The Effect of Different Intensities of Treadmill Exercise on FGF23 Gene Expression in Gastrocnemius and Soleus Muscles of Wistar Rats

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Abstract: Fibroblast growth factor 23 (FGF23) acts as a hormone that regulates phosphate metabolism associated with kidney function, and an inducer of left ventricle hypertrophy. But the role of FGF23 as a myokine has not yet to be confirmed. This research aims to investigate the effect of different intensities of treadmill exercise on FGF23 gene expression in gastrocnemius and soleus muscles of Wistar rats. Twenty male Wistar rats were given different intensities of treadmill exercise (low, moderate, and high) for as long as 8 weeks. FGF23 gene expression in gastrocnemius and soleus muscles was examined using semi-quantitative PCR. In this study, we obtained no change of relative FGF23 mRNA expression in gastrocnemius muscles ($p = 0.684$) compared to control. But interestingly, we found a significant increase of relative FGF23 mRNA expression in soleus muscles ($p = 0.030$). These results showed that different intensities of treadmill exercise do not stimulate FGF23 gene expression in gastrocnemius muscles of Wistar rats. While the low intensity of treadmill exercise does not increase FGF23 relative mRNA expression, moderate and high intensities of treadmill exercise increase FGF23 gene expression in the Wistar rat's soleus muscles.

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1 INTRODUCTION

Fibroblast Growth Factor (FGF) could be divided into three categories: intracrine, paracrine, and endocrine (Kyrou, Weickert, Gharanei, Randeva, & Tan, 2017; Ornitz & Itoh, 2015). The FGF family consisted of 22 families, with FGF15/19 is being ortholog in rodents and humans, while FGF23 is included as an endocrine FGF (Ho & Bergwitz, 2021; Ornitz & Itoh, 2015). As a hormone produced in osteoblasts and osteocytes, FGF23 is circulated into many organs such as the kidney, heart, and skeletal muscles (Faul et al., 2011; López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013; Vervloet, 2019). The action of FGF23 is mediated by Fibroblast Growth Factor Receptor (FGFR) together with the cofactor Klotho (Ornitz & Itoh, 2015).

FGF23 is an osteokine (a hormone produced in bone), with the kidney as its main target, where it inhibits calcitriol formation by suppressing 25-hydroxyvitamin D-1α-hydroxylase (Bacchetta et al., 2013; Ewendt, Feger, & Föller, 2021). Recent researches have shown that FGF23 is also produced by cardiomyocytes (Leifheit-Nestler & Haffner, 2018) and induces left ventricular hypertrophy (Faul et al., 2011). These proofs ensuring the role of FGF23 in the crosstalk between bone and muscle (Ewendt et al., 2021; Lara-Castillo & Johnson, 2020). But it is still unclear whether FGF23 could directly alter skeletal muscle function. A study by Avin et al proved that FGF23 is indirectly influenced the proliferation and differentiation of skeletal muscle cells in an ex vivo experiment (Avin et al., 2018).

Many factors might alter molecular mechanisms in skeletal muscle, including the physiological adaptation process to different intensities of exercise (MacInnis & Gibala, 2017). Exercise has been well known as a way to reduce chronic disease risk (Anderson & Durstine, 2019). American College of Sports Medicine (ACSM) recommended light to moderate exercise that may progress gradually to vigorous exercise, 30 minutes/day and for a minimum of 3 days a week, for people without cardiovascular, metabolic, or renal disease (Riebe et al., 2015). Exercise induces numerous substances secretion, known as myokines which conduce positive effects to prevent metabolic disease and sarcopenia (Son, Chae, Testroet, Du, & Jun, 2018).

It is still unclear whether FGF23 is a myokine or just an osteokine induced by the change in phosphate concentration (Lara-Castillo & Johnson, 2020; Takashi & Fukumoto, 2020). Some studies found increased serum levels of FGF23 after exercise in humans (Emrich, Dederer, et al., 2019; Kerschan-Schindl et al., 2021; G Lombardi et al., 2014). While in mice, a previous study has shown that 1 week of treadmill exercise upregulated FGF23 in blood, mRNA expression, and mitochondrial function in skeletal muscle (Li, Fu, Zhao, Ni, & Shen, 2016). Therefore, in this study, we aim to investigate the effect of different intensities of treadmill exercise on gene expression of FGF23 in the Wistar rat's skeletal muscles (gastrocnemius and soleus).

2 METHODS (AND MATERIALS)

2.1 Experimental Animals

We obtained twenty male rats, Wistar strain, aged about 6-8 weeks, weight about 200-220 grams from PT Biofarma, Bandung, Indonesia. Rats were divided into four groups (N=5 for each group) and put in a cage per group under constant photoperiod (light/dark cycle every 12 hours), temperature between 22-24°, and relative humidity. Standard chow diet (47.3% carbohydrate, 4% fat, 20% protein, 12% water, 4% fiber, 12% calcium, and 0/7% phosphorus) and water were provided ad libitum. We experimented on animals based on the use and care of laboratory animal guidelines (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, 2011). The rats were treated following an approved code of ethics from the Committee of the Faculty of Medicine, Maranatha Christian University- Immanuel Hospital Bandung Number 093/KEP/VI/2020.

2.2 Different Intensities of Treadmill Exercise

After two weeks of the adaptation period, the rats were trained to run on a treadmill with a gradual increase of speed and time for another two weeks for the initial adaptation. The protocol of treadmill exercise intensities was modified from the previous protocol, with lactate threshold as the basis for defining the intensities. The sub-lactate threshold is categorized as low intensity (10 meters per minute), lactate threshold as moderate intensity (20 meters per minute), and supra-lactate threshold as high intensity (30 meters per minute) (Lesmana et al., 2016). The treadmill exercise was conducted 5 times

a week, from Monday to Friday, 30 minutes per day, for 8 consecutive weeks. The sedentary group was used as a control group. At the end of the experiment, all the animals were sacrificed under inhalation anesthesia (5% isoflurane). We separated skeletal muscle tissues (gastrocnemius and soleus muscles), froze them at -80°C for RNA extraction.

2.3 RNA Extraction, Semi-quantitative PCR

RNA Trisure isolation reagent (Bioline, BIO-38032, London, UK) was used according to the protocol of RNA extraction from the manufacturer. After the extraction, RNA concentration and purity were quantified by measuring its absorbance in 260/280 nm (Tecan Infinite M200 Pro, No. 30050303, Switzerland). For the analysis of FGF23 and GAPDH gene expression, semi-quantitative PCR was conducted using a One-Step RT PCR Kit (Bioline, BIO-65409, London, UK). The process then continued with electrophoresis (Mupid Exu Submarine Electrophoresis System, Mupid Exu, Japan), and the result is visualized using Blupad Dual LED Blue/White Light Transilluminator (Bio-Helix, GeneDirex BP001CU, Taiwan). Quantification of the band was conducted using Image J. This procedure was adapted from the previous study (V. M. Tarawan et al., 2019). The primer sequences of FGF23 and GAPDH have been summarized in Table 1 (V. Tarawan, Gunadi, Subekti, Widowati, & Goenawan, 2019; Wang et al., 2017).

Table 1: Primer Sequence Used in This Study.

Gene	Primer Sequence Upper strand: sense Lower strand: antisense	Product Size (bp)
FGF23	CCTTCCTCTGCACTCGGTAG	301
	TGCCAGCTGCCAAGACGGTG	
GAPDH	GTTACCAGGGCTGCCTTCTC	
	GATGGTGATGGGTTTCCCGT	177

2.4 Statistical Analysis

Data are expressed as mean ± SEM. Differences between groups were evaluated by Analysis of Variance (ANOVA), followed by an LSD post hoc test. Statistical significance was set at $p<0.05$.

3 RESULTS AND DISCUSSION

As a result of this study, we found no difference in relative ratio of gastrocnemius's FGF23 mRNA expression in different intensities of treadmill exercise were: 0.628 ± 0.05 (low); 0.593 ± 0.03 (moderate); 0.647 ± 0.02 (high); compared to 0.602 ± 0.04 (control). This result is presented in figure 1.

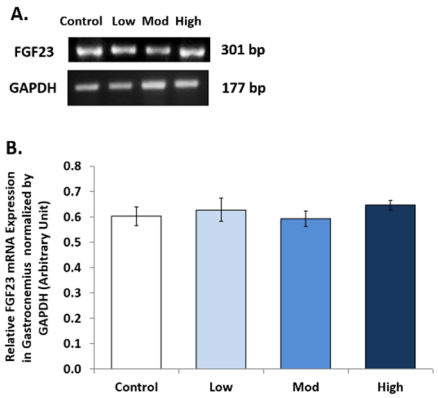

Figure 1: Relative Ratio of FGF23 mRNA Expression in Gastrocnemius Muscle of Wistar Rats After 8 weeks of Treadmill Exercise with Different Intensities. (Control = Sedentary, Low =low intensity (10 m/minute), Mod = moderate intensity (20 m/minute), High = high intensity (30 m/minute).

We found a significant increase of FGF23 mRNA expression in soleus muscles of Wistar rats ($p = 0.030$). Relative ratio of soleus's FGF23 mRNA expression in different intensities of treadmill exercise were: 0.583 ± 0.06 (low); 0.624 ± 0.03 (moderate); 0.648 ± 0.05 (high); compared to 0.552 ± 0.02 (control). This result is presented in figure 2.

In this study, we found no difference of FGF23 in gastrocnemius muscle of Wistar rats after 8 weeks of treadmill exercise with different intensities. But interestingly, we found a significant increase of FGF23 gene expression in soleus muscles of Wistar rats after 8 weeks of treadmill exercise with moderate and high intensities, while low intensity did not change FGF23 gene expression compared to control.

Recent research has proven that the effect of FGF23 on skeletal muscles was mediated by other endogenous substances that might act in concert with FGF23 (Avin et al., 2018). Our result (figure 2) suggested the increase of FGF23 in soleus muscles might be associated with exercise alteration of skeletal muscle metabolism, particularly with parathyroid hormone (PTH). PTH and FGF23 are modulating each other's secretion, FGF23 decreases

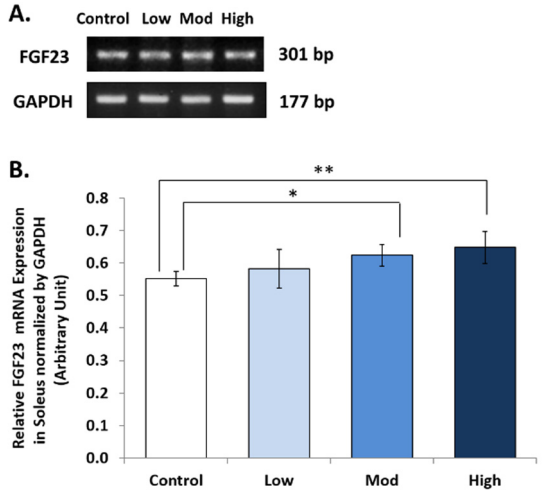

Figure 2: Relative Ratio of FGF23 mRNA Expression in Soleus Muscle of Wistar Rats After 8 weeks of Treadmill Exercise with Different Intensities. (Control = Sedentary, Low =low intensity (10 m/minute), Mod = moderate intensity (20 m/minute), High = high intensity (30 m/minute). * = significant ($p<0.05$), ** = very significant ($p<0.01$).

PTH secretion, and PTH increases FGF23 secretion (Giovanni Lombardi, Ziemann, Banfi, & Corbetta, 2020; Peacock, 2021). PTH responds to acute and chronic exercise, and its response might be influenced by some factors, such as VO_2max, vitamin D status, and hormonal status (Giovanni Lombardi et al., 2020). A study by Gardinier et al proved that PTH has a role in bone adaptation to exercise, and they found an increase of FGF23 mRNA expression in the tibia after 6 days of treadmill training (Gardinier, Al-Omaishi, Morris, & Kohn, 2016). But the crosstalk between PTH and FGF23 in bone and skeletal muscles needs further investigation. Effects exerted by PTH in muscle cells might be secondary to the effects on other tissue. (Lombardi, Ziemann, Banfi, & Corbetta, 2020).

The different results between gastrocnemius (figure 1) and soleus muscles (figure 2) might be associated with different properties of their mitochondria. Gastrocnemius and soleus muscles are two muscles that differ in their fiber types, while gastrocnemius muscle is fast type muscle fiber, the soleus muscle is slow type muscle fiber (Qaisar, Bhaskaran, & Van Remmen, 2016). Soleus muscles have a slow contraction speed and predominantly use oxidative metabolism for energy production, while gastrocnemius is fast-twitch and uses glycolytic metabolism (Qaisar et al., 2016). Therefore, soleus muscles have more and bigger mitochondria than gastrocnemius muscles (Sanchez, Li, Bragos, & Rutkove, 2014).

Different properties of gastrocnemius and soleus muscles could explain the different results of this study. A previous study concluded that exercise promotes FGF23 mRNA expression in skeletal muscle tissue by controlling mitochondrial function (Li et al., 2016). This is consistent with other studies that stated endurance training improved the function of muscle mitochondria which is essential for the homeostasis of energy in skeletal muscles (Zoladz, Koziel, Woyda-Ploszczyca, Celichowski, & Jarmuszkiewicz, 2016). These findings might explain why the increase of FGF23 was only found in soleus muscles (figure 2), but not in gastrocnemius muscles (figure 1).

The effect of FGF23 after exercise is still under debate because some studies have shown different results. Some studies claimed that FGF23 increased after exercise (Emrich, Dederer, et al., 2019; Kerschan-Schindl et al., 2021; Li et al., 2016; G Lombardi et al., 2014), while other studies found no change/decrease ((Buskermolen et al., 2019; Emrich, Baier, et al., 2019; Keshavarzi, Daryanoosh, Kooshki Jahromi, & Mohammadi, 2017; Neves et al., 2021). Different results of those studies might be affected by intensities, duration, and type of exercise, high altitude, and phosphate intake. There is a possibility that FGF23 would increase more after a long duration, high intensity, over strenuous exercise, and high phosphorus diet. The limitation of this study is we have no data regarding phosphate and PTH concentration which might be correlated with the increase of FGF23 in soleus muscles. For a better understanding of the FGF23 mechanism in the adaptation of skeletal muscles to exercise, we recommend a more detailed study, especially by investigating the crosstalk between FGF23 and PTH, the function of skeletal muscles mitochondria after exercise, and longer duration of exercise.

4 CONCLUSIONS

In summary, FGF23 gene expression in gastrocnemius muscles is not upregulated after 8 weeks of treadmill exercise with different intensities but upregulated in soleus muscles after 8 weeks of treadmill exercise with moderate and high intensities. But the role of FGF23 in skeletal muscle after exercise still needs further investigation.

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