

# Circulating FGF21 Levels in Human Health and Metabolic Disease

## Authors

Michaela Keuper<sup>1, 2, 3, \*</sup>, Hans-Ulrich Häring<sup>1, 2, 4, 5</sup>, Harald Staiger<sup>1, 2, 4, 6</sup>

## Affiliations

- 1 Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the Eberhard Karls University Tübingen, Tübingen, Germany
- 2 German Center for Diabetes Research (DZD), Neuherberg, Germany
- 3 Department of Molecular Bioscience, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden
- 4 Interfaculty Centre for Pharmacogenomics and Pharma Research at the Eberhard Karls University Tübingen, Tübingen, Germany
- 5 Department of Internal Medicine, Division of Endocrinology, Diabetology, Angiology, Nephrology, and Clinical Chemistry, University Hospital Tübingen, Tübingen, Germany
- 6 Institute of Pharmaceutical Sciences, Department of Pharmacy and Biochemistry, Eberhard Karls University Tübingen, Tübingen, Germany

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70469 Stuttgart, Germany

## Correspondence

Dr. Michaela Keuper

German Research Center for Environmental Health (GmbH),  
Ingolstaedter Landstraße 1,  
85764 Neuherberg,  
Germany

Tel.: +49 89 3187 3917, Fax: +49 89 3187 4589

michaela.keuper@su.se

## ABSTRACT

Human fibroblast growth factor 21 (FGF21) is primarily produced and secreted by the liver as a hepatokine. This hormone circulates to its target tissues (e. g., brain, adipose tissue), which requires two components, one of the preferred FGF receptor isoforms (FGFR1c and FGFR3c) and the co-factor beta-Klotho (KLB) to trigger downstream signaling pathways. Although targeting FGF21 signaling in humans by analogues and receptor agonists results in beneficial effects, e. g., improvements in plasma lipids and decreased body weight, it failed to recapitulate the improvements in glucose handling shown for many mouse models. FGF21's role and metabolic effects in mice and its therapeutic potential have extensively been reviewed elsewhere. In this review we focus on circulating FGF21 levels in humans and their associations with disease and clinical parameters, focusing primarily on obesity and obesity-associated diseases such as type-2 diabetes. We provide a comprehensive overview on human circulating FGF21 levels under normal physiology and metabolic disease. We discuss the emerging field of inactivating FGF21 in human blood by fibroblast activation protein (FAP) and its potential clinical implications.

## Introduction

### FGF21 as a metabolic regulator

Murine and human fibroblast growth factor 21 (FGF21) was identified and characterized at the cDNA level in 2000 by Nishimura et al. [1]. In 2005, using a glucose uptake assay to search for novel therapeutic targets to treat type 2 diabetes (T2DM), FGF21 was discovered as a metabolic regulator in mice with beneficial effects on

glucose and lipid metabolism as well as insulin-sensitivity in obese, diabetic mice [2]. Since then, it has been intensively investigated. Although the first clinical tests with FGF21 analogues failed to demonstrate beneficial effects on glucose homeostasis, they could reproduce important metabolic improvements, i. e. improvements in lipid metabolism and reduced body weight. Thus, several pharmaceutical companies are developing compounds to target the FGF21 signaling pathway, e. g., long-acting FGF21 analogues [3] or agonistic antibodies and small peptides targeting beta-klotho (KLB) [4], the crucial component of FGF21-receptor signaling complex.

\* Current address: Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 106 91 Stockholm, Sweden, email: michaela.keuper@su.se

## FGF21 is a hepatokine

Under basal conditions, the FGF21 gene in humans is considered to be nearly exclusively expressed in human liver but weak expression signals in the brain [5] and the pancreas [6] have also been reported. In line, FGF21 gene expression in mice under basal (thermoneutral) conditions is highest in the liver [7]. Thus, mainly the liver produces and releases the protein into the circulation. Although direct data in humans for extra-hepatic tissue contributing to circulating FGF21 levels are still missing, mouse data suggest at least autocrine and paracrine actions of FGF21 within adipose tissue and pancreas [7–10]. Additionally, in cold acclimated mice, brown adipose tissue (BAT) is a source for circulating FGF21, but not muscle or subcutaneous white adipose tissue (WAT) [7], and a contribution of human BAT to circulating FGF21 during cold exposure has been proposed [11]. Via the circulation, FGF21 reaches its target tissue which needs to be equipped with the FGF receptor and, most importantly, with beta-Klotho (KLB), an FGFR-binding single-pass transmembrane protein [12–16] to initiate intracellular signaling events (review of general FGF signaling [17]). A clear preference of FGF21 binding for FGFR1c-KLB and FGFR3c-KLB complexes has been demonstrated [14, 18]. In humans, FGFR1c and FGFR3c are ubiquitously expressed whereas KLB expression is restricted to liver, adipose tissue, breast, bone marrow and brain, and weak expression signals are observed in human pancreas but no signal in muscle [5]. FGF21 has also been detected in human cerebrospinal fluid, indicating that circulating FGF21 may enter the brain to act on the central nervous system [19]. Recent reviews have summarized the production and secretion sites of FGF21 [20, 21], the expression of its receptor components as well as FGF21 signaling pathway [22, 23], and the metabolic [20, 24] and pharmacological [25, 26] effects FGF21 in mice and men. Thus, in this review we will focus on FGF21 levels in the blood of healthy, obese and metabolically unhealthy individuals to provide an overview on the concentration levels in human blood. We will summarize factors that influence circulating FGF21 levels, clinical parameters associating with FGF21 levels, and recent results on fibroblast activation protein (FAP), a protease that inactivates human FGF21 in the blood.

## Circulating FGF21 Levels in Health and Disease

Reported median values for healthy individuals range mostly between 100–200 pg/ml [27–30] (► **Table 1**), but may be wider. For instance, ranges for FGF21 levels were reported to be: 21–5300 pg/ml (n = 76, 20–80 years, mean BMI 25.3 kg/m<sup>2</sup>) [27], 34–822 pg/ml (n = 115, 20–80 years, BMI < 30) [31] or 17–629 pg/ml (n = 160; only men, 30–79 years; mean BMI 24.1 kg/m<sup>2</sup>) [32] (see also ► **Table 1**). For children, a range of 61–1715 pg/mL (n = 138; in 38 samples FGF21 were below detection limit, non-obese) [33] and 31–595 pg/ml (n = 69, 5–12 years, 44 % obese) [31] was reported (► **Table 1**). Of note, data on FGF21 levels in children are limited and have not been as intensively studied as in adults yet. Collectively, serum concentrations of FGF21 exhibit considerable inter-individual variation with a right-skewed distribution ranging from levels below the de-

tection limit of conventional FGF21 ELISAs [34] up to 7100 pg/ml in one healthy individual [35]. In a monozygotic twin study, it has been estimated that the heritability of serum FGF21 level is 40 %, implying that there is a higher contribution of environmental factors to differences in FGF21 concentrations [36]. Physiological signals such as nutrients and hormones affect circulating FGF21, but also stress- and disease-related conditions associate with altered, almost exclusively increased, FGF21 levels in humans. These factors contribute to high variation in circulating FGF21 in humans and are summarized below and in ► **Fig. 1–2**.

### Nutrition (diet) alters serum FGF21 levels

In contrast to mouse models, common short-term fasting/refeeding regimens do not significantly increase FGF21 blood levels in humans [27, 28, 35]. Elevations of FGF21 levels have been reported after experimental starvation periods of seven days (by ~75 %) [27] or 10 days (4-fold increase) [28]. Recently, it has been shown that FGF21 levels decrease by ~30 % after 24h-fast in normal weight individuals (mean BMI 26) [37] and another group reported a ~55 % decrease in obese individuals (mean BMI 33) but no change in lean (mean BMI 22) after 60h-fast [38]. Ketogenic diets, which lead to robust increase in circulating FGF21 levels in mice, are either without impact, or even reduce FGF21 blood concentrations in humans, e. g., low FGF21 concentrations have been reported in humans with ketosis [27, 35, 39]. These reported differences seem mainly to be due to the different nutrient composition of ketogenic diet between mice and men (i. e., protein content). In line with this, recent murine data implicate that FGF21 levels are an indicator of low protein intake combined with high-carbohydrate intake [40]. An inverse correlation between protein intake and circulating FGF21 levels in humans has been reported [41] and T2DM patients with NAFLD demonstrate reduced FGF21 levels after protein-enriched diet together with the loss of hepatic fat [42]. Dietary protein restriction in humans leads to 1.7-fold increase in circulating FGF21 levels after 4 weeks [43] and to 2-fold increase after 6 weeks [44]. Moreover, FGF21 levels increase 3-fold when humans overfeed on a low-protein diet for 24h which is either combined with high carbohydrate or high fat content [37]. In that study, a total of 57 % of the variance in plasma FGF21 concentration was accounted by diet, 25 % by intra-individual factors and 17 % by inter-individual factors, suggesting that diet composition is the most important factor determining FGF21 levels [37].

Fructose ingestions raise FGF21 within 2 hours 3- to 4-fold in humans and a recent study showed a dose-dependent effect of fructose on FGF21 levels, with the highest fructose dose of 75g resulting in at least 100 % increase (2-fold) in all individuals that were studied [196]. This provides evidence for a negative feedback loop regulating sugar consumption, in coherence with mouse data showing that sugar ingestion suppresses FGF21 levels [45, 46]. This is further supported by large genome-wide association studies showing an association of single nucleotide polymorphisms (SNPs) in or near the human FGF21 gene with macronutrient intake in humans independently of BMI: SNP rs838145, about 10 kb upstream of the FGF21 gene, associates with higher energy intake from carbohydrates, lower energy intake from fat, and higher circulating FGF21 concentrations [47]. Carriers of the minor allele of SNP

► **Table 1** Reported fasting FGF21 levels in sera of healthy subjects

Study	ELISA (details see ► Table 4)	Sample number #	Age	FGF21 range (pg/ml)	Median (IQR) FGF21 (pg/ml)	comment
		(male/female)	(range, mean +/– SD or median (IQR))			
Gälman et al. 2008	developed in-house	76 (37/39)	20–80	21–5300	156	~11% over 1000 pg/ml
Fazeli et al. 2015	R&D Systems	11 (3/8)	22–48		124 (46, 491)	
Zhang et al. 2008	Biovendor	105 (53/52)	57.3 ± 12.6	45–795	208.7 (94.4, 325.7)	
Hanks et al. 2015	Millipore	115	20–80	34–822		
Taniguchi et al. 2014	R&D Systems	160 + 6 excluded	30–79	17–629		~2.4% < detection limit (17 pg/ml) ~1.2% > 1000 pg/ml
Li et al. 2009	Biovendor	134 (41/93)	46.0 ± 12.1		111.8 (58.0, 198.9)	
Kralisch et al. 2013	Biovendor	812 (327/485)	47.7 (22.1)		88.9 (117.7)	~17% < detection limit (7 pg/ml)
Dushay et al. 2010	Biovendor	20 (7/13)	31 ± 10	260–3190 <sup>+</sup>		
Dushay et al. 2010	Biovendor	11 (1/10)	25 ± 5	400–7100 <sup>+</sup>		
Jin et al. 2014	Biovendor	998 (752/246)	41 ± 10	16–4577	189	~3% > 1000 pg/ml
Woo et al. 2017	Antibody and Immunoassay Service	1257 (562/695)	49.8 ± 10.9		Women: 131.1 (78.1–233.1) Men: 155.3 (84.4–261.3)	
<b>Children</b>						
Hanks et al. 2015	Millipore	69 (45% obese)	5–12	29–595	156 (59, 254)	~6% < detection limit (set to 29 pg/ml)
Bisgaard et al. 2014	Biovendor	179 (65/114)	8–16	61–1715		~21% < detection limit (set to lowest standard 30 pg/ml)

# BMI < 30, if not otherwise stated; + in the original paper FGF21 levels are reported as "ng/ml"

rs838133 (population frequency: 45%) in exon 1 of the FGF21 gene exhibit reduced energy intake from protein diets and increased energy intake from carbohydrates diets [47–50]. This SNP has also been associated with higher consumption of alcohol and tobacco, representing other forms of reward-seeking behavior. Recently, the association of rs838133 with higher alcohol and sugar consumption has been reproduced [49]. In this study the authors additionally found that rs838133 associates stronger with lower total body fat content, a higher waist-to-hip ratio and higher blood pressure than it does with BMI and T2DM [49]. Furthermore, increased FGF21 levels after acute and sub-chronic alcohol consumption have been reported for humans [51–53]. There is no evidence that SNP rs838133 alters FGF21 expression in liver [49], and detailed data on how this synonymous SNP and other SNPs alter circulating FGF21 levels and/or function are scarce. It has been speculated, however, that the minor allele of rs838133 represents lower FGF21 function [49]. Interestingly, variations in KLB, the obligate FGF21 co-receptor, have also been associated with alcohol drinking in humans [54]. Thus, genetic variation in the FGF21 gene and its receptor may determine nutrient choices (i. e., negative regulator of alcohol and sugar intake) in humans by acting on the reward system. This further indicates that FGF21 mediates its metabolic effects (partially) via the central nervous system, which is supported by data in mouse models that lack KLB in the nervous system [54–56],

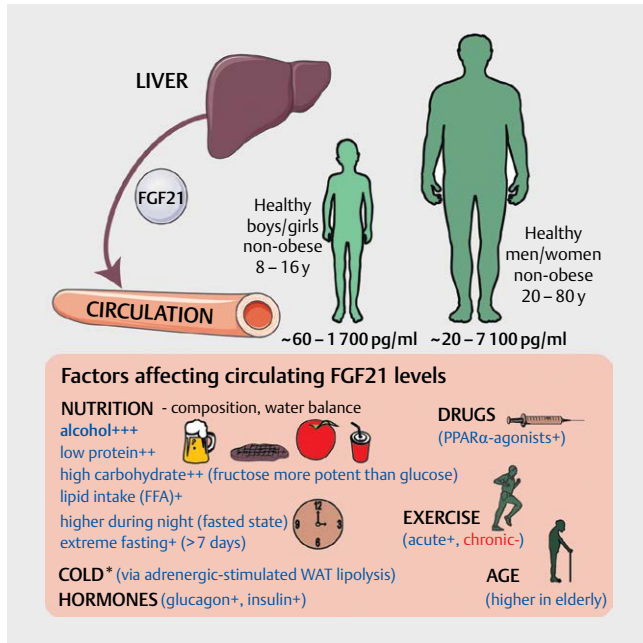
by mice that were infused with FGF21 via the lateral ventricle [56–58], and by food preference studies with FGF21 KO mice [45]. FGF21 induces corticosterone levels in mice [55] by inducing corticotropin-releasing hormone in the brain [56, 59], but if the interaction of FGF21 with the HPA axis drives food preferences in humans needs to be determined. Recent data suggest that FGF21 is an important regulator of body water balance by inducing thirst upon specific, dehydration-inducing nutrients. FGF21, however, does not stimulate water intake via stimulation of renin-angiotensin (-aldosterone) system (RAS/RAAS) but presumably by activating beta-adrenergic signaling pathways in the hypothalamus [53]. Thus, together with the suggested role in regulating proper hydration, the macronutrient balance in the food is one crucial factor determining FGF21 levels in healthy individuals. With a ~10-fold induction of circulating total FGF21 levels 2h–6h after ethanol ingestion [51, 52] compared to a 3–4-fold increase 2h after fructose consumption [46], 3-fold increase 24h after low protein diet [37] or a 6-fold increase after 7 days of low protein-high carbohydrate diet [60], alcohol is the most potent inducer of circulating FGF21 levels in humans identified so far.

### Circadian rhythm of circulating FGF21 levels

It has been reported that circulating FGF21 levels display a circadian rhythm, with high levels during the fasting state and low levels

during feeding [61, 62]. However, it has been suggested that free fatty acid (FFA) levels may explain the circadian rhythmicity of circulating FGF21 during feeding, as FFAs peak shortly before FGF21 levels begin to rise, an observation also confirmed by another re-

port [63]. Notably, FFAs have been reported to directly regulate FGF21 levels in healthy humans, i. e., FGF21 levels increase after lipid infusion [64]. In another study, neither FFA, nor glucose levels, nor the levels of insulin, glucagon and cortisol matched the pattern of FGF21 levels with peak levels at 02:30 a.m. and nadir levels at 08:30 a.m. during a 72 h fast. [65]. Thus the authors assumed that the periodicity of FGF21 is under direct control by the core clock machinery. The identification of a functional, evolutionarily conserved ROR $\alpha$ -binding site in the human FGF21 gene promoter supports this idea [66]. Therefore, without any nutritional/hormonal signal, FGF21 levels may display circadian rhythmicity, which is within the range of inter-individual variations and may be regulated by the core clock machinery. Fasting/feeding patterns, which come along with changes in circulating nutrients (e. g., glucose, FFA) and hormones (e. g., insulin, glucagon), are more important for the regulation of circulating FGF21 levels and presumably overwrite intrinsic circadian rhythm [61–63].

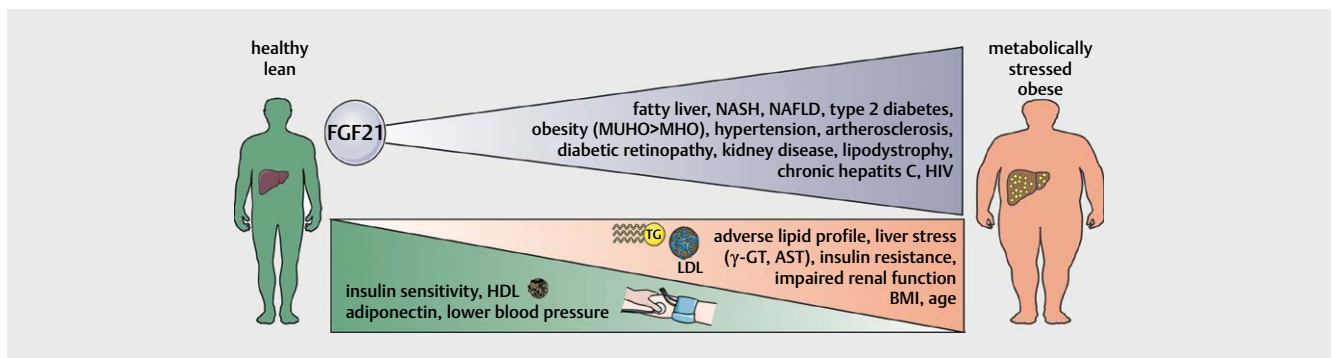


► **Fig. 1** Regulation of circulating human FGF21 levels by physiological factors. FGF21 is mainly produced and secreted by the liver and can be detected in human blood with high inter-individual variations ranging from below the detection limit (7 pg/ml) [34] up to 7100 pg/ml, e. g., for healthy non-obese children, a range of 60–1700 pg/ml [33] and for healthy, non-obese adults, a range of 21–5300 pg/ml (age 20–80 years, n = 76) [27] or 260–7100 pg/ml (age 18–60 years, n = 31) [35] has been reported. In most publications, the mean/median values range from 100–200 pg/ml in healthy, lean adults. The circulating FGF21 levels in humans are under complex control of nutritional and hormonal signals. The main regulator seems to be the individual nutrition (diet), potentially by influencing body's water balance, as alcohol is the most potent inducer identified so far followed by hormonal-induced changes upon nutrient intake, cold and exercise (catecholamine, insulin, glucagon). \* cold may render BAT a source for circulating FGF21; + increasing, - decreasing.

### Hormonal regulation of circulating FGF21 levels

Hormones regulating FGF21 levels have been studied in mouse models and include growth hormone (GH), glucocorticoids, glucagon-like peptide 1 (GLP1), insulin, and glucagon. No direct, acute effects of growth hormone on serum FGF21 levels (after 3 h) were observed in healthy humans, but 3 weeks of treatment slightly increased FGF21 levels about 3-fold, which was accompanied by increases in FFA levels [67]. This observation is in line with mouse studies suggesting that growth hormone induced FGF21 serum levels depend on increased adipose tissue lipolysis [68]. For cortisol, no associations with FGF21 levels could be found in patients with chronically elevated cortisol levels (Cushing syndrome) [69], or in stressed (healthy) humans [70]. Human data directly confirming or rejecting the regulatory effects of cortisol on FGF21 levels are yet missing.

In mice, a role for GLP1-FGF21 axis involving adipose tissue immune cells (invariant natural killer T (iNKT)) in regulating weight and glucose homeostasis by promoting WAT browning was shown [71]. In line, treating obese newly diagnosed T2DM patients for 8 weeks with a GLP1-receptor agonist (liraglutide) increased FGF21



► **Fig. 2** Higher FGF21 levels in metabolically stressed individuals and clinical correlates. FGF21 levels are increased in several disease conditions that are related to metabolic stress. The most consistent correlation between FGF21 levels and clinical parameters are related to lipid profile (TG, HDL, and LDL) and liver (liver fat content,  $\gamma$ -GT, AST) as well as insulin sensitivity/resistance (HOMA-IR, fasting insulin), including adiponectin and high blood pressure, BMI and age, but also kidney function.

plasma levels, decreased body weight and increased iNKT cell [71], providing evidence for a similar axis in humans.

Several groups demonstrated that insulin moderately increases the FGF21 concentration in blood [64, 72–74] and with glucose and insulin clamps, Samms et al. recently showed that insulin rather than glucose increases FGF21 levels [75]. Although insulin increases the mRNA levels of FGF21 in skeletal muscle and adipose tissue [72, 73, 76], a significant contribution of muscle and WAT to circulating FGF21 levels in humans has not been shown. Still, FFA levels seem to be more important nominators of FGF21 regulation than insulin [64].

Different exercise regimens stimulate FGF21 production in liver and increase blood FGF21 [77–80], and a recent study reports a 1-hour time delay between the peak levels of glucagon and FGF21 by endurance training [81]. Hansen et al. demonstrated that exercised-induced increase in circulating glucagon enhances hepatic FGF21 production [80, 82]. Injecting native glucagon in healthy, obese individuals increased circulating FGF21 levels [83], thus providing evidence for a muscle-pancreas-liver axis that plays a role in exercise-induced elevation of FGF21 blood levels. Another player in this axis may be WAT, as glucagon-induced WAT lipolysis may increase circulating FFA levels [84], which then would increase hepatic FGF21 expression and secretion. This mechanism may be similar to the one that has been postulated for GH, where the GH-induced increases of FGF21 levels are dependent on WAT lipolysis [67, 68]. Direct effects of FGF21 on WAT lipolysis are controversially discussed. Both pro-lipolytic (in mouse models [85, 86]) and anti-lipolytic (human adipocytes [87], in mouse models [68, 88]) actions of FGF21 have been shown. It appears that FGF21 effects on lipolysis depend on other signals as they differ between the fed and fasting state [86] and are differentially affected by acute or chronic treatments [87]. Insulin and glucagon, hormones which have opposing effects on metabolism, can both increase FGF21 levels, pointing towards a complex regulation of FGF21. These effects, however, are strongly influenced by the context (nutrition, obesity, diabetes).

Recently, Pan et al. showed that angiotensin-II increases FGF21 levels in mice and that FGF21 induces angiotensin-converting enzyme (ACE). Therefore, they suggested that FGF21 negatively regulates the renin-angiotensin system (RAS), where FGF21 exerts a protective function in Angiotensin-2 induced hypertension [89]. In humans, a link between FGF21 and renin-angiotensin-aldosterone system (RAAS) may exist. Patients treated with peritoneal dialysis show a significant decrease of 13% in FGF21 levels after six months of angiotensin receptor blockade therapy (n = 72) [90]. In another study subjects taking ACE inhibitors (median: 278; n = 113) displayed ~25% higher FGF21 levels than controls (median: 220; n = 661) [91]. There appears also to be a link between FGF21-induced water drinking via beta adrenergic signaling pathways in the brain [53], and FGF21 inducing diuresis due to increasing blood pressure which consequently leads to higher water intake [92]. In the light of this association, the interaction of FGF21 with the RAAS/RAS and the role of beta adrenergic signaling pathways (peripherally and centrally) may require further experiments for mechanistic insights. It may be noted that catecholamines (adrenergic signaling) may also play a role for exercise- [78] and alcohol-induced elevated FGF21 levels [93] via induction of adipose tissue

lipolysis. Catecholamines and cold exposure induce FGF21 levels and at least in mouse models, it has been reported that brown adipose tissue (BAT) becomes a source of circulating FGF21 [7, 8, 94]. In line, cold exposure of humans increases FGF21 levels, blunting the circadian rhythm of FGF21 levels [9, 11]. Based on these observations, a link between human BAT activity and FGF21 levels has been postulated [9, 11, 28, 95]. Interestingly, it has been shown that FGF21 increases systemic catecholamine levels by activating the SNS, and thereby increases adipose tissue lipolysis and FFA levels in mice [56, 93], which may then further elevate FGF21 levels. Furthermore, the central action of FGF21 to activate BAT via SNS has been suggested to be dependent on corticotropin-releasing factor [56] and FGF21 has been shown to increase HPA axis [59]. The functional link and direction between adrenergic activation (peripheral vs. centrally), interaction with HPA axis and the closely associated RAAS, WAT lipolysis, BAT activity (UCP1 levels), and FGF21 is a crucial and challenging future research area, which may bear the potential to explain controversial reports. In particular the different observations between mice and humans require explanation, as it is not known whether FGF21 analogues used in clinical trials are able to enter the brain.

### Drugs that impact circulating FGF21 levels

Several studies consistently demonstrated that treatment with PPAR $\alpha$ -activating fibrates increases FGF21 blood levels, suggesting a role of FFA-dependent transcription factors in human FGF21 gene induction that resemble murine FGF21 gene regulation, at least in this pharmacological setting [27, 39, 96–98]. The rise in FGF21 levels is rather marginal after 3 weeks of treatment, e. g., increasing by 28% in normal-weight, nondiabetic patients (n = 19) with primary hypertriglyceridemia [27]. After one year of fenofibrate treatment, however, FGF21 levels increased by 105% (n = 956) as compared to 11% in placebo (n = 963) treated T2DM [99].

In subjects who were either healthy or had impaired glucose tolerance, no effects were reported for thiazolidinediones, which are potent PPAR $\gamma$  agonists clinically used as insulin sensitizers [39, 64, 96]. In contrast, in patients with T2DM, rosiglitazone treatment improved insulin sensitivity and significantly decreased circulating FGF21. Notably, however, these patients were additionally on metformin therapy [100]. Treating T2DM patients for 6 months with metformin led to no significant changes in fasting glucose and insulin levels but increased circulating FGF21 levels [101]. Treating newly diagnosed T2DM patients for 12 weeks with metformin led to decreased FGF21 levels [102]. Thus, FGF21 data in relation to PPAR $\gamma$ -agonists and metformin are inconsistent, with metabolic effects that are probably secondary and depend largely on the context (healthy/unhealthy, non-obese/obese, combined therapy/single treatment), and improvements in glucose metabolism are accompanied by decreased FGF21 levels.

### Higher circulating FGF21 levels in metabolic diseases and its association with clinical parameters

Higher FGF21 levels are consistently found in obese as compared to lean individuals [35, 74]. FGF21 blood concentrations positively associate with body mass index (BMI) [29, 74, 96, 103, 104] and fat mass (visceral [32], pericardial [105], and epicardial [106]) (► **Table 2**). As FGF21 levels also raise with age [31], all human in vivo data on

FGF21 should be adjusted for confounding BMI and age, in particular if they are of correlative nature. Body weight and fat gain induced by overfeeding leads to elevated FGF21 concentrations in human blood (+50% after 3 days,  $n = 40$  [107], +31% after 7 weeks,  $n = 39$  [108]). A starvation period of 3 days leads to a significant decrease in FGF21 levels in obese ( $n = 8$ ) not lean ( $n = 7$ ) individuals [38], but in this report the changes in body weight or fat mass were not reported. Acute and pronounced weight and body fat loss induced by fasting or surgery, however, does not *per se* reduce FGF21 levels. No change in circulating FGF21 levels 1 year after Roux-en-Y gastric bypass (RYGB,  $n = 12$ , 16% weight loss) or sleeve gastrectomy (SG,  $n = 11$ , 13% weight loss) surgery were reported [109]. Instead, FGF21 levels are even increased by 75% after 7 days of fasting (7% weight loss,  $n = 5$ ) [27] and 2–3-fold increase after bariatric surgery (after 3 months: 17% body weight loss,  $n = 35$  [110], after 1 month: 11 kg fat mass loss,  $n = 24$  [111], after 3 months: ~15% weight loss,  $n = 16$  [112]). Body fat reductions induced either by diet ( $n = 28$ , -7% body fat), sleeve gastrectomy (SG,  $n = 20$ , -11% body fat) or RYGB ( $n = 66$ , -15% body fat), showed only reduced FGF21 levels with diet and SG, but not RYGB [113]. These observations argue against a general adiposity-dependent role of FGF21. It should be noted, however, that FGF21 effects, in particular those related to insulin sensitivity, have been linked to effects on WAT function in mouse models [114, 115], thus there are potential implications in cardiometabolic control and inflammation via interactions with adipokines and the WAT signaling network [116].

Notably, as mentioned in above, there are individuals with no detectable FGF21, e. g., in 142 out of 812 non-diabetic, lean donors (<7 pg/ml). These 17% of the donors showed significantly lower blood pressure and TG, higher HDL-cholesterol levels and higher insulin sensitivity, thus they were overall “metabolically healthier” [34]. Common obesity can be dissociated into two subtypes: ~20–40% of obese individuals show metabolically healthy obesity (MHO) without serious metabolic complications, whereas 60–80% display metabolically unhealthy obesity (MUHO), characterized by insulin resistance, increased visceral fat mass, ectopic fat deposition in skeletal muscle and liver, inflammation, and increased intima-media thickness of the carotid artery [117–120]. Reports on 2-fold higher levels of FGF21 in MUHO vs. MHO [121], and on BMI-independent positive associations of FGF21 levels with the metabolic syndrome [29], hyperinsulinemia [105], the development of diabetes [122], HOMA-IR and fasting insulin [123], abnormal glucose metabolism and insulin resistance [124] further support an adiposity-independent role of FGF21, suggesting FGF21 as marker for metabolic disease. FGF21 levels are higher during the metabolic syndrome [125, 126] and the number of criteria classifying the metabolic syndrome correlates with higher FGF21 levels in the patients [29].

Fatty liver is a hallmark of MUHO [117] and several studies have shown that concentrations of FGF21 are elevated in subjects with non-alcoholic fatty liver disease (NAFLD) [105, 125, 126] and steatohepatitis (NASH) [35, 127–131]. Indeed, liver fat content is the strongest BMI-independent determinant of hepatic FGF21 production and circulating FGF21 levels [35, 127–131], and the reduction of liver fat content leads to reduced circulating FGF21 levels [132, 133]. When HIV patients with high liver fat content are treat-

ed with tesamorelin (the synthetic form of growth-hormone-releasing hormone) for six months, this leads to the reduction of liver fat content associating with the reduction in FGF21 levels [132]. Additionally, combining pioglitazone treatment with exenatide (a GLP1 receptor agonist) demonstrated more pronounced reduction of liver fat content and of FGF21 levels after 12 months, which are not observed in T2DM therapy on pioglitazone treatment alone [133]. Accordingly, plasma FGF21 levels have been suggested as a potential diagnostic marker of NAFLD [35, 127]. The accuracy of FGF21 as a biomarker for NAFLD has a sensitivity of 73% and a specificity of 85% at a cut off value of 191 pg/ml, whereas the sensitivity and specificity of FGF21 as a biomarker for NASH is lower (sensitivity: 54%; specificity: 73%; cut-off value: 332 pg/ml) [134]. Additionally there is a stepwise increase in serum FGF21 levels with the histological steatosis score and serum FGF21 levels were the only independent predictor of hepatic steatosis scores in patients with NAFLD after stepwise linear regression analysis ( $n = 82$ ) [130]. For NASH diagnosis, combining FGF21 (cut-off  $\geq 332$  pg/ml) with two other circulating markers (Keratin 18 (CK-18) and Adipocyte fatty acid binding protein (AFABP); cut-off values:  $\geq 338$  U/L and  $\geq 15.0$  ng/ml) seems to be better, yielding an overall specificity of 95% and a positive predictive value of 90% [134]. In line, FGF21 is better for predicting the onset of simple steatosis, while other markers (such as CK-18) are better for predicting the prognosis of NAFLD patients [135]. FGF21 levels in liver disease are summarized in ► **Table 3** and Liu et al. summarized additional human NAFLD studies and mouse studies addressing FGF21 in the treatment of NAFLD [136].

Fatty liver plays a crucial role in the pathogenesis of T2DM [137], thus it is not unexpected that FGF21 blood levels are increased in T2DM [34, 74, 112, 122, 125, 138, 139] (► **Table 3**). A systematic review on FGF21 and gestational diabetes (GDM) found in four studies higher FGF21 levels in GDM, whereas another four reported no differences, thus allowing no firm conclusions yet [140]. Elevated FGF21 blood levels during the metabolic syndrome with increased intima-media thickness [138, 141], arterial stiffness [142, 143], atherosclerotic plaque formation [144, 145], coronary artery/heart disease [139, 146, 147], and acute myocardial infarction [148] point towards a complex relationship of FGF21 with vascular complications, i. e., liver fat-dependent and -independent. This is further supported by FGF21's association with hypertension [91], blood pressure [96, 122, 145, 149] and preeclampsia [150]. FGF21 levels were also suggested as biomarker for a subgroup of T2DM individuals with high risk of coronary heart disease [151]. A recent systematic review and meta-analysis (28 studies were included) reported that FGF21 not only predicts the risk of metabolic syndrome and disease incidence or progression and worsening renal failure in T2DM, but FGF21 also predicts cardiovascular mortality, the incidence of coronary artery disease, and all-cause mortality [152]. Higher circulating FGF21 concentrations associate with adverse lipid profiles, such as increased low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) cholesterol as well as increased plasma triglycerides in several reports [29, 30, 34, 96, 105, 106, 146, 147, 149, 153] (► **Table 2**). Among these, circulating FGF21 most robustly and consistently correlates with plasma triglycerides (TG) (20 of 26 studies referenced in ► **Table 2** found significant positive association, four reported no as-

► **Table 2** Reported associations between FGF21 levels and clinical parameters.

Study	Eto et al. 2010	Shen et al. 2013	Lee et al. 2015	An et al. 2012
Sample number (men/women)	139 (85/54)	253 (164/89)	1136	213 (117/96)
age	ns	-	*	ns
BMI	**	-	***	ns
gender	ns	-	-	ns
% body fat	-	-	-	-
HOMA-IR	ns	ns	-	ns
fasting insulin	ns	-	-	-
fasting glucose	ns	ns	ns	**
HbA1c	ns	ns	ns	-
triglycerides (TG)	***	***	***	ns
LDL	*	ns	ns	ns
HDL	ns	ns	***	ns
cholesterol (TC)	-	*	*	ns
systolic blood pressure	***	ns	*	-
diastolic blood pressure	0.08	ns	0.06	-
ALT	ns	-	-	ns
AST	ns	-	-	ns
γ-GT	-	-	-	-
liver fat	-	-	-	-
renal function (eGFR)	-	-	***	-
creatinine	**	-	***	0.09
hsCRP	-	ns	***	-
adiponectin	ns	-	***	-
ELISA	Biovendor	Antibody and Immunoassay Service	Antibody and Immunoassay Service	Millipore
comments		additionally adjusted for gender	additionally adjusted for gender	additionally adjusted for gender, associates with macrovascular-function
independent factors in multiple regression analysis	TG, creatinine, LDL, glucagon, platelet count, metabolic syndrome, use of fibrate		eGFR, adiponectin, gender, BMI, CRP, dyslipidemia, albuminuria	
Study	Li et al. 2010	Zhang et al. 2008	Giannini et al. 2013	Wu et al. 2017
Sample number (men/women)	348 (212/ 136)	232 (115/117)	217 (91/ 126)	808
age	ns	**	-	***
BMI	***	**	***	***
gender	-	ns	-	-
% body fat	ns	ns	*** visc	-
HOMA-IR	-	0.09	-	-
fasting insulin	-	*	-	-
fasting glucose	0.06	ns	-	ns
HbA1c	ns	-	-	ns
triglycerides (TG)	***	***	-	***
LDL	0.07	ns	-	ns
HDL	***	***	-	***
cholesterol (TC)	***	-	-	***
systolic blood pressure	0.09	ns	-	*
diastolic blood pressure	*	ns	-	***
ALT	***	-	***	*
AST	-	-	-	ns
γ-GT	***	-	-	***
liver fat	-	-	***	-
renal function (eGFR)	-	-	-	-
creatinine	-	-	-	-
hsCRP	-	ns	-	-
adiponectin	-	ns	-	***
ELISA	Biovendor	Biovendor	R&D Systems	Antibody and Immunoassay Service
comments	intrahepatic TG content correlates with FGF21 levels (n = 17)	sign higher level in obese	FGF21 associates with visceral obesity, and CK18 (marker for hepatic apoptosis)	correlates positively with metabolic syndrome and liver disease (steatosis grade, lobular inflammation, fibrosis, and NAS)
independent factors in multiple regression analysis	TG, γ-GT, waist circumference	Metabolic Syndrome, BMI and A-FABP		

► **Table 2** Continued.

Study	Chen et al. 2011	Lin et al. 2010	Kralisch et al. 2013	Lee et al. 2014
Sample number (men/women)	1900	196 (98/98)	670 (285/385)	189 (108/81)
age	***	ns	***	-
BMI	***	***	***	ns
gender	ns	ns	*	ns
% body fat	-	-	**	-
HOMA-IR	***	**	***	**
fasting insulin	***	*	***	*
fasting glucose	**	***	***	ns
HbA1c	-	-	-	ns
triglycerides (TG)	***	***	***	***
LDL	***	ns	**	***
HDL	***	***	***	*
cholesterol (TC)	***	ns	*	**
systolic blood pressure	***	-	***	ns
diastolic blood pressure	***	-	***	-
ALT	-	-	***	-
AST	-	-	***	-
γ-GT	-	-	***	-
liver fat	-	-	-	-
renal function (eGFR)	-	-	***	-
creatinine	-	-	ns	-
hsCRP	***	ns	0.08	ns
adiponectin	-	-	-	-
ELISA	Antibody and Immunoassay Service	Biovendor	Biovendor	Biovendor
comments	independent association of high FGF21 with T2DM development in a 5.4-year prospective study	adjusted to BMI, diabetes, hypertension	not adjusted for age and BMI	not adjusted for age and BMI
independent factors in multiple regression analysis	TG, age, hsCRP, systolic BP, LDL-C, waist circumference		systolic BP, TG and γ-GT	pericardial fat volume
Study	Cheng et al. 2011	Jin et al. 2014	Gómez-Ambrosi et al. 2017	Li et al. 2009
Sample number (men/women)	143 (70/ 73)	998 (752/246)	170 (90/80)	353 (116/237)
age	*	***	ns	***
BMI	ns	***	***	***
gender	-	ns	*	ns
% body fat	-	-	*	ns
HOMA-IR	*	-	***	-
fasting insulin	ns	-	***	-
fasting glucose	***	**	***	-
HbA1c	***	ns	-	-
triglycerides (TG)	ns	***	*	***
LDL	ns	ns	ns	*
HDL	ns	ns	*	ns
cholesterol (TC)	ns	*	ns	***
systolic blood pressure	**	***	ns	-
diastolic blood pressure	*	***	*	-
ALT	-	-	-	ns
AST	-	-	-	-
γ-GT	-	-	-	***
liver fat	-	-	-	-
renal function (eGFR)	-	-	-	-
creatinine	-	-	-	-
hsCRP	***	-	*	-
adiponectin	-	-	-	-
ELISA	Adlitteram Diagnostic Laboratories	Biovendor	Biovendor	Biovendor
comments	not adjusted for age and BMI	not adjusted for age and BMI	not adjusted for age and BMI	
independent factors in multiple regression analysis	fasting insulin, HOMA-IR, LDL-C and hsCRP	TG, systolic BP		TG, cholesterol, γ-GT and age



► Table 2 Continued.

Study	Esteghamati et al. 2017	Chow et al. 2013	Ong et al. 2015	Stein et al. 2010
Sample number (men/women)	130 (67/63)	670 (285/385)	9697 (6078/3619)	120 pregnant women
age	ns	***	***	ns
BMI	*	***	***	ns
gender	-	ns	***	-
% body fat	-	-	-	-
HOMA-IR	-	-	***	***
fasting insulin	-	-	***	**
fasting glucose	***	*	***	ns
HbA1c	***	**	*	-
triglycerides (TG)	-	***	***	***
LDL	ns	***	***	ns
HDL	0.06	***	***	***
cholesterol (TC)	ns	-	*	ns
systolic blood pressure	ns	***	***	ns
diastolic blood pressure	ns	***	***	ns
ALT	***	-	-	-
AST	**	-	-	-
γ-GT	-	-	-	-
liver fat	-	-	-	-
renal function (eGFR)	***	-	***	-
creatinine	***	***	***	ns
hsCRP	-	***	-	ns
adiponectin	-	-	-	***
ELISA	Cusabio Biotech	Biovendor	Antibody and Immunoassay Service	Biovendor
comments	not adjusted for age and BMI	not adjusted for age and BMI	higher levels in women, not adjusted for age and BMI	not adjusted for age and BMI
independent factors in multiple regression analysis		carotid intima-medial thickness in women, not men	positive: Age, BMI, WHR, fasting glucose, HOMA-IR, TG, creatinine negative: diabetes duration, LDL-C, HbA1c	
Study	Akyildiz et al. 2015	Mraz et al. 2009	Taniguchi et al. 2014	Zhang et al. 2015
Sample number (men/women)	86 (0/86)	69 (0/69)	160 (160/0)	504 (221/283)
age	-	-	ns	ns
BMI	***	**	0.09	ns/*
gender				
% body fat	***	-	*** visc	-
HOMA-IR	***	ns	ns	-
fasting insulin	-	ns	ns	-
fasting glucose	***	**	ns	ns
HbA1c	-	-	ns	ns
triglycerides (TG)	***	ns	***	***
LDL	*	-	ns	ns
HDL	***	-	0.09	ns/*
cholesterol (TC)	0.09	ns	-	ns
systolic blood pressure	-	-	-	ns/*
diastolic blood pressure	-	-	-	ns/*
ALT	***	-	*	-
AST	ns	-	ns	-
γ-GT	***	-	***	-
liver fat	-	-	-	-
renal function (eGFR)	-	-	-	-
creatinine	-	-	-	-
hsCRP	ns	-	-	***/*
adiponectin	-	*	-	-
ELISA	Aviscera	Biovendor	R&D Systems	Antibody and Immunoassay Service
comments	not adjusted for age and BMI	not adjusted for age and BMI		not adjusted for age and BMI; FGF21 correlates with femoral inter-media thickness in women only
independent factors in multiple regression analysis	BMI, TG, epicardial fat thickness		visceral fat area, TG	

► **Table 2** Continued.

Study	Xiao et al. 2015	Cuevas-Ramos et al. 2010
Sample number (men/women)	212 (107/105)	210 (81/129)
age	ns	* / *
BMI	ns	* / ***
gender		ns
% body fat	-	ns
HOMA-IR	-	ns
fasting insulin	ns	ns
fasting glucose	* / *	* / **
HbA1c	ns	-
triglycerides (TG)	* / *	ns
LDL	ns	-
HDL	ns	ns
cholesterol (TC)	-	-
systolic blood pressure	ns	-
diastolic blood pressure	ns	-
ALT	-	-
AST	-	-
γ-GT	-	-
liver fat	-	-
renal function (eGFR)	-	-
creatinine	-	-
hsCRP	-	ns
adiponectin	-	ns/ *
ELISA	Antibody and Immunoassay Service	Biovendor
comments	not adjusted for age and BMI; FGF21 correlates with carotid inter-media thickness in women only	
independent factors in multiple regression analysis		BMI, glucose, uric acid, and physical activity
<p><b>p adjusted for age and BMI, if not otherwise stated in the comment, - not reported; ns not significant; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001, black text on white background: positive association, white text on black background: negative association; gray background: only one gender or gender stratified analysis</b> ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT: gamma-glutamyl transferase; BMI, body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; eGFR: estimated Glomerular Filtration Rate.</p>		

sociation, and in 2 reports TG were not analyzed), possibly reflecting the strong association with hepatosteatosis. Metabolic diseases including obesity, hyperglycemia, insulin resistance, dyslipidemia, and metabolic syndrome have been linked to oxidative stress, thus an association of FGF21 with oxidative stress has been suggested [154].

Of note, a role for FGF21 in kidney disease has been proposed by several reports (► **Table 3**), and human studies investigating the relationship between serum FGF21 concentration and renal function are summarized in [155]. However, more detailed studies addressing the link between FGF21 and renal function are certainly required, considering FGF21's role in drinking behavior, as potential negative regulator of the RAS/RAAS pathway [53, 89] and FGF21's association with diabetic kidney disease [156, 157]. One recent report suggests that FGF21 regulates glucose homeostasis partially by reducing glucose reabsorption in the kidney [158].

Clinical conditions (► **Table 3**) and parameters (► **Table 2**) that associate with FGF21 levels in humans are summarized in ► **Fig. 2**. Overall, clinical data suggest that FGF21 is a key marker for metabolic stress, in particular for diseases involving liver fat accumulation. A twin study suggests that 23 % of the variation of FGF21 levels in monozygotic twins can be explained by differences in liver fat, and in dizygotic pairs 10 % of variation in FGF21 levels could be explained by triglyceride levels [36]. This is further emphasized by several other reports showing that FGF21 levels consistently correlate with liver parameters (fat content, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase) and an adverse lipid profile (TG and HDL/LDL levels). Additionally, FGF21 levels correlate with markers of insulin resistance (HOMA-IR, glucose and adiponectin levels), and with BMI, age, and to some extent with body fat. Gender-dependency have been found for associations of FGF21 levels with BAT activity [95] and femoral inter-media thickness [141, 145], but a general gender difference of circulating FGF21 levels has not yet been established. In children (pre-puberty), a gender difference in FGF21 levels has been reported with girls having higher FGF21 levels [33], contrasting no significant gender difference in many reports on adults ► **Table 2**. A gender-specific role for FGF21 under certain conditions, however, cannot be excluded at this stage, as gender specific-associations of FGF21 levels with femoral inter-media thickness, HDL and blood pressure have been reported (► **Table 2**).

### Stability of human FGF21 in the blood

Only limited information on the stability of FGF21 in the blood is available. Using human recombinant E.-coli-derived, i. e., non-glycosylated, FGF21, Xu et al. reported a half-life of about 1.5–2 h after intravenous injection into C57BL/6 mice [159]. In other studies, half-lives of 20–30 min (CD-1, Swiss Webster mice, cynomolgus macaques [160, 161]) and of 1.2 h (Sprague Dawley rats [162]) were measured with the identical protein, and recently a plasma half-life ( $T_{1/2}$ ) of 2 h for native FGF21 in lean minipigs has been published [163]. Thus, it can be assumed that human (recombinant) FGF21 has a half-life of less than 2 h, while the in vivo stability of endogenous FGF21 is currently unknown. This relatively short-half live presumably results from two processes, renal clearance due to its small size (~22 kDa) [164, 165] and/or proteolytic degradation which may be mediated by fibroblast activation protein (FAP) as discussed in later sections.

### Increased stability of pharmacological FGF21 analogues

A major effort of pharmaceutical companies focuses on increased stability of FGF21. Three long-acting derivatives have been engineered for potential future therapeutically use: (1) LY2405319 is a human FGF21 molecule modified by deletion of four N-terminal amino acids of the signal peptide, introduction of an additional disulfide bond, and elimination of an O-linked glycosylation site (Ser167) [166]. Testing this compound in T2DM patients revealed after four weeks of LY2405319 treatment reduced plasma triglycerides, total and LDL cholesterol concentrations, and increased plasma HDL cholesterol, β-hydroxybutyrate, and serum adiponectin concentrations as well as 50 % decrease in plasma FGF21, but no

► **Table 3** Reported FGF21 levels in obesity, diabetes, liver and kidney disease.

Study	Control group			disease	Disease group		
	n (male/female)	mean ±SD or median (IQR) or [range]			n (male/female)	mean ±SD or median (IQR) or [range]	
Novotny et al. 2014	136 (74/62)	222	± 299.9	MetSy	73 (31/42)	471.4 <sup>***</sup>	± 531.7
Bobbert et al. 2013	298 (85/213)	213	[19.8–1296]	MetSy	54 (26/28)	326 <sup>***</sup>	[97.8–625]
Bobbert et al. 2013	339 (110/229)	226.4	[19.8–1296]	T2DM	35 (14/21)	315.6 <sup>***</sup>	[109.7–1081.0]
Mraz et al. 2009	32	213	± 23	T2DM obesity	11 26	395 <sup>*</sup> 323 <sup>*</sup>	± 56 ± 35
Lips et al. 2014	12 women only	140 <sup>+</sup>	± 20	obesity T2DM	27 27	250 <sup>++</sup> 470 <sup>***+</sup>	± 40 ± 60
Kralisch et al. 2013	670 (285/385)	88.9	(117.7)	T2DM	101 (42/59)	141.8 <sup>*</sup>	(174.4)
Li et al. 2009	134 (41/93)	111.8	(58.0, 198.9)	IFG IGT	101 (33/68) 118 (42/76)	126.9 164.6 <sup>**</sup>	(79.8, 221.9) (89.7, 261.0)
Chen et al. 2011	1044 (446/597)	123	(75.9–230.0)	T2DM	298 (149/148)	234 <sup>***</sup>	(144.6–365.7)
Chow et al. 2013	262	191.1	(125.6–329.7)	T2DM	112	288 <sup>***</sup>	(172.9–450.0)
Kim et al. 2015	30 (23/7)	141	(73.9–180.9)	T2DM	30 (9/21)	224 <sup>*</sup>	(146.8–337.4)
Stein et al. 2010	80	102.9	± 144.9	gestational DM	40	97.5	± 166.1
Esteghamati et al. 2017	42	99	(126.75)	T2DM	44	103.5	(75.75)
Dushay et al. 2010	6 (3/3)	300 <sup>+</sup>	± 100	NAFLD NASH	6 (2/4) 9 (2/7)	7700 <sup>++</sup> 2500 <sup>++</sup>	± 2900 ± 800
Yilmaz et al. 2010	77 (37/40)	93	(70–180)	NAFLD	82 (38/44)	200 <sup>***</sup>	(87–410)
Li et al. 2010	124 (70/54)	198.62	(134.96, 412.62)	NAFLD	224 (142/82)	402.38 <sup>**</sup>	(242.03, 618.25)
Shen et al. 2012	74 (40/34)	104	(70–161)	NAFLD	146 (81/65)	291 <sup>***</sup>	(167–478)
Shen et al. 2013	183 (121/62)	273.3	(164.9–383.7)	NAFLD	70 (43/27)	388 <sup>**</sup>	(253.0–655.4)
Wu et al. 2017	442 (159/283)	222.44	(136.06–351.47)	NAFLD	123 (59/64)	357.5 <sup>***</sup>	(230.67–592.66)
Lin et al. 2010	61 (31/30)	131	(70.4–249.5)	CHD	135 (67/68)	326.5 <sup>***</sup>	(210.6–661.9)
Shen et al. 2013	47 (23/24)	256.1	(150.6–351.9)	CAD	136 (98/38)	277.8 <sup>*</sup>	(171.1–406.6)
Kim et al. 2015	30 (23/7)	141	(73.9–180.9)	CAD	30 (21/9)	277.1 <sup>***</sup>	(155.6–476.6)
Esteghamati et al. 2016	44 (T2DM)	103.5	(75.75)	retinopathy	46 (T2DM)	225.0 <sup>***</sup>	(106.9)
Esteghamati et al. 2017	44 (T2DM)	103.5	(75.75)	microalbuminuria	44 (T2DM)	269.5 <sup>***</sup>	(188.5)
Stein et al. 2009	30 NGT/30 T2DM	201.91	(275.5)	chronic hemodialysis	28 NGT/32 T2DM	3710 <sup>***</sup>	(5541.3)
Han et al. 2010	63 (30/33)	86.9	± 60.2	peritoneal dialysis	72 (37/35)	754.2 <sup>***</sup>	± 463.5
Semba et al. 2013	509	208	(117,335)	hypertension	235	269 <sup>***</sup>	(161,457)

+ in the original paper FGF21 levels are reported as “ng/ml”; MetSy: metabolic syndrome; NAFLD: non-alcoholic fatty liver disease, NASH: non-alcoholic steatohepatitis; CAD: coronary artery disease; CHD: coronary heart disease; NGT: normal glucose tolerance; T2DM: type 2 diabetes mellitus; IGT: impaired glucose tolerance; IFG: impaired fasting glucose; \* p<0.05, \*\* p<0.01; \*\*\* p<0.001 vs. control group.

significant effects on blood glucose levels [167]. (2) PF-05231023 which is formed by covalent conjugation of two modified human FGF21 molecules (desHis FGF21 Ala129Cys) to the F<sub>ab</sub> regions of a monoclonal scaffold antibody is an artificial macromolecule [161]. This stable analogue evoked similar effects on plasma lipids and no effects on blood glucose after a single intravenous dose to T2D patients [168]. In a four-week randomized placebo-controlled phase-1b trial in T2D patients, additional effects were seen as significantly decreased body weight, increased blood adiponectin concentrations, reduced blood markers of bone formation, increased markers of bone resorption and elevated blood IGF1 levels [169]. In obese subjects with and without T2DM, this compound increased blood pressure and heart rate [170]. (3) PEGylated FGF21 is a FGF21 form stabilized by conjugation with a 30-kDa polyethylene glycol (PEG) residue. This compound has not yet been tested in humans [162].

Besides these three long-acting FGF21 analogues (1–3), there are other strategies to target FGF21 signaling in metabolic disease [3, 4].

### Inactivation of FGF21 in human blood by fibroblast activation protein (FAP)

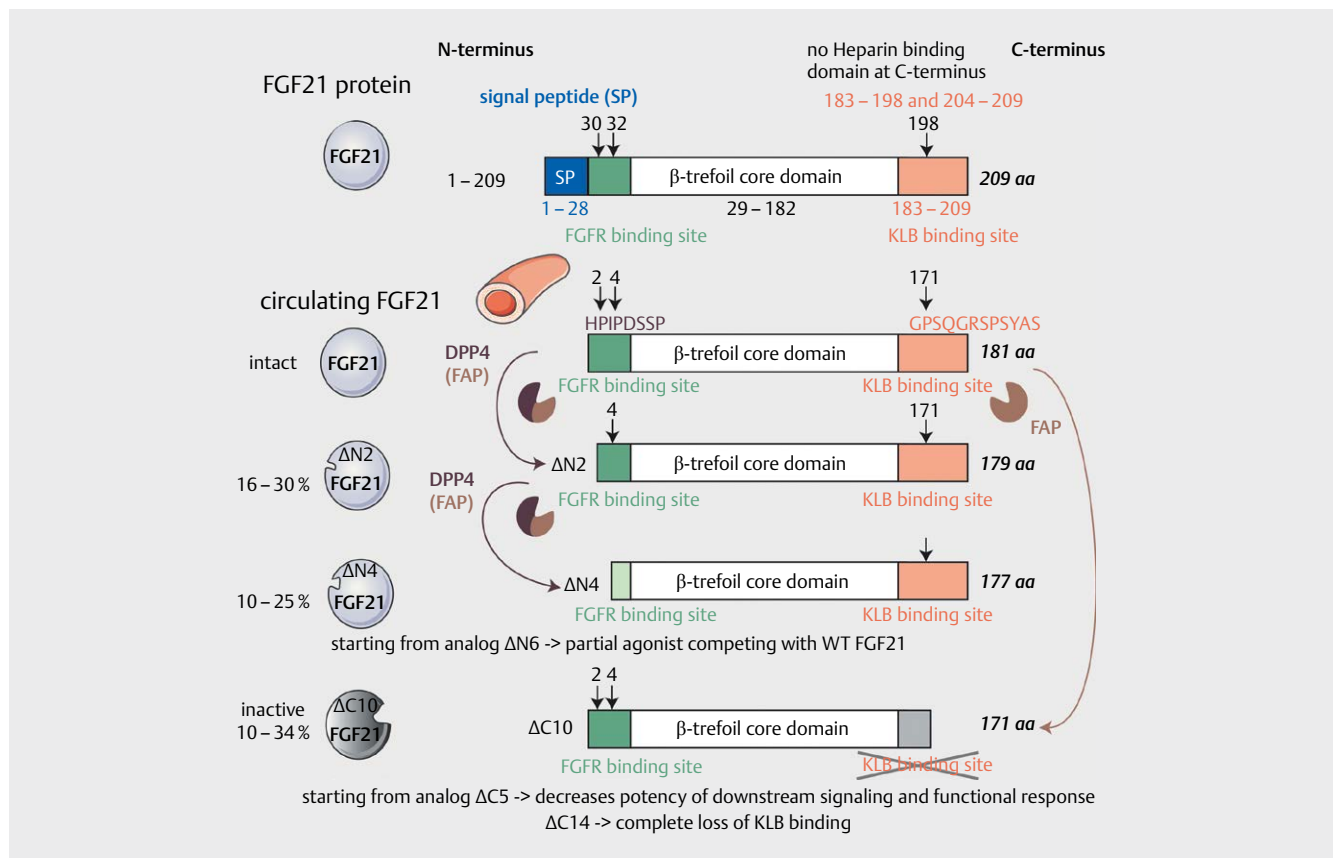
In 2016, several groups independently reported on fibroblast activation protein (FAP) that catalyzes the cleavage of FGF21 at the C-terminal end behind proline 171, cutting off 10 amino acids [171–173]. The loss of the last 10 amino acids by FAP cleavage robustly impairs KLB-binding of the remaining FGF21 protein. Thus, ΔC10-FGF21 is biologically inactive [171–173]. FAP can also cleave at two sites at the N-terminal HPIP sequence, resulting in ΔN2- and ΔN4-FGF21. This cleavage, however, does not per se render FGF21 inactive but leads to decreased potency inducing downstream signaling pathways and functional responses [174, 175]. The FGF21

variant lacking 17 amino acids at the N-terminus antagonizes FGF21 signaling, and Agrawal et al. recently showed that the minimal fragment of FGF21 to antagonize FGF21 (and FGF19) signaling consists of 25 residues at the C-terminus (containing the KLB binding site) [176, 177]. Endogenous levels of the major FGF21 degradation forms,  $\Delta$ N2-FGF21,  $\Delta$ N4-FGF21 and  $\Delta$ C10-FGF21, were estimated in vivo in human blood with abundances of 16–30%, 10–25% and 10–34% respectively [171]. The abundance in plasma samples from healthy humans of all FGF21 forms with intact N-terminus ranged from 50 to 75%, and for FGF21 with intact C-terminus ranged from 70 to 90% [171]. Another study estimated that 65% of total FGF21 was present as the active form of FGF21 [178]. Whether the relative distribution of the different FGF21 fragments in blood of donors is dependent on the donor's metabolic status should be addressed in future studies. Of note, although 40% of the N-terminal tetrapeptide (YPIP) in mouse FGF21 is clipped by FAP, the C-terminus of mouse FGF21 is protected from FAP cleavage due to G170E mutation in rodents FGF21 sequence [171]. However, it has been suggested that in mice, the cleavage at the N-terminus may represent a primary signal that affects clearance of this protein [179].

Since FAP belongs to the same di-peptidyl peptidase (DPP) sub-family as DPP4, one could hypothesize that administration of DPP4 inhibitors, in clinical use as anti-diabetic incretin-stabilizing drugs since 2007, may also stabilize FGF21 and increase its blood concentrations. This notion remains to be tested in humans, but recent mouse data using talabostat (non-selective DPP4 peptidase inhibitor) showed promising effects [179]. With the caveat of non-existing cleavage at the C-terminus of mouse FGF21 as discussed above, this effect may be attributable to the inhibition of DPP4 rather than FAP and independent of FGF21 as recently suggested [180]. Notably, FAP activity levels differ between species, with mice having ~15-fold greater FAP activity than humans [181]. Thus, using mouse models to gain insights into the metabolic role for FAP-dependent FGF21 cleavage in humans seems limited. The protein structure of human FGF21 and its secreted form together with its truncated form detectable in blood are depicted in ► Fig. 3.

### FAP as a biomarker

FAP is a serine protease that is constitutively active. It exists as a dimer located on the cell surface as well as a soluble, circulating form in the blood [182]. FAP has both dipeptidase and endopeptidase ac-



► **Fig. 3** Protein structure and potential cleavage sites of human FGF21 and its resulting truncated forms in blood. Human FGF21 consists of 209 amino acids (aa) with a signal peptide (SP) at the N-terminus. Circulating FGF21 consists of 181 amino acids (aa) and it can be cleaved at two sites at the N-terminus by DPP4 (and/or FAP), resulting in  $\Delta$ N2- or  $\Delta$ N4-FGF21, both are still able to induce intracellular signaling and function. The abundances of these two forms are estimated to range between 10 and 30% in human blood. Fibroblast activation protein a (FAP) can cleave a 10 aa fragment from the C-terminus of hFGF21 resulting in  $\Delta$ C10-FGF21 which has been estimated to account for 10–34% of total circulating FGF21 in healthy, human blood.  $\Delta$ C10-FGF21 represents the inactive form since it can no longer efficiently bind KLB, the crucial co-factor for activating intracellular signaling pathways.

tivity and besides FGF21, it can cleave denatured collagen with specificity for type I collagen,  $\alpha$ 2-antiplasmin and neuropeptides (i. e., neuropeptide Y (NPY), peptide YY, B-type natriuretic peptide and substance P) [183–185]. FAP is considered generally absent from normal adult tissues, but it is upregulated during embryogenesis and present at sites of wound healing and tissue damage such as cancers, fibrosis, and inflammation [182]. FAP is up-regulated in stromal fibroblasts in over 90% of malignant epithelial tumors but not in benign tumors [186]. Therefore FAP has been suggested as a biomarker and therapeutic target for tumor stroma [187, 188]. Other reports show “healthy” FAP<sup>+</sup>-cells which play important roles in bone marrow and muscle, questioning to target FAP<sup>+</sup>-cells as cancer therapy [189, 190]. FAP activity is very low in normal, healthy human liver, but it becomes significant in two distinct liver diseases (alcoholic liver disease and primary biliary cirrhosis) [181]. FAP levels are increased in cirrhotic livers and correlate with the histological severity of liver fibrosis [191]. FAP may be a highly promising biomarker for liver disease, as both tissue and circulating levels are low in healthy individuals, but elevated in the diseased state [181, 191], which may result in the higher ratio of inactive to active FGF21. In line, low serum FAP level may be used diagnostically to exclude severe, clinically relevant liver fibrosis in obese, diabetic patients [192]. Plasma FAP concentrations range from 50 to 250 ng/ml and FAP activity of these samples (range from 1.3 to 7 nmol/min per  $\mu$ l) appears to correlate well with FAP protein levels [171]. In the aforementioned report, no correlation between FAP protein and BMI was found, but another study reported on the correlation between the activity levels of FAP and BMI, and found higher FAP activity levels in men as compared with women [193]. FAP-deficient mice are leaner and metabolically healthier (insulin sensitive, glucose tolerant) than wildtype controls on a high-fat diet [179, 193]. Thus, elevated circulating FAP may associate with BMI and FAP may affect metabolic functions by inactivating FGF21. So far, it cannot be excluded that the phenotype is partially related to FAP's other substrates such as NPY [194]. To date, the published facts on FAP's role in metabolism and metabolic disease are too limited for drawing further conclusions in particular as there are robust differences between mice and men in the FAP-FGF21 axis (i. e., FAP activity and different amino acid sequence at the FAP-cleavage site of FGF21s C-terminus). This, however, may stimulate further research addressing FAPs role for human metabolism in the near future.

## FAP-specific proteolysis of FGF21 as a biomarker and therapeutic target

Targeting FAP to increase the active form (intact) of FGF21 has been proposed as a treatment option for metabolic disease [172, 179], but the definite determination as to whether the balance between active and inactive FGF21 matters in health and metabolic disease, remains to be resolved. Future human studies that measure total and active forms of FGF21 as well as FAP activity in relation to metabolic disease will help to shed light on this issue. In healthy, lean individuals, the plasma concentration of total and active FGF21 correlates well ( $n = 34$ ,  $r = 0.972$ ;  $p < 0.001$ ), but are expectedly lower (range of total: 42 to 462 pg/ml; range of active: 11 to 399 pg/ml) [195]. Recently, a constant ratio of intact to total FGF21 of 50% in the fasting state of lean, healthy individuals has been reported, which remained stable upon a 75g fructose challenge [196]. Recent data further indicate an impaired postprandial rise in the ratio of bioactive to total FGF21 in T2D [75] and that exercise increased circulating FAP levels but not FGF21 levels (neither total nor active) [178]. Additionally, alcohol-induced increase in total FGF21 follows the same temporal pattern as the increase in active FGF21, but the fold-change of total FGF21 is 3 times greater [51]. ELISAs that are commonly used (► **Table 4**), however, detect all forms (active and truncated). As total FGF21 ELISAs detect the intact FGF21 form with a 2-fold better efficiency than the truncated forms [172], the concentration of truncated FGF21 in human blood samples may be underestimated. These observations may become important in the view of studies suggesting N-terminally truncated FGF21 as partial agonist, binding to the KLB-FGFR complex without or with weak downstream signaling. So far, only ELISAs exist that are specific for the intact form, but ELISAs specific for the inactive and cleaved forms are still missing. The reliable determination of endogenous FGF21 in its intact or truncated forms by mass spectrometry seems to be currently limited [172], emphasizing the development of methods to distinguish FGF21 isoforms in human blood as a challenging, but rewarding future effort.

## Summary

The clinical relevance of FGF21 in humans is still unclear, owing to the lack of knowledge on the cause and consequence of higher circulating FGF21 levels in metabolic disease. FGF21 levels in human

► **Table 4** Overview of human FGF21 ELISAs.

Company	Sensitivity	intra-Assay CV	inter-Assay CV	
R&D Systems	8.69 pg/ml	<4%	<5%	
Biovendor	7 pg/ml	<5%	<5%	
Millipore	10 pg/ml	<6%	<9%	
Antibody and Immunoassay Service	30 pg/ml	4–5%	3.5–10.2%	
Cusabio Biotech	3.9 pg/ml	<8%	<10%	
Adlitteram Diagnostic Laboratories	(1–1000 pg/ml)	5.1%	7.2%	reported in Cheng et al. 2011
Abcam	30 pg/ml	4.7%	7.2%	
Eagle (total FGF21)	10.4 pg/ml	<10%	<7%	
Eagle (intact FGF21)	1.7 pg/ml	<6%	<7%	
If not stated otherwise information is according to datasheet provided by the companies				

blood display high variation (< 7pg/ml to 7 ng/ml) and are impacted by several physiological factors. So far, most important for the induction of human FGF21 levels appears to be nutrition (diet) and alcohol is the most potent inducer identified so far putatively relating to FGF21's proposed role in regulating water balance. FGF21 levels do not only correlate with several clinical parameters of the metabolic syndrome (mostly TG and liver fat content) but also with AST, a general marker of tissue breakdown. Thus, FGF21 levels may not only mark metabolic derangements in the liver but also the loss of tissue integrity. As FGF21 seems to be a general stress hormone, the examination of multiple markers of metabolic function may be required to examine the overall metabolic health of obese individuals and to determine their risk for obesity-associated metabolic disorders, besides liver fibrosis and steatosis.

An open question for future research will be to delineate the role of endogenous FGF21 and its analogues on the central nervous system, in particular how FGF21 signaling regulates, and is regulated by the SNS-WAT/BAT-energy expenditure axis in humans, how FGF21 regulates human drinking behavior and hydration levels via the beta-adrenergic signaling pathways, which indeed may act simultaneously to, or counteract RAS/RAAS and the interaction with HPA axis. In line with this, detailed studies on whether and how FGF21 mediates effects via the kidney are required, e. g., by regulating excretion of glucose as recently suggested [158].

Open topics also include the question: Why is this hormone that is known for metabolic improvements, elevated during metabolic disease? One hypothesis is obesity-induced FGF21 resistance, as obese mice and humans display reduced expression of KLB in WAT [197, 198]. This hypothesis is, however, controversially discussed [199, 200] and another report argues against FGF21 resistance and found even higher response to FGF21 in two obese mouse models [201]. Another hypothesis arises from recent data suggesting that FAP levels and its activity in human blood may add valuable information to elucidate the physiological relevance of human FGF21.

## Conclusion

The potential of FGF21 levels as a biomarker for specific metabolic derangements should be further explored, in particular if the ratio of active/total FGF21 and/or FAP levels and activity matter in human metabolic disease. Another important aspect for future research topic include the dissection of central vs. peripheral effects of FGF21, and the interaction of FGF21 with blood pressure, thirst and renal function via RAAS and HPA.

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## Conflict of Interest

Authors declare that they have no conflict of interest.

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