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Review

Effects of aerobic exercise on fibroblast growth factor 21 in overweight and obesity. A systematic review

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ABSTRACT

Fibroblast growth factor 21 (FGF21) has been suggested to improve metabolism during aerobic exercise in obesity. However, the variability of exercise interventions gives rise to discrepancies in the field. Therefore, we aimed to systematically review the available literature regarding the effects of aerobic exercise on FGF21 in the context of overweight and obesity. Our search included original articles published between 2009 and November 2021 found in PubMed, Science Direct, and Medline. Clinical and preclinical studies were included. Studies, where subjects or animals presented with other conditions (e.g., cancer, stroke), were excluded. From an initial 43 studies, 19 (clinical studies = 9; preclinical studies = 10) were eligible for inclusion in this review. The main findings were that acute exercise tended to increase circulatory levels of FGF21. In contrast, chronic exercise programs (≥4 weeks) had the opposite effect along with inducing mRNA and protein increases of FGF receptors and β-klotho in adipose tissue, liver, and skeletal muscle. In conclusion, both clinical and preclinical studies showed that aerobic exercise exerts changes in circulatory and tissue FGF21, along with its receptors and co-receptor. Future research is needed to elucidate the mechanisms, along with the physiological and clinical implications of these changes.

1. Introduction

Obesity is a global health problem characterized by an increase in adipose tissue depots, and contributing to the development of hypertension, insulin resistance, and type 2 diabetes [1]. In recent decades, scientific efforts have focused on finding specific mediators behind this relationship, designing effective therapeutic strategies and/or interventions [2].

Such mediators include fibroblast growth factor 21 (FGF21), which was first described in 2000 in the liver and thymus of mouse embryos [3]. In 2005, the metabolic functions were described such as increasing glucose uptake in adipocytes [4]. FGF21 is a protein with a small molecular weight (~20 kDa) and recognized as a key factor in energy homeostasis, given its ability to regulate glucose uptake [5] (in conjunction with insulin), stimulate fatty acid oxidation, and inhibit lipogenesis. Comprehensive reviews regarding FGF21 function are published elsewhere [6–9]. Briefly, FGF21 exerts its actions through binding with FGF receptors (FGFR1 and FGFR2), along with its obligate coreceptor β-klotho (KLB). In the absence of FGF21, FGFRs and KLB (present in the plasmatic membrane) bind to each other forming a 1:1 heterocomplex, however, in the presence of FGF21, FGFRs form a homodimer, establishing a 1:2 heterocomplex with KLB [10] changes that trigger its signalling cascade (Fig. 1). Therefore, FGF21 can act in tissues where FGFR and KLB are present, such as white adipose tissue, skeletal muscle, heart, pancreas, and brown adipose tissue [7]. Normally, the concentration of circulating FGF21 is low, however, stimuli such as fasting, stress, and metabolic disorders increase its expression and secretion mainly by the liver. However, FGF21 is also expressed in adipose

Abbreviations: FGF21, fibroblast growth factor 21; FGFR, fibroblast growth factor receptor; KLB, β-klotho; Akt, protein kinase B; VO2 peak, peak oxygen consumption; OLEFT, Otsuka Long-Evans Tokushima Fatty; mRNA, messenger ribonucleic acid; HIIT, high-intensity interval training; MICT, moderate-intensity constant training; MAPK, mitogen-activated protein kinase; ERK, extracellular response-stimulated kinase; PI3K, phosphatidylinositol 3-OH kinase; HbA1c, glycated hemoglobin; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; HFD, high-fat diet; TGR5, G-protein-coupled bile acid receptor; BMI, body mass index.

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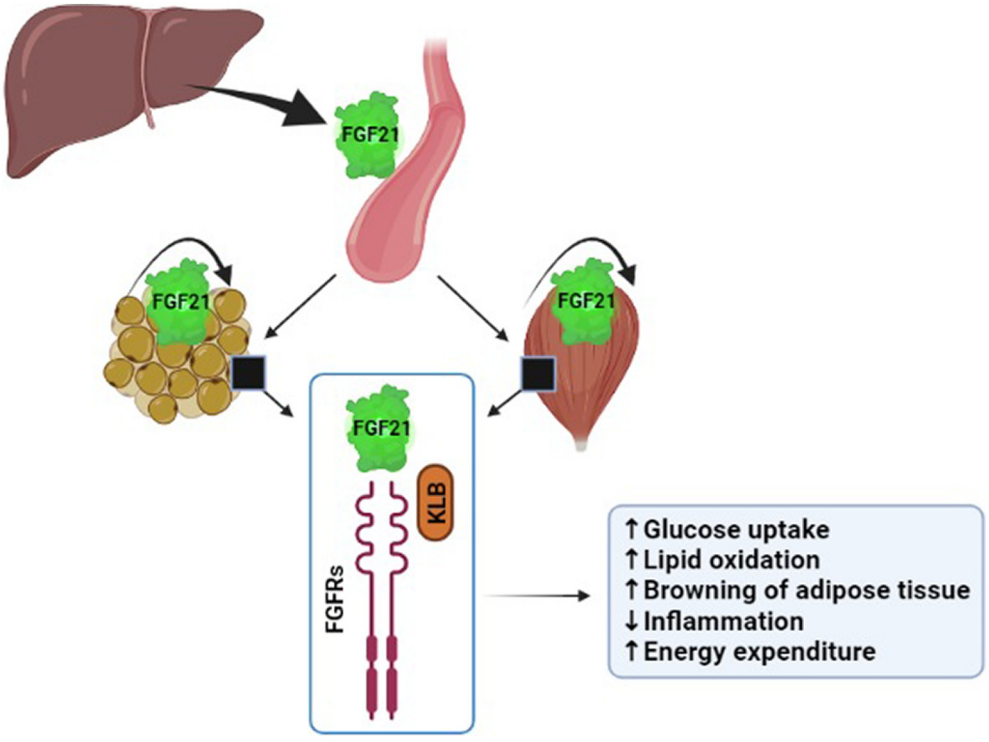

Fig. 1. Summary of FGF21 sources and its metabolic functions. FGF21 is mainly secreted by the liver into the circulation. There, it can act in tissues where FGFRs and its co-receptor KLB are present, such as adipose tissue and skeletal muscle. These tissues, also have the ability to produce FGF21 in an autocrine/paracrine manner, to further induce its metabolic functions, such as regulating glucose uptake, lipid oxidation, browning of adipose tissue, inflammation, and energy expenditure. FGF21: fibroblast growth factor 21; FGFRs: fibroblast growth factor receptors; KLB: β-klotho. Figure created in BioRender.com.

tissue, skeletal muscle, brain, and pancreas being classified as a myokine, adipokine, and hepatokine [11–13]. In that context, higher circulating FGF21 levels have been consistently reported in subjects with obesity [14] and type 2 diabetes [15], changes that have nurtured the hypothesis of the development of a FGF21 resistant state [16]. The mechanism behind this condition is elusive [17]; however, the down-regulation of FGFR and KLB in insulin-sensitive tissues has been proposed [18].

As a therapy against obesity-related metabolic disorders, the benefits of physical activity and exercise are well-described [19]. However, studies that examine the mechanisms behind those benefits are limited. There is emerging evidence that has investigated the effects of exercise on FGF21 levels and signalling pathways. For instance, Tanimura et al. investigated the acute effects of one exercise session on FGF21 levels in lean humans and mice. Interestingly, they found that aerobic exercise increased serum FGF21 concentration in both humans and mice. Moreover, increases in mRNA and protein FGF21 levels were seen in the liver and gastrocnemius muscle of mice, findings that were associated with higher levels of pAkt/Akt levels in muscle [20], suggesting that these changes are functionally relevant, particularly from a glucose uptake point of view. However, when exercise and FGF21 have been investigated in obesity and metabolic dysfunction, the outcomes differ. Indeed, Slusher et al. found a ~20% increase in circulating FGF21 in subjects with obesity after one exercise session [21]. However, Yang et al. described opposite results (>50% decrease in plasma FGF21) after 12 weeks of exercise training in overweight participants [22]. Furthermore, others have failed to describe any changes in FGF21 levels whatsoever [23–25].

Therefore, considering the discrepancies in terms of results from the studies in the field, this study aims to systematically review the evidence regarding the effects of aerobic exercise on FGF21 levels in the context of overweight/obesity, to identify the available knowledge in this field, as well as the gaps that could direct the intention of future studies.

2. Methods

Literature searches were conducted in three databases: PubMed, ScienceDirect, and Medline. Original articles published between 2009 and November 2021, written in English, were considered. Eligible studies included preclinical studies that used obesity models and clinical studies that included participants with overweight, obesity or type 2 diabetes between 18 and 60 years old. Studies where other associated conditions were included, such as cancer, heart failure, or stroke were excluded.

Searches were conducted as follows: PubMed: "Exercise" [Mesh] AND "Fibroblast growth factor 21" [Supplementary concept] AND "Overweight" [Mesh]; Science Direct: (Exercise OR physical activity OR aerobic exercise) AND (FGF21 OR FGF-21 OR fibroblast growth factor) AND (overweight OR Obesity); MEDLINE: (MM "Exercise+") AND "fibroblast growth factor 21" AND (MM "Overweight+"),

3. Results

From an initial 43 studies found, 19 articles were included in this review, from which 9 were clinical and 10 preclinical, as described in Fig. 2. It is worth mentioning that one study was added by bibliography search [26]. To facilitate the interpretation of the results from the selected studies, these were further classified depending on whether the exercise effects studied were acute (only one exercise session) or chronic (longer than 4 weeks of training).

3.1. Clinical studies

From the 9 studies included, 4 were focused on the acute effects of exercise on FGF21 levels (one exercise session), whereas 5 involved exercise training programs lasting between 5 and 12 weeks, with a prescription ranging from 3 to 5 times/week. In 6 studies, the participants

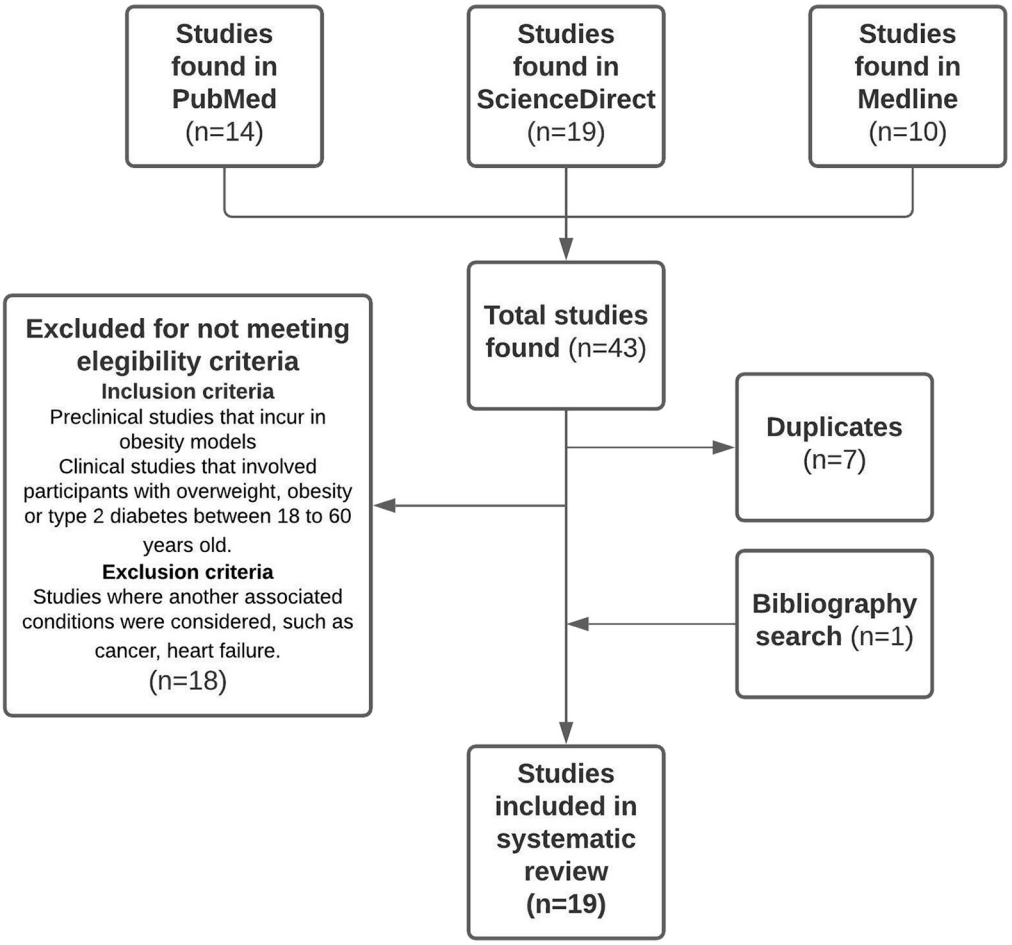

Fig. 2. Flowchart of search and article selection.

were classified as healthy and sedentary overweight/obese, whereas in three of them, subjects with overweight/obesity and type 2 diabetes were included.

For studies examining the acute effects of exercise on FGF21, two studies found a plasma FGF21 increase levels of about 20% compared with baseline values (16, 21). One of them also reported increases in vastus lateralis FGF21 mRNA levels [27]. In contrast, two studies that had similar exercise prescriptions (60% of VO_2 peak, for 60 min in cycle ergometer) did not find any significant changes [24,28].

For studies examining the chronic effects of exercise on FGF21, only one reported a significant change in plasma FGF21 where its levels decreased by over 50% compared to pre-training levels [22]. The clinical studies included in this review are listed in Table 1.

3.2. Preclinical studies

All the preclinical studies included in this review (n = 10) focused on investigating the chronic effects of exercise, where training programs lasted from 4 to 36 weeks. Four out of 10 studies in this category included voluntary wheel running as intervention [29–32], which is similar to "physical activity" with its lack of prescription (e.g. intensity, duration). Animals used in these studies were mostly C57BL/6 mice [26,30–37], one involved OLEFT rats [29], and another did not report the mice strain [31].

75% (6/8) of these studies reported decreases in circulating levels of FGF21 after exercise training (unchanged to ~20–80%) [26,29,32–35]. Three also reported decreases in liver FGF21 mRNA levels (~0.2 to 0.35-fold) [29–31], which are also the ones that involved voluntary wheel running. Two studies compared different exercise regimes (high-intensity interval training (HIIT) vs moderate-intensity constant training (MICT)), finding similar effects on circulating FGF21 between training regimes [26,35]. However, Xiong et al. analyzed FGF21 local tissue changes after HIIT and MICT, finding that the latter induced transcriptional increases in the liver, brown adipose tissue, and quadriceps. Also, a ~3.5-fold increase in FGF21 protein was seen in brown adipose tissue [35]. 40% (4/10) of studies also described exercise-induced increases in transcriptional and translational levels of FGFR1, FGFR2, and/or KLB in the liver, epididymal fat, brown adipose tissue, and skeletal muscle [29,33,35,36]. The preclinical studies included in this review, which reported chronic exercise training effects in murine models, are listed in Table 2.

A summary of the results presented here is described in Fig. 3.

4. Discussion

This systematic review aimed to analyze the available evidence, in clinical and preclinical studies, regarding the effects of aerobic exercise on FGF21 in the context of metabolic disease. The main findings indicate that the FGF21 response against exercise depends on several factors, such as length of intervention (acute vs chronic) and where FGF21 was measured (circulation vs tissues). Remarkable, single exercise sessions were sufficient to increase circulatory FGF21 levels. However, studies involving exercise training programs (mostly preclinical) reported decreases in plasma/serum FGF21, decreases in the transcriptional and translational levels of liver FGF21, along with increases in the mRNA and protein levels of FGFRs and its co-receptor KLB (Fig. 1).

Table 1
Clinical studies (n = 9).

A/C	Authors	Year	Participants	Exercise program	Changes in FGF-21	Secondary outcomes
A	Slusher et al. [21]	2015	24 healthy and sedentary men and women. Mean age: 22.7 years old. Mean BMI: 35.5 kg/m^2	Treadmill One session of 30 min at 75% of VO_2max	↑ Plasma (1.2-fold)	↑ Plasma IL-6 (1.2-fold)
A	Sargeant et al. [28]	2017	11 healthy, sedentary, or moderately active men and women. Mean age: 45 years old. Mean BMI: 29.2 kg/m^2	Treadmill. One session of 60 min at 60% of VO_2peak.	↔ Plasma	↔ Plasma follistatin ↔ Plasma LECT2 ↔ Fetuin A
A	Sabaratnam et al. [27]	2018	13 males with type 2 diabetes without complications. Mean age: 55.4 years old. Mean BMI: 29.7 kg/m^2	Cycle ergometer. One session of 60 min at 70% of VO_2max.	↑ Vastus lateralis mRNA (~1.5-fold) ↑ Plasma (~1.2-fold) ↓ Plasma (0.75-fold 3 h post-exercise)	↑ Vastus lateralis IL-6 mRNA (~50-fold) ↑ Plasma IL-6 (3.5-fold) ↑ Vastus lateralis ANGPTL4 mRNA (6-fold and 12-fold at 3 h recovery) ↑ Plasma ANGPTL4 (1.2-fold and 2-fold at 3 h recovery) ↑ Vastus lateralis CYR61 mRNA (5-fold)
A	Garneau et al. [24]	2020	11 healthy and sedentary women. Mean age: 30.0 years old. Mean BMI: 35.4 kg/m^2	Cycle ergometer 60 min at 60% VO_2peak	↔ Plasma	↑ IL-6 (1.5-fold)
C	Yang et al. [22]	2011	40 healthy and sedentary women. Mean age: 45.3 years old. Mean BMI: 27.6 kg/m^2	Treadmill and cycling 12 weeks of combined training. Aerobic component: 45 min at 60–75% HRmax, 5 days/week, for 12 weeks.	↓ Plasma (0.45-fold)	↓ Plasma TGs ↓ BMI ↓ Systolic and diastolic blood pressure
C	Basse-Patin et al. [23]	2014	11 healthy and sedentary men Mean age: 35.4 years old. Mean BMI: 32.6 kg/m^2	Cycling and running 8 weeks, 5 times/week 85% VO_2max, 45–60 min	↔ Plasma	↑ Apelin (~2-fold mRNA Vastus lateralis) ↔ Adiponectin ↔ Leptin ↔ IL-6
C	Kong et al. [25]	2016	18 healthy and sedentary men and women. HIIT group Mean age: 19.8 years old Mean BMI: 25.5 kg/m^{2}MICT group Mean age: 19.9 years old Mean BMI: 26.2 kg/m^2	HIIT Cycle ergometer, 60 cycles of 8 s at 90% of VO_2peak intercalated by 12 s of active restMICT Cycle ergometer, 40 min at 65% VO_2peakBoth training programs for 5 weeks, 4 times/week.	↔ Serum	↑ VO_2peak (HIIT and MICT) ↔ Body composition ↔ Leptin ↔ Blood glucose
C	Banitalebi et al. [50]	2019	14 men and women with type 2 diabetes and sedentary. Mean age: 55.4 years old. Mean BMI: 29.3 kg/m^2	Cycle ergometer. 10 weeks, 3 times/week. 4 bouts of 30 s at all-out intensity	↔ Serum	↓ IL-15 ↓ IL-6 ↓ Insulin ↓ HbA1c ↓ Fasting glucose
C	Motahari Rad et al. [51]	2020	30 males with type 2 diabetes, sedentary, without diabetic complications. Mean age: 44.4 years old Mean BMI: 29.3 kg/m^2	Concurrent training Aerobic component: Treadmill walking/running, 10 min at 75–95% HRmax. 3 sessions per week, for 12 weeks.	↔ Serum	↑ Serum irisin (1.2-fold) ↑ Serum follistatin (1.1-fold) ↓ Serum myostatin (0.75-fold)

Abbreviations: BMI: body mass index; HRmax: maximum heart rate; TGs: triglycerides; VO_2max: maximum oxygen consumption, IL-6: interleukin 6; HIIT: high-intensity interval training; MICT: moderate-intensity constant training; VO_2peak: peak oxygen consumption; LECT2: leukocyte cell-derived chemotaxin-2; ANGPTL4: angiopoietin-like 4; CYR61: cysteine-rich angiogenic inducer 61; IL-15: interleukin 15; HbA1c: glycated hemoglobin; ↑: increase; ↓: decrease; ↔: no change.

In terms of exercise training protocols, the most recurrent modalities in clinical studies were treadmill running and cycling, with 1 (acute) or 3–5 sessions per week (chronic), 30–60 min per session, from 5 to 12 weeks. In preclinical studies (mice or rats), only chronic effects of exercise were investigated, where voluntary wheel and treadmill running were the most recurrent modalities, 2–5 sessions per week, 20–60 min per session, from 4 to 36 weeks.

Since it was first described, FGF21 has gained attention from the scientific community given its role in energy homeostasis by, for instance, promoting free fatty acid oxidation and glucose tolerance [3,4]. Therefore, results described from early studies regarding the FGF21 levels in the context of obesity were somewhat surprising. Zhang et al. in 2008 were one of the first to describe higher circulatory FGF21 levels in subjects with overweight/obesity, compared to normal-weight controls. Moreover, these results were consistent with the ~3–4 fold increases in Fgf21 mRNA levels in liver and adipose tissue of *db*/*db* mice [14], suggesting that this response might be compensatory for the obesity/related metabolic disturbances, or a sign for possible FGF21 resistance development, a hypothesis which was subsequently confirmed by Fisher et al. proposing that obesity promoted a FGF21 resistant state [16]. More recent studies in animal models have described that subcutaneous injections with recombinant human FGF21 were enough to improve glucose tolerance in HFD-fed mice [38], highlighting that further increases in circulating levels of FGF21 levels during obesity might still confer metabolic benefits. Interestingly, significant increases of circulating, skeletal muscle, and liver FGF21, has been previously described in lean mice and humans after a single exercise bout [20], findings that are concurrent with the results reported in two different studies included in this review, where a ~20% increase in FGF21 circulatory levels in humans after a single session of aerobic exercise at a moderate intensity [21,27], changes that were concurrent with FGF21 mRNA increases in vastus lateralis muscle [27]. This could indicate that even when obesity triggers a compensatory metabolic response by increasing FGF21 secretion, exercise can induce a summative response. The physiological implications/relevance of these changes is still unknown, given that no concurrent signalling pathway measurements (e.g. MAPK, ERK, Akt-

Table 2
Preclinical studies (n = 10).

A/C	Authors	Year	Animal	Exercise program	FGF-21	Secondary outcomes
C	Fletcher et al. [29]	2012	OLEFT rat (4-weeks old; n = 7–8)	Voluntary wheel running 36 weeks.	↓ Serum (~0.2-fold) ↓ Liver mRNA (~0.35-fold) ↓ Liver protein (~0.5-fold)	↑ FGFR2 hepatic mRNA (1.6-fold)
C	Hao et al. [30]	2016	C57BL/6 male mice (4 weeks old; n = 9) fed with HFD (60% kcal from fat) for 16 weeks.	Voluntary wheel running 16 weeks.	↓ Liver mRNA (~0.2-fold)	↓ Body weight ↓ Liver TGs
C	Loyd et al. [31]	2016	Mice (strain not reported - 10 week-old; n = 5) fed with HFD (fat content not reported) for 12 weeks.	Voluntary wheel running 16 weeks.	↔ Plasma ↓ Liver mRNA (~0.35-fold)	↓ Liver mass ↓ Liver cholesterol ↓ Liver TGs
C	Geng et al. [33]	2019	C57BL/6 male mice (10 week-old; n = 5–12) fed with HFD (45% kcal from fat) for 12 weeks.	Treadmill running. 10° inclination, 5 days/week, for 4 weeks at 12 m/min, 20 min.	↓ Serum (~0.4-fold) ↓ Liver mRNA (~0.2-fold)	Epididymal fat ↑ FGFR1 protein (~4-fold) ↑ KLB protein (~3-fold)Brown fat ↑ FGFR1 protein (~3-fold) ↑ KLB protein (~5-fold)
C	Yang et al. [36]	2019	C57BL/6 male mice (8 week-old; n = 10) fed with HFD (60% kcal from fat) for 12 weeks.	Treadmill running. No inclination. 5 days/week at 12 m/min for 50 min, for 12 weeks.	↔ Serum ↔ Liver mRNA ↑ WAT mRNA (1-fold)	↑ Serum total adiponectin (1.8-fold) ↑ Serum HMW adiponectin (2-fold) ↑ Epididymal fat adiponectin mRNA (2-fold) ↑ Epididymal fat KLB mRNA (3-fold) ↑ Epididymal fat FGFR1 mRNA (2-fold) ↑ Epididymal fat FGFR2 mRNA (4-fold)
C	Martínez-Huenchullán et al. [26]	2019	C57BL/6 male mice (10 week-old; n = 10–12) fed with HFD (45% kcal from fat) for 20 weeks.	Treadmill running. No inclination. 3 days/week for 10 weeks. HIIT 8 bouts at 20 m/min (2.5 min each) intercalated by active rest periods at 10 m/min (2.5 min/each).MICT 15 m/min for 40 min.	↓ Plasma (0.5-fold, for both HIIT and MICT)	↓ Liver collagen 1 protein (0.7-fold, only MICT)
C	Xiong et al. [35]	2020	C57BL/6 male mice (3 week-old; n = 6) fed with HFD (60% kcal from fat) for 20 weeks.	Treadmill running. No inclination. 5 days/week, for 8 weeks. HIIT: 10 bouts at 25–27 m/min (1 min each) intercalated by active rest periods at 14–16 m/min (2 min each). MICT: 14 m/min for 45 min.	↓ Serum (0.8-fold, both MICT, and HIIT) ↑ Liver mRNA (12-fold, only MICT) ↑ BAT mRNA (10-fold, only MICT) ↑ BAT protein (3.5-fold, only MICT) ↑ Quadriceps mRNA (10-fold, only MICT)	↑ Liver KLB mRNA (5-fold, only MICT) ↑ BAT KLB mRNA (14-fold, only MICT) ↑ Quadriceps KLB mRNA (40-fold, only MICT) ↑ Liver KLB protein (3.5-fold, only MICT) ↑ BAT protein (2.5-fold, only MICT) ↑ Quadriceps KLB protein (2-fold, only MICT)-
C	Wang et al. [34]	2021	C57BL/6 male mice (4 week-old; n = 6) fed with HFD (60% kcal from fat) for 20 weeks.	Treadmill running. No inclination. 5 days/week, for 8 weeks at 14 m/min, 60 min per session.	↓ Serum (0.8-fold)	↓ Serum KIM-1 (0.8-fold)
C	Power Guerra et al. [32]	2021	C57BL/6 female mice (4 week-old; n = 13) fed with HFD (60% kcal from fat) for 12 months.	Treadmill running. No inclination. 2 days/week, for 36 weeks at 60% of MRC, 60 min per session.	↔ Plasma	–
C	Claycombe-Larson et al. [37]	2021	C57BL/6 female mice (8 week-old; n = 5–8) fed with HFD (45% kcal from fat) for 12 weeks.	Voluntary wheel running. 10,000 m/day maximum, for 12 weeks.	↔ WAT protein	–

Abbreviations: OLEFT rat: Otsuka–Long–Evans–Tokushima Fatty rat; FGFR2: fibroblast growth factor receptor 2; HFD: high-fat diet; TGs: triglycerides; FGFR1: fibroblast growth factor receptor 1; KLB: β-klotho; HMW: high molecular weight; WAT: white adipose tissue; HIIT: high-intensity interval training; MICT: moderate-intensity constant training; BAT: brown adipose tissue; KIM-1: kidney injury molecule-1; MRC: maximum running capacity; ↑: increase; ↓: decrease; ↔: no change.

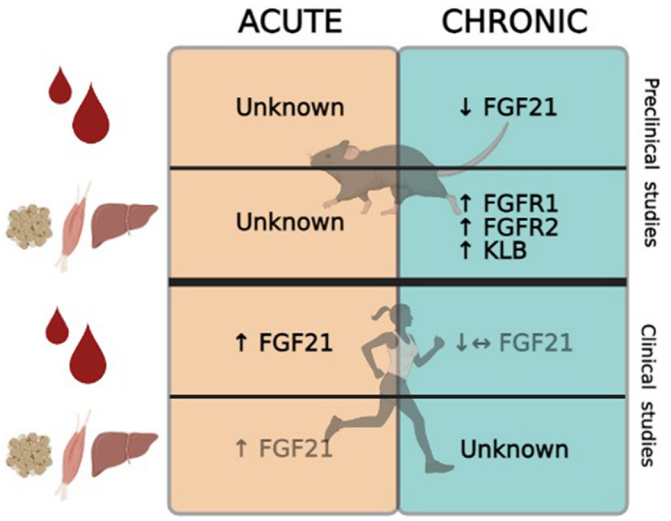

Fig. 3. Summary of the effects of aerobic exercise on fibroblast growth factor 21 (FGF21), its receptors, and co-receptor. The figure is divided by acute (left) and chronic (right) effects of exercise on circulating (blood vessel) and tissue (adipose, muscle, and liver) levels of FGF21, FGFRs, KLB, found in preclinical (upper half) and clinical (lower half) studies. The faded text indicates that incipient data suggest that change. ↑: increase; ↓: decrease; ↔: no change; FGFR1: fibroblast growth factor receptor 1; FGFR2: fibroblast growth factor receptor 2; KLB: β-klotho. Figure created in BioRender.com.

PI3K) have been conducted to date. In the future, exploration of these pathways using, for example, clinical designs where circulatory and tissue-specific changes of FGF21 after exercise (acute and/or chronic) are concurrently measured could clarify the clinical impact of the different acute changes promoted by exercise in an obesity state. In this context, an advantage when comparing studies focused on the acute effects of exercise on circulatory FGF21 is that the methodology used for sample extraction and protein measurements (serum/plasma and ELISA-based experiments respectively) is comparable between them, increasing the reproducibility of the results presented here. However, there is a fair amount of variability in terms of timepoints chosen when measuring circulatory FGF21, ranging from immediately after the exercise bout [21,24,27,28] to 24 h after [24]. Among the studies reviewed here, FGF21 increases were seen immediately after [21,27,28], 30 min [28], and 1 h [21] after the exercise bout, whereas significant decreases have been described after 3 h during the recovery period [27]. Interestingly, no changes have been described at 12 and 24 h after exercise [24], therefore, the measurement times after the exercise bouts is a sensible factor that has to be taken into account when analyzing acute effects of exercise on circulatory FGF21.

Preclinical studies have been particularly focused on the chronic effects of exercise on FGF21. In general, a decrease in circulatory FGF21 levels has been reported after exercise [26,33–35] and physical activity-based [29] interventions. This change might seem counterintuitive considering the effects of single sessions of exercise on FGF21 circulating levels which depend mainly on the secretion by the liver [6,7], a phenomenon that is associated with liver stress and/or damage in subjects with obesity [15,16]. Interestingly, 40% (4/10) studies included in this review agreed that exercise/physical activity-induced transcriptional and/or translational reductions of liver FGF21 [29–31,33], which were also associated with lower liver triglycerides [30,31], findings that could indicate that reductions of circulatory FGF21 could be associated with lower obesity-related hepatic steatosis. In agreement with these findings, Tok et al. recently described the effects of diet with or without physical activity on the circulatory levels of several myokines and adipokines, including FGF21, in overweight adults with impaired glucose metabolism. They described that diet, only when paired with physical activity, induced a significant reduction of circulatory FGF21, compared with diet alone, even when both interventions had similar effects on fasting glucose, HbA1c, total cholesterol, and triglycerides [39]. This highlights a potential differential effect of exercise on FGF21 over diet in an obesity context. However, the mechanism behind this hypothesis is elusive, therefore, future studies should be undertaken.

In parallel, chronic exercise also induced changes in adipose tissue, liver, and skeletal muscle, and several studies reviewed here reported increases in mRNA and protein levels of FGFRs and KLB [29,33,35,36], findings that could be associated with a normalization of the previously described FGF21 resistance [16]. These results are particularly interesting considering the physiological relevance of KLB in FGF21 signalling and its derived metabolic effects. In this regard, recently Moure et al. described a reduced thermogenic response to β-adrenergic activation with an adrenergic agonist, in brown and beige adipocytes from mice with total (KLB-null) or partial (KLB-heterozygotes) ablation of KLB, a response that was similar to what was reported from brown and beige adipocytes from FGF21-null mice [40]. Moreover, FGF21-null mice had increased body weight with excessive adiposity, higher serum cholesterol, insulin resistance, and hyperglycemia [41]. In contrast, the relevance of circulatory levels of FGF21 on adipose tissue is challenged by a similar study, where the inguinal adipose tissue from mice with a specific FGF21 ablation on the liver (which eliminates circulatory FGF21) was still responsive to β-adrenergic activation browning [42]. Thus, these findings highlight that FGF21 signalling, particularly in adipose tissue, has a relevant autocrine feature. Interestingly, FGF21 has been considered as a key regulator of the differentiation to brown adipocytes where, following exposure to exercise, FGF21 induces upregulation of local peroxisome proliferator-activated receptor gamma co-activator.

(PGC)-1-alfa promoting thermogenesis in adipose tissue and skeletal muscle [41].

In the liver, the relevancy of FGF21 signalling has also been highlighted given that FGF21-null mice have worsened fibrosis and hepatocellular carcinoma (HCC) incidence rates after 52 weeks of high sucrose high fructose diet, findings that emphasize the protective role of FGF21 in liver metabolism in an obesogenic context [43]. Complementarily, in children with NAFLD, the KLB variant rs17618244, which is related to lower levels of circulatory KLB, is also associated with a higher risk of ballooning and lobular inflammation [44], results that again link KLB with metabolic health in obesity-related impairments. However, surprisingly, protection against HFD-induced obesity has been described in KLB-null mice, where crosstalk between the liver, microbiota, and brown adipose tissue has been hypothesized [45], stating that the higher hepatic production of cholic acid in these mice is associated with the accumulation of microbiota-derived deoxycholic acid, which in turn induces the activation of TGR5 receptor, a process that stimulates thermogenic activity in brown adipose tissue. Considering these somewhat contradictory results, further research should be conducted to fully understand the effects of exercise on FGF21 signalling, particularly in the liver.

In skeletal muscle, the literature regarding FGF21 signalling during obesity and exercise is limited. Early studies pointed out that elevated circulatory levels of FGF21 were associated with muscle insulin resistance in humans [46]. Later, Yeon et al. investigated levels and signalling of FGF21 in skeletal muscles from subjects with type 2 diabetes or impaired glucose tolerance, finding that even when the protein levels of FGF21 were higher than the vastus lateralis of healthy volunteers, the phosphorylated FGF21 receptor levels were lower. Moreover, they reported similar results from the soleus muscle of diet-induced obese mice where higher FGF21 proteins levels were found, concomitantly with lower levels of KLB [47]. Therefore, the previously described exercise-derived translational increases in muscle KLB [35] are promising, however, further studies are required to test if these changes are physiologically and clinically significant.

Lately, a strong neuroprotective role for FGF21 has been postulated, which is interesting considering the brain expression of this factor and also its ability to cross the blood-brain barrier by simple diffusion [11]. The crosstalk between FGF21 and autophagy is one of the postulated pathways explaining this protective role but FGF21 may also role as a modulator of neuroinflammation and oxidative stress [48,49]. Whether

exercise-induced modifications to circulate levels of FGF21 are a way to explain the beneficial effects of exercise in preventing/delaying neurodegenerative disorders as well as the signalling pathways that regulate these effects is a matter for investigation.

Moreover, when possible, potential effects of external interventions (e.g. exercise) on FGF21 signalling should be studied considering both, circulatory and tissue-specific levels of FGF21, along with its receptors and co-receptor, to have a deeper understanding of the potential metabolic effects of those interventions. Finally, it is worth considering that most of the tissue-specific exercise-derived changes present here were derived from studies conducted in animals (rats and mice), therefore these results should be carefully interpreted.

The strengths of this systematic review are related to the expansion from only analyzing changes in the circulatory levels of FGF21 to its transcriptional and translational changes in tissues, along with the effects of exercise on FGF21 receptors and co-receptor, which are key for its biological functions triggering, moreover, the differentiation between acute and chronic effects of exercise also contributes to organize the information available and identify the potential gaps in the field. However, this review has a few limitations due to variability in exercise prescriptions and the heterogeneity in patient cohorts. These include that exercise prescription differs significantly in terms of intensity, duration, and frequency, hindering the possibility of extrapolating these findings into a broader scenario, even more when some studies used physical activity (wheel running) as opposed to a forced exercise regimen. Moreover, the age (from young to almost older adults) and BMI of the participants (from slightly overweight to severely obese >35 kg/m^2) in the clinical studies also have wide variability, which could explain the lack of agreement between studies with similar interventions. Even considering these weaknesses, the results presented here could be useful to further support the relevancy of implementing aerobic exercise training programs for the management of metabolic dysfunctions derived from overweight and obesity, even in the absence of dietary interventions focused on body weight reduction/normalization.

This is the first known systematic review that aims to elucidate the effects of aerobic exercise on FGF21 in a context of overweight/obesity, which highlights the need for further efforts in the field, considering that the most consistent finding was located locally in active metabolic tissues (e.g., liver, adipose tissue, skeletal muscle), instead of the circulatory levels of FGF21. Moreover, FGF21 receptors and co-receptor, instead of FGF21 itself could be the factors that more actively react to exercise, a hypothesis that needs further clarification in the future.

In conclusion, findings in clinical and preclinical studies support that aerobic exercise exerts changes in circulatory and tissue FGF21, along with its receptors and co-receptor. Interestingly, these responses are dependent on the length of the intervention (acute vs chronic). Therefore, this factor must be considered when studying the metabolic effects of exercise, particularly in terms of FGF21 expression and signalling in the context of overweight/obesity. Future studies should focus on testing different exercise prescriptions (e.g., HIIT vs MICT) to find which subtype of aerobic exercise exerts the highest benefits in subjects with metabolic dysfunction.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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