ORIGINAL ARTICLE

Skeletal muscle size and circulating IGF-1 are increased after two weeks of twice daily "KAATSU" resistance training

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Int. J. Kaatsu Training Res. 2005; **1**: 6-12

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This study investigated the effects of twice daily sessions of low-intensity resistance training (LIT, 20% of 1-RM) with restriction of muscular venous blood flow (namely "LIT-Kaatsu" training) for two weeks on skeletal muscle size and circulating insulin-like growth factor-1 (IGF-1). Nine young men performed LIT-Kaatsu and seven men performed LIT alone. Training was conducted two times / day, six days / week for 2 weeks using 3 sets of two dynamic exercises (squat and leg curl). Muscle cross-sectional area (CSA) and volume were measured by magnetic resonance imaging at baseline and 3 days after the last training session (post-testing). Mid-thigh muscle-bone CSA was calculated from thigh girth and adipose tissue thickness, which were measured every morning prior to the training session. Serum IGF-1 concentration was measured at baseline, mid-point of the training and post-testing. Increases in squat (17%) and leg curl (23%) one-RM strength in the LIT-Kaatsu were higher ($p<0.05$) than those of the LIT (9% and 2%). There was a gradual increase in circulating IGF-1 and muscle-bone CSA (both $p<0.01$) in the LIT-Kaatsu, but not in the LIT. Increases in quadriceps, biceps femoris and gluteus maximus muscle volume were, respectively, 7.7%, 10.1% and 9.1% for LIT-Kaatsu ($p<0.01$) and 1.4%, 1.9% and -0.6% for LIT ($p>0.05$). There was no difference ($p>0.05$) in relative strength (1-RM / muscle CSA) between baseline and post-testing in both groups. We concluded that skeletal muscle hypertrophy and strength gain occurred after two weeks of twice daily LIT-Kaatsu training.

Key words: muscle hypertrophy, training frequency, muscle volume, magnetic resonance imaging

INTRODUCTION

Human skeletal muscle is responsive to acute and chronic stimuli associated with resistance training. The nature of the phenotypic adaptation is dependent upon how the specific variables of the resistance-training regime (training intensity, volume, frequency, and recovery, etc.) are combined. Several societies [ACSM, 1998; NSCA, 2003] have published their guidelines for optimizing muscle hypertrophy and strength gains. In general, a training intensity of over 65% of one repetition maximum (1-RM) is required to achieve substantial muscle hypertrophy [Campos et al., 2002; Kraemer et al., 2004; McDonagh et al., 1984]. Training below an intensity of 65% of 1-RM rarely produces increases in muscle size or strength [Kraemer et al., 2004]. In contrast, previous published studies have reported that low-intensity resistance training (LIT, 20-50% of 1-RM) combined with restriction of muscular venous blood flow (namely "LIT-Kaatsu" training) can increase muscle cross-sectional area (CSA) and strength in men [Burgomaster et al., 2003; Shinohara et al., 1998; Takarada et al., 2002] and women [Takarada et al., 2000b]. The LIT-Kaatsu training produces similar increases in muscle CSA as traditional high-intensity resistance training (HIT, 80% of 1-RM) and ~3 times the growth hormone (GH) secretion as HIT [Kraemer et al., 1991; Takarada et al., 2000a; Viru et al., 1998]. Interestingly, LIT-Kaatsu does not require a long recovery time between training sessions [Abe, 2004] due to very low mechanical stress and minimal muscle damage [Takarada et al., 2000a] produced when a load of only 20% of 1-RM is used. Therefore, high frequency training is possible with LIT-Kaatsu training. In the previous HIT studies [Abe et al., 2000; Jones and Ratherford, 1987; Staron et al., 1991], substantial muscle hypertrophy was observed after approximately 24 sessions of the training (e.g., 8 weeks and 3 days per week). We hypothesized that substantial muscle hypertrophy may be achieved following a short period of high frequency LIT-Kaatsu training. Thus, the purpose of this study was to investigate the effects of twice daily sessions of LIT-Kaatsu training for two weeks (6 days / week, total 24 sessions) on skeletal muscle size and circulating insulin-like growth factor-1 (IGF-1) level.

METHODS

Subjects

Sixteen healthy men [mean (SD) age 23.6 (6.5) years, height 172.4 (6.5) cm, body mass 64.3 (9.8) kg] volunteered to participate in the study. All subjects led active lives, with 8 of 16 participating in regular aerobic exercise. However, none of the subjects had participated in a regular resistance exercise program for at least 6 months prior to the start of the study. The subjects were randomly divided into two training groups: a low-intensity resistance training with Kaatsu (restriction of muscular venous blood flow) group [LIT-Kaatsu, n=9] and a low-intensity resistance training without Kaatsu group [LIT, n=7]. All subjects were informed of the procedures, risks, and benefits, and signed an informed consent document before participation. The study was approved by the Ethics Committee for Human Experiments, Tokyo Metropolitan University.

Training protocol

The subjects in both LIT-Kaatsu and LIT groups participated in two weeks of supervised resistance training. Training was conducted twice per day (morning and afternoon sessions, with at least 4 hours between sessions) 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 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 [Takarada et al., 2002]. 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 Day 1, the cuff pressure was 160 mmHg and the pressure was increased 10 mmHg each day until a final training cuff pressure of 240 mmHg was reached. The cuff pressure of ~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 restricts arterial blood flow [Takarada et al., 2000b; Takarada et al., 2002]. The estimated coefficient of variation (CV) of this pressure measurement was 2.2%. The restriction of muscular blood flow was maintained for the entire exercise session (including rest periods, about 10 min total training time) 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

One week prior to training, the subjects were familiarized with testing and training equipment. Proper lifting technique was demonstrated for each of the two exercises (squat and leg curl) and all subjects performed practice lifts prior to attempting maximal lifts. Maximum dynamic strength (1-RM) was assessed prior to (baseline) and 3 days after the final training (post-testing) for each exercise. 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 only when 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 (Abe et al., 2000).

Muscle-bone cross-sectional area estimation

An anthropometric method (Mid-thigh CSA = π [r - (Q-AT + H-AT) / 2]^2) was used to estimate the muscle-bone cross-sectional area (CSA) for the mid-thigh [Gurney and Jelliffe, 1973]. Where r was the radius of the thigh calculated from mid-thigh girth of the right leg, Q-AT and H-AT were ultrasound-measured [Abe et al., 1994] anterior and posterior thigh adipose tissue thickness, respectively. The estimated CV of this measurement was 1.2 %. This measurement was carried out each morning prior to the training session and prior to the post-testing.

Body composition

Body density was measured by the hydrostatic weighing technique with simultaneous measurement of residual lung volume by oxygen dilution at baseline and post-testing [Abe et al., 1994]. Body fat percentage was calculated from the body density using the equation of Brozek et al [1963]. Fat-free mass was estimated as body mass minus fat mass.

MRI-measured muscle CSA and volume

Magnetic resonance imaging (MRI) images were prepared 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. The intervertebral space between the fourth and fifth lumbar vertebrae was used as the

origin point and contiguous transverse images with 1.0 cm slice thickness (0 cm interslice gap) were obtained from the fifth lumbar vertebrae to the ankle joints for each subject. All MRI scans were segmented into four components (skeletal muscle, subcutaneous adipose tissue, bone, and residual tissue) by a highly trained analyst, and then traced. For each slice, the skeletal muscle tissue CSA was digitized, and the muscle tissue volume (cm^3) per slice was calculated by multiplying muscle tissue area (cm^2) by slice thickness (cm). Muscle volume of the individual muscle was defined as the summation of the slices of muscle. 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. This measurement was completed at baseline and post-testing.

Blood sampling and biochemical analyses

Venous blood was drawn from each subject at three time points: at baseline, at the mid-point of the training, and at post-testing. All blood samples were obtained at the same time of day following an overnight fast (12-13 hours). The subjects were counseled to refrain from ingesting alcohol and caffeine for 24 hours prior to blood collection and not to perform any strenuous exercise except training sessions. Serum IGF-1 concentrations were determined using a commercially available radioimmunoassay (Daiichi Radioisotope Laboratory, Chiba, Japan). Radioactivity was measured using an automated gamma counter (ARC-950, Aloka, Tokyo, Japan). Plasma activity of creatine phosphokinase (CPK) was measured with spectrophotometry for NADPH formed by a hexokinase and D-glucose-6-phosphate-dehydrogenase-coupled enzymic system. Plasma concentrations of lipid peroxide and myoglobin were measured by spectrofluorimetry using the reaction product of malondialdehyde and thiobarbituric acid [Yagi, 1976] and using a commercially available radioimmunoassay (Daiichi Radioisotope Laboratory, Chiba, Japan).

Table 1. Body composition and 1-RM strength at baseline for the low-intensity resistance training combined with restriction of muscular blood flow (LIT-Kaatsu) and low-intensity resistance training alone (LIT) groups.

	LIT-Kaatsu	LIT
N	9	7
Age (yr)	23.9 (8.4)	23.1 (3.1)
Standing height (cm)	171.5 (7.9)	173.6 (4.5)
Body mass (kg)	65.4 (10.6)	62.8 (9.1)
Body fat (%)	14.9 (5.1)	14.4 (3.3)
Fat-free mass (kg)	55.7 (7.8)	54.1 (6.7)
Mid-thigh girth (cm)	51.1 (3.9)	50.6 (4.6)
Muscle-Bone CSA (cm^2)	176 (26)	174 (30)
Squat 1-RM (kg)	99 (12)	95 (19)
Leg curl 1-RM (kg)	51 (19)	60 (11)

Statistical Analyses

Results are expressed as means ± standard deviations (SD) for all variables. A two-way ANOVA with repeated-measures (group and time) was utilized to evaluate the effect of the Kaatsu training independent of the changes in the LIT alone. Post-hoc testing was performed by a Fisher's least significant differences test. Baseline differences between LIT-Kaatsu and LIT and percentage changes between baseline and post-testing were evaluated with a one-way analysis of variance (ANOVA). Statistical significance was set at $P < 0.05$.

RESULTS

Baseline measurements

There were no statistically significant differences in body composition, muscle-bone CSA, 1-RM strength (Table 1), mid thigh musde CSA, or muscle volume (Table 2) between LIT-Kaatsu and LIT at baseline.

Relative change in estimated muscle-bone CSA

Muscle-bone CSA gradually increased ($p<0.01$) in the LIT-Kaatsu but not in the LIT. The muscle-bone CSA increased 7% at the end of the first week in the LIT-Kaatsu. By post-testing, the muscle-bone CSA had increased 9% in the LIT-Kaatsu. In LIT, muscle-bone CSA increased 3% ($p>0.05$) at the end of the first week, and was similar (~2%) to baseline at post-testing (Figure 1).

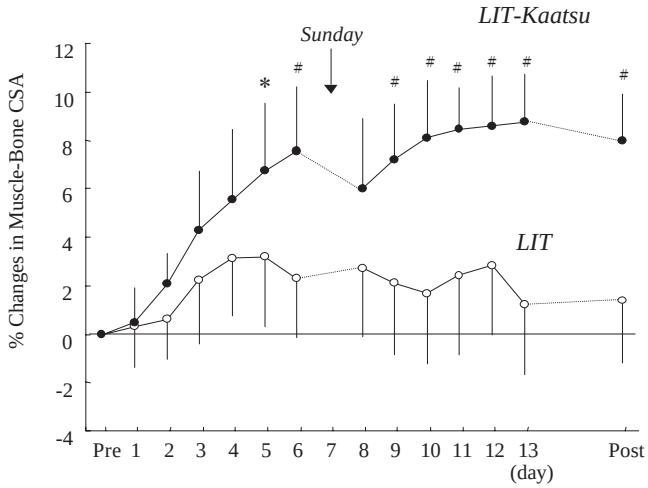

Figure 1. Percent change in estimated muscle-bone cross-sectional area (CSA) for the low-intensity resistance training combined with restriction of muscular blood flow (LIT-Kaatsu, filled symbols) and low-intensity resistance training alone (LIT, unfilled symbols) groups measured before, during (every morning prior to the training session), and after the training period. Values are mean ± SD. *$P < 0.05$ and #$P < 0.01$ between LIT-Kaatsu and LIT.

Changes in MRI-measured muscle CSA and volume

Mid-thigh muscle CSA increased ($p<0.01$) by 8.5% in the LIT-Kaatsu but not (1.8%, $p>0.05$) in the LIT. Quadriceps and biceps femoris muscle volumes increased (both $p<0.01$) 7.7% and 10.1%, respectively in the LIT-Kaatsu but only 1.4% and 1.9% ($p>0.05$), respectively in the LIT. Gluteus maximus muscle volume increased ($p<0.01$) 9.1% in the LIT-Kaatsu, but did not change in the LIT (-0.6%) (Figure 2 and Table 2).

Baseline

Post-testing

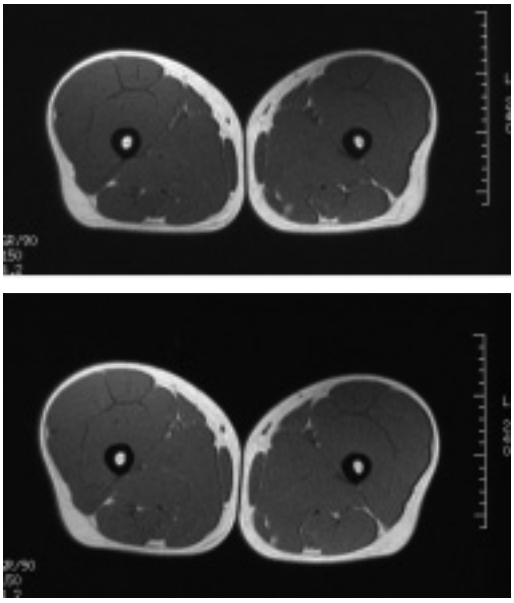

Figure 2. Typical magnetic resonance images showing transverse sections of the mid-thigh taken before (baseline) and after (post-testing) the two weeks of low-intensity resistance training combined with restriction of muscular blood flow. The images show identical sections, mid-way along the femur in the same subject.

Changes in absolute and relative strength

Squat strength increased in both LIT-Kaatsu (16.8%, $p<0.01$) and LIT (8.9%, $p<0.05$). However, leg curl strength increased (22.6%, $p<0.01$) in the LIT-Kaatsu but not (1.3%, $p>0.05$) in the LIT. The relative percentage changes in squat and leg curl strength were larger ($p<0.05$) in the LIT-Kaatsu compared to the LIT (Figure 3). The 1-RM squat strength per unit quadriceps muscle CSA was similar ($p>0.05$) at baseline and at post-testing in both groups. The 1-RM leg curl strength per unit hamstrings muscle CSA was also similar ($p>0.05$) at baseline and post-training in both groups (Figure 4).

Change in serum IGF-1

In the LIT-Kaatsu group, serum IGF-1 increased progressively and reached significance ($p<0.05$) after 2 weeks of training. There was no change ($p>0.05$) in serum IGF-1 in LIT (Table 3).

Biochemical parameters

At baseline, all subjects had a normal CPK, lipid peroxide and myoglobin concentrations. During and after the training, those values were unchanged ($p>0.05$) in both groups (Table 3).

DISCUSSION

The major finding of the present study was that two weeks of twice daily LIT-Kaatsu produced increases in skeletal muscle size (7-8%) that were similar in magnitude to those reported in traditional HIT of 3-4 months [Abe et al., 2000; Jones and Ratherford, 1987]. Previous published studies [Jones and Ratherford, 1987; Staron et al., 1991; Staron et al., 1994] have reported that a substantial increase in skeletal muscle and fiber CSA in the thigh is not observed earlier than six weeks of HIT. To the best of

Table 2. Changes in muscle cross-sectional area (CSA) and muscle volume for the low-intensity resistance training combined with restriction of muscular blood flow (LIT-Kaatsu) and low-intensity resistance training alone (LIT) groups measured before (baseline) and after (post-testing) the training period.

	LIT-Kaatsu (N=9)			LIT (N=7)		
	Baseline	Post-testing	%Δ	Baseline	Post-testing	%Δ
Mid-thigh muscle CSA (cm^2)						
QF	72.9 ± 9.9	78.6 ± 9.2 †	8.0	72.6 ± 9.7	73.6 ± 8.0	1.8
HAM	20.8 ± 4.1	23.0 ± 4.9 †	10.7	21.6 ± 4.3	21.9 ± 4.4	1.5
ADD	40.1 ± 4.6	43.2 ± 4.3 †	8.0	37.8 ± 7.9	37.8 ± 7.6	0.2
Total	141.3 ± 17.8	152.9 ± 17.1 †	8.5	142.0 ± 22.0	144.3 ± 20.8	1.8
Muscle volume (cm^3)						
QF	1790 ± 294	1924 ± 288 †	7.7	1787 ± 266	1809 ± 257	1.4
BF	235 ± 47	257 ± 45 †	10.1	239 ± 52	244 ± 58	1.9
GM	1602 ± 353	1737 ± 334 †	9.1	1604 ± 303	1594 ± 298	-0.6

QF, quadriceps femoris; HAM, hamstrings; ADD, adductors; BF, biceps femoris; GM, gluteus maximus

† $p<0.01$ Baseline vs. Post-testing

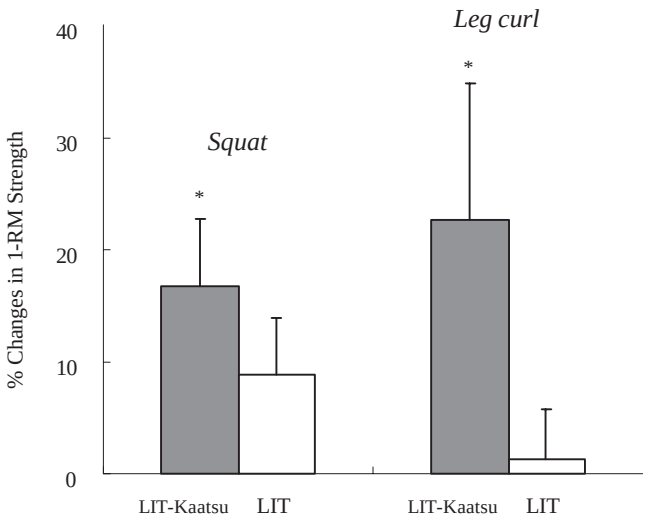

Figure 3. Percent change in 1-RM strength for the low-intensity resistance training combined with restriction of muscular blood flow (LIT-Kaatsu, filled bars) and low-intensity resistance training (LIT, unfilled bars) groups measured before and after the training period. *P < 0.05 LIT-Kaatsu vs. LIT.

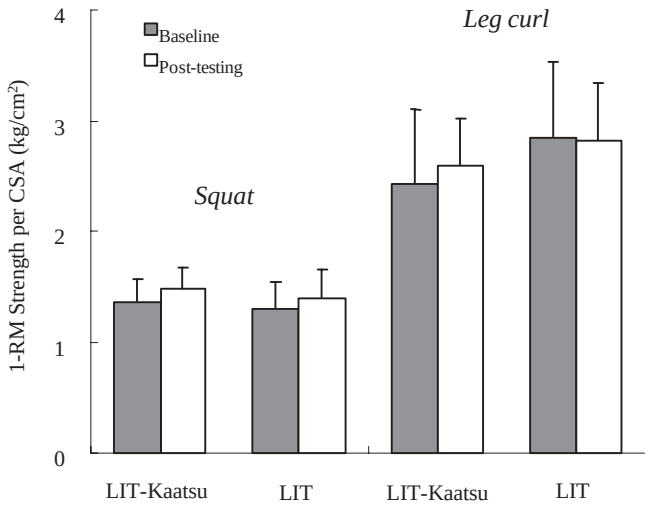

Figure 4. Relative 1-RM strength (squat / quadriceps CSA and leg curl / hamstrings CSA) of the low-intensity resistance training combined with restriction of muscular blood flow (LIT-Kaatsu) and low-intensity resistance training (LIT) groups measured before (baseline) and after (post-testing) the training period.

Table 3. Changes in serum IGF-1 and blood makers for muscle damage and oxidative stress in the low-intensity resistance training combined with restriction of muscular blood flow (LIT-Kaatsu) and low-intensity resistance training (LIT) groups.

	LIT-Kaatsu			LIT		
	Baseline	Mid-point	Post-testing	Baseline	Mid-point	Post-testing
IGF-1 (ng/ml)	323 ± 38	373 ± 98	400 ± 75 †	276 ± 74	256 ± 61	281 ± 95
CPK (IU/l)	212 ± 173	384 ± 240	223 ± 134	283 ± 236	342 ± 147	165 ± 78
MYO (ng/ml)	59 ± 22	74 ± 28	64 ± 20	63 ± 5	65 ± 16	62 ± 4
LP (nmol/ml)	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	0.8 ± 0.3	0.8 ± 0.3

MYO, myoglobin; LP, lipid peroxide
† p<0.05; Baseline vs. Post-testing

our knowledge, there are no published data that have reported a significant increase in thigh muscle size following only two weeks of HIT [Akima et al., 1999]. In most of the previous 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 the 2 weeks of training. Optimal training frequency is based on the theories of "supercompensation" and "over-training" which attempt 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 [Takarada et al., 2000a], less recovery time is necessary [Abe, 2004], and therefore training frequency may be increased. The data from the present study demonstrated that substantial skeletal muscle 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 LIT-Kaatsu.

The present study showed that plasma markers for muscle damage (CPK activity and myoglobin) and oxidative stress (lipid peroxide) were not elevated during or after the training in both LIT-Kaatsu and LIT. These results are consistent with data reported by Takarada and colleagues [Takarada et al., 2000a], who showed that plasma markers for muscle damage and oxidative stress did not increase considerably following acute LIT-Kaatsu exercise. Taken together, the results of the present study along with the previous acute study suggest that the rapid response to skeletal muscle hypertrophy following LIT-Kaatsu is not associated with muscle damage and/or inflammation of the muscle as measured by the plasma markers.

Myogenic regulatory factors and GH / IGF-1 pathway have been indicated to play important roles in resistance training-induced skeletal muscle hypertrophy [Florini et al., 1996; McPherron et al.,

1997]. In line with these observations, two weeks of LIT-Kaatsu training produced a 24% increase in circulating IGF-1 and this change was similar in magnitude to the elevation in circulating IGF-1 following HIT [Borst et al., 2001; Marx et al., 2001]. Moreover, the elevation in circulating GH 15-min following LIT-Kaatsu exercise is elevated ~3-fold larger than the increase in GH following HIT [Kraemer et al., 1991; Takarada et al., 2000a; Viru et al., 1998]. The resistance training-induced increase in GH has been reported to increase hepatic production of IGF-1 and results in elevated circulating IGF-1. Circulating IGF-1 stimulates muscle protein synthesis [Borst et al., 2001; Marx et al., 2001]. In addition, circulating GH directly stimulates endogenous muscle production of IGF-1 [Florini et al., 1996]. Therefore, the increase in circulating IGF-1 may have contributed to muscle hypertrophy and strength gains during the two weeks of twice daily LIT-Kaatsu training.

An interesting and surprising finding of the present study was that LIT-Kaatsu training-induced muscle hypertrophy occurred not only in the thigh muscle but also in the gluteus maximus muscle. During the squat exercise, mainly the knee and hip extensor muscles are activated. Since a training intensity of 20% of 1-RM was used in the present study, it would seem reasonable that the load on the gluteus maximus muscle during the squat would be insufficient to produce the muscle hypertrophy. However, this was not the case as significant hypertrophy was observed in the gluteus maximus. The reasons for the muscle hypertrophy of the gluteus maximus muscle after the LIT-Kaatsu are unclear, but several possibilities exist. During LIT-Kaatsu exercise, high lactate accumulation in the muscle fibers [Takarada et al., 2000b] of exercised thigh muscles may inhibit muscular contraction. Consequently, additional motor unit recruitment may be required in order to maintain sufficient force generation. Previously published studies have reported that the mean integrated electromyographical muscle activity during LIT-Kaatsu is almost equal to that of HIT (80% of 1-RM) exercise [Takarada et al., 2000b]. Under these conditions, synergistic action of the thigh and hip muscles may occur during the squat and this would increase the training intensity of the hip muscles. Subsequently, additional motor units would be recruited by the hip muscles and this could explain the muscle hypertrophy seen in the gluteus maximus muscle. If so, this would suggest that the fast-twitch fibers and their higher threshold motor units are recruited for a sustained period of time during LIT-Kaatsu. In support of this hypothesis, fast-twitch fibers demonstrated a larger degree of hypertrophy than the slow-twitch fibers following LIT-Kaatsu training [Yasuda et al., 2004].

There were no statistical changes in relative strength and the magnitude of the changes were relatively small compared to previous strength training studies [Narici et al., 1996]. However, this was consistent with previous LIT-Kaatsu studies [Takarada et al., 2000b; Takarada et al., 2002]. Increases in relative strength during resistance training, particularly the early phase of the training is highly variable and subject to much debate. For the most part, neural activation increases with training [Moritani and de Vries, 1979], however, this is not always true and extremely well motivated subjects often display full motor unit activation, even before training [Narici et al., 1996]. Additional factors may contribute to the increase in relative strength, such as changes in the co-contraction of the antagonist muscles, density of contractile elements, the muscle architecture and/or increases in motor unit synchronization of the trained muscles [Narici et al., 1996]. Subjects in the present study appeared well motivated, but it is unclear whether antagonist co-activation and/or motor unit synchronization are altered in response to low intensity (20% of 1-RM) resistance training with blood flow restriction.

In conclusion, two weeks of twice-daily LIT-Kaatsu produced increases in skeletal muscle size that were similar in magnitude to those reported in traditional HIT of 3-4 months. Increases in circulating IGF-1 may have contributed to the skeletal muscle hypertrophy and strength gain. Therefore we concluded that skeletal muscle hypertrophy and strength gain occurred after two weeks of twice daily LIT-Kaatsu training.

ACKNOWLEDGEMENTS

The authors thank the students who participated in this study. We also thank the Sato KAATSU Training Research Foundation (to NI) and the Ministry of Education, Science, Sports and Culture of Japan (Grant #15300221 to TA) for their generous support.

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