*ORIGINAL ARTICLE*

# Administration of Oral Curcumin to Resistance Exercise after Immobilization Does Not Affect Skeletal Muscle Fiber Diameter in Rattus Norvegicus

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## ABSTRACT

**Introduction:** The aim of this study was to explore the effect of adding oral curcumin to resistance exercise after immobilization on the diameter of skeletal muscle fiber in *Rattus Norvegicus*.

**Methods**: This was a post-test only study design on animal model. Subjects of the study were male *Rattus norvegicus strain Wistar*, age 10-12 weeks old, weigh between 150-300 g, were immobilized at soleus muscle for 2 weeks, then randomly allocated to 3 groups: (i) control group, (ii) resistance exercise, (iii) oral curcumin + resistance exercise. After 4 weeks of intervention, the diameter of the muscle fibers was measured.

**Result**: The results of this study showed a significant difference on the diameter of skeletal muscle fiber between control group and resistance exercise, as well as control group and resistance exercise + oral curcumin ($p<0.05$). There was no significant difference between resistance exercise only and resistance exercise + oral curcumin ($p>0.05$).

**Conclusion**: Administration of oral curcumin to resistance exercise after immobilization does not affect skeletal muscle fiber diameter in *Rattus Norvegicus*.

**Keywords** : curcumin, good health and well-being, immobilization, resistance exercise, skeletal muscle.

## ABSTRAK

**Pendahuluan:** Tujuan penelitian ini adalah untuk mengetahui pengaruh penambahan kurkumin oral pada latihan resistensi setelah imobilisasi terhadap diameter serat otot rangka *Rattus Norvegicus.*

**Metode:** Penelitian ini menggunakan bentuk desain *post-test only* pada model hewan. Subjek penelitian adalah *Rattus Norvegicus strain Wistar* jantan, umur 10-12 minggu, berat badan antara 150-300g, diimobilisasi pada otot soleus selama 2 minggu, kemudian secara acak dibagi menjadi 3 kelompok: (i) kelompok kontrol, (ii) latihan resistensi, (iii) kurkumin oral + Latihan resistensi. Setelah 4 minggu intervensi, diameter serat otot diukur.

**Hasil:** Hasil penelitian ini menunjukkan perbedaan yang signifikan pada diameter serat otot rangka antara kelompok kontrol dan latihan resistensi, serta kelompok kontrol dan latihan resistensi + kurkumin oral ($p<0.05$). Tidak ada perbedaan yang signifikan antara latihan resistensi saja dan latihan resistensi + kurkumin oral ($p>0.05$).

**Kesimpulan:** Pemberian kurkumin oral pada latihan resistensi setelah imobilisasi tidak mempengaruhi diameter serat otot rangka pada *Rattus Norvegicus.*


**Kata kunci:** imobilisasi, kesehatan yang baik dan kesejahteraan, kurkumin, latihan resistensi, otot rangka.

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## INTRODUCTION

Disuse atrophy is one of the conditions that accompany patients with prolonged immobilization, where the cause of immobilization is generally trauma and neuromuscular disorders (paralysis in stroke or spinal cord injury), orthopedic casts, body jackets, and splints or after trauma, fractures, diseases that require bed rest, or prolonged lying position.[1,2]

Immobilization causes muscle atrophy where there is a decrease in the diameter of skeletal muscle fibers.[3,4] This decrease in muscle fiber diameter causes a weakening of muscle strength and becomes a problem because within 3 to 5 weeks of immobilization will reduce muscle strength by 50%. Whereas muscle strength that is reduced in 1 week takes 4 weeks to return to initial strength through an intensive strengthening exercise program.[5]

In a state of immobilization, there is a decrease in protein synthesis, hypoproteinemia and a decrease in the volume of myofibril fibers.[6,7] Meanwhile, the stimulus response at the cellular level includes activation (Tumor Necrosis Factor-alfa) TNF-and transcription factor (Nuclear Factor-kappaB) NF-B which causes muscle protein degradation[8] and inhibit myogenesis and muscle regeneration.[9,10] Muscle

atrophy also occurs due to increased gene expression Atrogin-1 and MuRF-1.[11,12]

Several studies have shown that the process of decreasing protein synthesis and degradation can be inhibited by administering curcumin. Larger muscle mass and greater force production were displayed between the prolonged dietary curcumin and pair-fed groups for thirty-two-month-old male F344xBN rats.[13] Moreover, greater nuclear levels of Nrf2 and lower oxidative protein damage were observed in curcumin-fed animals.[13] Curcumin is a yellow pigment found in plants of the genus Curcuma longa (turmeric) and Curcuma xanthorrhiza Robx (temulawak) which has been widely used in traditional medicine. Curcumin is an inhibitor of NF-B transcription factor where inhibition of this NF-B pathway will stimulate myogenesis activity and skeletal muscle regeneration. Resistance exercise is a strengthening exercise using a load of 60-80% repetition maximum (RM) which will reduce the expression of the Atrogin-1 and MuRF-1 genes according to the intensity of muscle contraction. Specific objectives of this study is to explore the effect adding oral curcumin to resistance exercise after immobilization on the diameter of skeletal muscle fiber in Rattus Norvegicus.

## METHODS

This was an experimental study using post-test only study design. The animal model of this study were Rattus norvegicus strain Wistar male, age 10-12 weeks old, weighing between 150-300g.

The Rattus norvegicus were immobilized on the soleus muscle for 2 weeks. Immobilization was applied using thermoplastic splint from proximal knee joint to distal ankle, with the ankle at maximal plantar flexion, so the soleus could not contract but still able to walk. Then, they were randomly allocated in one of the three groups: intervention group A (exercise + curcumin), intervention group B (exercise), and control group. Each groups comprised of minimum 7 rats. Subjects were dropped out from the study if the died or if the splint became loose during immobilization or if there was contracture on the knee and ankle joint after immobilization that can hinder the exercise. All experimental procedures conformed with WHO Animal Ethics.

The first intervention group (A) was given oral curcumin (turmeric extract) at a dose of 500 mg/rat 3 times/week + resistance exercise 3 times/week. The second intervention group (B) was given resistance exercise 3 times/week. The resistance exercise protocol as follows: (i) measuring the weight (25-35% of body weight), and then attached the weight to its body, (ii) training of resistance exercise by climbing 6 steps of stairs of 40cm height and 45° angle, as depicted in Figure 1 (iii) repeated exercise for 3 times. The third group was the control group (C).

After 4 weeks of intervention, the subjects were terminated using enflurane, one of the commonly used inhalant gasses to kill laboratory mice in the most humane manner. The histological examination of the soleus muscle fibers of the three groups was then carried out. The soleus muscle was stained using the Hematoxylin-Eosin, the diameter of the muscle fibers was measured using a micrometer under 400x microscopic magnification.

**Figure 1.** Resistance exercise protocol

## RESULTS

The overview of the mean skeletal muscle weight and skeletal muscle fibers diameter of the experimental animals in all 3 groups are shown in Table 1. In Figure 2 comparison of the mean diameter of skeletal muscle fibers between groups is depicted. The mean diameter of skeletal muscle fiber of the resistance exercise group increased by 41.37% compared to the control group. In the resistance exercise + oral curcumin group experienced an increase in the average diameter of muscle fibers by 40.11% compared to the control group.

**Table 1. Overview of experimental animals (Rattus Norvegicus)**

| Groups | n | Skeletal Muscle Weight | Skeletal Muscle Fiber Diameter |
|---|---|---|---|
| | | Mean ± SD (g) | Mean ± SD (µm) |
| Control (C) | 8 | 233.0 ± 13.54 | 26.50+4.54 |
| Resistance Exercise (B) | 10 | 233.4 ± 38.42 | 45.20+5.61 |
| Resistance Exercise + Curcumin (A) | 8 | 239.4 ± 36.36 | 44.25+3.81 |

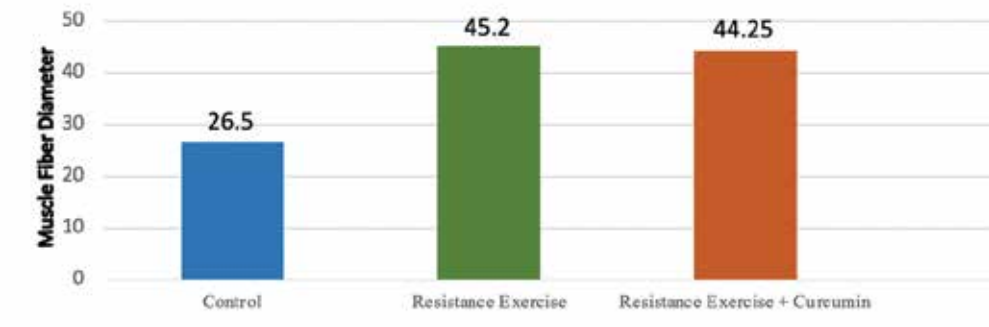


**Figure 2.** Mean Diameter of Skeletal Muscle Fibers Diameter

The ANOVA (Analysis of Variance) test showed significant difference (p=0.000) in the mean diameter of skeletal muscle fibers between groups (Table 2).

**Table 2. Skeletal Muscle Fiber Diameter ANOVA Test**

| | Sum of Squares | df | Mean Square | F | p |
|---|---|---|---|---|---|
| Between groups | 1854.285 | 2 | 927.142 | 40.303 | 0.000* |
| Within group | 529.100 | 23 | 23.004 | | |
| Total | 2383.385 | 25 | | | |

The level of significance (alpha) is set at 0.05 (5%)

* Significant if p < 0.05, ** not significant if p > 0.05

The multiple comparison test was conducted to analyze in which group the difference in the mean diameter of skeletal muscle fibers was found. In Table 3 the mean difference in the diameter of skeletal muscle fibers between the resistance exercise + curcumin and the resistance group only showed no significant difference (p=0.909). A significant difference were found in the diameter of skeletal muscle fibers between control group and resistance exercise, and also between control group and resistance exercise + curcumin (p=0.000).

**Table 3. Multiple Comparison Test of Skeletal Muscle Fiber Diameter**

| Groups (I) | Groups (J) | Mean difference (I-J) | p |
|---|---|---|---|
| Control | Resistance exercise | -18.70000* | 0.000* |
| | Resistance exercise + Curcumin | -17.75000* | 0.000* |
| Resistance exercise | Control | 18.70000* | 0.000* |
| | Resistance exercise + Curcumin | 0.95000 | 0.909 |
| Resistance exercise + Curcumin | Control | 17.75000* | 0.000* |
| | Resistance exercise | -0.95000 | 0.909 |

The level of significance (alpha) is set at 0.05 (5%)

* Significant if p < 0.05, * non significant if p > 0.05

* Mean difference is significant at 0.05 level

## DISCUSSION

Muscle atrophy results from increased muscle protein degradation over protein synthesis. This relationship between anabolic and catabolic signals causes skeletal muscle mass to increase or decrease.[14,15] In this study, the soleus muscle was made atrophic by being immobilized for 2 weeks, before performed exercise in the form of resistance exercise with a load of 25% - 35% body weight to stimulate hypertrophy of the muscles.

Muscles that perform resistance exercise will experience trauma to the muscle fibers, causing muscle injury. Disruption of muscle cell organelles will activate satellite cells that are outside the muscle fibers located between the basal lamina and the sarcolemma to proliferate at the trauma site. Biological attempts to repair or replace damaged muscle fibers begin with the fusion of satellite cells into the muscle fiber to increase the cross-sectional area or hypertrophy. Satellite cells replicate and differentiate and combine with muscle fibers to form new myofibrils and/or repair damaged fibers. Myofibrils will continue to increase in thickness and number. After fusion, satellite cells will become a source of new cell nuclei to increase muscle fiber growth.[16,17] Activation or phosphorylation of Akt is an anabolic signal and a dominant inhibitor of catabolic signals. Activation of Akt is mediated by the insulin-like growth factor 1 (IGF-1)/phosphatidylinositol-3 kinase (PI3K) pathway. The IGF-1/PI3K pathway is triggered by increased muscle load and encoding IGF-1 gene expression.[15,18]

Previous study showed that giving oral curcumin 400mg/rat every week for 2 weeks in an immobilized soleus muscle can reduce muscle atrophy.[19] Curcumin can act directly on muscle precursor cells to stimulate proliferation and differentiation under favorable conditions. NF-kappaB regulates myogenesis and its modulating activity in muscle which is beneficial for muscle repair. Curcumin also shows anti-inflammatory effects by downregulating NF-κB expression and suppressing COX-2 production, both of which have a vital role in the inflammatory cascade.[20]

In this study, administration of oral curcumin to resistance exercise did not show a significant different in muscle diameter compared to resistance exercise only. It can be analyzed that curcumin-activated myogenesis in the form of stimulation of muscle cell proliferation is not sufficient to increase the diameter of muscle fibers. However, the immunohistochemistry analysis was not performed in this study that might explain the beneficial effect of oral curcumin in skeletal muscle metabolism. Furthermore, resistance exercise alone can increase muscle mass in human by stimulating intracellular anabolic signals. These intracellular anabolic signals further increase protein synthesis and reduce protein degradation.[21] Both intervention groups in this study that performed resistance exercise, with or without oral curcumin, showed significant difference in increased muscle fiber diameter compared to control group.

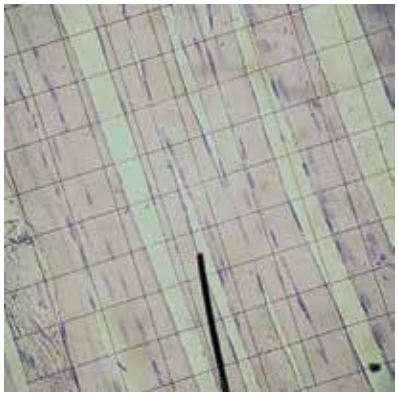

**Figure 3.** Example of a histopathological longitudinal cross section of soleus muscle of the control group (Magnification x 400)

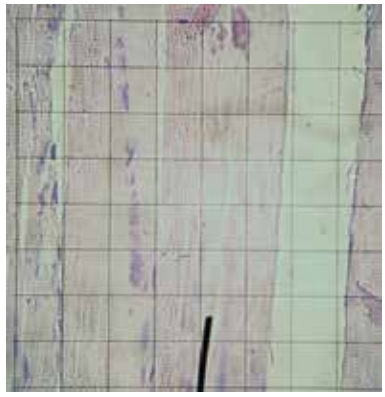

**Figure 4.** Example of a histopathological longitudinal cross section of soleus muscle from the resistance exercise group (Magnification x 400)

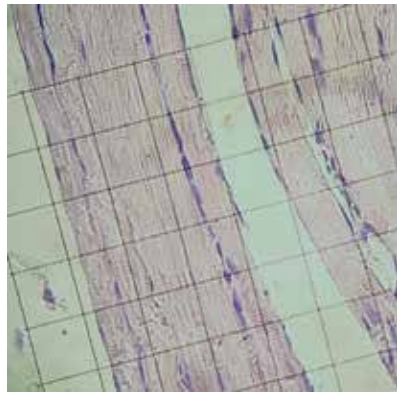

**Figure 5.** Example of a histopathological cross-section of the soleus muscle from the resistance exercise group plus oral curcumin (Magnification x 400)

## CONCLUSION

Administration of oral curcumin to resistance exercise for 4 weeks after immobilization did not affect skeletal muscle fiber diameter in *Rattus Norvegicus*. Resistance exercise alone was sufficient to cause an increase in skeletal muscle fiber diameter compared to control group in *Rattus Norvegicus*. Further studies with immunohistochemistry analysis are needed to evaluate the effect of adding oral curcumin in skeletal muscle metabolism.

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