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The Effect of Interval versus Continuous Training on Actin and Myosin Heavy Chain Levels in Adult Rat Skeletal Muscle

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Abstract

Objective: Exercise helps maintain and improve contractile proteins and muscle mass. It is necessary to determine which type of exercise and its intensity yields maximal benefits. This study compared the effects of interval versus continuous training on levels of actin and myosin heavy chain (MHC) in the gastrocnemius muscles of adult Wistar rats. **Materials and Methods:** Twenty male Wistar rats aged 12 months were evenly and randomly divided into three groups: (1) the control group, (2) the interval training group, which consisted of four rounds of running for 4 min (with 1 min of active rest between each run), and (3) the continuous training group, which consisted of 40 min of continuous running. The treatments were given for 8 weeks, 5 days per week. Actin and MHC levels in gastrocnemius muscle were measured using the ELISA. **Results:** Actin levels in the continuous training group were significantly higher than the interval training group ($P = 0.039$). We found actin levels in continuous training group were significantly higher than the control group ($P = 0.016$), but there was no significant difference between interval training and control group ($P = 0.624$). There were no significant differences in MHC levels between the continuous and the interval training groups ($P = 0.231$). **Conclusion:** We found that continuous training was more effective than interval training in stimulating actin proteins in the gastrocnemius muscle.

Keywords: Actin, aging, continuous training, interval training, myosin heavy chain, sarcopenia

Introduction

The process of aging leads to deterioration in the physiological functions of various body systems, especially in the musculoskeletal system. The decrease in skeletal muscle mass and strength occurs with aging,[1] a condition described as sarcopenia[2] which causes muscle weakness and disability in elderly people.[1] Muscle weakness can be caused by a decrease in skeletal muscle mass.[3] The decrease in muscle mass generally occurs after 30 years of age[3,4] and mainly occurs in the lower limbs.[5] The reduced number of muscle fibers can be described as a decline of skeletal muscle mass.[4] The decline in muscle mass during the aging process is a multifactorial process.[6,7] There is evidence that a decrease of myosin heavy chain (MHC) synthesis occurs with aging. Research by Thompson showed that there is a decrease in MHC content in aged semimembranosus muscles.[2] The force generation of skeletal muscle mass can be altered if the amount of MHC is decreased and/or if there is an insufficient amount of actin for cross-bridge formation.[8]

Various types of exercise may prevent a decrease in muscle mass during the aging process and help maintain muscle mass in elderly people, but which type of exercise and what intensity yield maximal benefits is still under study.[9] The WHO and American College of Sports Medicine recommend a minimum of 150 min of moderate-intensity physical exercise (40%–60% of VO_2 maximum) or 75 min of vigorous-intensity physical exercise (60%–85% VO_2 max) per week for healthy adults to maintain or improve health. Although the benefits of moderate-to-vigorous exercise have been known, 31.1% of adults worldwide have failed to meet the minimum physical exercise guidelines. One of the main advantages of interval training compared to continuous training is that interval training requires lesser time with similar benefits.[10] Interval training is one of the recommended physical exercises to prevent the

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aging of skeletal muscles because it is efficient in achieving the benefits of exercise. Interval training is a type of exercise with intermittent periods of intense exercise separated by periods of recovery.[11] This exercise can cause adaptations such as endurance and strength.[11] Research studies show that interval training can induce myofibrillar protein synthesis.[12] This study compared the effects of interval training and continuous training on actin and MHC levels in the gastrocnemius muscles of adult Wistar rats.

Materials and Methods

This research was approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia (No. 0889/UN2. F1/ETIK/2018).

Animals and housing

This study was an experimental study using 15 male adult Wistar strain *Rattus novergicus* (300–400 g) aged 12 months. The age of 12 months in rats is equivalent to 30 years in humans.[13] We used this age because muscle mass generally starts to decrease after 30 years of age in humans.[3,4] The rats were obtained from Biofarma Bandung. The animals were housed under controlled temperature conditions (23°C), were kept on a light-dark cycle of 12 h and received water and food *ad libitum*. The animals were maintained according to the code of conduct of the animal handling commission in the use of experimental animals. The acclimatization period was performed before treatment to introduce the animals to the environmental enrichment cage, treadmill, and other research conditions. The animals were divided randomly into three groups: (1) the control group, (2) the interval training group, and (3) the continuous training group.

Interval training protocol

To introduce the training program in the acclimatization phase and to minimize the stress in subsequent experiments, the animal treadmill was set to low speed (15 m/min for 10 min) for 5 days. The interval training was performed according to a protocol slightly modified from Hafstad *et al*. and Arabmomeni *et al*.[14,15] The interval training was performed every morning on Monday until Friday (5 days/week) for 8 weeks. The interval training consisted of 4 min of running interspersed with 1 min of active recovery, and it was repeated four times. The treadmill speed for running was increased each week from 16 m/min in the 1st week of exercise to 25 m/min in the last week of exercise. The animals performed a 5-min warm-up and a 5-min cool-down at the rate of 6 m/min [Figure 1].

Continuous training protocol

To introduce the training program in the acclimatization phase and to minimize the stress in subsequent experiments, the animal treadmill was set to low speed (15 m/min for 10 min) for 5 days. The continuous training was performed every morning on Monday until Friday (5 days/week) for 8 weeks. The continuous training consisted of 40 min of continuous running. The running speed was increased gradually from 9 m/min in the 1st week of exercise to 15 m/min in the last week of exercise. The animals performed a 5-min warm-up and a 5-min cool-down at the rate of 6 m/min [Figure 2].

Tissue preparation

After the completion of 8-week training program, the rats were sacrificed and the gastrocnemius muscles were excised. The muscles were trimmed of visible fat and connective tissue, frozen in liquid phosphate-buffered saline, and kept at −80°C until further processing.

Measurement of myosin heavy chain and actin levels

Gastrocnemius muscles were homogenized. The whole protein concentrations were measured with the Bradford protein assay and compared to MHC and actin concentrations that were measured with the ELISA kit. The Rat myosin heavy chain (MYH) Elisa kit (MBS755779) from MyBio Source, USA and the Rat ACTα1 Elisa kit (E-EL-R2583) from Elabscience was used.

Statistical analyses

The Shapiro–Wilk test was used to verify data normality. One-way ANOVA was used to analyze actin and MHC levels. The value of $P < 0.05$ was used as a criterion for statistical significance. Statistical analyses were performed using the IBM SPSS Statistic Program version 24, IBM, US.

Results

Actin levels

Figure 3 shows a comparison of actin protein levels between the control group, the continuous training group, and the interval training group. The study shows that actin levels were 2.88 ± 1.42 ng/mg in the control group, 5.17 ± 1.47 ng/mg in the continuous training group, and 3.29 ± 0.88 ng/mg in the interval training group [Table 1]. Based on statistical testing,

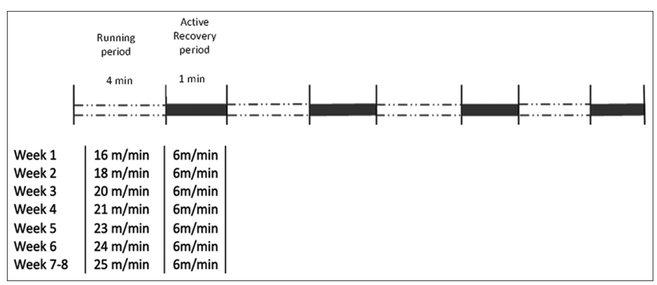

Figure 1: Interval training scheme

Table 1: Levels of actin alpha 1 and myosin heavy chain after 8 weeks of treatment

	Control	Continuous training	Interval training	Significant
ACTα1 (ng/mg total protein)	2.88±1.42	5.17±1.47	3.29±0.88	0.035*
MHC (ng/mg total protein)	18.83±3.18	19.34±3.50	16.63±3.40	0.433

*Significant $P < 0.05$, Data mean±SD, significant if $P<0.05$. SD: Standard deviation, MHC: Myosin heavy chain, ACTα1: Actin alpha 1 there were significant differences in actin levels between the control group and the continuous training group ($P = 0.016$), but there were no significant differences between the control group and the interval training group ($P = 0.624$). On the other hand, there were significant differences in actin levels between the continuous training group and the interval training group ($P = 0.039$).

Myosin heavy chain levels

Figure 4 shows a comparison of MHC protein levels between the control group, the continuous training group, and the interval training group. Our study shows that MHC levels were 18.83 ± 3.18 ng/mg in the control group, 19.34 ± 3.50 ng/mg in the continuous training group, and 16.63 ± 3.40 ng/mg in the interval training group [Table 1]. Based on statistical testing, there were no significant differences in MHC levels between the control group and the continuous training group ($P = 0.814$), between the control group and the interval training group ($P = 0.327$), and between the continuous training group and the interval training group ($P = 0.231$).

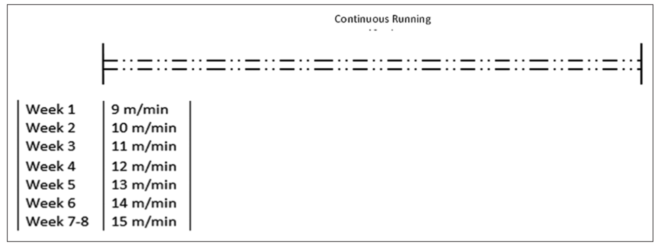

Figure 2: Continuous training scheme

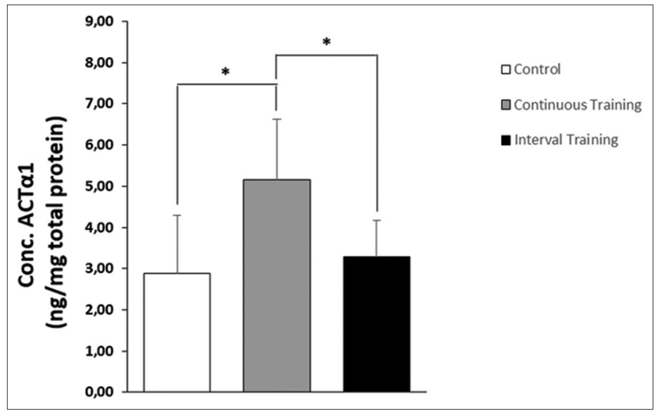

Figure 3: Actin alpha 1 protein levels in the gastrocnemius muscles of control group, continuous training group, and interval training group. Data are represented as mean ± standard deviation. *$P < 0.05$

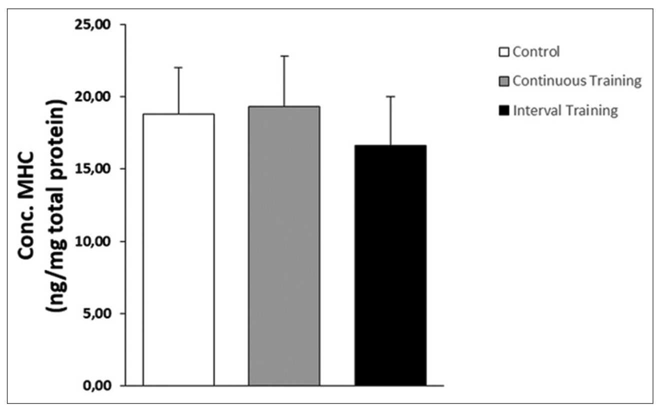

Figure 4: Myosin heavy chain protein levels in the gastrocnemius muscles of control group, continuous training group, and interval training group. Data mean ± standard deviation. $P = 0.433$ (not significant)

Discussion

The reduction of muscle mass is characteristic of aging skeletal muscles. Regular physical exercise is recommended to counteract the decrease in muscle mass due to the aging process. For this reason, it is very important to determine the most effective and efficient type of exercise.[2] In the present study, we examined actin and MHC isolated from the gastrocnemius muscles to compare the effects of interval training and continuous training on actin and MHC levels in adult rat skeletal muscles. Force generation of skeletal muscle contraction depends on myofibrillar proteins, especially actin and myosin.[2] A decline in muscle mass during aging is caused by reduced size and number of muscle fibers. The cause of decreased muscle mass is a multifactorial process that includes physical activity, hormonal changes, lack of nutrition, and oxidative stress.[5] Physical exercise is one of the most efficient methods of counteracting the loss of skeletal muscle mass with aging.[16]

In the present study, we found differences in actin protein levels between the control group and the continuous training group. As shown in Figure 3, actin levels in the continuous group were higher than in the control group. This finding is supported by data showing that skeletal muscle hypertrophy can occur through aerobic exercise.[17] Recent studies suggest that inducing skeletal muscle hypertrophy requires continuous training that achieves a high volume of muscle contractions and a low load on skeletal muscles with an appropriate intensity of 70%–80% HRR and duration of 30–45 min, 4–5 days/week.[17,18] On the other hand, our study showed that there were no significant differences in actin levels between the control group and the interval training group, but we found differences in actin protein levels between the continuous training group and the interval training group [Figure 3]. These data suggest that continuous training has a better potential to stimulate myofibrillar protein synthesis (particularly actin protein synthesis) than interval training does.

The present study showed no significant differences in MHC levels between the control group and the continuous training group. There were no significant differences in MHC levels between the control group and the interval training group [Figure 4]. This finding is supported by a study from Konopka *et al*., which observed reduced mRNA expression for MHC IIb after aerobic training and unaltered MHC IIa mRNA expression after 12 weeks of aerobic exercise training.[19] Further research is needed to examine the MHC isoform proportion related to continuous training and interval training.

The results of this study indicate that continuous training may promote muscle fiber hypertrophy and may contribute to enhanced skeletal muscle mass. The reason for increased levels of actin and MHC in the gastrocnemius muscle may be the longer duration (high volume) of continuous training compared to interval training. In contrast, a study by Bell *et al*. shows that high-intensity interval training can increase the rate of myofibrillar protein synthesis 24-h post bicycle ergometer cycling exercise (10×1 min, 95% maximal heart rate max).[12] The protocol of interval training used in this study was of lower dose than the protocols used by Bell *et al*. The intensity of interval training used in this study may not have been enough to stimulate much myofibrillar protein synthesis. Therefore, it is necessary to determine the right dose of interval training to stimulate myofibrillar protein synthesis. The exercise stimulus to the protein synthetic response involves the activation of numerous signaling pathways. The Akt-mTOR is the main pathway for protein synthesis regulation. Various anabolic signals, one of which is produced by physical activity, can stimulate the Akt-mTOR pathway. Research shows that there is an increase in the mTOR levels in an age group after 6 weeks of running at the intensity of 60% VO_2 max.[20]

It has been shown that physical training can stimulate myofibrillar protein synthesis, and hence, it could potentially increase skeletal muscle mass during the aging process. Type, intensity, duration of exercise, and nutritional intake affect the result of myofibril synthesis in exercise.[21] The elderly are known to be able to produce protein synthesis after doing physical exercise,[12] but dose-response information is lacking. Aerobic exercise is assumed not to have a large effect on increasing skeletal muscle mass, and hence, there is a little scientific investigation on it compared to resistance training. Myofibrillar protein synthesis following aerobic exercise has been studied.[17,18] Previous research suggests that aerobic exercise programs can counteract the decrease of muscle mass in the aging process, with the recommended intensity of 60%–80% $VO_{2\ max}$ being an important aspect of aerobic exercise that can increase muscle mass.[17] A variety of different training programs were applied in each study, resulting in highly variable adaptations. This could be the reason some studies have not observed an increase of myofibrillar proteins, particularly actin and MHC. In conclusion, our study finds that continuous training was more effective than interval training in stimulating actin protein synthesis.

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Conflicts of interest

There are no conflicts of interest.

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