Original Research

Comparison of Hamstrings and Quadriceps Femoris Muscle Thickness Increment between Agonist-Antagonist Paired Set and Traditional Set Resistance Training in Untrained Healthy Subjects

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ABSTRACT

Background: Resistance training is an effective way to increase muscle mass. Resistance training with agonist-antagonist paired set method can be an alternative to increase muscle mass within a relatively short training time.

Aim: To compare the increase in hamstrings and quadriceps femoris muscle thickness between agonist-antagonist paired set (APS) and traditional set (TS) resistance training in untrained healthy subjects.

Material and Methods: This study was an experimental study on 16 untrained healthy men which were randomly assigned to the APS and the TS group. Each group got leg curl and leg extension exercises with equal training volume for 6 weeks. For the APS group, 1 set of leg curls was followed by 1 set of leg extensions, repeated for 3 sets. For the TS group, 3 sets of leg curls were followed by 3 sets of leg extensions. Muscle thickness was compared from pre- to post-training and between the intervention groups using B-mode ultrasound.

Results: Muscle thickness of the hamstrings and quadriceps femoris increased significantly from pre- to post-training in both groups (p<0.05). The increase in muscle thickness between the two groups was not significantly different (p> 0.05).

Conclusion: Resistance training with the APS method did not give a higher increment of hamstrings and quadriceps femoris muscle thickness compared to the TS method in healthy untrained subjects.

Keywords: agonist-antagonist paired set, muscle thickness, resistance training, traditional set.
Introduction

Inadequate muscle strength can contribute to major functional losses of even the most basic activities of daily living.\(^1\)

Many factors affect muscle strength. One of them is muscle size. There is a clear positive relationship between the cross-sectional area of muscles and muscle strength, greater cross-sectional area correlated with greater strength capacity.\(^2\)

Resistance training is an effective way to increase muscle mass. Progressive resistance training positively stimulates intracellular anabolic signals due to mechanical tension, muscle damage, inflammatory response, and metabolic stress. These intracellular anabolic signals further increase protein synthesis and reduce protein degradation.\(^3\)

Over time, the summation of these responses causes muscle thickness increment through increasing the number of sarcomeres.\(^4\) Muscle imbalance that can cause injury should be avoided. Therefore, agonist and antagonist muscle groups exercise, such as hamstrings and quadriceps femoris, should be included in routine resistance training.\(^4\)

The amount of muscle damage and inflammatory response after resistance training is influenced by the type of muscle contraction, load intensity, and rest period during resistance training. The greater the load intensity and the shorter the rest period between sets, the greater the muscle damage and the inflammatory response that will occur.\(^6\) The American College of Sport Medicine (ACSM) recommendation for increasing muscle strength and hypertrophy is to prescribe high intensity load resistance training with a minimum load of 70% 1 RM (repetition maximum).\(^5\)

The hypertrophic response of resistance training can be maximized by manipulating appropriate training variables. However, most resistance training use traditional method (multiple sets with a rest interval 2-3 minutes/set).\(^6\) A 3-5 minute rest period is recommended for maximum or near maximum load exercises. Often, 1 type of exercise is done with a certain number of sets before proceeding to the next type of exercise. This training method is time consuming due to the long rest period.\(^7\) The agonist-antagonist paired set training, which is resistance training in the agonist-antagonist relationship carried out alternately, has been demonstrated to be more efficient than the traditional technique of performing sets for each exercise independently by significantly reducing the total time of a resistance training session.\(^8\)\(^9\)

Studies examining chronic adaptation of the APS method are still limited. These studies are only performed on trained subjects and do not measure muscle hypertrophy/muscle thickness. Therefore, the aim of this study is to compare the increase in hamstrings and quadriceps femoris muscle thickness between agonist-antagonist paired set (APS) resistance training and traditional set (TS) resistance training in untrained healthy subjects. We hypothesized that high intensity resistance training using the APS method results in higher increment of hamstrings and quadriceps femoris muscle thickness compared to the TS method in untrained healthy subject.

Material and Methods

This study was an experimental study using randomized pre- and post-test design on Dr. Soetomo General Hospital Physical Medicine and Rehabilitation resident physicians, Surabaya, Indonesia who met the inclusion criteria and did not have any of the exclusion criteria. The research team consisted of 1 resident and 3 specialists of
Department of Physical Medicine and Rehabilitation, Dr. Soetomo General Hospital, Surabaya, Indonesia. Inclusion criteria were untrained healthy men aged 18-40 years with body mass index 18.5-24.99 kg/m², agreed to be the study subject, and followed the protocol by signing the informed consent. Exclusion criteria were participation in resistance training at least 6 months preceding the start of the experiment, history of injury, fracture, or previous surgery in the non-dominant lower limb, hypertension, ischemic heart disease, and peripheral artery disease. Drop out criteria: subject discontinuation and unwillingness to continue the programs, inability to complete the training according to the study protocol, complaint of joint/muscle pain in active movement (no load) or signs of inflammation that appeared suddenly during training, and complaint of chest pain and dyspnea during or after a training session.

Sixteen participants were recruited using consecutive sampling technique. Participants were divided into 2 groups, namely the antagonist-agonist paired set (APS) group and the traditional set (TS) group, through simple randomization using a sealed envelope. Each group received hamstrings (leg curl) and quadriceps femoris (leg extension) resistance training on non-dominant limb using Quadriceps Bench (Enraf Nonius, Rotterdam, Netherland) with an intensity load of 70% 1-RM, 12 reps/sets, 3 sets, 2 times/week. The rest interval for each set for the same type of exercise was 2 minutes. Determination of 1-RM was done every week. For the APS group, 1 set of leg curls was followed by 1 set of leg extensions, alternating to 3 sets for each muscle. For the TS group, 3 sets of leg curls were followed by 3 sets of leg extensions. Training sessions were conducted and supervised by research resident under the direction of Physical Medicine and Rehabilitation specialists, which all of whom were members of the research team. Both participants and trainer were not blind to group allocation. Determination of leg dominance was done by kicking the ball test.

The participants underwent a familiarization process for 1 session before beginning the training. The participants sat up straight as relaxed as possible with 90° flexion of the hip and knee. The distance between the edges of the chair to the popliteal was 5 cm. Resistance arm was placed just above the ankle. The movement speed was regulated by a metronome. Verbal encouragement could be given during training session.

**Figure 1. Schematic representation of the traditional method.**

A = leg curls and B=leg extensions.
Figure 2. Schematic representation of the agonist-antagonist paired set method.
A = leg curls and B = leg extensions.

Before and after 6 weeks of training, the muscle thickness of hamstrings and quadriceps femoris were measured with B-mode ultrasound (Logiq P6) on the anterior [50% of thigh length (TL)] and posterior (50% and 70% of TL) aspects of the non-dominant thigh. Thigh length was measured using anatomical landmarks (the distance between the most proximal side of the major trochanter and the most distal side of the lateral femoral condyle), and the measurement sites were marked using a marker pen. Participants were asked to lie down as relaxed as possible. The surface of the transducer was positioned to the skin surface at minimum pressure to obtain a clear image. All images were taken in the longitudinal plane relative to the examined limb. Muscle thickness was defined as the distance between the bone-muscle interface and the adipose-muscle interface. Measurements were made from the most superficial side of the bright line representing the bone-muscular cortex interface to the most superficial side of the bright line representing the interface of the outer fascial layer of muscle and subcutaneous adipose tissue. Three images from each location were evaluated and the average value of each location was used for data analysis. Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS version 23.0). The characteristics baseline were compared using independent sample t test. We evaluated the differences of muscle thickness before and after the intervention of both groups. If the data were normally distributed, we use paired sample t test. However, if the data were distributed abnormally, we use Wilcoxon test. We also compared the group differences (delta) of muscle thickness using independent sample t test. The differences were considered statistically significant at \( p < 0.05 \). All study subjects had signed the informed consent form and this study had ethical clearance from the ethical committee of Dr. Soetomo General Hospital (ethical approval certificate number: 1914/KEPK/ III/2020).

### Results

Fourteen subjects completed the training sessions and study protocol. Two of 16 subjects were dropped off because they did not attend the training session more than two times. None of the subjects reported any adverse effects during or after the training session. The homogeneity test of subject’s characteristics, whether age, height, body weight, body mass index, and muscle thickness before the intervention between both of the groups, found no significant differences (Table 1) so they did not influence the result of the study. The muscle thickness of quadriceps femoris and hamstrings in APS and TS groups are shown in figure 3 and 4.

| Characteristics | Agonist-antagonist paired set group (n=7) | Traditional set group (n=7) | \( P \) value* |
|-----------------|------------------------------------------|----------------------------|---------------|
| Age (year)      | 31.57 ± 3.95                             | 34.71 ± 4.03               | 0.166         |
| Bodyweight (kg)     | 62.86 ± 9.89 | 65 ± 7.68 | 0.659 |
|-------------------|--------------|-----------|-------|
| Height (m)        | 1.71 ± 0.06  | 1.69 ± 0.07 | 0.582 |
| Body mass index (km/m²) | 21.50 ± 2.01 | 23.00 ± 2.16 | 0.205 |
| Muscle thickness quadriceps (Pre) (mm) | 35.98 ± 9.34 | 40.05 ± 5.14 | 0.332 |
| Muscle thickness hamstrings (Pre) (mm) | 43.09 ± 5.71 | 40.14 ± 8.06 | 0.445 |

* Independent sample t test

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**Table 2. Comparison of training time efficiency between the two groups (Mean ± SD)**

| Variable | APS group | TS group |
|----------|-----------|----------|
| a        | b         | a        | b       | a        | b       | a        | b       |
| 1        | 2         | 1        | 2       | 1        | 2       | 1        | 2       |

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**Figure 3. Quadriceps femoris muscle thickness (a) before exercise (b) after 6 weeks of training.** 1 = the APS group, 2 = TS group

**Figure 4. Hamstrings muscle thickness (a) before exercise (b) after 6 weeks of training.** 1 = APS group, 2 = TS group.

In this study, the APS method required about 7 minutes on each session. The total training time was 84 minutes or 1.4 hours. Meanwhile, the TS method required about 13 minutes on each session. The total training time was 156 minutes or 2.6 hours. The efficiency of training time in the APS group has a large effect size (Table 2).
Absolute gain (mm) | Time (hour) | Efficiency (mm/hour) | Absolute gain (mm) | Time (hour) | Efficiency (mm/hour) | Effect size
---|---|---|---|---|---|---
Muscle thickness of *quadriceps femoris* | 4.33 ± 2.38 | 1.4 | 3.09 ± 1.7 | 3.05 ± 2.85 | 2.6 | 1.17 ± 1.1 | 1.34 (large)
Muscle thickness of *hamstrings* | 9.88 ± 3.20 | 1.4 | 7.06 ± 2.29 | 9.51 ± 3.46 | 2.6 | 3.66 ± 1.33 | 1.82 (large)

There were significant increases in hamstrings 1-RM in the APS group (2.17 ± 0.45 kg, p = 0.003) and TS group (2.56 ± 0.46 kg, p = 0.001). There were significant increases in quadriceps femoris 1-RM in the APS group (4.50 ± 0.47 kg, p = 0.000) and TS group (5.01 ± 0.68 kg, p = 0.000) (Figure 5). There was no significant difference of quadriceps femoris (p=0.548) and hamstrings (p=0.557) 1-RM differences between the APS group and the TS group (Figure 6).

![Figure 5](image)

**Figure 5.** 1-RM of the non-dominant quadriceps femoris and hamstrings after the intervention. Notes: green bar = pre training; purple bar = post training. *Difference between pre and post training (p < 0.05). Values are means ± standard deviation of the mean.
Figure 6. 1-RM differences between two groups after training (p>0.05).

Notes: green bar = quadriceps femoris; purple bar = hamstrings.

Values are means ± standard deviation of the mean.

There were significant increases in hamstrings muscle thickness in the APS group (9.88 ± 3.20 mm, p = 0.000) and TS group (9.51 ± 3.46 mm, p = 0.000). There were significant increases in quadriceps femoris muscle thickness in the APS group (4.33 ± 2.38 mm, p = 0.003) and TS group (3.05 ± 2.85 mm, p = 0.030) (Figure 7). There was no significant difference of the quadriceps femoris (p=0.306) and hamstrings (p=0.842) muscle thickness delta between the APS group and the TS group (Figure 8).

Figure 5. Muscle thickness of the non-dominant quadriceps femoris and hamstrings after the intervention. Notes: blue bar = pre training; red bar = post training. *Difference between pre and post training (p < 0.05). Values are means ± standard deviation of the mean.
Figure 6. The muscle thickness differences between two groups after training (p>0.05).

Notes: blue bar = quadriceps femoris; red bar = hamstrings.

Values are means ± standard deviation of the mean.

Discussion

Subjects in this study were clinically proven healthy men, thus they considered to be feasible to participate in resistance training with high intensity. Homogenization of subjects’ gender was carried out because gender affected changes in post-training muscle hypertrophy. The average age of subjects in both groups were 31 and 34 years which showed that the subjects were still in the productive age, which enabled them to participate in resistance training with a relatively smaller injury risk and still have good awareness. In addition, hypertrophic response to resistance training also decreased with age. The subjects had normal anthropometric profiles suitable with inclusion criteria.

In this study, measurement of the muscle thickness was performed using ultrasound. A systematic review conducted by Abe et al. shows that measurement of the muscle thickness using ultrasound has high intra-rater and inter-rater reliability. Abe et al. also reported a strong correlation between the anterior mid-thigh muscle thickness and the cross sectional area of quadriceps femoris and a strong correlation between the posterior mid-thigh muscle thickness and the cross sectional area of hamstrings.

The muscle thickness increment in this study was a response to physiological adaptation of high-intensity resistance training in the form of muscle hypertrophy and, to a lesser extent, muscle hyperplasia. Mechanical stress from high intensity resistance training activated Protein Kinase B (PKB)/Akt and further increased muscle protein synthesis. In addition, high intensity resistance training resulted in the occurrence of myotrauma which could release growth factors for proliferation and differentiation of satellite cells. The muscle thickness increment in the TS group after 6 weeks of resistance training showed that the training protocol was sufficient to provide mechanical stress to trigger muscle hypertrophy.

In the APS group, there was also an increase in the muscle thickness of the quadriceps femoris and hamstrings, although it did not differ significantly between the two groups. The volume load of both methods were made equal in this study so that the level of fatigue might be somewhat similar. In addition, when resistance training are below the level of muscle failure, the training response may be more heterogeneous due to
differences in the level of fatigue experienced by each individual during training.\textsuperscript{16}

The APS method generally produced a greater training volume in a shorter time which could increase muscle fatigue.\textsuperscript{17} Higher volume load was needed to maximize the response of muscle hypertrophy in diverse populations.\textsuperscript{18} Study conducted by Paz et al.\textsuperscript{17} showed an increase in bench press and seated row volume loads and an increase in the fatigue index of latissimus dorsi, biceps brachii, pectoralis major, and triceps brachii during seated row training in APS method. In contrast to the study of Paz et al.\textsuperscript{17}, this study used 12 repetitions/set and avoided muscle failure so that the volume load between both groups could not be compared.

Although the subjects’ satisfaction index was not assessed objectively, the researchers observed that satisfaction of the APS group subjects was less compared to the TS group subjects. This may have occurred due to the characteristic of the APS method which was more fatiguing. Although the rest interval between the same types of training was similar in both groups, the hamstrings and quadriceps femoris in the APS method did not fully rest during the rest interval. Giving quadriceps femoris resistance training in the middle of hamstrings rest interval resulted in early muscle co-activation. In this case, the hamstrings continued to contract eccentrically to control the contractions of the quadriceps femoris and contract concentrically to restore the position of the knee joint after quadriceps femoris contractions.

In this study, there was an increase in hamstrings muscle thickness that was higher than quadriceps femoris in both groups. This might be caused by the characteristic of the hamstrings muscle itself. Liu et al.\textsuperscript{19} stated that hamstrings muscle contained a relatively high proportion of type II fibers compared to quadriceps femoris. Type II muscle fibers had higher concentration or activity of glycolytic enzymes than Type I muscle fibers. This will support the rapid formation of ATP for muscle contraction through anaerobic glycolysis with lactate as a metabolic byproduct. Thus, hamstrings muscle was more prone to lactate buildup compared to quadriceps femoris muscle. This lactate accumulation could further affect muscle hypertrophy through mechanism mediated by anabolic hormone and cytokine enhancement.\textsuperscript{20}

In this study, the APS group produced an increase in muscle thickness similar to the TS group with the total training time in the APS group almost half of the TS group. The efficiency calculation also showed that the APS method had higher time efficiency than the TS method with large effect size. Thus, the APS method could be an alternative method for resistance training of hamstrings and quadriceps femoris especially for individuals who had limited exercise time to get muscle hypertrophy similar to traditional resistance training.

This study has several limitations. First, this study only took certain untrained healthy male subjects so that they could not be generalized. Second, the resistance training only involved 2 muscles so the accumulation of metabolic byproducts was less compared to the resistance training using several types of antagonists and agonists muscles. Third, researchers had difficulty in monitoring physical activities and daily food intake that could affect the study results. Last, lactate level during the intervention was not assessed in this study. Research conducted by de Souza et al.\textsuperscript{21} on 10 trained subjects using 8RM loads until reaching muscle failure showed lactate level and fatigue scale (RPE) were higher in the superset and APS groups compared to the TS group with the superset group showing the highest increase. An increase in lactate level could indicate an increase in metabolic stress during exercise in response to low energy. Metabolic stress has
an important role in the release of hormones and anabolic cytokines, recruitment of additional motor units, cell swelling, and production of reactive oxygen species. All of these processes can produce anabolic signals that stimulate muscle protein synthesis.\textsuperscript{22}

Conclusion

The APS method did not result in a higher increment of hamstrings and quadriceps femoris muscle thickness compared to the TS method in healthy untrained subjects. However, the agonist-antagonist paired set method has higher training time efficiency compared to the traditional set method.

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