Exercise-stimulated FGF23 promotes exercise performance via controlling the excess reactive oxygen species production and enhancing mitochondrial function in skeletal muscle

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Exercise-stimulated FGF23 promotes exercise performance via controlling the excess reactive oxygen species production and enhancing mitochondrial function in skeletal muscle

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Abstract

Objective: Physical exercise induces many adaptive changes in skeletal muscle and the whole body and improves metabolic characteristics. Fibroblast growth-factor 23 (FGF23) is a unique member of the FGF family that acts as a hormone regulating phosphate metabolism, calcitriol concentration, and kidney functions. The role of FGF23 in exercise and skeletal muscle is largely unknown yet.

Materials and Methods: C57BL/6J mice were exercised on a motor treadmill. Mice serum FGF23 levels; FGF23 mRNA expression in various organs including the liver, heart, skeletal muscle tissue, and thyroid; and FGF23 receptor Klotho mRNA expression were examined using enzyme-linked immunosorbent assay, real-time polymerase chain reaction, and immunoblotting, respectively, after a single bout of acute exercise (60 minutes), exhaustive exercise, and chronic prolonged exercise (60 minutes every day for one week). C57BL/6J mice were injected with recombinant FGF23 (100 mg/kg, twice per day, i.p.) or vehicle control (saline) for 3 days, and then the exercise performance, reactive oxygen species (ROS), H_2O_2 production, and mitochondrial functional biomarkers in muscle (gene expression of sirtuin 1, PPAR-δ, PGC-1α and mitochondrial transcription factor A [TFAM], and citrate synthase activity) were assayed.

Results: Three forms of exercise, acute exercise, exhaustive exercise, and chronic exercise, increased serum FGF23 levels. However, only chronic exercise upregulated FGF23 mRNA and protein expression in skeletal muscle. FGF23 mRNA expression in

the heart, liver, and thyroid was not affected. FGF23 protein was mainly located in the cytoplasm in skeletal muscle tissue and the localization of FGF23 was not altered by exercise. Exogenous FGF23 treatment significantly extended the time to exhaustion and reduced the exercise-induced ROS and H_2O_2 production. FGF23 treatment increased the mRNA level of PPAR-δ and citrate synthase activity, but did not influence the mRNA expression of sirtuin 1, PGC-1α, and TFAM in skeletal muscle.

Conclusion: These results demonstrate that exercise-stimulated FGF23 promotes exercise performance via controlling the excess ROS production and enhancing mitochondrial function in skeletal muscle, which reveals an entirely novel role of FGF23 in skeletal muscle.

Keywords: FGF23; exercise; skeletal muscle; PPAR-δ; mitochondrial function

Abbreviation list

DCFH, 2',7'-dichlorofluorescein-diacetate

FGF, fibroblast growth factor

PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α

PPAR-δ, peroxisome proliferator-activated receptor-δ

ROS, reactive oxygen species

TFAM, mitochondrial transcription factor A

1. Introduction

Over the last 30 years, the combination of both a sedentary lifestyle and excessive food availability has led to a significant increase in the prevalence of obesity and aggravation of rates of type 2 diabetes mellitus. Physical exercise is one of the most effective therapeutic strategies for metabolic disorders [1]. Exercise induces many physiological changes, including improving metabolic status, enhancing insulin sensitivity, and reducing risk of cardiovascular disease [2-5]. However, physical exercise may induce excess reactive oxygen species (ROS) in skeletal muscle, which may lead to muscle damage [6]. Thus, understanding the exercise-induced intramuscular and whole-body responsive adaptations is beneficial for alleviating the unfavorable effects of exercise, and it may help to develop new and more effective means in combating metabolic and cardiovascular disorders.

Fibroblast growth factors (FGFs) are a large family of secreted factors composed of at least 23 members, some of which exist in different isoforms. They are structurally related and characterized by high affinity to heparin[7]. FGFs play critical roles in regulation of metabolism and endocrine function [8-10]. FGF23 was identified as the last member of the FGF family. FGF23 is a unique member of the FGF family because it acts as a hormone that derives from bone[11]. Circulating FGF23 regulates serum phosphorus, calcitriol concentration, and kidney functions, whereas most other FGF family members are thought to regulate various cell functions at a local level[11]. Gene knockout of FGF23 in mice induces aging-like features, including

shortened life span, growth retardation, hypogonadism, cognition impairment, hearing loss, vascular calcification, and cardiac hypertrophy[11]. Further, FGF23 has been found to be a potential biomarker in cardiovascular and renal diseases, besides its role in phosphate homeostasis and bone biology. High blood FGF23 level is associated with chronic kidney diseases [12] and coronary artery disease [13]. Moreover, FGF23 increases distal renal tubular Na^+ uptake and leads to volume expansion [14], and underlies some metabolic action of leptin [15]. Nevertheless, the role of FGF23 in skeletal muscle was rarely investigated, although previous studies have shown the expression of FGF23 in skeletal muscle tissue [16].

Thus, we speculated that FGF23 might play an important role in skeletal muscle. In the present study, we examined the effects of three forms of physical exercise on serum FGF23 concentrations and FGF23 expression in skeletal muscle in mice. Moreover, we evaluated the effects of FGF23 treatment on exercise endurance, intramuscular ROS/H_2O_2 production, and several mitochondrial function-related markers (including sirtuin 1, peroxisome proliferator-activated receptor [PPAR]-δ, peroxisome proliferator-activated receptor γ coactivator 1 α [PGC-1α], mitochondrial transcription factor A [TFAM], and citrate synthase activity) in mice.

2. Materials and Methods

2.1 Animals

The C57BL/6J mice were purchased from the Animal Center of our University. They were maintained in an animal facility and cared for in accordance with the institutional guidelines for animal welfare. All experiments on mice were approved by the Institutional Animal Care and Use Committee of Tongji University.

2.2 Physical exercise

Exercise training was performed on a motor treadmill at a speed of 5 m/min for 10 min and then increased by 5 m/min to a maximum speed of 20 m/min. Three forms of exercise (acute, exhaustive, and moderately chronic) were applied. For a single bout of acute exercise, the treadmill exercise lasted for 60 min. For exhaustive exercise, the treadmill exercise lasted until exhaustion was observed in the mice. *Exhaustion* was defined as the inability of the animal to remain on the treadmill despite mechanical prodding. The time to exhaustion was recorded and considered to be an index of exercise endurance performance. For moderately chronic exercise, the mice were exercised for one week (60 min/ per day).

2.3 Blood and tissue sampling

Before and after exercise training (one week), mice were fasted for 8h and were then anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Their blood (0.5 ml) was

obtained, and skeletal muscle tissues (gastrocnemius and gluteus maximus) were carefully isolated and washed in phosphate-buffered saline (PBS) 3 times to remove blood. Separated samples were frozen at –80^{o}C for subsequent determination.

2.4 Blood parameter determination

Serum glucose measurement was made with Ames One Touch Glucometer (LifeScan, Johnson and Johnson, New Brunswick, NJ). Serum glycerol was determined by using the Colorimetric Assay Kit (Cayman, Cayman chemical (Ann Arbor, MI)). Serum insulin was measured using an insulin enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden) with a negligible cross-reaction with C-peptide of <0.01%, sensitivity<6 pmol/l. The serum FGF23 concentration was determined using the FGF23 ELISA kit (Millipore, Bedford, MA) according to the instructions from the manufacturer. The optical density was read at 450 nm to determinate the standard curve.

2.5 FGF23 Treatment

For FGF23 treatment, recombinant FGF23 (FGF23, 100 mg/kg/day, Novoprotein Scientific, Short Hills, NJ) and vehicle control (saline) were given intraperitoneally twice daily to C57BL/6J mice for 3 days. The animals were not allowed to exercise during the FGF23 treatment. Three days later, the two groups of mice were subjected to treadmill exercise to assess their endurance performance, and then anesthetized

for tissue sampling to assay intramuscular ROS production. In another set of experiments to evaluate the effect of FGF23 treatment on sirtuin 1, PPAR-δ, PGC-1α, and gene expression, the mice were given FGF23 for 3 days and then anesthetized for tissue sampling without exercise.

2.6 Real-time PCR

Real-time quantitative PCR was performed as described previously[17, 18]. Total RNA for real-time quantitative RT-PCR was isolated from frozen tissues using RNAiso Reagent (TaKaRa, Tokyo, Japan) and reverse-transcripted into the double-strand cDNAs. The primers for FGF23, sirtuin 1, PPAR-δ, PGC-1α, TFAM, and β-actin are listed in **Table 1**. Quantification of mRNA was performed using the ABI Prism 7500 (Applied Biosystems) with PrimeScript™ RT-PCR Kit (TaKaRa, Tokyo, Japan)[19]. The specificity of the real-time quantitative PCR assays was assessed by melting point analysis and gel electrophoresis. The relative quantities of FGF23 were calculated using the ΔCT method[20]. β-actin was used as a housekeeping gene for real-time PCR.

2.7 Immunochemistry

Immunochemistry in skeletal muscle tissue was performed as described previously[21, 22]. The frozen skeletal muscle tissue was cut into sections (6μM). Frozen sections were fixed with buffer containing 4% paraformaldehyde and 0.1%

Triton X-100 at room temperature for 20 min and then blocked by 4% normal goat serum, followed by incubation with primary antibody against FGF23 (sc-50291, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at 37°C for 4 h, and incubation with Alexa Fluor 488-conjugated secondary antibody. DAPI was used to stain nuclei[23]. Fluorescence density was measured with Olympus FV1000 confocal microscopy and analyzed with FV1000 software (Olympus, Japan).

2.8 Immunoblotting

Immunoblotting analyses were performed as described previously[24, 25]. Tissues or cells were lysed with RIPA buffer with protease inhibitors (PMSF, 2 mmol/L; leupeptin, 50 μg/ml; pepstatin A, 5 μg/ml; and dithiothreitol (DTT, 2 mmol/L) and phosphatase inhibitor cocktail 2 (Sigma). After determination of protein concentration using the Enhanced BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Haimen, China), a ~30 μg sample was loaded and subjected to 10% SDS-PAGE, and transferred onto PVDF membranes at 100V for 1 h. After being blocked in blocking buffer with 5% (w/v) nonfat milk and 0.1% (v/v) Tween 20 in PBS for 4 hours, the membrane was incubated with specific primary antibody (FGF23, 1: 500 dilution; β-actin, 1:2000 dilution) and washed three times by PBST (5 min each). Then, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Quantitation of blots was carried out using the Quantity One software system (Bio-Rad Laboratories, Inc.). Each experiment was repeated at least three

times. β-actin was used as a loading control.

2.9 ROS production

2',7'-Dichlorofluorescin (DCFH) was used as a fluorescent probe to measure the ROS according to the method described previously with some modification[26]. About 1 mg protein of muscle homogenate was added to 50 μl (1.25 mM) methanol with 5 μM DCFH-diacetate, and then incubated at 37°C for 30 min to allow the DCFH-diacetate to cross the membrane and be cleaved by esterase. The excitation wavelength of 488 nm and emission at 530 nm were recorded to calculate the ROS level based on the standard curve set by a group of known DCF concentrations. The level of H_2O_2 was evaluated using Hydrogen Peroxide Assay Kit (Abcam, Cambridge, MA) according to the manufacturer's instruction. In the presence of horseradish peroxidase, the OxiRed Probe reacts with H_2O_2 to produce a product with color (λmax = 570 nm)[27].

2.10 Citrate synthase activity

Citrate synthase activity was determined using a commercial kit from Abcam (Cambridge, UK) as described previously[28, 29]. Frozen skeletal muscle samples were thawed and homogenated with extraction buffer from the kit. Then, assays were performed according to the manufacturer's instruction. Finally, the absorption at OD = 412 nm was measured to calculate the CS activity.

2.11 Statistical analysis

Data are expressed as mean ± SEM. Differences were evaluated by two-tailed Student's *t*-test or analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical significance was set at $P < 0.05$.

3. Results

3.1 Acute exercise increases serum FGF23 levels in mice

To study the effect of exercise on FGF23 systemic levels, we challenged eight C57BL/6 mice with short acute exercise, running on a treadmill for 60 min. The plasma parameters of mice were analyzed before and after the treadmill exercise. The serum glucose levels after exercise were significantly lower than those before exercise (**Fig. 1A**). Accordingly, the serum insulin levels were also decreased after exercise (**Fig. 1B**). By contrast, the serum glycerol levels in mice after exercise were higher compared with those before exercise (**Fig. 1C**). Interestingly, we found that serum FGF23 levels were significantly increased in exercised mice compared with mice before exercise (**Fig. 1D**). The FGF23 mRNA expression in the liver, heart, skeletal muscle, and thyroid was not changed. The FGF23 receptor Klotho RNA expression in skeletal muscle was also not affected by acute exercise.

3.2 FGF23 gene expression in skeletal muscle is upregulated by chronic exercise

We next studied the effect of exhaustive and moderately chronic exercise on FGF23 expression and serum levels. Both exhaustive and moderately chronic exercise enhanced serum FGF23 levels (**Fig. 2A and 2D**). Chronic exercise increased FGF23 mRNA only in skeletal muscle (**Fig. 2E**). By contrast, exhaustive exercise failed to modulate FGF23 expression in any organs (**Fig. 2B**). Accordingly, FGF23 receptor Klotho expression in skeletal muscle was increased by chronic exercise (**Fig. 2F**) but

not by exhaustive exercise (**Fig. 2C**).

3.3 Effect of exercise on FGF23 protein localization and level in skeletal muscle

Currently, there is no information about the FGF23 expression pattern in skeletal muscle. Thus, we further detected FGF23 expression and localization in gastrocnemius using immunohistochemistry and immunoblotting. We found that the FGF23 protein was mainly located in the cytoplasm in skeletal muscle tissue, and the localization of FGF23 was not altered by exercise (**Fig. 3A,** left panel). The optical density of FGF23 protein in skeletal muscle of exercised mice was higher than that before exercise (**Fig. 3A,** right panel). We next studied the effects of physical exercise on FGF23 protein expression in the gastrocnemius and gluteus maximus. Immunoblotting analysis confirmed that FGF23 protein levels in both the gastrocnemius and gluteus maximus were significantly increased in exercised mice (**Fig. 3B**).

3.4 FGF23 promotes exercise endurance

To explore the role of the upregulated FGF23 in exercise, we treated mice with recombinant FGF23 or saline (control) for 3 consecutive days and then subjected them to treadmill exercise. There was no significant change in body weight between vehicle-treated and FGF23-treated mice (data not shown). The baseline of exercise performance before treatment was comparable between these two groups of mice

(**Fig. 4A**). However, FGF23 treatment significantly extended the time to exhaustion (**Fig. 4B**). This result indicates that FGF23 promotes exercise endurance.

3.5 FGF23 protects against exercise-induced ROS and H_2O_2 production in skeletal muscle

Next, we evaluated the effect of FGF23 treatment on the ROS and H_2O_2 production in skeletal muscle in the exhaustive exercised mice. We found that the ROS level in skeletal muscle tissue (gastrocnemius) from FGF23-treated mice was significantly lower than that in skeletal muscles from control mice (**Fig. 5A**). In addition, the H_2O_2 content in skeletal muscle from FGF23-treated mice was lower than that in skeletal muscles from control mice (**Fig. 5B**).

3.6 Enhanced mitochondrial function may contribute to the ergogenic action of FGF23 in exercise

To explore further the molecular mechanisms for the exercise performance of FGF23, we measured the mitochondrial function in skeletal muscle in FGF23-treated or control mice without physical exercise. In the gastrocnemius and gluteus maximus, we observed that the mRNA levels of PPAR-δ were enhanced by FGF23 treatment (**Fig. 6B and 6E**). However, no changes of sirtuin 1 **(Fig. 6A and 6D)** and PGC-1α **(Fig. 6C and 6F)** were found at mRNA levels. FGF23 treatment significantly increased citrate synthase activity in both the gastrocnemius **(Fig. 7A)** and gluteus

maximus **(Fig. 7B)**. However, FGF23 did not change the TFAM mRNA levels in the gastrocnemius **(Fig. 7C)** and gluteus maximus **(Fig. 7D).**

4. Discussion

We investigated the effects of exercise on FGF23 levels in mice and obtained the following findings: First, both acute and chronic exercise can increase circulating blood FGF23 level, whereas only chronic exercise upregulates FGF23 mRNA expression in skeletal muscle. Second, treatment of FGF23 in mice significantly improved the exercise performance. Third, FGF23 enhanced mitochondrial function in skeletal muscle. All of these results demonstrate the critical role of muscle FGF23 in exercise.

Previous data demonstrate that FGF23 is a hormone released primarily by osteocytes that regulates phosphate and vitamin D metabolism[30]. Studies on the potential effect of FGF23 in skeletal muscle are rather rare. The first study on FGF23 used semiquantitative reverse transcription PCR analysis to show that FGF23 mRNA is mainly expressed in the heart, liver, and thyroid, whereas the expression of FGF23 in skeletal muscle is weak [31]. Southern blotting analysis confirmed these results [32]. However, a later investigation using real-time quantitative PCR to detect FGF23 mRNA expression found that the rank order of FGF23 expression in normal mouse tissues was bone > thymus >brain > heart > skeletal muscle > spleen > skin > lung >testes, with nearly undetectable levels in the liver and kidneys[16]. In this study, immunohistochemistry and immunoblotting analyses demonstrated that FGF23 protein is indeed expressed in skeletal muscle tissue. It should be noted that Klotho, which is the receptor/cofactor of FGF23 and is necessary for FGF23 signaling, has

been shown to be abundantly expressed in skeletal muscle [33, 34]. Thus, these results support the important role of FGF23 in skeletal muscle biology. Moreover, the elevated FGF23 after exercise increases the possibility of FGF23 being an exercise-induced myokine. Recently, novel myokines, such as irisin [35-41] and dermcidin[42], have attracted great interest because of their crucial roles in metabolism and endocrinology regulation.

The elevated oxygen consumption in skeletal muscle tissue during exercise causes abundant ROS in mitochondria[43]. Although the primary tissues responsible for ROS production during exercise is not completely known, compelling evidence indicates that NADPH oxidase, PLA2-dependent processes, and xanthine oxidase contribute to contraction-induced ROS production in muscle in contracting skeletal muscle fibers[43].ROS production also is an important inducer of muscular fatigue during prolonged and intense exercise. Previous studies have explored the role of antioxidants in preventing muscular fatigue during endurance exercise in humans[43]. However, this notion is still controversial because an optimal level of ROS-mediated stimulation seemed to be required for the normal signaling response to sprint exercise in humans[44]. Lombardi *et al.* showed that FGF23 increased 50% after a 3-week stage race; however, they did not investigate any biological role of FGF23 in skeletal muscle [45]. Our results on the serum FGF23 levels after exercise are apparently in line with their data. Phelps *et al.* also showed that running endurance was markedly reduced in Klotho knockout mice[33]. Because Klotho is the receptor of

FGF23, our findings apparently support the notion that the FGF23–Klotho axis in skeletal muscle plays a beneficial role in exercise.

Mitochondrial function in skeletal muscle has a critical role in exercise-related biology [46-48]. Sirtuin 1, PPAR-δ, and PGC-1α are three master transcriptional regulators of oxidative metabolism and mitochondrial function[49-52]. They are activated after acute exercise and thought to be molecular mechanisms underlying the beneficial effects induced by physical exercise [49-51]. In our study, we only observed an enhancement of PPAR-δ mRNA expression, but not sirtuin 1 or PGC-1α mRNA expression, in the skeletal muscle of FGF23-treated mice. Narkar et al. showed that activation or overexpression of the transcription factor PPAR-δ in muscle resulted in an increase in mitochondrial biogenesis [51]. Moreover, activation of PPAR-δ seems to be increase running endurance and protect against diet-induced obesity and type II diabetes[51]. Interestingly, PPAR-δ agonist mimics an exercise response by promoting gene regulatory responses in cultured human skeletal muscle[53]. Thus, activation of the PPAR-δ pathway by FGF23 may contribute to the action of FGF23 on exercise endurance. Moreover, the enhanced citrate synthase activity by FGF23 may also contribute to the ergogenic action of FGF23 on skeletal muscle. An increase in citrate synthase activity is a common index to confirm the exercise training effect[54]. Further, several recent findings also show that the activated mitochondrial function in the brain may mediate the beneficial effects of exercise [55, 56]. However, it seems that this observation is still debatable[57]. In

addition, although the increase of citrate synthase activity is always considered to indicate mitochondrial biogenesis[54], Larsen *et al.* reported that citrate synthase activity did not necessarily occur because of new mitochondrial biogenesis[58]. Because we also found that TFAM mRNA levels, a good indication of a change in mitochondrial DNA copy number, were not significantly altered by FGF23 in skeletal muscle, it is safe to conclude that FGF23 improves exercise performance through enhancing mitochondrial function. Nevertheless, the effects of FGF23 on mitochondrial structure and biogenesis are still open questions.

There are several limitations in our work. First, we did not measure the effects of FGF23 on the oxygen transport system, which might have caused the increase in endurance performance [59]. Determination of metabolic or VO_2 or lactate during submaximal exercise may provide more information about the biological function of FGF23. Second, acute exposure of primary cardiomyocytes to FGF23 resulted in elevated intracellular Ca^{2+} [60], suggesting that FGF23 may increase exercise endurance via increasing circulating volume and following positive effects on preload and cardiac output. Third, direct release of FGF23 from the muscle vascular bed was not determined. Thus, whether FGF23 is released from skeletal muscle during exercise is still an unanswered question.

In conclusion, we demonstrated that physical exercise increased blood concentration of FGF23 and expression of FGF23 in skeletal muscle. Supplementing exogenous FGF23 improved endurance performance in physical exercise via

controlling the excess ROS production and enhancing mitochondrial function. These results suggested an important role of FGF23 in muscle biology and exercise performance.

Authors' contribution(s)

D.J.L. performed experiments, designed experiments, interpreted data, and wrote the manuscript. H.F. performed experiments. T.Z. and M.N. discussed the study and interpreted data. F.M.S. conceived the idea of the study, designed experiments, and wrote and edited the manuscript.

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Conflict of Interest

The authors have no competing financial interests to declare.

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Figure legends

Fig. 1

Effects of a single bout of acute exercise (60 minutes) on serum glucose, insulin, glycerol and FGF23 levels, FGF23 mRNA expression in liver, heart, skeletal muscle and thyroid, and FGF23 receptor Klotho mRNA expression in muscle. (A-D) Serum glucose, insulin, glycerol and FGF23 levels after acute exercise. (**E**) FGF23 mRNA expression in liver, heart, skeletal muscle and thyroid. (**F**) FGF23 receptor Klotho mRNA expression in muscle. *P < 0.05 versus Pre-Ex. N = 8 per group. Pre-Ex, pre exercise; Post-Ex, post exercise. NS, no significance.

Fig. 2

Effects of a single bout of exhaustive exercise and one-week moderately chronic exercise on serum FGF23 concentration, FGF23 mRNA expression in liver, heart, skeletal muscle and thyroid, and FGF23 receptor Klotho mRNA expression in muscle. (A-C) Serum FGF23 concentration (**A**), FGF23 RNA expression in liver, heart, skeletal muscle and thyroid (**B**), and Klotho mRNA expression in muscle **(C)** after a single bout of exhaustive exercise. **(D-F)** Serum FGF23 concentration (**D**), FGF23 RNA expression in liver, heart, skeletal muscle and thyroid (**E**), and Klotho mRNA expression in muscle **(F)** after one-week moderately chronic exercise. *P < 0.05 versus Pre-Ex. N = 8 per group. Pre-Ex, pre exercise; Post-Ex, post exercise; NS, no significance.

Fig. 3

Unchanged distribution and increased expression of FGF23 in skeletal muscle tissues after exercise for one week. (A) Immunohistochemistry showed the distribution of FGF23 in skeletal muscle tissues before and after exercise. *P < 0.05 versus Pre-Ex. N = 8 per group. (**B**) Comparison of FGF23 protein level in gastrocnemius and gluteus maximus before and after exercise by immunoblotting. *P < 0.05 versus Pre-Ex. N = 8 per group.

Fig. 4

Effects of FGF23 treatment on exercise endurance in mice. Comparison of the time to exhaustion in treadmill exercise before **(A)** and after FGF23 treatment**(B)** in two groups of mice. *P < 0.05 versus control. NS, no significance. N = 8 per group.

Fig. 5

Effects of FGF23 treatment on exercise-induced ROS and H_2O_2 production in skeletal muscle. The intramuscular ROS (**A**) and H_2O_2 (**B**) levels before and after exercise were determined. *P < 0.05 versus control. Pre-Ex, pre exercise; Post-Ex, post exercise. N = 8 per group.

Fig. 6

Effects of FGF23 treatment on mRNA levels of sirtuin 1, PPAR-δ andPGC-1α in skeletal muscle in mice. The skeletal muscle tissues from mice received FGF23 treatment for 3 days or vehicle (control) were isolated and used to isolate cDNA for real-time PCR assay. (**A-C**) Comparison of the mRNA levels of sirtuin 1, PPAR-δ andPGC-1α in gastrocnemius between Control and FGF23-treated mice. *P < 0.05 versus control. N = 4 per group. (**D-F**) Comparison of the mRNA levels of sirtuin 1, PPAR-δ andPGC-1α in gluteus maximus between control and FGF23-treated mice. *P < 0.05 versus control. N = 4 per group.

Fig. 7

Effects of FGF23 treatment on mitochondrial function in mouse skeletal muscle. **(A-B)** Effects of FGF23 treatment on citrate synthase activity in gastrocnemius and gluteus maximus**. (C-D)** Effects of FGF23 treatment on TFAM mRNA expression in gastrocnemius and gluteus maximus**.** *P < 0.05 versus control. N = 6 per group.

Table 1. Primers for real-time PCR analysis

Gene	Forward	Reverse
FGF23	TGGGCACTGCTAGAGCCTAT	CTTCGAGTCATGGCTCCTGT
sirtuin 1	AAAATGCTGGCCTAATAGACTTG	GCACCGTGGAATATGTAACGA
PPAR-δ	GTTTGCTGTCAAGTTCAATGCG	ACTGGCTGTCAGGGTGGTTG
PGC-1α	TGTTCCCGATCACCATATTCC	GTATTCATCCCTCTTGAGCCTTT
TFAM	AACCTTTGACACTCAGTTCATTTTTCT	CAAGGCTCAAAAGGTCAACAGA
β-actin	CCCATCTATGAGGGTTACGC	TTTAATGTCACGCACGATTTC

Fig. 1

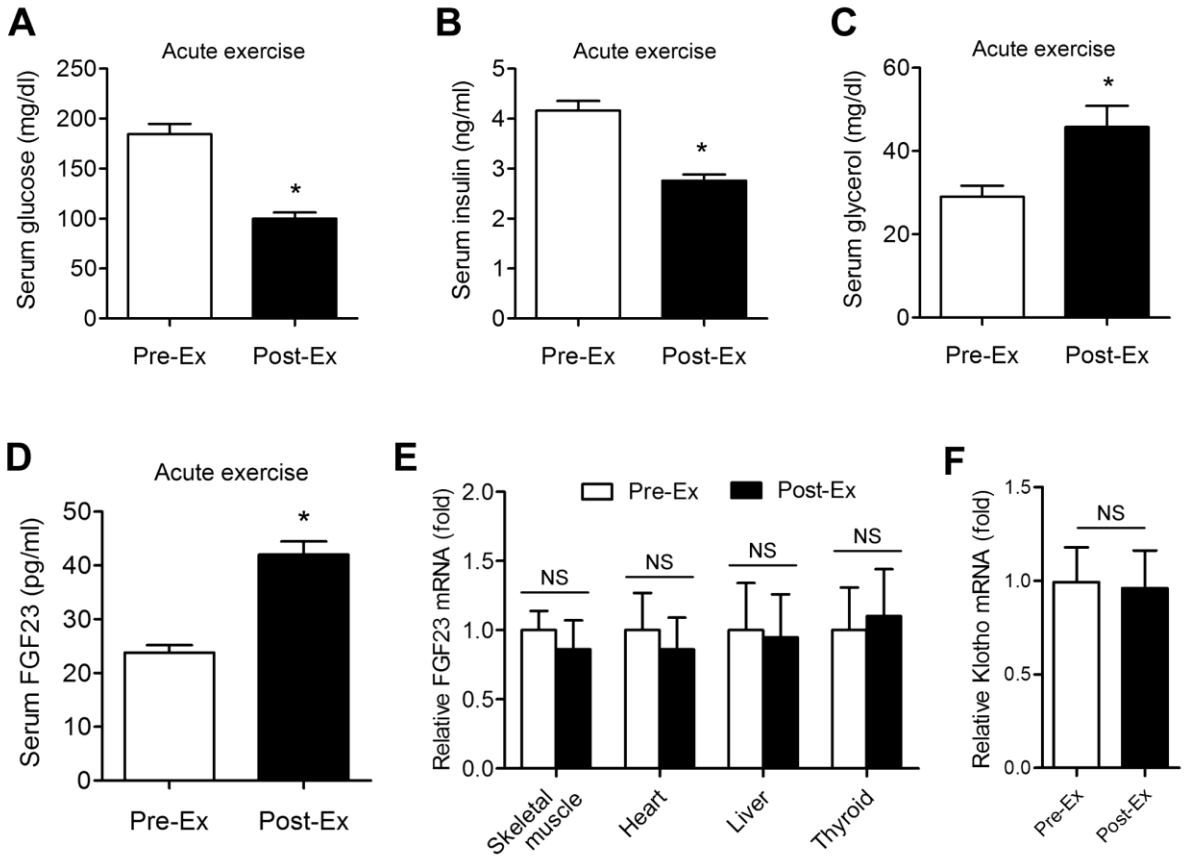

Fig. 2

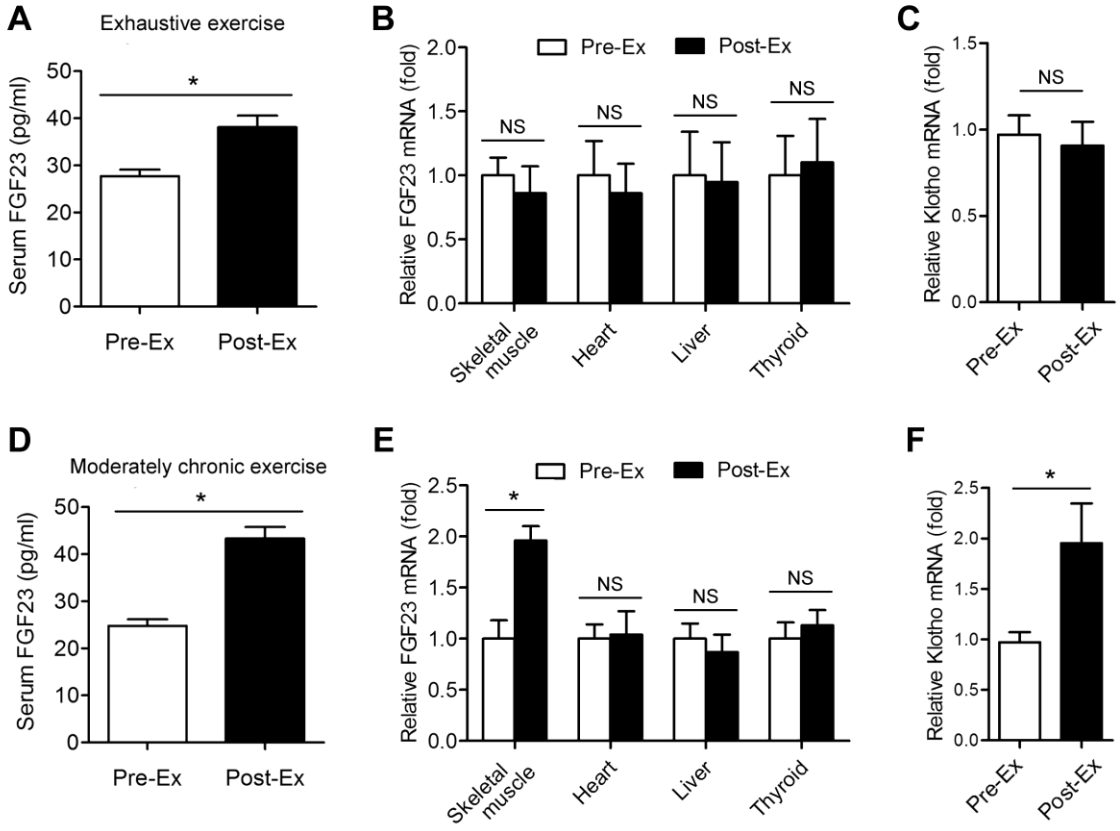

Fig. 3

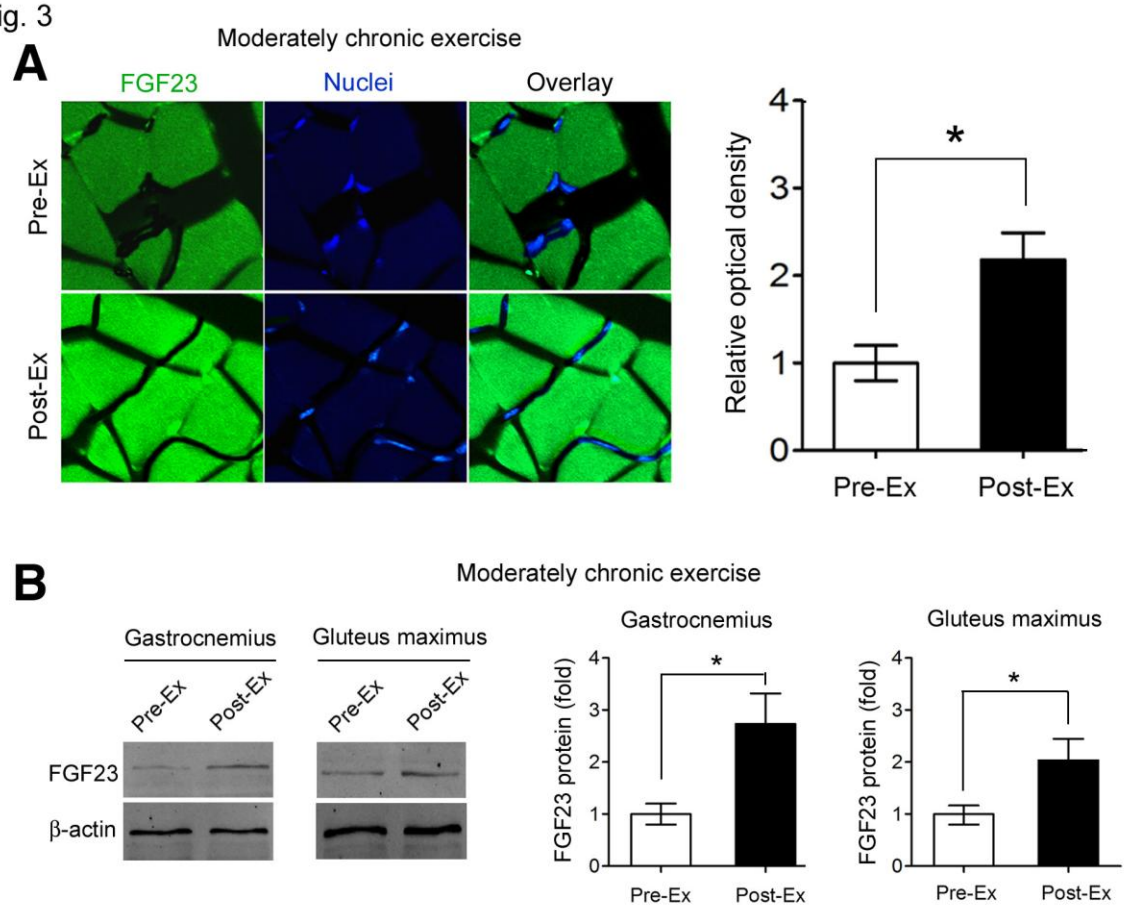

Fig. 4

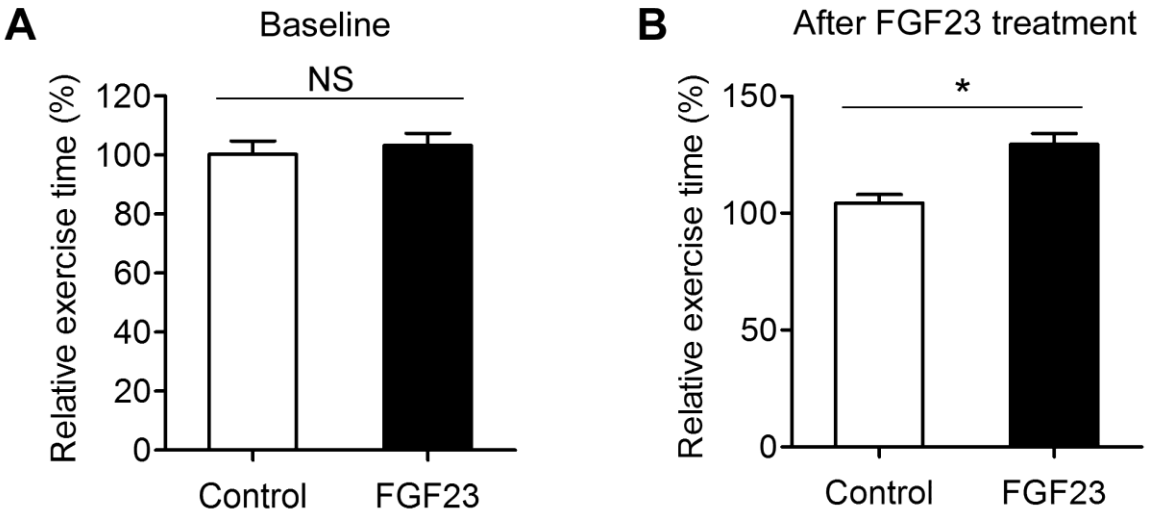

Fig .5

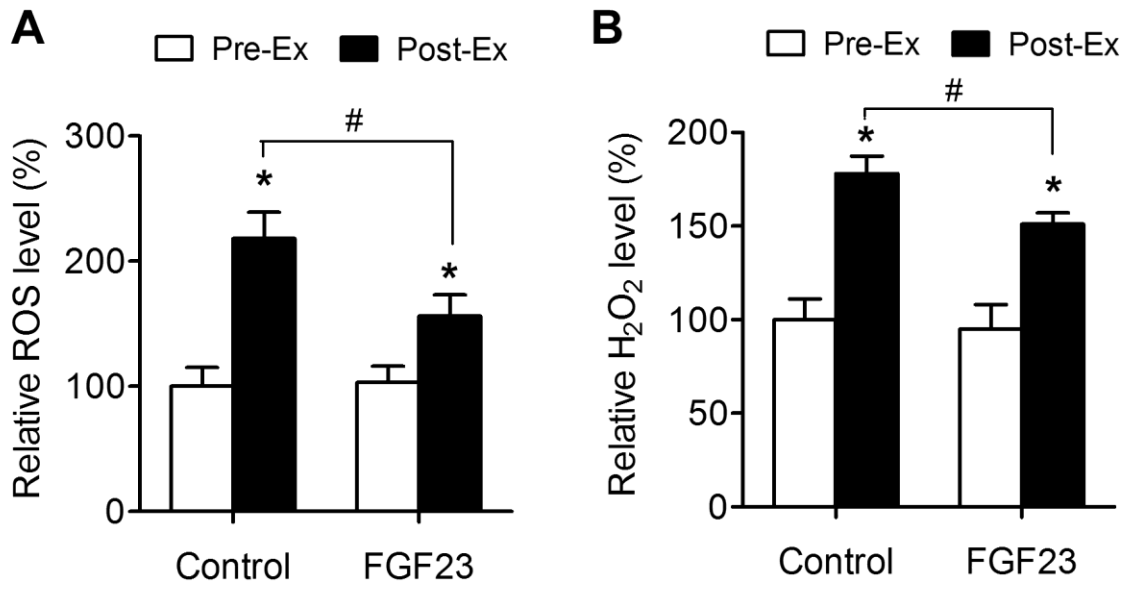

Fig. 6

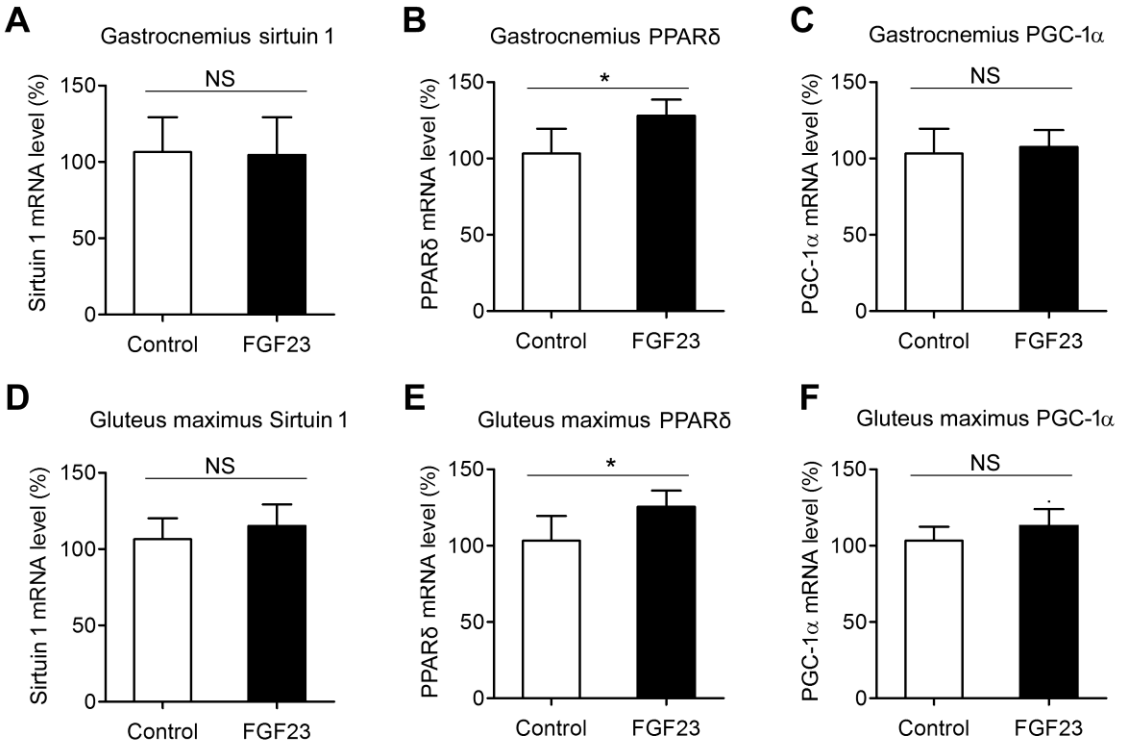

Fig. 7