

## Effect of Acute Aerobic Physical Activity on Skeletal Muscle MyoD Gene Expression of Wistar Rats

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### Abstract

Aerobic physical activity is known to induce skeletal muscle adaptation. Some genes, including MyoD, are known to have a major role in the process of muscle adaptation. Several studies has stated that the expression of MyoD increases during the aerobic activity process; however, the studies were carried out in a single bout. Studies on the effects of acute phase exercise (<2 weeks) are still rare, especially those regarding the difference effect of acute training phase at different times. This study was performed in 2018 at the Central Laboratory and Animal Physiology Laboratory, Faculty of Medicine, Universitas Padjadjaran. The aim of this study was to determine the different effects of MyoD gene expression in a two-week acute aerobic physical activity. The effects were measured in the skeletal muscle in day 3, 7, and 14 of the activity. Twenty four male rats (*rattus novergicus*) were divided into 4 groups. Those rats in the treatment groups were run on an animal treadmill (P1=3 days, P2=7 days, and P3=14 days). On the last day of ran, rats were sacrificed and the soleus and gastrocnemius muscles were dissected. The expression of the MyoD gene in both muscles was then amplified using reversed PCR and detected by agarose gel electrophoresis. This study showed that there were differences in MyoD gene expression levels in both muscles and that the level increased with days of treatment, although it was statistically insignificant. This study concludes that the MyoD gene expression level is not significantly affected by an acute aerobic physical activity in certain periods (3, 7 and 14 days).

**Key words:** Acute, aerobic physical activity, gastrocnemius, MyoD, rats, soleus

## Perbedaan Pengaruh Aktivitas Aerobik Akut Intensitas Sedang terhadap Ekspresi Gen IGF1 dan *MyoD* pada Otot Rangka Tikus Galur Wistar

### Abstrak

Aktivitas fisik aerobik diketahui menginduksi adaptasi otot rangka. Beberapa gen diketahui memiliki peran utama dalam proses adaptasi otot termasuk *MyoD*. Dalam beberapa penelitian diketahui bahwa ekspresi *MyoD* meningkat selama proses aktivitas aerobik, tetapi penelitian dilakukan dalam pertarungan tunggal. Penelitian tentang efek latihan fase akut (<2 minggu) masih belum banyak dilakukan, terutama mengenai perbedaan efek fase latihan akut pada periode tertentu (3, 7, dan 14 hari). Penelitian ini dilakukan pada tahun 2018 di Laboratorium Sentral dan Laboratorium Fisiologi Hewan Fakultas Kedokteran Universitas Padjadjaran. Tujuan penelitian ini adalah mengetahui perbedaan efek ekspresi gen *MyoD* dalam aktivitas fisik aerobik akut pada berbagai hari (3, 7, 14 hari) pada otot rangka. 24 tikus jantan *Rattus novergicus* dibagi menjadi 4 kelompok. Kelompok perlakuan dijalankan dengan *treadmill* hewan (P1=3 hari, P2=7 hari, dan P3=14 hari). Pada hari terakhir, tikus dikorbankan dengan soleus dan otot gastroknemius dibedah. Ekspresi gen MyoD pada kedua otot kemudian diamplifikasi menggunakan PCR terbalik dan dideteksi dengan elektroforesis gel agarosa. Penelitian ini menunjukkan perbedaan level ekspresi gen MyoD pada kedua otot, hasil ini menunjukkan bahwa ada peningkatan level perawatan per hari, walaupun secara statistik tidak signifikan. Penelitian ini menyimpulkan bahwa pada otot gastrocnemius tidak terlihat efek yang berbeda dari durasi aktivitas fisik aerobik terhadap tingkat ekspresi gen *MyoD*, hanya otot soleus menunjukkan perubahan dan peningkatan ekspresi konsisten terlihat pada hari ke 14 (3, 7, dan 14 hari).

**Kata kunci:** Aktivitas fisik aerobik, Akut, gastroknemius, MyoD, soleus, tikus

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## Introduction

Adult muscle consists of multinucleated myofibers which can undergo changes in size (atrophy/hypertrophy) and type. Muscles will also experience some minor damage and lesions due to daily mechanical stimulation.

Under normal conditions, adult skeletal muscle is a stable tissue. However, in response to injury or damage due to physical activities or direct trauma, skeletal muscle has a special ability to regenerate themselves, prevent loss of muscle mass and/or delay the appearance of clinical symptoms of muscle disease. This is caused by the presence of mononucleate cells that are passively localized in the gap formed by the sarcolemma of muscle fibers adjacent to the basal lamina which are known as the satellite cells.<sup>1,2</sup>

Aerobic physical activity, or commonly referred to as endurance activity, is known to be able to primarily induce skeletal muscle adaptation. As a result there will be a disruption of homeostasis in the muscles that have never been trained until they undergo muscle hypertrophy. Increased mitochondrial density and muscle cell respiration capacity are also included. One of the most effective examples of aerobic exercise is running.

Muscles that experience injury or damage due to severe aerobic physical activity will experience rapid myofiber necrosis. Disorders that occur in the sarcolemma produce an increase in serum level of muscle cytosolic proteins such as creatinine kinase. In response, muscles will become inflamed then passive satellite cells will become active and repair the muscle cells. The satellite cells will re-enter the cell cycle and form new passive satellite cells or differentiate into myoblasts until physiological adaptation of muscle hypertrophy occurs. This adaptation process plays an important role in body health or in increasing the ability of athletes to get the desired goal.<sup>1-4</sup>

In response to stimuli such as injury or exercise, active satellite cells will express myogenic regulatory factors (MRFs) such as Myf5, MyoD, myogenin, and Mrf4. MRFs are transcription factors of basic helix-loop-helix (bHLH) which regulates the myogenesis process. MyoD is the key gene that regulates myogenesis of the four genes in MRFs. In addition, there are many MyoD in type II muscle fibers that are located mostly in the main muscles of the leg. Among the main muscles of the leg that are very much involved in aerobic activities, such as

running, are soleus and gastrocnemius muscles. Previous studies also strongly support that MyoD is expressed on acute endurance activities until a hypertrophic adaptation process occurs.<sup>1,5,6</sup> Until recently, literatures on the effects of acute phase exercise (<2 weeks) are still scarce, especially those that discuss the difference effect of acute training phase at a certain period. Therefore, the aim of this study was to determine the difference pattern of MyoD gene expression in acute aerobic physical activity period (3, 7, and 14 days) in rattus skeletal muscle.

## Methods

This study was approved for the methodology and other ethical by the Research Ethics Committee of the Faculty of Medicine Universitas Padjadjaran with the issuance of the ethical clearance Number 1385/UN6.KEP/EC/2018. This study was carried out at the Central Laboratory and Animal Physiology Laboratory, Faculty of Medicine, Universitas Padjadjaran, in 2018.

Twenty four male Wistar rats (10 weeks) weighed 200–300g were randomly divided into 3 treated groups (P1, P2, P3) and 1 control group (K). Before the study began, rats used in the study had been acclimatized/adapted for 1–2 weeks in a 25–27 °C temperature. Each group consisted of 4 rats and was placed in a 50x47x45 cm cage with ad libitum access to food and water. The rats were obtained from Biofarma Inc, Cisarua, West Bandung, Indonesia, and maintained at the Department of Physiology, Faculty of Medicine, Universitas Padjadjaran, Jatinangor, Indonesia.

This study used a 5-lanes animal treadmill. Before being given the first exercise, rats were habituated for 2 weeks. The treatment group were given aerobic physical activities in the form of running on animal treadmill. The treatment group P1, P2, and P3 rats ran on the treadmill at a speed of 20 m/minute for 30 minutes (moderate intensity), while the control group rats were kept in the cage. P1 ran for 3 days, P2 ran for 7 days, and P3 ran for 14 days. The inclination angle of the animal treadmill is set at 0°. The duration of the run will be measured using stopwatch.

On the last day the rats were run, rats were sacrificed for sample collection. Before being sacrificed, rats were anesthetized using isoflurane gas. Afterwards, rats were sacrificed by cervical dislocation. Legs of the mouse were opened until the soleus and gastrocnemius muscles are exposed. The muscle sample was

**Table 1 Primers Used for PCR in this Study**

Gene	Sequences	Tm (°C)	Product (bp)
MyoD	F: CGA CTG CCT GTC CAG CAT AG R: GGA CAC TGA GGG GTG GAG TC	57,5	174
GAPDH	F: GTTACCAGGGCTGCCTTCTC R: GATGGTGATGGGTTTCCCGT	61	177

immediately dissected, weighed, and inserted into the eppendorf tube separately. The tube was then stored in the -80°C refrigerator.

RNA isolation of the soleus and gastrocnemius muscles was done separately. Muscle samples were taken to be pulverized and homogenized with a 200 µl TRIzol™ reagent. This RNA isolation was performed by using acid phenol method. Isolated RNA concentration in each sample was quantified with TECAN. If the ratio was ~2, it was categorized as pure/uncontaminated and can be used.

The polymerase chain reaction (PCR) of MyoD was conducted using a kit from Bioline and PCR Gradient Sensoquest. RNA isolates then underwent reverse transcription and amplification in PCR machine. The MyoD primers were obtained from Genbank and Primer BLAST (National Center for Biotechnology Information (NCBI)).

Polymerase chain reaction products were run on agarose gel that had been mixed with TAE solution in a 250 mL erlenmeyer dish, with 1.4 gr agarose for each dish, and the mixture was then heated in an oven with 70p for 2 minute. Afterwards, 7 µL of fluorescence dye SYBR Safe DNA Gel Stain were added to 2% agarose mixture and then inserted into the gel mold with comb slot installed in the chamber. The agarose gel was then soaked with TAE buffer solution until it reached the solid state. PCR product

was then run in 100V for 30 minutes. Protein bands in agarose gel were then observed and photographed under BluPad. The photographed bands were quantified using *Image J* software.

Results were expressed as mean±SEM. Data were analyzed with one way ANOVA with 95% Confidence Interval using SPSS version 22.

## Results

In soleus muscle, the mean MyoD gene expression were 0.447±0.089, 0.502±0.196, and 0.408±0.045 for P1(3 days), P2 (7 days), and P3(14 days) respectively and 0.353±0.070 for the control group. For the gastrocnemius muscle, the mean were 0.757±0.055, 0.751±0.044, and 0.928±0.082 for P1 (3 days), P2 (7 days), and P3 (14 days) respectively and 0.693±0.102 for the control group.

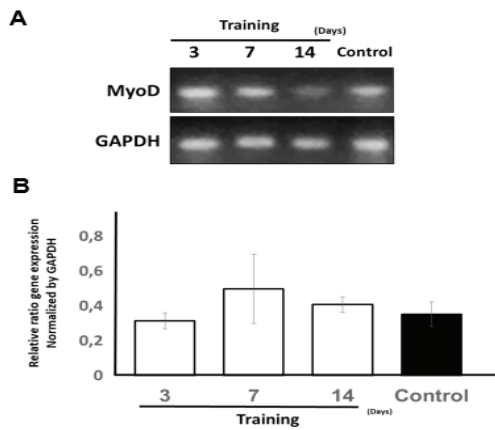
Table 2 presents the difference levels of MyoD gene expression in soleus and gastrocnemius muscle which tends to increase with days of treatment in a given period. Although statistically the increase in gene expression was not significant in either muscles ( $p>0.05$ ).

## Discussion

Based on the results obtained from this study, it

**Table 2 Results of MyoD Gene Expression in Soleus and Gastrocnemius After Given Different Period of Aerobic Physical Activity**

	MyoD				P Value
	K	P1	P2	P3	
Soleus					
Mean	0.353	0.447	0.502	0.408	0.747
±SEM	0.070	0.089	0.196	0.045	
Gastrocnemius					
Mean	0.693	0.757	0.751	0.928	0.16
±SEM	0.102	0.055	0.044	0.082	

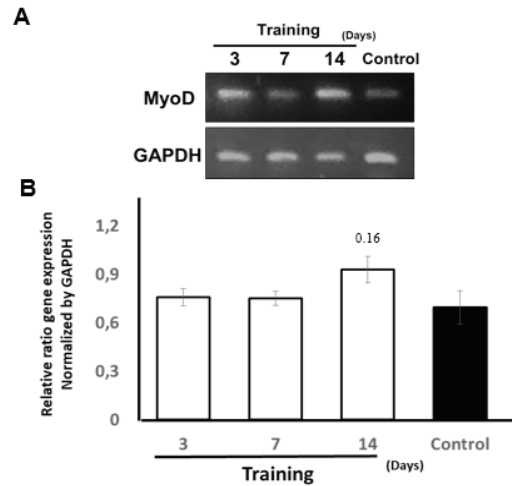


**Figure 1 MyoD Bands Visualized by Electrophoresis (Bluepads) (A), mRNA Expressions in Soleus (B). Values are expressed as Mean±SEM (p=0.747)**

is obvious that there are different levels of MyoD gene expression in rats after a certain period of acute aerobic physical activity (3, 7, and 14 days), with the MyoD gene expression level tends to increase per period, albeit statistically insignificant. MyoD is a family of proteins known as myogenic regulation factors (MRFs) that plays a major role in regulating the differentiation of myoblasts towards hypertrophy. An explanation of the increase in MyoD gene expression is because MyoD will be activated in satellite cells in response to physical activity or damage to muscle cells.<sup>1,2,5</sup> these cells become activated and express myogenic regulatory factors (MRFs<sup>7,8,9</sup>

Normally, quiescent satellite cells will express Pax 3/Pax 7 during survival and inhibit muscle differentiation. However, in response to aerobic physical activities or damages, Lepper et al. has demonstrated that Pax7-expressing satellite cells can allow muscle repair after several bouts of acute injury, and then expresses MyoD.<sup>7,10</sup>

At first, muscles will become inflamed then passive satellite cells will become active and perform repair of muscle cells. MyoD-positive cells then exits from the cell cycle and form new passive satellite cells or differentiate into myoblasts until physiological adaptations occur towards muscle hypertrophy.<sup>1,11</sup> Based on previous research, an increase in the expression of the MyoD gene is induced by several biomolecular pathways including release of MyoD from the inhibitory effect of



**Figure 2 MyoD Bands Visualized by Electrophoresis (Bluepads) (A), Mrna Expressions in Gastrocnemius (B). Values are expressed as Mean±SEM (p=0.16)**

PHB2, activation of mTOR pathway that inhibits proteolysis in MyoD, and activation of P70S6K pathway<sup>1,12,13</sup>

Insignificant increased level of MyoD gene expression showed in this study proves that there is an increase of MyoD gene expression with time. The control group did not express that high amount of MyoD because there was less physical activities or damages to induce the activation of quiescent satellite cells, especially in the soleus and gastrocnemius muscle, in this control group.

With time, the expression of MyoD also increased. MyoD as the main regulator protein induces the expression of another MRFs family (myogenin, myf5, mrf4) to join the interplay for myogenesis and finally induce hypertrophy. There are some reasons that may explain the statistically insignificant results in this study. The duration, intensity, number of samples, or maybe the age of the rats may contribute to this result. Hence, to obtain the significant results, further study with increased intensity and time of exercise per day as well as longer duration and different age of animals in the sample is needed. This study has shown that different periods of time in acute phase lead to different expressions of MyoD and that longer period of time can induce a higher expression of the gene.

This study concludes that acute aerobic physical activities in a certain period (3, 7 and 14 days) create different effects on MyoD gene expression

although statistically insignificant. This study also suggests that the longer the period is, the higher the MyoD gene expression level.

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