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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 min), exhaustive exercise, and chronic prolonged exercise (60 min 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.

Abbreviations: 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.

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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/day).

2.3. Blood and Tissue Sampling

Before and after exercise training (one week), mice were fasted for 8 h 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 °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