats, and cynomolgus macaques (18, 35–37). Thus, a half-life of <2 hours can be assumed for human recombinant FGF21. The *in vivo* stability of endogenous glycosylated FGF21 in animals and humans is currently unknown. However, as glycosylation generally confers relative resistance to proteolysis, we anticipate that the physiologically occurring FGF21 form has an appreciably longer half-life in the circulation compared with the bacterially produced recombinant protein.

Recently, Zhen *et al.* (38) characterized major cleavage events during proteolytic inactivation of human FGF21 in the circulation: these include two N-terminal cleavage events after proline residues 2 and 4 catalyzed by dipeptidyl peptidase IV and/or fibroblast activation protein (FAP) and one C-terminal cleavage event after proline 171 probably catalyzed by FAP (39). As the C terminus is important for KLB binding and overall activity of FGF21 (40, 41), inhibitors targeting FAP may increase full-length, active FGF21 blood concentrations. This, however, remains to be tested.

Regulation of FGF21 in Mice and Humans

In humans, FGF21 is considered to be nearly exclusively produced by liver, and data from liver-specific knockout animals suggest that circulating FGF21 in mice mainly derives from liver (42). FGF21 is, however, also expressed in several key metabolic tissues (see "Tissue specificity of FGF21 and FGF21 receptor gene expression"), and certain physiological stimuli and pathological conditions provoke considerable increments in these extrahepatic expression sites that may influence this hormone's circulating levels. Additionally, there may be metabolically relevant roles of locally produced FGF21, for example, in pancreas, brain, muscle, or adipose tissue (43, 44).

Nutritional regulation

Nutrient deprivation/fasting, lipid intake via suckling, and consumption of ketogenic diets (*i.e.*, a high-fat low-carbohydrate diets designed to simulate the fasting state) result in a several-fold raise of FGF21 serum levels in mice that seems to be a result of increased blood concentrations of free fatty acids (FFAs) that activated peroxisome proliferator-activated receptor (PPAR)α–dependent FGF21 gene induction in liver (29, 45–47). Contrasting the FGF21 induction by FFAs in mice, elevation of plasma FFAs in humans does not increase, but rather decreases circulating FGF21 concentrations, as shown in two larger studies of healthy volunteers during a lipid tolerance test (48, 49). Furthermore, humans demonstrate a huge interindividual range of FGF21 levels, and the effects of fasting (up to 48 hours) on FGF21 blood concentrations are not consistent, either showing no effect, only modestly increased levels, or even a drop in FGF21 levels (30, 31, 33, 50). One explanation for this discrepancy between mice and humans might be the overall higher metabolism of mice as compared with humans. Accordingly, elevations of FGF21 are only seen in humans after prolonged fasting periods of at least 7 days (30, 31). In agreement, mice fasted for 8 hours demonstrate no difference in FGF21 expression (29). Therefore, the physiological role for human FGF21 in adaptation to starving is still under debate.

Although the contribution to circulating FGF21 levels is questionable, starvation regulates FGF21 expression in extrahepatic tissues: gene and protein expressions of FGF21 in the pancreas are reduced upon fasting in mice (51). Pancreatic FGF21 expression, however, supposedly does not contribute to circulating levels. A recent murine study rather suggested that pancreatic FGF21 acts in an autocrine/paracrine manner as a pancreatic secretagogue to prevent endoplasmic reticulum (ER) stress/protein overload (43). Human data are still missing. Muise *et al.* (52) demonstrated that fasting and a high-fat diet (HFD), probably via FFA-mediated PPARγ activation, induce the FGF21 gene in murine adipose tissue. Although not a predominant expression site, FGF21 is upregulated in skeletal muscle upon fasting (53). Jiang *et al.* (54) investigated the role of FGF21 in testes and found that, in contrast to liver, testicular FGF21 expression is not regulated by fasting. Regarding thymic FGF21 (major expression site in mice), a recent mouse study demonstrated that the age-related decline in thymic FGF21 expression could be restored by caloric restriction (55).

Sugar ingestion (high-carbohydrate diets), in particular fructose, acutely provokes changes in FGF21 blood levels and hepatic expression in mice and humans (56–60). Within 2 hours after fructose ingestion, FGF21 concentrations raise 3.4-fold in humans (60), and after 1 hour in mice a twofold increase of FGF21 has been reported, which seems to be dependent on ChREBP (58, 59). Glucose led only to a modest and delayed increase in FGF21 levels in humans (60). The robust effect of fructose on FGF21 levels in mice and humans is intriguing. Together with mouse data showing FGF21-induced suppression of sugar ingestion (59) and that a preference for sweets induced by FGF21 is dependent on KLB expression in the brain (61), the findings provide evidence for a novel negative feedback loop along the liver–brain axis that regulates sugar consumption. A similar mechanism by which FGF21 acts on the reward system in the brain to regulate food intake has now been indicated in humans (62).

A ketogenic diet (KD) strongly induces FGF21 in liver and increases its circulating levels in mice (29). In humans (healthy and obese/diabetic), a KD does not increase FGF21 serum levels (30, 33, 50) and even decreases its levels in obese patients when a KD was combined with low calorie intake (63). The observation that upon long-term fasting, ketone bodies appear in the circulation days before FGF21 levels begin to rise (30) additionally argues against a regulating role for FGF21 in ketogenesis in humans. One has to consider, however, that KDs used for mouse studies have lower protein content than do control (chow) diets (9.5% vs 23.5% wt/wt) (29, 64–66), whereas human KDs are usually well controlled for adequate protein content and sometimes are even combined with high protein intake (63). Accordingly, supplementation of the murine KD with methionine almost completely prevented the KD-induced FGF21 induction (67). Several studies demonstrated that hepatic FGF21 production is robustly induced by amino acid deprivation and protein restriction, both of which are mediated by the eukaryotic translation initiation factor (eIF)2α– activating transcription factor (ATF)4–CHOP axis of the ER stress response in mice (66, 68–71). Just as in mice, FGF21 blood levels increase in humans in response to dietary protein restriction (1.7-fold after 4 weeks (66) and approximately twofold after 6 weeks) (72). Although the human FGF21 gene was identified as a target for ATF4 in cell culture experiments (71), the involvement of ER stress involving the eIF2α–ATF4 pathway *in vivo* has not yet been addressed in humans. Recently, with a nutritional modeling platform using data of mice that were fed with one of 25 diets varying in protein, carbohydrate, fat, and total energy density, a major role for low protein intake driving FGF21 expression/secretion has been demonstrated with a maximal FGF21 induction when low protein content was coupled to high carbohydrates (57). It appears that FGF21 levels and its metabolic actions are strongly coupled to the macronutrient composition of the mouse diet, an observation that should be considered in human studies where diet cannot be controlled as tight as in murine studies.

"Exercise promotes hepatic FGF-21 production probably via adipose tissue lipolysis and subsequent fatty acid signaling and ER stress in liver."

Exercise

Interestingly, Kim *et al.* (73) reported that acute exercise elevates FGF21 blood levels in mice and humans, and this was associated with a rise in circulating FFAs and enhanced hepatic expression of FGF21, PPARα, and ATF4, but not with altered FGF21 gene expression in skeletal muscle or adipose tissue. Thus, exercise promotes hepatic FGF21 production probably via adipose tissue lipolysis and subsequent fatty acid signaling and ER stress in liver. In humans, different exercise regimens stimulate FGF21 production in liver and increase blood FGF21 (74–77). Hansen *et al.* (76, 77) demonstrated that circulating glucagon that rises during exercise enhances hepatic FGF21 production in humans, indicating a muscle–pancreas–liver axis being responsible for elevated FGF21 blood levels upon exercise.

Hormonal regulation

As mentioned previously, hepatic FGF21 expression and circulating FGF21 levels are increased by glucagon (via adenosine 5′-monophosphate–activated protein kinase and PPARα) in mice and humans (78, 79). Additionally, insulin moderately increases the FGF21 blood concentration in mice under hyperinsulinemic/euglycemic clamp technique (80–83). In agreement, human skeletal muscle does express appreciable amounts of FGF21 under hyperinsulinemia (82–84). This increased expression, however, does not significantly contribute to circulating FGF21 levels (82, 84). Interestingly, human data indicate suppression of FGF21 secretion from liver by insulin, which contrasts mouse data showing no difference in FGF21 levels in liver-specific insulin receptor knockout mice (85). Growth hormone (GH) acutely increases FGF21 serum levels (2.5-fold after 2 hours, 10-fold peak at 6 hours) in mice, but this seems to be dependent on adipose lipolysis (86). In healthy humans, GH had no acute effect on serum FGF21 levels (after 3 hours) (87). In mice, additional hormonal stimuli of hepatic FGF21 expression include thyroid hormones (via thyroid hormone receptor β, retinoid X receptor, and PPARα) (88, 89) and glucocorticoids (via glucocorticoid receptor) (90). The effect of these hormones on FGF21 levels in humans has not been addressed in detail so far. No difference in FGF21 in hyperthyroidism or after treatment with the liver-selective thyroid hormone analog eprotirome argues against regulation of FGF21 levels by thyroid hormones in humans (91).

Circadian rhythm

A circadian rhythmicity of circulating FGF21 with high levels during the fasting state and low levels during feeding has been reported for both mice and men (92, 93). Andersen *et al.* (94) reported circadian rhythmicity of human FGF21 blood levels during a 72-hour fast with peak levels at 02:30 AM and nadirs at 08:30 AM. As there are three circadian-responsive elements (E-box, D-box, and a retinoic acid receptor–related receptor-response element site) that are hallmarks of a classical circadian-regulated gene and are located in the FGF21 promotor (93, 95, 96), direct control of FGF21 levels by the core clock machinery is possible. In primary murine hepatocytes, insulin induced circadian output protein (*i.e.*, E4-binding protein 4), which is a repressor of the FGF21 promoter (92). Nevertheless, circadian FGF21 expression in murine liver seems to be dependent on PPARα in mice (93), and in humans oscillating FFA levels match those of FGF21 (97). Thus, insulin and FFAs are physiological signals that may explain the circadian rhythmicity of circulating FGF21 with high levels during the fasting state (during the night) and low levels during feeding (92, 93, 97).

Cold exposure

In BAT and WAT, but not in liver, cold exposure and adrenergic signaling potently induce FGF21 gene expression in mice and humans (98–102). The pathway of cold-induced FGF21 induction includes cyclic adenosine monophosphate (cAMP), protein kinase A, p38 mitogen–activated protein kinase, and ATF2 (100). Notably, some reports show an increase in serum FGF21 levels indicating that under certain conditions adipose tissue may contribute to circulating levels in mice and humans (100, 101). No direct mouse and human data are available, but in rats a difference in arteriovenous concentrations of plasma FGF21 across interscapular BAT has been demonstrated, further arguing for BAT as a source of circulating FGF21 (100). In agreement, BAT of cold-exposed uncoupling protein (UCP)1 knockout mice is the source of circulating FGF21 (103), and thus at least in rodents, adipose tissue might contribute to circulating levels under distinct conditions.

PPAR agonists

Treatment with PPARγ agonists (thiazolidinediones), clinically used as insulin sensitizers, does not alter human FGF21 blood levels in humans (50, 80, 104). In agreement, PPARγ agonist–treated mice have increased FGF21 protein levels in WAT but no elevated circulating levels (27). Treatment with PPARα-activating fibrates increases human FGF21 blood levels, pointing to a role of this FFA-dependent transcription factor in FGF21 gene induction reminiscent of that in mice, at least in this pharmacological setting (30, 50, 104, 105). Although selective PPARγ agonists induce FGF21 in adipose tissue but not in liver, selective PPARα agonists do this in liver but not in adipose tissue (27, 52), reflecting the tissue specificity of these PPARs. Although one mouse study demonstrates that PPARγ agonist treatment results in FGF21 secretion of WAT concomitantly with elevations in plasma FGF21 (52), future studies are needed to explore whether and under which condition WAT is

contributing to circulating levels in particular in humans. It is certain that WAT-FGF21 acts locally as an autocrine/paracrine factor in mice and humans.

In summary (Fig. 2), the main expression and secretion site in mice and humans is the liver. Besides the previously-mentioned stimuli, hepatic FGF21 expression is regulated by bile acids (via farnesoid X receptor) (108) and dietary supplements/drugs such as all-*trans* retinoic acid (via retinoic acid receptor β) (109), α-lipoic acid (via cAMP response element binding protein H) (110), and resveratrol (via SIRT1) (111, 112). This list demonstrates that hepatic FGF21 expression in mice and humans is under complex nutritional and hormonal control and is regulated by multiple transcription factors in a combinatorial way with a network of nuclear receptors being of central importance. As to the regulation of circulating FGF21, mice and humans share the following physiological stimuli: nutrition (protein restriction, fructose ingestion), exercise (via pancreatic insulin and glucagon), and, to a lesser extent, circadian clock machinery.

FGF21 in Metabolic Disease

Several metabolic disorders are associated with increased FGF21 levels in mice and humans. In the following section, we discuss metabolic diseases that demonstrate altered FGF21 levels with a special focus on human data. The reason for increased serum FGF21 in these pathological conditions is largely unknown (potential FGF21 resistance will be discussed at the end of the section).

Obesity

Genetic and diet-induced murine models of obesity, such as *ob*/*ob* and HFD-fed C57BL/6 mice, display several-fold increased serum FGF21 concentrations (up to 3 ng/mL) that are accompanied by increased FGF21 gene expression in liver and to a lesser extent in WAT (19, 113). In humans, FGF21 blood concentrations positively associate with body mass index (BMI) and whole-body, visceral, pericardial, and epicardial fat mass and are elevated in the obese state (32, 33, 81, 104, 114–118). Overfeeding-induced gain of weight and body fat result in elevated human FGF21 concentrations (119, 120). In contrast, acute and pronounced weight and body fat losses due to bariatric (Roux-en-Y gastric bypass) surgery do not lead to reductions in circulating FGF21 (121, 122), showing that adipose tissue is not a source of circulating FGF21 in humans.

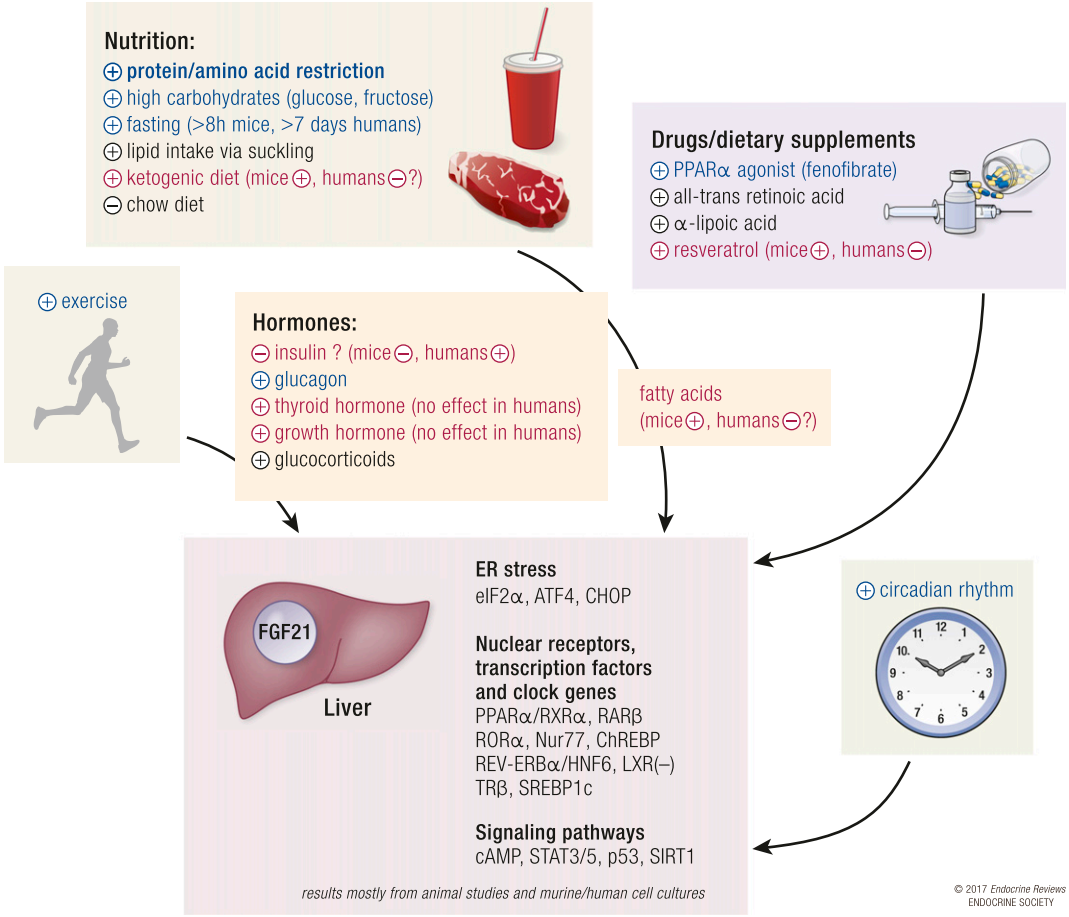

Figure 2. Regulation of hepatic FGF21 production in mice and humans. Data on signaling and nuclear/transcriptional factors [see reviews in Adams and Kharitonenkov (106) and Bae *et al.* (107)] are mainly derived from genetic mouse studies and cell culture systems. Stimulatory effects are indicated by plus signs (+), the inhibitory effects by minus signs (−). Stimuli/mediators in mice and humans are indicated in blue, stimuli/mediators that are different between mice and humans are indicated in red, and stimuli/mediators with only mice data available are indicated in black. Figure produced with graphics from Servier Medical Art (smart.servier.com) under Creative Commons BY 3.0 license.

Metabolically unhealthy obesity

Common obesity can be dissociated into two subtypes: metabolically healthy obesity without serious metabolic complications (~20% to 40%), with the remainder being metabolically unhealthy obesity (MUHO) characterized by insulin resistance, increased visceral fat mass, ectopic fat deposition in skeletal muscle and liver, inflamed adipose tissue and liver, and increased intima-media thickness of the carotid artery (123–126). Recently, Berti *et al.* (127) demonstrated that FGF21 blood levels are more than twofold higher in MUHO as compared with body fat–matched metabolically healthy obese subjects, and the authors suggested that this reflects an adiposity-independent role of FGF21 in the metabolic derangements of MUHO. This is in agreement with studies, for example, reporting BMI- and body fat mass–independent positive associations of FGF21 levels with insulin resistance (128, 129).

Mouse models of obesity-associated diseases, such as nonalcoholic fatty liver disease, chronic hyperglycemia, and atherosclerosis, consistently reveal increased FGF21 blood levels (130–133). Also in humans, FGF21 levels are increased in several obesity-associated disorders and the metabolic syndrome (32, 115, 134, 135).

Fatty liver and diabetes

The strongest BMI-independent determinant of hepatic FGF21 production and circulating FGF21 concentrations is liver fat content (136–139), and fatty liver is a hallmark of MUHO (123). Accordingly, FGF21 blood concentrations are consistently elevated in patients with nonalcoholic fatty liver disease and steatohepatitis (33, 136–138, 140–142). With respect to the prominent role of fatty liver in the pathogenesis of T2D [for review, see Stefan *et al.* (143)], it is not unexpected that FGF21 blood levels are increased in prediabetic dysglycemia (34, 144–146), T2D (81, 144–149), gestational diabetes (150, 151), and diabetic retinopathy (152, 153). Additionally, in the blood of patients with (diabetic) nephropathy, elevated FGF21 concentrations were measured, which may derive from reduced glomerular filtration rates (154–159).

Lipid profile and vascular complications

Higher circulating FGF21 concentrations associate with atherogenic lipid profiles, that is, increased plasma triglycerides, total and low-density lipoprotein (LDL) cholesterol, and decreased high-density lipoprotein cholesterol (32, 34, 104, 115, 116, 139, 160–164). Among these lipids, circulating FGF21 most robustly correlates with plasma triglycerides, possibly reflecting its strong association with hepatosteatosis and steatosis-related increased very-low-density lipoprotein secretion.

The associations of elevated FGF21 blood levels with metabolic syndrome, increased intima-media thickness, arterial stiffness, and atherosclerotic plaque formation (149, 165–169), as well as with coronary artery/heart disease (160, 163, 170) and acute myocardial infarction (171), argue for a complex, that is, liver fat–dependent and –independent, relationship of this hormone with vascular complications. This is additionally strengthened by the association of FGF21 with hypertension (128, 160, 162, 164, 167, 172) and preeclampsia (173).

Bone diseases

In keeping with the inhibitory action of FGF21 on bone formation in mice (174), Hanks *et al.* (175) reported an inverse relationship between circulating FGF21 and bone mineral density measured by dual energy X-ray absorptiometry in humans. Moreover, elevated FGF21 levels are associated with reduced bone strength and worsened bone microarchitecture (decreased trabecular number and increased trabecular separation in the radius) (176) and with bone loss in knee osteoarthritis (177).

Muscle diseases

In mouse models, manipulations inducing metabolic dysregulation in muscle lead to the induction and release of FGF21 from muscle: Izumiya *et al.* (53) reported that muscle-specific transgenic Akt1 overexpression increases FGF21 expression and blood concentrations, providing evidence that muscle-derived FGF21 can be of systemic relevance. Mitochondrial myopathy, a stress situation accompanied by Akt activation, is associated with FGF21 gene induction in skeletal muscle (178). Additionally, muscle-specific autophagy knockouts (ATG7) demonstrate mitochondrial stress/ATF4–dependent increased FGF21 expression in muscle, but not in liver, WAT, or BAT, and an increase in serum FGF21 levels (179). Similar regulation of the FGF21 gene is seen in heart muscle: fasting and ER stress, with the latter resulting from intracellular triglyceride overload due to whole-body deficiency of adipose triglyceride lipase, provoke marked increases in FGF21 expression (180). Induction of muscular ER stress (evidenced by eIF2α and ATF4 activation) by ectopic expression of uncoupling protein 1 results in markedly enhanced FGF21 gene expression in muscle, but not in liver or adipose tissue, and in fivefold higher FGF21 blood levels even in the absence of myopathy (181). Likewise, mice accumulating intramyocellular triglycerides due to skeletal muscle-specific transgenic perilipin 5 overexpression also exhibit pronounced FGF21 expression in muscle and concomitantly increased circulating FGF21 concentrations (182). Also, human myopathy (mitochondrial and iron–sulfur cluster scaffold homolog) patients have higher expression of FGF21 in muscle and higher FGF21 serum levels (183), indicating some similarities between mice and humans. It further indicates that under extreme

metabolic disarrangements, muscle might contribute to circulating FGF21 levels in mice and humans.

Mitochondrial diseases

Mitochondrial diseases represent a heterogeneous group of rare genetic and acquired metabolic disorders characterized by mitochondrial dysfunction [for review, see Magner *et al.* (184)]. Several groups demonstrated markedly elevated FGF21 blood levels in mitochondrial diseases (183, 185–190). Although the molecular link is currently unclear, these findings are in line with increased FGF21 levels observed in common diseases associated with mitochondrial dysfunction, that is, insulin resistance, nonalcoholic fatty liver disease, myopathy, and T2D (191–193).

Pancreatitis

One of the major FGF21 mRNA expression sites in mice is pancreas. Pancreatic FGF21 gene expression derives from endocrine α- and β-cells as well as exocrine acinar cells (43, 194–196). In the latter, FGF21 is induced in experimental models of pancreatitis in mice (43, 195, 196). In agreement, FGF21 is elevated in serum of patients with pancreatitis (197).

Lipodystrophies

Patients with human immunodeficiency virus–associated, congenital, and acquired lipodystrophies, that is, pathologies characterized by reduced adipose tissue mass, marked ectopic lipid deposition, and insulin resistance demonstrate elevated FGF21 levels (198, 199).

FGF21 resistance

With respect to the beneficial effects of FGF21 on murine glucose and lipid metabolism, the elevated FGF21 blood concentrations observed in murine and human diseases with disturbed glucose and lipid metabolism are completely unexpected. This has prompted the hypothesis of FGF21 resistance, as it is comparable to high circulating insulin and leptin concentrations in insulin- and leptin-resistant states, respectively. Accordingly, treating diet-induced obese mice with recombinant human FGF21 leads to no significant decline in blood glucose concentrations and only a small decrease in plasma FFA concentrations together with attenuated ERK signaling and impaired induction of the FGF21 target genes early growth response-1 and c-Fos in liver and adipose tissue (19, 200). In agreement, human obesity is accompanied by elevated FGF21 levels and reduced levels of KLB in WAT (201). A recent study demonstrated impaired FGF21-induced ERK phosphorylation in WAT of obese mice, which could not be restored by maintaining KLB expression in WAT, indicating FGF21 resistance in WAT is downstream of KLB (202). Murine studies further indicate that thiazolidinediones may increase KLB expression, thus potentially enhancing FGF21 signaling (203), and recently it has been demonstrated that dietary fish oil increased hepatic FGF21 sensitivity by increasing KLB (204). However, there are also mouse studies arguing against FGF21 resistance: Hale *et al.* (113) tested HFD-fed obese and genetically obese *ob/ob* mice and found that even though WAT expression of KLB and FGFR1c are reduced, dose-response curves with recombinant human FGF21 reveal no right-shifted ERK phosphorylation in liver or adipose tissue. Moreover, these obese animals were more responsive to glucose- and weight-lowering effects of FGF21 than were lean mice (113). Whether these contradictory results derive from the different obesogenic diets or mouse strains used remains to be determined. More work is needed to prove or reject the hypothesis of FGF21 resistance in particular in human obesity and its associated metabolic complications.

In summary, any changes (physiological or pathophysiological) in metabolism (whole body or just tissue specific) are characterized by induction of FGF21 in mice and humans. Thus, FGF21 emerges as an energy (nutritional) stress–induced factor not only in liver (Fig. 2) but also in muscle, BAT/WAT, and pancreas (Fig. 3). The contribution, however, of extrahepatic expression sites (adipose tissue and muscle) to circulating levels has only been suggested for some mouse models/conditions, and direct data in humans are missing. Furthermore, it is not known how certain conditions provoke FGF21 release from the tissue into the circulation whereas others do not.

Metabolic Effects of FGF21 in Mice and Humans

Effects on whole-body glucose and lipid metabolism

Administration of (human or murine) recombinant FGF21 to nutritional (HFD-fed) or genetic (*i.e.*, *ob/ob* and *db/db*) mouse models of obesity and diabetes increases fat utilization and energy expenditure and reduces body weight, whole-body fat mass, and liver triglyceride content (6, 205, 206). Furthermore, FGF21 administration provokes resistance to HFD-induced weight gain, improves glucose tolerance and hepatic and peripheral insulin sensitivity (without triggering hypoglycemia), and normalizes hyperinsulinemia and hypertriglyceridemia (6, 205, 206). *In vivo*, FGF21-mediated glucose transporter 1 induction and glucose uptake in WAT is only seen in lean, but not in obese, mice, suggesting that the robust reduction in blood glucose concentration observed in acutely FGF21-treated obese mouse models results from the indirect suppressive effect of FGF21 on hepatic glucose output (18, 200, 206). In the apolipoprotein E–deficient mouse model of atherosclerosis, FGF21 inhibits atherosclerotic plaque formation in part by suppressing hepatic expression of the transcription

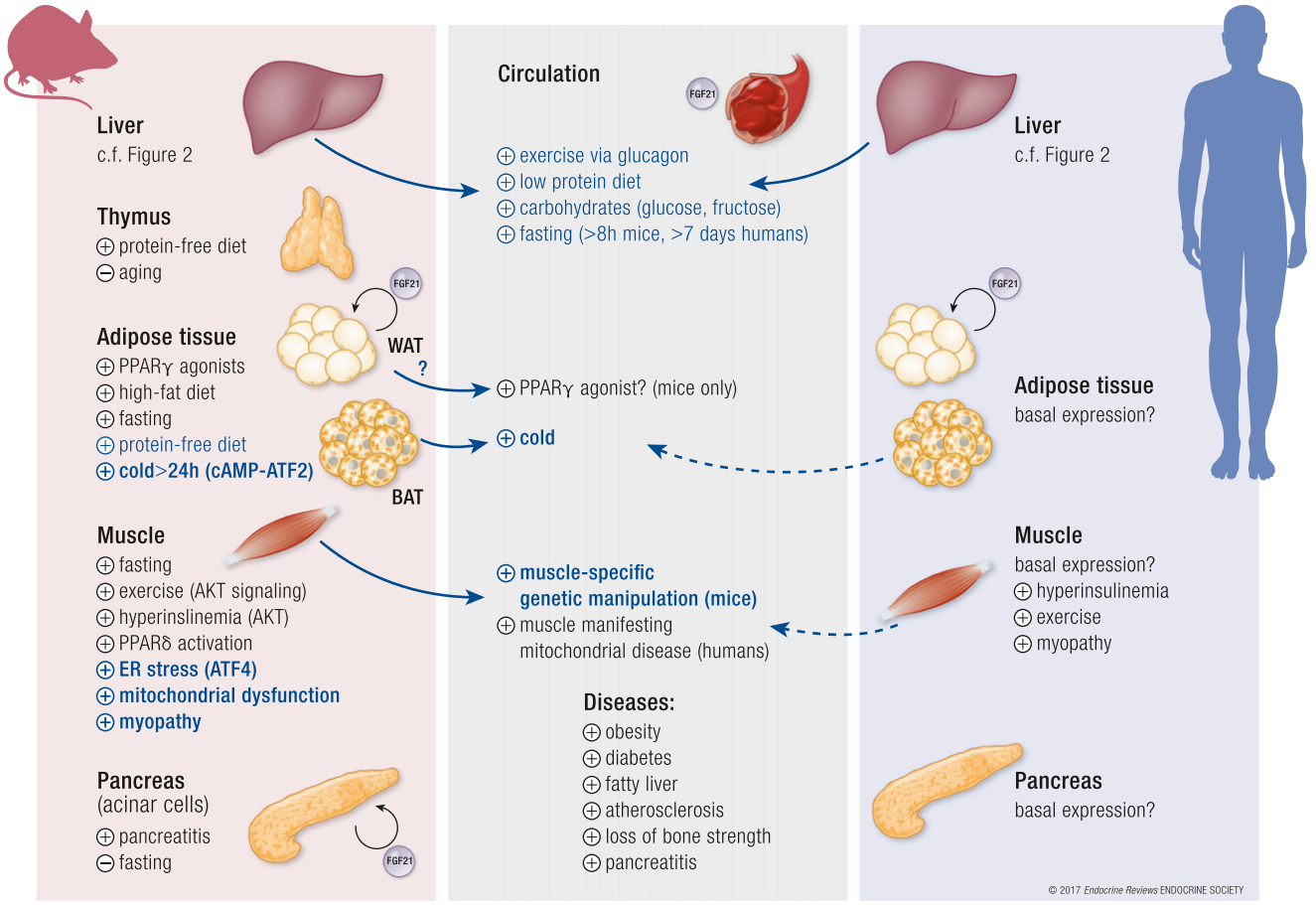

Figure 3. Regulation of extrahepatic production and circulating FGF21 in mice and humans. Stimulatory effects are indicated by plus signs (+), the inhibitory effects by a minus sign (−). Stimuli rendering extrahepatic tissues as source of circulating FGF21 levels are indicated in blue. Figure produced with graphics from Servier Medical Art (smart.servier.com) under Creative Commons BY 3.0 license.

factor sterol regulatory element binding protein 2, thereby attenuating hepatic cholesterol synthesis and improving hypercholesterolemia (133). Conversely, whole-body FGF21 deficiency due to genetic knockout promotes weight gain, hepatosteatosis, and glucose intolerance upon ketogenic diet (64). The findings in mice about improvements in lipid and glucose metabolism prompted pharmaceutical companies around the world to develop FGF21-based novel therapies for metabolic diseases, especially for T2D.

Owing to the instability of recombinant non-glycosylated FGF21 in the circulation, for humans, only results with stabilized FGF21 analogs (LY2405319 and PF-05231023) are available (207–210). LY2405319 represents a human FGF21 molecule modified by introduction of an additional disulfide bond, deletion of four N-terminal amino acids, and elimination of an *O*-linked glycosylation site (211); PF-05231023 is an artificial macromolecule formed by covalent conjugation of two modified human FGF21 molecules (desHis FGF21 Ala129Cys) to the Fab regions of a monoclonal scaffold antibody (36). LY2405319 was already tested in T2D patients in a randomized placebo-controlled double-blind proof-of-concept trial (207): 4 weeks of LY2405319 treatment reduced plasma triglycerides and total and LDL cholesterol concentrations and increased plasma high-density lipoprotein cholesterol, β-hydroxybutyrate, and serum adiponectin concentrations, but it did not reveal a significant effect on blood glucose levels. Similar effects on plasma lipids as well as a lack of effect on blood glucose were also observed for PF-05231023 after a single intravenous dose administered to T2D patients (208). Talukdar *et al.* (209) performed a 4-week randomized placebo-controlled phase 1b trial with twice-weekly administration of PF-05231023 (5 to 140 mg) in T2D patients and reported, apart from improvements in plasma lipids, significantly decreased body weight, increased blood adiponectin concentrations, reduced blood markers of bone formation, increased markers of bone resorption, elevated blood insulin-like growth factor (IGF)-1 levels, and no effect on plasma glucose upon treatment. Another study using once-weekly injection of PF-05231023 (25 to 150 mg) in obese hypertriglyceridemic subjects reported reduced TG levels and increased adiponectin levels without changes in body weight, but again no improved glucose metabolism (210). It is unclear whether the observed weight loss in the study of Talukdar *et al.* (209) is due to side effects, that is, diarrhea and nausea (seen in 29% and 26% of PF-05231023–treated patients, respectively) that may

have impaired food intake. These human studies recapitulated the beneficial effects of FGF21 on lipid metabolism found in mouse studies, but they revealed one crucial deviation: FGF21 analogs failed to lower blood glucose in humans. Note, however, that analogs [for overview of existing analogs, see Zhand and Li (212) and Degirolamo *et al.* (213)] do not represent wild-type (regular) human FGF21. Therefore, it is currently unknown whether the reported effects of analogs reflect physiological functions of the endogenous protein.

Effects on growth and lifespan

FGF21 transgenic mice are markedly smaller than their wild-type littermates (214), and FGF21 causes hepatic GH resistance by blunting GH signaling at the extracellular and intracellular level (214). Additionally, transgenic FGF21 overexpression extends lifespan of C57BL/6 mice by 36% by interfering with GH/IGF-1 signaling in liver without affecting food intake, physical activity, energy expenditure, or adenosine 5′-monophosphate–activated protein kinase, mammalian target of rapamycin, and sirtuin signaling in liver, muscle, and adipose tissue (215). Recently, it has been demonstrated that an increase in lifespan by FGF21 overexpression involves the prevention of age-induced loss of naive T cells (55). Given that these observations have been made with a mouse model that is hypermetabolic and growth-restricted due to transgenic overexpression of FGF21, an effect of FGF21 on lifespan and growth in humans is very speculative. A study investigating the association of FGF21 and growth or IGF-1 in obese children revealed no connection (118), contrasting another study demonstrating an inverse relationship of FGF21 with linear growth rate in infancy (216). Thus, FGF21 as a negative regulator of human growth has not been established, but awaits further studies.

Effects on glucose and lipid metabolism mediated by adipose tissue

As WAT has high FGFR1c and KLB expression and the FGFR1c/KLB complex is the preferred signaling complex for FGF21, adipose tissue is considered the major target of FGF21. In HFD-fed mice, adipose tissue–selective ablation of either KLB or FGFR1 impairs FGF21 effects, such as weight loss, insulin sensitization, and improvement of glucose tolerance, hyperinsulinemia, and hypertriglyceridemia (14, 217, 218). Additionally, the beneficial effects of FGF21 are absent in a mouse model of lipodystrophy but restored after WAT transplantation (219), indicating a central role for WAT in mediating FGF21 improvements on whole-body metabolism.

It has become clear that FGF21 plays a role in WAT lipolysis, but there are conflicting reports on whether FGF21 promotes or inhibits lipolysis. In particular, cell culture experiments using FGF21 treatment or gain- and loss-of-function studies in human or murine primary adipocytes or murine 3T3-L1 adipocytes demonstrated inconsistent results (220–222). *In vivo* studies suggest a difference between chronic vs acute effects: administration of a single dose of recombinant human FGF21 acutely lowered plasma FFA concentrations and WAT hormone–sensitive lipase expression in lean and *ob*/*ob* mice (19, 222). In contrast, mouse models with chronically altered FGF21 levels (knockout, overexpression, and FGF21 administration) support the lipolysis-promoting effect of FGF21 with increased expression of lipases (hormone-sensitive lipase, adipose triglyceride lipase), reduced adipocyte size, and elevated plasma FFA concentrations (45, 64, 223). This argues for a more indirect and context-dependent effect of FGF21 on WAT lipolysis, which might also explain inconsistent cell culture results using different conditions. Recent mouse data indicate that inflammation (*e.g.*, mediated by interleukin-6) might affect FGF21-mediated lipolysis (224), which is commonly observed in obesity and its associated metabolic disorders.

FGF21 acutely affects the production and release of the adipocyte-derived hormone (adipokine) adiponectin: a single FGF21 dose doubles adiponectin concentration in the blood (217). Adiponectin is an insulin-sensitizing, anti-inflammatory, and atheroprotective adipokine with a major role in glucose and lipid metabolism (225, 226). When adiponectin is genetically ablated, HFD-induced and genetic mouse models of obesity are refractory to FGF21-induced improvements in hyperglycemia, hypertriglyceridemia, hepatic and muscle insulin resistance, and hepatosteatosis (227, 228). Of note, the protective function of FGF21 on vascular inflammation and atherosclerotic plaque formation in apolipoprotein E–deficient mice is at least in part dependent on its adiponectin-elevating properties (133). In humans, administration of the FGF21 analog LY2405319 or PF-05231023 led to increased adiponectin levels in obese/diabetic patients (207, 209, 210), indicating that also human metabolism might benefit indirectly from FGF21 administration by increased adiponectin levels.

FGF21 stimulates glucose uptake in murine and human adipocytes (6), and Ge *et al.* (200) demonstrated that this is due to transcriptional activation of the glucose transporter 1 gene via ERK1/2, serum response factor, and Ets-like protein-1. FGF21 effects on glucose uptake are more pronounced in BAT: *in vivo*, injecting native FGF21 into diet-induced obese mice increased glucose uptake in WAT but to a much higher extent in BAT (229, 230), and mice having no circulating FGF21 (liver-specific FGF21 knockout mice) show reduced glucose uptake specifically in BAT, not WAT, muscle or heart (42).

Schlein *et al.* (231) reported that WAT and BAT contribute to FGF21-stimulated reductions in plasma triglyceride concentrations by enhanced

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clearance of triglyceride-rich lipoproteins in these depots. Additionally, Coskun *et al.* (205) reported that chronic FGF21 administration in obese mouse models (HFD-fed and *ob*/*ob* mice) reduces body weight and adiposity via increased energy expenditure and fat utilization, suggesting the involvement of BAT (and/or browning of WAT) in these fat mass–regulating FGF21 effects. Adipose tissue browning (conversion of white adipocytes into brown-like UCP1-positive cells) (232) depends, at least in part, on FGF21, as FGF21-deficient mice display significantly diminished browning capacity (98). Furthermore, chronic FGF21 treatment and FGF21 gene transfer to the mouse liver are accompanied by induction of thermogenic genes (*e.g.*, UCP1) and of genes favoring β-oxidation of fatty acids (*e.g.*, carnitine palmitoyltransferase-1α and -1β) in BAT (52, 205, 233). Finally, induced hepatic FGF21 production may also be involved in activation of thermogenesis during the fetal-to-neonatal transition, a critical period where newborns have to compensate for a dramatic drop in ambient temperature (47). There is uncertainty whether FGF21-induced adipose tissue browning is exclusively a direct effect on adipocytes: recent evidence, based on central KLB knockout and lateral ventricle infusion of FGF21, suggests that hypothalamic FGF21 signaling stimulating sympathetic nerve activity contributes to adipose tissue browning (234, 235). Of note, two studies support the notion that pharmacological FGF21 effects are independent of WAT browning (236, 237). Although there seems to be a connection between FGF21 and BAT in humans as well (238), the rather low capacity for browning and the low amount of BAT in humans (usually at thermoneutrality) as compared with mice (commonly housed not at thermoneutrality) might explain at least partly the differences of the effects of FGF21 on glucose metabolism between the two species.

Effects on glucose and lipid metabolism mediated by liver

Among the earliest findings about FGF21 effects in mice was the stimulation of hepatic fatty acid oxidation (via induction of PPARγ coactivator-1α and -1β) and ketogenesis (via induction of ketone body–synthesizing enzymes) in the fasting state and under fasting state–mimicking conditions (ketogenic diets) (29, 45). Additionally, FGF21 suppresses hepatic *de novo* lipogenesis (via repression of the lipogenic genes encoding sterol regulatory element–binding protein 1c and fatty acid synthase) (29, 239, 240). Thus, FGF21 transgenic mice display decreased hepatic triglyceride contents (45), and FGF21 knockout mice store fatty acids from fasting-associated adipose tissue lipolysis as triglycerides in the liver (29, 64).

Chronic FGF21 treatment led to increased Akt phosphorylation, decreased diacylglycerol concentrations, and reduced protein kinase Cε activity in liver specimens, indicating insulin sensitization at the hepatocellular level (239, 241). In agreement, FGF21 knockout mice demonstrate hepatic insulin resistance and elevated hepatic glucose production (242). Data obtained from liver-specific insulin receptor knockout mice demonstrate that FGF21 effects on glucose metabolism are not due to direct effects on liver but to activation of BAT and browning of WAT, leading to increased energy metabolism and substrate use (85). These data further suggest that insulin action in the liver is not required for FGF21 to correct hyperglycemia but to mediate its effect on lipid metabolism in diabetic mice (85).

Berglund *et al.* (206) and Xu *et al.* (18) demonstrated that chronic FGF21 treatment suppressed hepatic glucose output and enhanced hepatic glycogen storage. In contrast, transgenic FGF21 mice demonstrated enhanced gluconeogenesis already during the fed state, and acute FGF21 treatment leads to PPARγ coactivator-1α–independent induction of the key gluconeogenic enzymes glucose-6-phosphatase and phosphoenol pyruvate carboxykinase, reflecting the prominent role of FGF21 during fasting (20, 243).

However, a direct effect of FGF21 on liver *in vivo* has been questioned, as FGFR4 is the main FGF receptor isoform expressed in liver and FGF21 does not activate downstream signaling through the FGFR4/KLB complex (no phosphorylation of ERK) (10). Besides indirect effects of FGF21 on liver, another explanation for a more direct FGF21 effect on liver has recently been proposed: FGF21 antagonizes the effect of FGF15/19 on the hepatic FGFR4/KLB complex, thereby increasing the bile acid pool (244). Several studies on FGF21 effects on human liver used HepG2 cells as a model for hepatocytes, but in contrast to primary hepatocytes, HepG2 cells do express high amounts of FGFR1c, and therefore these data cannot be extrapolated into the human *in vivo* context. Nevertheless, human and murine liver do express FGFR1c, although to a much lower degree than adipose tissue, and hepatic FGFR3c expression is even higher than in adipose tissue (245). Notably, the regulating effect of FGF21 on cholesterol metabolisms has been suggested to be at least partly mediated via the FGFR2/KLB complex in the liver (133), and FGFR2 is the second most abundant FGFR isoform in liver (10).

Effects on pancreas

Pancreatic acinar and islet cells are FGF21 targets, and FGF21 treatment triggers ERK signaling in both cell types (51). Wente *et al.* (246) demonstrated that short-term FGF21 treatment of healthy C57BL/6 mice and diabetic *db/db* mice lowers blood insulin concentrations after an oral glucose load. In contrast, constant long-term infusion of FGF21 in *db/db* mice raises insulin (246). In the absence of enhanced islet cell proliferation, long-term treatment provokes increments in pancreatic islet number and insulin content per islet (246). FGF21 knockout mice display distortion of islet morphology and impaired glucose-stimulated insulin

secretion, with the latter possibly due to unblocked GH signaling in the islets (247). Moreover, HFD-fed FGF21-deficient mice develop islet hyperplasia and periductal lymphocytic inflammation (51). Notably, FGF21 has recently been discovered as a pancreatic secretagogue that mainly functions in an autocrine/paracrine manner to alleviate ER stress that can occur in pancreas under either physiological conditions such as fasting/refeeding or pathological conditions such as pancreatitis (43). Collectively, these findings point to islet-protective functions of FGF21. Interestingly, hyperglycemia in *db/db* mice and high glucose concentrations *in vitro* downregulate KLB expression and FGF21 signaling in pancreatic islets, providing preliminary evidence of hyperglycemia-induced FGF21 resistance in the pancreas of diabetic mice (248). No published *in vivo* data on FGF21 expression or direct action on human pancreas are available.

Effects on brain

Very recently, it was demonstrated that FGF21 is expressed in different murine brain regions, including substantia nigra and striatum (249), and in cerebellar neurons upon treatment with cell adhesion molecule L1 (250). FGF21 expression in the brain in mice and most importantly in humans still needs further confirmation, and so far nothing is known about potential regulatory stimuli or mechanisms. Notably, however, FGF21 is able to pass the murine blood–brain barrier (251) and this seems to be true also for humans (252). In the brain of C57BL/6 mice, FGFR1c and FGFR3c, the two major FGFRs, are broadly expressed, whereas KLB expression is restricted to the suprachiasmatic nucleus of the hypothalamus, where the circadian pacemaker is located, and the dorsal vagal complex and nodose ganglia of the hindbrain (253). FGF21 action in the murine brain via KLB increases corticosterone levels, lowers insulin levels, inhibits growth, and alters circadian rhythm, all representing features of starvation (253, 254). Additionally, central FGF21 action is needed for its effect on energy expenditure (via WAT browning), weight loss, and lowering cholesterol in mouse models (234, 235, 253, 254). Using FGF21 knockout and intracerebroventricular FGF21 injection, Liang *et al.* (254) demonstrated that acute stimulation of hepatic gluconeogenesis by FGF21, at least in part, is caused by FGF21's activation of the hypothalamic–pituitary–adrenal axis triggering adrenal corticosterone release (via ERK–cAMP response element binding protein-induced corticotropin-releasing hormone gene expression in hypothalamic neurons). Another central action of FGF21 has very recently been demonstrated in a mouse study: FGF21 suppressed consumption of simple sugars and noncaloric sweeteners, but not of complex carbohydrates, proteins, or lipids via hypothalamic neurons (59). Human genetic data (see the following section) point toward the existence of similar mechanisms in humans (62). These observations constitute a novel and multifunctional liver–brain axis with the hepatokine FGF21 as a crucial player in mice and humans. Some controversial reports about actions of FGF21 in mice and humans may be attributable to central effects, as it has not been shown for FGF21 analogs whether or how efficient they cross the blood–brain barrier. Additionally, although there are indications of KLB, FGFR1, and FGFR3 expression in human brain (*cf.* www.proteinatlas.org), restricted expression to specific areas within the hypothalamus, for instance, has not been demonstrated so far.

Effects on bone

The negative regulation of bones by FGF21 is one of the adverse effects that may jeopardize the use of FGF21 as a therapeutic (69, 174). Even though the presence of KLB in osteoblasts and/or osteoclasts remains to be proven, FGF21 has effects on bones: using transgenic overexpression and pharmacological application of FGF21, Wei *et al.* (174) reported striking decreases in bone mass together with *ex vivo* assay data demonstrating inhibition of osteoblastogenesis and stimulation of adipogenesis in bone marrow–derived mesenchymal stem cells by FGF21. In contrast, FGF21 knockout mice exhibit a high–bone mass phenotype (174) and are protected from transient loss of bone mass during lactation (255). Even though FGF21 has no direct impact on osteoclasts, it promotes IGF-binding protein-1 release from liver, and IGF-binding protein-1 stimulates bone resorption *in vivo* (256). Thus, FGF21 promotes bone loss via direct inhibition of bone formation and indirect enhancement of bone resorption. In humans, the FGF21 analog PF-05231023 leads to changes in bone biomarkers with body weight changes (209) but also without body weight changes (210). Thus, adverse effects on bones need to be carefully addressed in future human studies.

Figure 4 summarizes effects (chronic and acute) of regular, that is, wild-type, FGF21 on metabolically relevant tissues/organs in mice and humans as derived from treatment studies and, for mice, from genetic manipulation as well: in brief, very similar FGF21 effects on lipid and glucose metabolism are reported in the two species with respect to adipocyte glucose uptake and mitochondrial oxidative capacity, fat cell browning, as well as hepatic fatty acid oxidation and *de novo* lipogenesis. However, obvious differences exist between mice and humans when looking closer at the effects of FGF21 on adipocyte lipolysis and hepatic ketogenesis. BAT seems to be the major tissue mediating FGF21 effects on glucose uptake, and this might represent the reason for the missing effect of FGF21 on glucose in obese patients, which have only low or even no BAT (257, 258).

Human Genetic Data

In line with a potential role of FGFR2-KLB signaling in liver being responsible for FGF21 effects on

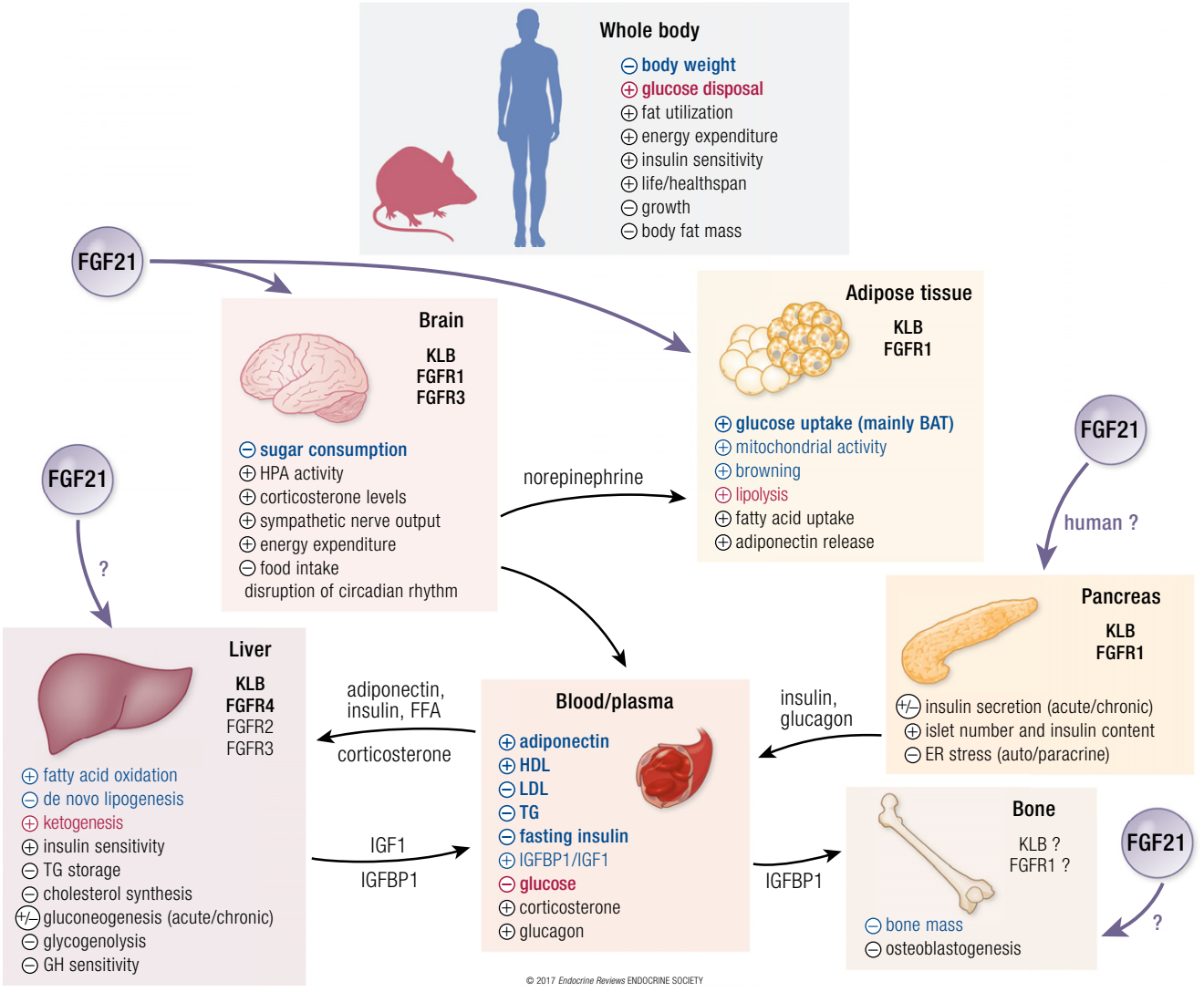

Figure 4. FGF21 effects on metabolism in mice and humans. Stimulatory effects are indicated by plus signs (+), the inhibitory effects by minus signs (−). Effects that are similar in mice and humans are indicated in blue, effects that are different between mice and humans (or controversial in the literature) are indicated in red, and effects for which human data are missing are indicated in black. Figure produced with graphics from Servier Medical Art (smart.servier.com) under Creative Commons BY 3.0 license.

cholesterol metabolism (133), a genetic variant of *FGFR2* [single nucleotide polymorphism (SNP) rs2071616] is associated with LDL cholesterol in humans (257). Consistent with a potential role of FGF21 in the brain, as proposed by mouse data, two large genome-wide association studies provided evidence that SNPs in or near the human FGF21 gene modulate macronutrient intake in humans independently of BMI: minor allele carriers of the synonymous SNP rs838133 in exon 1 of the FGF21 gene exhibit reduced energy intake from protein and increased energy intake from carbohydrates (258); and the minor allele of SNP rs838145, ~10 kb upstream of the FGF21 gene and in moderate linkage disequilibrium with rs838133 (r^2 = 0.7), is associated with higher energy intake from carbohydrates, lower energy intake from fat, and higher circulating FGF21 concentrations (259). The SNP rs838133 has recently been associated not only with sugar preference but also with more consumption of alcohol and tobacco, thus other forms of reward-seeking behavior. Interestingly, variations in KLB, the obligate FGF21 coreceptor, have also been identified to be associated with alcohol drinking in humans (260). Even though functional data on how the SNPs alter FGF21 function/expression are lacking, these data suggest that genetic variation in the FGF21 gene and its receptor determines nutrient choices in humans by acting on the reward system.

Summary and Conclusions

FGF21 represents a fascinating hormone with impressive implications in whole-body homeostasis and several metabolically relevant pathways, in particular in lipid and glucose metabolism in both mice and humans. This is why most published work hitherto was primarily driven by pharmacological interests and not by academic interests in this hormone's role in physiology and pathophysiology.

Since 2000, the year of its discovery, FGF21 has been intensely studied in mice, and a wealth of very robust data has been generated in mice that, with the exception of a negative impact on bone metabolism, consistently documents favorable roles of FGF21 in lipid and glucose metabolism. The last 10 years of *in vivo* and *ex vivo* analyses in humans have also created a considerable body of data complicating the translation of several of the mouse findings to the human situation. Human treatment studies assessing the systemic effects of regular FGF21 are currently lacking due to the instability of recombinant nonglycosylated FGF21 in the circulation, but results from three treatment studies using FGF21 analogs are available (207–209). These studies revealed one crucial deviation from what is seen in mice: FGF21 analogs failed to lower blood glucose, and this is the reason why all pharmaceutical companies engaged in the development of FGF21-based antidiabetic drugs [such as FGF21 analogs and activating anti-FGFR1c/KLB antibodies; for review, see Zhang and Li (212) and Degirolamo *et al.* (213)] have now stopped their programs.

Based on all what is hitherto known about FGF21, it is insufficient to explain the observed discrepancies of the pharmacological effect of FGF21 analogs on blood glucose between mice and humans merely by limited availability of human data or by the ultimately unproven existence of FGF21 resistance. Rather, it is more obvious that the differences have species-specific reasons and derive from technical limitations associated with the use of mice as model organism for human physiology and disease. As species-inherent limitations, divergences due to adaptations of mice and humans to their specific habitats, dietary habits, and environmental constraints are conceivable. With regard to metabolism, it is well known that mice considerably differ from humans in lipoprotein metabolism and susceptibility to atherosclerosis (261) as well as in inflammatory responses to different traumata (262). A possible technical limitation of mouse studies may arise from housing temperatures that exert dramatic effects on inflammatory and atherosclerotic events (263) and BAT/WAT biology. As BAT and WAT are major targets of the beneficial effects of FGF21 on metabolism, we assume that this is the reason for the divergent findings between mice and humans in particular on glucose metabolism. In agreement, it has been shown in a mouse model that the glucose lowering effect of FGF21 is blunted when KLB is specifically ablated in UCP^+ cells (264). There is no beneficial effect of FGF21 on glucose clearance in UCP1-deficient mice (265), and UCP1 knockout mice treated with FGF21-Fc (another long-acting FGF21 analog) demonstrated no reduction in plasma glucose (236). The relative small and varying amount of $UCP1^+$ cells in humans may explain the divergent effect of FGF21 on glucose metabolism in mice and humans.

We stress that the vast majority of human *in vivo* data are of a correlational nature. If correctly adjusted for known confounders, these data can help unmask real relationships. However, we note that correlational data cannot give mechanistic insights or solve the problem of causation/reverse causation (the "hen and egg" problem) inherent to all association analyses. Additionally, only very few FGF21 treatment studies that harbor the potential to provide mechanistic clues have hitherto been reported in humans. Apart from these limitations, an interesting observation is the robust positive correlation of FGF21 levels with many human metabolic disorders and diseases, for example, metabolically unhealthy obesity, nonalcoholic hepatosteatosis, gestational diabetes, and T2D, coronary artery/heart disease, preeclampsia, myopathy, lipodystrophy, and mitochondrial disease. Although FGF21 as an antidiabetic drug may not be feasible in humans, FGF21 might be a good biomarker and/or predictor for muscle-related mitochondrial diseases (185, 266) or arteriosclerosis (267).

We should not abandon further exploration of the biology of FGF21, but follow-up investigations are required to ultimately solve the FGF21 puzzle. Given the prominent role of FGF21 in lipid metabolism in mice and humans, patients suffering from metabolic disorders other than diabetes, such as atherosclerosis, might benefit from FGF21 therapies (133). However, future studies need to carefully address issues such as the kind (*e.g.*, murine vs human recombinant FGF21), dose, and timing of FGF21 treatment. Perhaps new animal models closer to humans, for example, omnivores such as (mini)pigs, will provide functional and mechanistic data that can better be translated to the human situation. With respect to the emerging role of the brain in metabolic diseases (268–270), one of the most exciting and also most challenging areas of future research is certainly the assessment of the efficacy of FGF21 and its analogs in the brain.

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Abbreviations

aa, amino acid; ATF, activating transcription factor; BAT, brown adipose tissue; BMI, body mass index; cAMP, cyclic adenosine monophosphate; eIF, eukaryotic translation initiation factor; ER, endoplasmic reticulum; ERK, extracellular signal–regulated kinase; FAP, fibroblast activation protein; FFA, free fatty acid; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GH, growth hormone; HFD, high-fat diet; IGF, insulin-like growth factor; KD, ketogenic diet; KLB, β-Klotho; LDL, low-density lipoprotein; mRNA, messenger RNA; MUHO, metabolically unhealthy obese; PPAR, peroxisome proliferator-activated receptor; SNP, single nucleotide polymorphism; T2D, type 2 diabetes mellitus; UCP, uncoupling protein; WAT, white adipose tissue.