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Effect of Acute Physical Exercise with Moderate Intensities on FGF23 Gene Expression in Wistar Rat Heart

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Abstract

A myokine is one of the proteins that are produced and released by myocytes in response to muscular contractions when doing physical exercise. One protein that is thought to function as myokine is FGF23.The purpose of this study was to determine the effect of acute physical exercise with moderate intensity on the expression of FGF23 gene in Wistar rat heart. This was an animal experimental study using 24 male Wistar rats that were divided into 4 groups:treatment groups that performed 30 minute acutephysical exercise with moderate intensity (20 m/min) for 3 days, 6 days, and 15 days and a control group without physical exercise. The study was conducted in the Animal Laboratory and Central Laboratory of Universitas Padjadjaran during the period of February to July 2019. Data observed were the FGF23 gene expressions in Wistar rats heart. Data were analyzed using Kruskal-Wallis and Mann-Whitney tests. The results from the Kruskal-Wallis test showed that acute physical exercise with moderate intensity did not increase the FGF23 gene expression in Wistar rat heart ($p>0.05$), and the average of relative ratios of FGF23/GAPDH gene expression were as follows: control (0.970±0,03), 3 days (0.992±0.03), 6 days (1.014±0.05), and 15 days (1.056±0.02). GAPDH was used in this study as a housekeeping gene since its expression is very constant. This study proves that FGF23 is more likely to take a role in the cardiac remodeling process, especially those associated with cardiac hypertrophy after chronic exercise with no effect observed after acute physical exercise with moderate intensity in Wistar rat heart.

Key words: Acute, FGF23, moderate intensity, myokine, physical exercise

Pengaruh Latihan Fisik Akut Intensitas Sedang terhadap Ekspresi Gen *FGF23* pada Jantung Tikus Galur Wistar

Abstrak

Miokin diproduksi dan dilepaskan oleh miosit sebagai respons terhadap latihan fisik. Salah satu protein yang diduga berfungsi sebagai miokin adalah FGF23. Penelitian ini bertujuan mengetahui pengaruh latihan fisik akut intensitas sedang terhadap ekspresi gen *FGF23* pada jantung tikus galur Wistar. Penelitian ini menggunakan desain eksperimental dengan tikus galur Wistar jantan yang berjumlah 24 tikus. Tikus dibagi menjadi 4 kelompok untuk diberi perlakuan latihan fisik intensitas sedang (20 meter/menit) dengan durasi 30 menit, selama 3 hari, 6 hari, dan 15 hari serta kelompok tanpa latihan fisik. Penelitian ini dilakukan di Lab Hewan dan Lab Sentral Universitas Padjadjaran pada bulan Februari hingga Juli 2019. Ekspresi gen *FGF23* pada jantung tikus galur Wistar dilihat dengan PCR. Analisis data menggunakan Uji Kruskal-Wallis dan Mann-Whitney. Uji statistik tidak mendapatkan peningkatan ekspresi gen *FGF23* setelah dilakukan latihan fisik akut intensitas sedang selama 3 hari, 6 hari dan 15 hari ($p>0,05$) dan didapatkan rasio relatif ekspresi gen *FGF23/GAPDH* otot jantung pada : kontrol (0,970±0,03), hari 3 (0,992±0,03), hari 6 (1,014±0,05), hari 15 (1,056±0,02). GAPDH digunakan dalam studi ini sebagai gen '*housekeping*' karena ekspresinya yang sangat konstan. Studi ini membuktikan bahwa FGF23 mungkin lebih berperan dalam proses *remodeling* jantung, terutama yang berhubungan dengan hipertrofi jantung setelah latihan fisik kronik. Simpulan, tidak terdapat pengaruh latihan fisik akut intensitas sedang terhadap ekspresi gen *FGF23* pada jantung tikus galur Wistar.

Kata kunci: Akut, *FGF23*, intensitas sedang, latihan fisik, miokin

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Introduction

The Center for Disease Control (CDC) stated that of all deaths in United States (US) 300,000 deaths per year are likely to be caused by the lack of physical exercise and poor eating habits.[1] Therefore, one of the preventive efforts to avoid this is by doing regular physical exercises. One of the ideal physical exercises recommended for cardiovascular health is the 30-minute aerobic physical exercise per day, 5 times per week. According to the World Health Organization (WHO), physical exercise is a part of physical activities that is planned, structured, repetitive, and has a specific purpose to maintain or improve physical fitness.[2] Moderate intensity physical exercise has a measurement limit of 40–59% VO_2max and 3–6 MET.[3] Examples of moderate-intensity physical exercise recommended by the American Heart Association (AHA) are brisk walking (at least 40 km/hour per hour), tennis (double), and cycling <16 km/hour.[2]

During regular treadmills, many changes occur in body organs in response to adaptation to exercise. This change is caused by myokines that are produced and released by muscle cells (myocytes) in response to muscle contraction during physical exercise.[4] Myokines are one of several hundred cytokines or other small proteins that have autocrine, paracrine, or endocrine effects. Receptors for myokines are found in muscle, fat, liver, pancreas, bone, heart, immune, and brain cells.[5] The location of these receptors reflects the fact that myokines have many functions. Mainly, they are involved in metabolic changes associated with physical exercise as well as in metabolic changes after exercise adaptation. Myokines also play a role in tissue regeneration and repair, healthy body function maintenance, immunomodulation, and cell signaling, expression, and differentiation.[4] Acute physical exercise could be defined as a short term periodization describing manipulating of daily exercise variables over a few days up to a few weeks.[6]

One protein that might have a function as myokine is the fibroblast growth factor-23 (FGF23).[7] FGF23 is a hormone secreted by osteocytes that functions to regulate phosphate metabolism and vitamin D.[8] FGF23 gene expression can be found in bone, thymus, brain, skeletal muscle, spleen, and heart muscle.[9] Lombardi et al.[10] examined the levels of FGF23 in bicycle athlete's blood at the Giro Championships in Italy for 3 weeks and found an increase of 50% on day 21. Previous research conducted by Li et al.[7] also showed that physical exercise for 60 minutes and 1 week can increase the level of fibroblast growth factor 23 (FGF23) in mouse blood and that physical exercise for 1 week can increase the expression of FGF23 mRNA in the skeletal muscle of mice. In addition, FGF23 also controls the production of reactive oxygen species (ROS) and enhances mitochondrial function in skeletal muscle. A study by Faul et al.[17] has proven that FGF23 induces left ventricular hypertrophy in chronic kidney disease, but the role of FGF23 in physiological adaptation to exercise in still unclear. Therefore, studying acute exercise will be more appropriate compared to chronic exercise to understand the change of FGF23 gene expression from days to weeks, as a part of adaptation to exercise.

Studies on FGF23 association with physical exercise in the heart with physiological contexts is still limited. Grabner et al.[11] suggested that FGF23/FGFR4 signaling increases cardiac contractility and plays a role in reversible cardiac remodeling. It was also mentioned that there was a possibility that the FGF23 in the heart is initially physiological and beneficial but after a certain time the concentrations could turn into unfavorable as seen,for example, in animal models with chronic kidney disease.The actual role of FGF23 in cardiovascular, whether only as a biomarker or as a target of therapy, is still unclear.[12]

Therefore, this study explored the effect of acute physical exercise with moderate intensity for 3, 6, and 15 days on the expression of the FGF 23 gene in the heart of Wistar strain rats. This study used GAPDH as one of the most commonly used reference genes as an internal control, because its expression is very constant.[13] The purpose of this study was to determine whether acute intensity moderate physical exercise to the expression of the FGF23 gene in Wistar rat heart.

Methods

Subject of this study were twenty-four male Wistar rats (Rattus Norvegicus) from Biofarma Laboratory. The rats were 8–10 weeks old and weighed 250–300 grams. The rats were habituated for 4 weeks to adjust to the laboratory environment and for physical exercise preparation. Animals were kept in room temperature with 12 light/dark cycles. Rats were given food and drink ad libitum. This study was approved by the Medical Ethics Commission of the Faculty of Medicine, Maranatha Christian

Table Primers Used for Semi-quantitative PCR Analysis

Gene Symbol	Primer Sequence (5' to 3') *Upper strand : sense* *Lower strand : antisense*	Product Size (Bp)	Annealing (°C)	Cycle	Reference
FGF23	GCCAGTGGACGCTAGAGAAC CCTTCCTCTGCACTCGGTAG	301	58	36	[15]
GAPDH	GTTACCAGGGCTGCCTTCTC GATGGTGATGGGTTTCCCGT	177	61	35	[16]

FGF23: fibroblast growth factor 23; GAPDH: glyceraldehyde 3-phosphate dehydrogenase

University, with the issuance of the ethical clearance number 133/KEP/V/2019.

Prior to training period, the rats were habituated to the treadmill for 2 weeks. Training in treadmill were gradually increased until it reached the target speed. After adaptation period, rats were randomly divided into 4 groups: control group, 3 days group (run at 20 m/min for 3 days), 6 days group (run at 20 m/min for 6 days), and 15 days group (run at 20 m/min for 15 days). Treadmill speed of 20 m/minute is considered as having a moderate intensity based on the lactate threshold according to a previous study.[14] Treatment duration were 30 minutes per session, 5 times a week. By the end of training, rats were euthanized and heart was isolated. The heart was kept in a temperature of -80ºC until use. The study was conducted in Animal Laboratory of Universitas Padjadjaran and Central Laboratory of Universitas Padjadjaran during the period of February to July 2019.

Total mRNA was isolated from the left heart ventricle tissue using TRIsure reagent (Bioline, United Kingdom). RNA purification were read using Multimode Microplate Reader spectrophotometry (M200 Pro, Tecan, Morrisville, NC) at 268/280 nm. Semi quantitative PCR using One Step RT PCR Kit (Bioline, United Kingdom) was performed to amplify FGF23 mRNA and GAPDH as the internal control. PCR results were visualized usingthe BluePad Detection. PCR band quantification were performed using Image J software (NIH,USA). Primer sequence used in this study was provided in Table.

Data normality was tested with the Shapiro-Wilk test and homogeneity of the data was tested with the Levene test. One-way ANOVA/Kruskal-Wallis test were used, followed by Mann Whitney test if the data were not normally distributed and Post Hoc LSD with α=0.05 if the data were normally distributed. Differences were considered statistically significant if p value≤ 0.05.

Results

Subjects of this study were 24 male Wistar rats. Subjects were run on a treadmill at 20 m/min speed for 30 min/day, 5 times per week. The treatment group was divided into four different groups based on the length of treatment into 3 days, 6 days, and 15 days group with another group as the control. The rats were sacrificed on different days according the group and the left ventricle myocardium was isolated. RNA from heart muscle were extracted and subjected into reverse transcriptase PCR using FGF23 primer. The FGF23 mRNA band is presented in Figure 1. PCR band density was analyzed using Image J. The ratio of band density were obtained from FGF23 band and were divided by GAPDH as the internal control. Normality and homogenity tests were performed and Kruskal-Wallis statistical analysis was used to evaluate the statistical significance of the FGF23 mRNA expression between groups. No significant change was observed among the groups as presented in Figure 1 ($p>0.05$).

Discussion

Despite the absence of statistical difference in the FGF23 mRNA expression between groups after acute physical exercise of moderate intensity for 3 days, 6 days and 15 days, an increase in FGF23 gene expression was observed on day 3, 6, 15 when compared to control. This

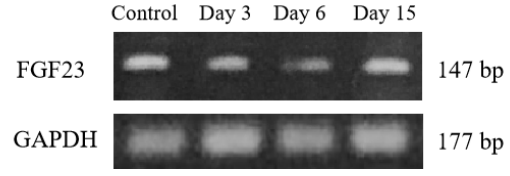

Figure 1 FGF23 mRNA Expression in Heart Muscle After Physical Exercise

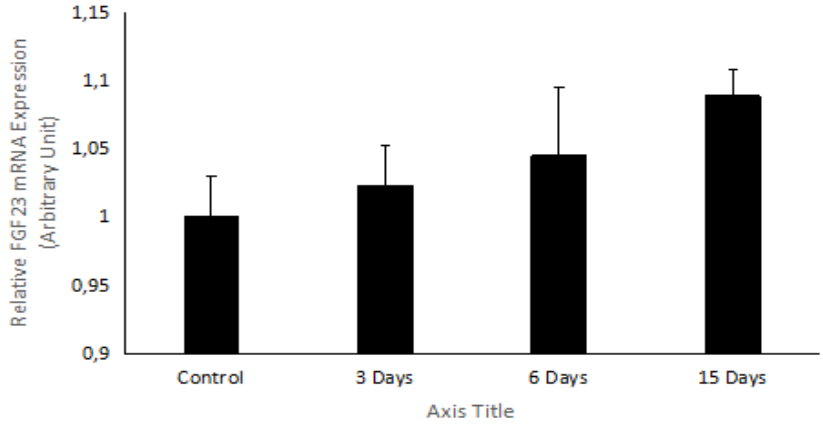

Figure 2 FGF23 mRNA Expression in left Ventricle Myocardium After Different Intensity Training

proves that FGF23 is more likely to take a role in the cardiac remodeling process, especially those associated with cardiac hypertrophy.[17] The process of cardiac hypertrophy due to physical exercise most likely occur at 4 weeks and will become plateau from 6 to 8 weeks.[18] There is a possibility that FGF23 is started to increase after 15 days because this study found that the FGF23 gene expression increased after 15 days, but not significantly, when compared to control. It is possible that if the exercise is performed in a longer period, more significant changes would be obvserved.

The results of this study were supported by Emrich et al. who stated that acute physical exercise for 60 minutes with sub-maximal intensity and high intensity does not increase plasma FGF23 levels and by Li et al.[7] who suggested that physical exercise for 1 week does not increase FGF23 gene expression in the heart, liver, and thyroid.

Contrary to our result, FGF23 level in the blood increased by 50% in 9 bicycle athletes after 3 weeks following a bicycle racing championship in Italy. The results discrepancies might be due to the fact that the bicycle athletes received a high calorie intake per day (6,000 kcal) with a calcium intake of 1799.91 mg/day and phosphate of 3,874.97 mg/day. There is a possibility that the increase in FGF23 expression is conceivably due to high phosphate intake in these athletes, and might has no correlation with the 3 weeks bicycle activity.[10] As mentioned earlier, FGF23 can be induced by hyperphosphatemia, high parathyroid hormone, or increased vitamin D. Therefore, further study is needed to elucidate the role of calcium and phosphate role in FGF23 expression level during exercise. Other studies conducted on mice also found increased FGF23 gene and protein expression in gastrocnemius and gluteus maxima muscles after 1 week of physical exercise. The results also show that FGF23 prevents excess ROS production and increases mitochondrial function in skeletal muscle.[7]

The difference with this study presumably occurs because the heart muscle is different from the skeletal muscle that changes in skeletal muscle are not always the same as changes in heart muscle. Cardiac muscle is an involuntary muscle, while skeletal muscle is a voluntary muscle. The sarcomeres in skeletal muscle are arranged in regular and parallel manner while cardiac muscle connects in irregular and non-parallel manner, known as intercalated disc. Skeletal muscle fibers aredivided into type I/ slow twitch and type II/fast twitch, but the cardiac muscle mainly consists of type I.[19] Exercise can cause fiber shifting from slow twitch to fast twitch in skeletal muscle but in cardiac muscle, the shifting is between αMHC and βMHC that are mainly found in type I. [19, 20] This differences might explain why the change of FGF23 in skeletal muscle is not the same as in cardiac muscle; however, further studies are still needed to prove this assumption.

The limitation of this study is that it does not examine the expression of the FGF23 gene in chronic physical exercise, nor does it examine ROS and mitochondrial function in the heart muscle. Hence, the conclusion regarding the role of FGF23 as a myokine remains as an unanswered

question.

In conclusion, moderate acute physical exercise does not change the expression of the FGF23 gene in the heart of the Wistar strain rat.

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