CASE REPORT

Muscle fiber cross-sectional area is increased after two weeks of twice daily KAATSU-resistance training

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Int. J. KAATSU Training Res. 2005; **1**: 65-70

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The purpose of this study was to examine the effect of low-intensity (20% of 1-RM) resistance training (LIT) combined with restriction of muscular venous blood flow (KAATSU) on muscle fiber size using a biopsy sample. Three young men performed LIT-KAATSU (restriction pressure 160-240 mmHg), and two young men performed LIT alone. Training was conducted twice daily for 2 weeks using 3 sets of two dynamic lower body exercises. Quadriceps muscle CSA was measured by magnetic resonance imaging at midpoint of the thigh. Muscle biopsies were obtained from the vastus lateralis (VL) muscle using a needle biopsy. Mean relative change in 1-RM squat strength was 14% in the LIT-KAATSU and 9% in the LIT after two weeks of the training. Mean changes in quadriceps muscle CSA was 7.8% for LIT-KAATSU and 1.8% for LIT. Changes in muscle fiber CSA was 5.9% for type-I and 27.6% ($p<0.05$) for type-II in the LIT-KAATSU, and -2.1% and 0.5%, respectively, in the LIT. Mean fiber CSA changed 17.0% in the LIT-KAATSU, but not in LIT (-0.4%). We concluded that skeletal muscle and fiber hypertrophy, especially type-II fiber, occur after high frequency KAATSU training.
Key words: muscle hypertrophy, fast-twitch muscle fiber, Kaatsu training

INTRODUCTION

Low-intensity (20% of 1-RM) resistance training combined with restriction of muscular venous blood flow (KAATSU training) produces muscle hypertrophy and strength gain (Takarada et al., 2000; 2002). Muscle CSA is increased to a similar degree as traditional high-intensity (HIT, 80% of 1-RM) resistance training when KAATSU training is performed at a similar training volume and frequency (Takarada et al., 2000). Recently, Abe et al. (2005) found that magnetic resonance imaging (MRI)-measured thigh muscle volume and circulating insulin-like growth factor-1 (IGF-1) increased after two weeks of high frequency (twice a day) KAATSU training. In that study, thigh muscle size increased as much as previously reported (Jones and Rutherford 1987; Young et al. 1983) in HIT of 3-4 months (~8%). However, it is unclear whether this skeletal muscle hypertrophy is attributed to fiber hypertrophy or any other extracellular tissue/liquid gain.

During KAATSU training exercise, both the venous outflow from and arterial inflow to the exercising muscle is considerably suppressed. The resulting hypoxic and acidic intramuscular environment may induce additional muscle fiber recruitment of both small (type I fibers) and large motor units (type II fibers) in order to maintain a given level of force (Takarada et al. 2000). Previous studies (Moritani et al. 1992; Sundberg 1994) have suggested that the type II muscle fibers might be recruited preferentially during exercise when blood flow is suppressed. Therefore we hypothesized that fiber hypertrophy would be larger in the type II fibers than in the type I fibers following KAATSU training. In the present study, we examined the effects of KAATSU training on muscle fiber cross-sectional area (CSA) using a muscle biopsy sample.

METHODS

Subjects

Three men [age 20-47 yrs, height 164-179 cm] performed low-intensity resistance training combined with restriction of muscular venous blood flow (LIT-KAATSU), and two men [age 23 and 27 yrs, height 173 and 176 cm] performed low-intensity resistance training without blood flow restriction (LIT). All subjects were informed of the procedures, risks, and benefits, and signed an informed consent document before participation. The study was approved by the Human Experiment Ethics Committees, University of Tokyo and Tokyo Metropolitan University.

Training protocol

The subjects in the both LIT-KAATSU and LIT groups participated in two weeks of individualized resistance training. Training was conducted twice per day (10:00-12:00 and 16:00-18:00) for 12 consecutive days (excluding one Sunday). Following a warm up, the subjects performed 15 repetitions of squat and leg curl exercises using an isotonic training machine (Nippyo). The intensity of exercise was 20% of one repetition maximum (1-RM) for both

LIT-KAATSU and LIT groups. The subjects performed three sets of exercise in each exercise session, with 30 seconds rest between sets and exercises. The exercise intensity was determined during the initial stage of training and remained constant for the duration of the training period. A specially designed elastic belt (Sato Sports Plaza Ltd., Tokyo, Japan) was placed around the most proximal portion of both legs during the exercise session in the LIT-KAATSU group (Abe et al., 2005; Takarada et al., 2000). The belt contained a small pneumatic bag along its inner surface that was connected to an electronic pressure gauge that monitored the restriction pressure (MPS-700, VINE, Tokyo, Japan). On the first day for the training (Day 1), the cuff pressure was 160 mmHg and the pressure was increase 10 mmHg each day until a final training cuff pressure of 240 mmHg (Day 9) was reached. The restriction pressure of 160-240 mmHg was selected for the occlusive stimulus as this pressure has been suggested to restrict venous blood flow and cause pooling of blood in capacitance vessels distal to the cuff, and ultimately arterial blood flow. The restriction of muscular blood flow was maintained for the entire exercise session (including rest periods) and was released immediately upon completion of the session. The LIT group performed the same exercises at the same intensity but without the restriction of muscular blood flow.

Maximum strength measurements

Maximum dynamic strength (1-RM) was evaluated by using an isotonic squat machine. All subjects performed practices lifts prior to attempting maximal lifts. One-RM was assessed prior to (pre-testing) and 3 days after the final training (post-testing). After warming up, the load was set at 80% of the predicted 1-RM. Following each successful lift, the load was increased by 5% until the subject failed to lift the load through the entire range of motion. A test was considered valid if the subject used proper form and completed the entire lift in a controlled manner without assistance. On average, five trials were required to complete a 1-RM test. Approximately 2-3 min of rest was allotted between each attempt to ensure recovery.

MRI-measured skeletal muscle cross-sectional area

Magnetic resonance imaging (MRI) images were captured using a General Electric Signa 1.5 Tesla scanner (Milwaukee, Wisconsin, USA). A T1 weighted, spin echo, axial plane sequence was performed with a 1500 millisecond repetition time and a 17 millisecond echo time. Subjects rested quietly in the magnet bore in a supine position with their legs extended. A 1.0-cm slice thickness scan was taken at the midpoint between the greater trochanter of the femur and the lateral condyle of the tibia. MRI scans were segmented into four components (skeletal muscle, subcutaneous adipose tissue, bone, and residual tissue) by a highly trained analyst, and then traced. These traced images were scanned into a personal computer and the quadriceps muscle cross-sectional area was measured by image-analysis software (NIH image ver.5.0). The estimated CV of this measurement was 2.1% (Abe et al. 2003). The average value of the right and left sides of the body was used.

Muscle biopsy sampling

Biopsy samples were obtained from each subject at 2 weeks before the start of the training and 4 days after the final training session. Muscle biopsy samples were extracted from the superficial region (depth of 3-4 cm) of the vastus lateralis muscle (approximately 60% distal of the thigh) using the percutaneous needle biopsy technique (Ryushi & Fukunaga, 1986). The muscle samples were removed from the needle, immediately frozen in isopentane cooled by liquid nitrogen to -159°C, and stored at -74°C until further analyses could be performed. The pre-training and post-training biopsy sites were far enough apart so that the insertion of the first biopsy needle and extraction of tissue did not affect the area of the second biopsy. The frozen biopsy specimens were thawed to -24°C and sectioned serially (12 μm thick) for histochemical analysis. To determine the muscle fiber-type composition, myofibrillar adenosine triphosphatase (mATPase) histochemistry was performed using preincubation pH values of 4.3, 4.6, and 9.4 (Khan et al., 1972). Muscle fibers were subsequently classified into only two types, as type I (ST) and type II (FT) fibers. Cross-sectional area was determined on 30 fibers for both type I and type II per biopsy sample using NIH imaging software program.

Statistical Analyses

For comparison of changes in muscle fiber individually, a one-way analysis of variance (ANOVA) was used, using each of the 30 fibers sample between baseline and post-testing. Because of the low number of subjects, post-hoc power testing was done using SPSS SamplePower 2. Statistical significance was set at $P<0.05$.

RESULTS

Mean relative change in 1-RM squat strength was 14.0% in the LIT-KAATSU and 9.1% in the LIT after the training. Change in quadriceps muscle CSA was 7.8% in the KAATSU and 1.8% in the LIT. *Post-hoc* power analysis of the changes in quadriceps muscle CSA revealed $\beta = 0.24$. The squat strength per unit quadriceps muscle CSA was similar between pre- and

Table 2. Effects of two weeks of KAATSU training on muscle fiber size

	Subject	PRE	POST	Differences	change(%)
Mean fiber CSA	LIT-KAATSU group				
($\mu m^2 \times 10^2$)	A	82.4 (13.4)	97.0 (24.8)**	14.6	17.7
	B	50.8 (12.8)	54.5 (14.7)	3.7	7.4
	C	44.0 (9.4)	55.4 (13.2)*	11.4	25.8
	Mean	59.1	69.0	9.9	17.0
	LIT group				
	D	52.3 (12.0)	53.2 (10.1)	0.9	1.6
	E	43.8 (12.3)	42.7 (6.7)	-0.9	-2.5
	Mean	48.1	48.0	-0.1	-0.4
FT fiber CSA	LIT-KAATSU group				
($\mu m^2 \times 10^2$)	A	72.5 (10.9)	95.7 (22.8)**	23.2	32.0
	B	48.7 (10.8)	55.7 (16.0)*	7.0	14.5
	C	41.5 (7.5)	56.2 (13.5)**	14.7	35.3
	Mean	54.2	69.2	15.0	27.6
	LIT group				
	D	48.9 (10.4)	50.7 (9.6)	1.8	3.7
	E	44.3 (11.2)	43.1 (6.7)	-1.2	-2.8
	Mean	46.6	46.9	0.3	0.5
ST fiber CSA	LIT-KAATSU group				
($\mu m^2 \times 10^2$)	A	88.2 (15.0)	98.0 (26.6)	9.8	11.1
	B	54.5 (16.3)	52.3 (12.2)	-2.2	-4.1
	C	49.2 (13.2)	53.0 (12.4)	3.8	7.7
	Mean	64.0	67.8	3.8	5.9
	LIT group				
	D	60.4 (15.9)	58.2 (11.2)	-2.2	-3.7
	E	42.1 (13.9)	41.8 (6.7)	-0.3	-0.6
	Mean	51.3	50.0	-1.3	-2.1

**$p<0.01$, *$p<0.05$; Baseline vs. Post-testing

DISCUSSION

It is well known that substantial skeletal muscle hypertrophy has been observed following regular frequency (Takarada et al., 2000, 2002) and high frequency (Abe et al., 2005) KAATSU training. However, it is unclear whether KAATSU-induced skeletal muscle hypertrophy is attributed to an increase in muscle fiber size or any other extracellular tissue/liquid gain. The finding of the present study was that two weeks of KAATSU training produced increases in quadriceps muscle CSA while in LIT alone, there was no change. Fiber CSA increased by a mean of 27% for the type-II and 6% for the type-I in the KAATSU. The magnitude of the increases in fiber size were consistent with previous studies (Campos et al., 2002; Staron et al., 1994), which have reported increases of approximately 10-25% in muscle fiber CSA following 2-3 months of traditional HIT. It is possible that a sufficient number of subjects would have produced a significant increase in muscle fiber CSA in the present case study. Our preliminary data indicated that muscle fiber hypertrophy can occur after two weeks of KAATSU training, especially in the type II fibers.

Previous published studies (Jones and Rutherford, 1986; Abe et al., 2000) have reported that substantial increase in skeletal muscle and fiber CSA in the quadriceps are not observed earlier than six weeks of HIT. In those studies, subjects exercised 2-3 times per week during the study, thus only 4-6 sessions are completed during the first 2 weeks of the training. Our subjects, however, performed 24 sessions of resistive exercises during 2 weeks training. Optimal training frequency is based on the theory of "supercompensation" that attempts to generate the greatest growth stimulus while still allowing for sufficient rest between exercise sessions (Kraemer, 2000). Since a training intensity of 20% of 1-RM produces minimal muscle damage, less recovery time is necessary, and therefore training frequency may be increased (Abe et al., 2005). The data from the present study demonstrated that substantial skeletal muscle and fiber hypertrophy can occur more rapidly than previously reported. This rapid time-course in hypertrophy may be associated with the higher training frequency and smaller recovery period that is possible with KAATSU.

An interesting finding in the present study was that KAATSU-induced relative change in fiber CSA was greater in type-II than in type-I. Both the venous outflow from and arterial inflow to the exercising muscle would have been considerably suppressed during KAATSU. Consequently, the hypoxic and acidic intramuscular environment may be induced an additional muscle fiber recruitment to keep a given level of force. Averaged iEMG measured during KAATSU exercise was equivalent to that seen during HIT exercise (Takarada et al., 2000). Although previous studies (Moritani et al. 1992; Sundberg 1994) have suggested that type II fibers are recruited when muscle blood flow is suppressed, there are published data demonstrating that type-II fiber hypertrophies under this condition. The present case study reports for the first time that hypertrophy occurs in type-II muscle fibers during low-intensity (20% of 1-RM) resistance training combined with restriction of muscular blood flow.

In summary, two weeks of KAATSU training produced increases in skeletal muscle and fiber size that were similar in magnitude to those reported in traditional HIT of 2-3 months. Additional studies with greater statistical power are needed to confirm these results but these preliminary data demonstrate that type II muscle fiber hypertrophy occurs following high frequency KAATSU training.

ACKNOWLEDGEMENTS

The authors thank the students who participated in this study. The study was supported in part by the Ministry of Education, Science, Sports and Culture of Japan (grant No. 15300221).

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