Resistance training restores muscle sex steroid hormone steroidogenesis in older men

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ABSTRACT Skeletal muscle can synthesize testosterone and 5α-dihydrotestosterone (DHT) from dehydroepiandrosterone (DHEA) via steroidogenic enzymes in vitro, but hormone levels and steroidogenic enzyme expression decline with aging. Resistance exercise has been shown to increase in plasma sex steroid hormone levels. However, it remains unclear whether resistance training can restore impaired steroidogenic enzyme expressions in older individuals. Six young and 13 older men were recruited, and muscle biopsies were taken from the vastus lateralis at basal state. The same group of older subjects underwent resistance training involving knee extension and flexion exercises for 12 wk, and post-training biopsies were performed 4–5 d after the last exercise session. Muscular sex steroid hormone levels and sex steroidogenesis-related enzyme expressions were significantly lower in older subjects than younger ones at baseline, but 12 wk of resistance training significantly restored hormone levels (DHEA: 432±26 at baseline, 682±31 pg/μg protein, DHT: 6.2±0.9 at baseline, 9.8±1.4 pg/μg protein). Furthermore, the steroidogenesis-related enzymes such as 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD, and 5α-reductase expressions were significantly restored by resistance training. We conclude progressive resistance training restores age-related declines in sex steroidogenic enzyme and muscle sex steroid hormone levels in older men.—Sato, K., Iemitsu, M., Matsutani, K., Kurihara, T., Hamaoka, T., Fujita, S. Resistance training restores muscle sex steroid hormone steroidogenesis in older men. FASEB J. 28, 000–000 (2014). www.fasebj.org

Key Words: skeletal muscle • exercise • aging • muscle strength

Steroid sex hormones, which are secreted mainly by the ovary, testis, and adrenal cortex, regulate diverse physiological processes in target tissues, including reproductive organs, bone, liver, cardiovascular system, brain, and skeletal muscle (1). As precursors of sex steroid hormones, dehydroepiandrosterone (DHEA) and its sulfate derivates (DHEA-S) play critical physiological roles in maintaining steroidogenesis in peripheral tissues (1). DHEA is converted to testosterone by 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD and testosterone then converted to 5α-dihydrotestosterone (DHT) by 5α-reductase (1). Aging leads to reduced serum levels of DHEA (2), which are significantly correlated with increased risks of metabolic syndrome (3). Thus, it seems critical to prevent the aging-induced attenuation of steroidogenesis for healthy aging.

In our previous studies, we demonstrated that skeletal muscle can synthesize testosterone, estradiol, and DHT from DHEA locally in cultured skeletal muscle and rat muscle tissue models (4, 5). In a recent study by Vingren et al. (6), it was found that acute resistance exercise for young human subjects did not change muscular steroidogenesis-related enzymes. Moreover, another study reported that gene expression of steroidogenic enzymes were detected in female subjects, and they declined with aging, specifically 3β-HSD and P450 aromatase (2). In addition, a significant correlation was found between serum DHEA level and muscle force per cross-sectional area (CSA; ref. 2). However, the effect of aging on steroidogenic enzymes and muscular sex steroid hormone levels in men has not been investigated. If it is possible to improve the aging-induced decrease in muscular steroidogenesis, this improvement may contribute to augmentation of muscle mass. Exercise, especially resistance exercise, is characterized by contracting skeletal muscles and has been shown to increase plasma DHT levels in the older adults (7). The program of chronic resistance exercise combined with endurance training increased serum levels of testosterone and estradiol, whereas resistance training itself only increased serum DHT level according to the previous study (8). Thus, resistance exercise may reverse aging-induced impairment of

Abbreviations: CSA, cross-sectional area; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone; HRP, horseradish peroxidase; HSD, hydroxysteroid dehydrogenase; IGF-1: insulin-like growth factor 1; MRI, magnetic resonance imaging; mTOR, mammalian target of rapamycin; 1-RM, 1-repetition maximum; RPE, rating of perceived exertion

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steroidogenesis in skeletal muscle with concomitant increase in muscle mass and function in older individuals. However, it remains unclear whether chronic resistance exercise induces changes in muscle sex steroid metabolism in aging men.

Therefore the aim of this study was to investigate whether 12 wk of progressive resistance training can enhance muscle steroidogenesis and muscle sex steroid hormones in older men. To achieve this, we measured seroidogenic enzymes such as 3β-HSD, 17β-HSD, and 5α-reductase protein expressions, as well as muscular sex steroid hormone levels especially, DHEA, free-testosterone, and DHT levels, which were measured before and after resistance training in older subjects. We hypothesized that 12 wk resistance training enhanced impaired muscular steroidogenesis in older men.

MATERIALS AND METHODS

Subjects

Thirteen older men (mean age: 67.2±1.8 yr) and 6 young men (mean age, 24.3±1.3 yr) volunteered to participate in this study. All volunteers provided written informed consent before participating in the study, which was approved by the Ethics Committee of Ritsumeikan University and was conducted in accordance with the Declaration of Helsinki. The older subjects were examined by a physician to confirm that none had medical problems that might preclude participation or affect the results. None of the subjects regularly performed resistance exercise, but they were moderately active. Their physical activities included walking and jogging. Subjects were instructed to continue their normal activities of daily living and usual diets throughout the experimental period.

One-repetition maximum (1-RM) strength tests were performed every 4 wk to adjust training intensity. Isokinetic peak torque was assessed in the knee extensors before and after training using a dynamometer (Biodex System 4; Biodex Medical Systems, Shirley, NY, USA). To avoid a possible learning effect, a 1-RM test was performed twice at least 3 d after the first 1-RM measurement. The same investigator supervised all training sessions to ensure that proper technique and progression were being used in each exercise session. Each exercise included 2 exercises; bilateral knee extension involving the leg extensors, and bilateral knee flexion exercising the leg flexors. The starting weight used during the resistance exercise portion of this study was 70% of each subject’s predetermined 1-RM for 3 sets of 10 repetitions using weight-stack machines (Life Fitness, Tokyo, Japan). The rest period between sets was 3 min. The weight was increased for each subject when his rating of perceived exertion (RPE) was <16 for the 10th repetition of the 3rd set. Determination of 1-RM was repeated every 4 wk to adjust the training weights to 70% of 1-RM.

Magnetic resonance imaging (MRI)

MRI was used to determine the muscle CSA of the quadriceps. A 1.5-T magnetic resonance system (Signa HDxt; GE Medical Systems) was used to obtain a series of axial slices from the superior border of the patella to the anterior superior iliac spine, encompassing the entire quadriceps femoris muscle group. The images were obtained from 10-mm-thick slices. Multislice T1-weighted spin-echo images were acquired to guide the positioning of the volume of interest and used for measuring muscle CSA at the level of the midthigh (9). Subjects were instructed not to drink or eat after midnight on the night before the scans, which were performed between 8 and 10 AM.

Muscle biopsies

Muscle biopsies were obtained from the lateral portion of the vastus lateralis using a Core biopsy instrument (Bard Max-Core; Bard Peripheral Vascular, Tempe, AZ, USA) under sterile conditions with local anesthesia (1% lidocaine). The subjects were fed the same standard dinner (1800 kcal) at 1800 h and were allowed only water ad libitum after 2200 h. All subjects participated in an overnight fast under basal conditions and refrained from exercise for 24 h before study participation. The biopsy for older subjects after 12 wk of resistance training was performed 4–5 d after the last exercise session to minimize its acute effects. The muscle sample was quickly rinsed with ice-cold saline, blotted, and then frozen at –80°C until analysis (10).

Immunoblot analysis

Muscle specimens were homogenized with 20 mM Tris-HCl, pH 7.8; 300 mM NaCl; 2 mM ethylenediaminetetraacetic acid; 2 mM dithiothreitol; 2% nonidet P-40 (Nonidet P-40); 0.2% sodium lauryl sulfate; 0.2% sodium deoxycholate; 0.5 mM phenylmethylsulfonyl fluoride; 60 μg/ml aprotinin; and 1 μg/ml leupeptin. The homogenate was gently mixed for 30 min at 4°C and then centrifuged at 12,000 g for 15 min at 4°C. The protein concentration of the resulting supernatant was determined. Samples (40 μg protein) were denatured at 96°C for 7 min in Laemml buffer. Western blot analysis was performed essentially as described previously (11). Briefly, muscle samples were separated using 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were then treated for 24 h at 4°C with blocking buffer (5% skim milk in phosphate-buffered saline with 0.1% Tween 20). Next, the membranes were probed with antibodies against 17β-HSD, 3β-HSD, and the androgen receptor (Cell Signaling Technology, Beverly, MA, USA), all diluted 1:1000 with blocking buffer. Anti-5α-reductase (Abnova, Taipei, Taiwan) was used at 1:500 dilution. The membranes were washed 3 times with PBS-T and then incubated for 1 h at room temperature with a horseradish peroxidase (HRP)-conjugated secondary antibody and anti-rabbit immunoglobulin (Cell Signaling Technology), diluted 1:3000 in blocking buffer. Next, the membranes were washed 3 times with PBS-T. Finally, 17β-HSD, 3β-HSD, 5α-reductase, and androgen receptor proteins were detected using an enhanced chemiluminescence plus system (GE Healthcare Biosciences, Piscataway, NJ, USA) and visualized using an LAS4000 imager (GE Healthcare Biosciences).
Of resistance exercise induced significant increases in maximal isokinetic extension strength and quadriceps 
CSA in older subjects.

**Serum hormone levels**

Serum sex steroid hormone and IGF-1 levels were significantly lower in older compared with young subjects 
at baseline. Resistance exercise was associated with increased serum DHEA and DHT levels (P<0.01). Although 
there was no statistically significant change in either serum free testosterone (P=0.052) or IGF-1 (P=0.055) 
concentrations, there was a tendency toward increased serum levels with resistance exercise (Table 2).

**Muscle sex steroid hormone levels**

DHEA and DHT levels in skeletal muscle were significantly lower in older as compared with young subjects 
at baseline. However, DHEA levels increased significantly in the older subjects after the training period 
(P<0.01). Baseline muscle free testosterone levels were also significantly lower in the older subjects than the 
young subjects, but free testosterone levels increased significantly after training. Moreover, muscle DHT 
levels were lower in older compared with young subjects. In contrast, 12 wk of training were associated with 
significant increases in muscle DHT levels in older subjects. Resistance training restored muscular sex ste-
roid hormones in older subjects to levels seen in the young subjects (Fig. 1).

**Sex steroidogenic enzyme expression in muscle**

Expression of steroidogenic enzymes such as 3β-HSD and 17β-HSD were significantly lower in older sub-
jects before training. However, resistance training was associated with significant increases in the ex-
pression of steroidogenic enzymes (P<0.01; Fig. 2). 5α-Reductase and androgen receptor expression was 
also higher after 12 wk of resistance training in older subjects (P<0.01; Fig. 3). In addition, significant 
correlation was seen between percentage change of muscular DHEA and DHT levels (r=0.721, P<0.001). 
Furthermore, 5α-reductase protein expression was

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young pre</th>
<th>Old pre</th>
<th>Old post</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>24.3 ± 1.3*</td>
<td>67.2 ± 1.81</td>
<td>67.4 ± 1.82</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.2 ± 1.6</td>
<td>167.2 ± 1.3</td>
<td>167.1 ± 1.3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>66.6 ± 1.51</td>
<td>63.9 ± 1.24</td>
<td>64.5 ± 1.38</td>
</tr>
<tr>
<td>BMI</td>
<td>22.6 ± 0.9</td>
<td>22.9 ± 0.4</td>
<td>23.1 ± 1.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>13.2 ± 1.2*</td>
<td>22.8 ± 1.3</td>
<td>21.0 ± 1.4</td>
</tr>
<tr>
<td>Isokinetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>extension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(60°/s, Nm)</td>
<td>210.6 ± 7.3*</td>
<td>127.9 ± 6.3</td>
<td>155.2 ± 6.2*</td>
</tr>
<tr>
<td>Quadriceps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>muscle CSA (cm²)</td>
<td>166.1 ± 2.7*</td>
<td>84.3 ± 2.5</td>
<td>90.3 ± 2.5*</td>
</tr>
</tbody>
</table>

Young pre, young subjects at baseline; old pre, older subjects at baseline; old post, older subjects after training. Values are means ± SE. *P < 0.01 vs. old pre.

### Table 2. Serum hormone concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young pre</th>
<th>Old pre</th>
<th>Old post</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>DHEA (pg/ml)</td>
<td>1569 ± 398*</td>
<td>666 ± 85</td>
<td>841 ± 117*</td>
</tr>
<tr>
<td>Free-testosterone</td>
<td>18.2 ± 1.6*</td>
<td>8.18 ± 0.49</td>
<td>10.6 ± 0.89</td>
</tr>
<tr>
<td>DHT (pg/ml)</td>
<td>132 ± 14*</td>
<td>47 ± 24</td>
<td>56 ± 35*</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>201.6 ± 16.4*</td>
<td>112.1 ± 8.4</td>
<td>120.3 ± 7.5</td>
</tr>
</tbody>
</table>

Young pre, young subjects at baseline; old pre, older subjects at baseline; old post, older subjects after training. Values are means ± SE. *P < 0.01 vs. old pre.
significantly correlated with muscular DHT level ($r=0.887$, $P<0.001$).

Relationship between sex steroid hormone concentrations and muscle strength and mass

No significant correlations were found between percentage changes in serum hormone concentrations and isokinetic strength or between percentage changes in serum hormone concentrations and muscle CSA ($P>0.1$). However, the percentage change of intramuscular DHEA and free testosterone levels was significantly correlated with isokinetic strength. Moreover, muscular DHT levels were significantly correlated with both muscle power ($P<0.001$) and CSA ($P=0.018$; Table 3). In addition, 5α-reductase protein expression was significantly correlated with both isokinetic strength ($P<0.001$) and CSA ($P<0.001$).

DISCUSSION

Here we report for the first time that 12 wk progressive resistance training appeared to increase or partially reverse the age-associated reduction in muscle sex steroid hormone levels and muscular steroidogenic enzyme protein expression in men. Although protein expression of steroidogenic enzymes such as 3β-HSD, 17β-HSD, 5α-reductase, and androgen receptor protein expressions in muscle were significantly lower in older men compared with younger men.
counterparts at baseline, 12 wk of resistance training significantly increased levels of these enzymes and restored both serum and muscle levels of DHEA, free testosterone, and DHT to levels seen in young subjects. Interestingly muscular steroid hormone levels significantly correlated with muscle strength and CSA. Thus, progressive resistance training seems to restore muscle sex steroid hormone levels via enhancement of steroidogenesis-related enzyme expressions in the skeletal muscle and may partly contribute to the increase in muscle strength and CSA.

Aging is associated with decreases in serum DHEA levels (12). Consequently, reductions in sex steroid hormone concentrations in blood would result in an increased risk of the metabolic syndrome (3, 13, 14, 15) and accelerated sarcopenia (15, 16). Using in vitro and in vivo animal models, we have previously demonstrated that testosterone and DHT can be synthesized from DHEA in muscle (4, 5) and acute and chronic aerobic exercise increases sex steroid hormones in muscle with concomitant elevations of serum steroid hormones in rats (11, 17). Furthermore, exercise, especially resistance exercise, is characterized by contracting skeletal muscles and increased sex steroid hormone levels in the older adults (7). In the present human study, the serum and muscle levels of sex steroid hormones were significantly lower in older as compared with younger men. However, resistance training restored both plasma and muscular sex steroid hormone levels in older subjects. Importantly, restoring DHT has a more potent effect on target tissue than DHEA and testosterone because of its greater affinity to the androgen receptor (1). Therefore, resistance training-induced increases in muscular DHT and androgen receptor expressions may have contributed to the training effect in older subjects.

A previous study reported gene expression of steroidogenic enzymes declines with aging, especially 3β-HSD and P450 aromatase in women (2). In the present study, steroidogenic enzyme expressions such as 3β-HSD, 17β-HSD, and 5α-reductase significantly were lower in older men compared with young at baseline. However, 12 wk resistance training significantly increased muscular steroidogenic enzyme expressions; therefore, the training-induced restoration of impaired muscular steroidogenic enzymes in older men may have increased the synthesis and intramuscular levels of sex steroid hormones.

### Table 3. Association of systemic and local hormone levels with muscle power and CSA in percentage changes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Isokinetic strength</th>
<th>Muscle CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum hormone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHEA</td>
<td>0.328</td>
<td>0.371</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>0.411</td>
<td>0.102</td>
</tr>
<tr>
<td>DHT</td>
<td>0.463</td>
<td>0.064</td>
</tr>
<tr>
<td>IGF-1</td>
<td>−0.164</td>
<td>0.671</td>
</tr>
<tr>
<td>Muscle hormone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHEA</td>
<td>0.493</td>
<td>0.034</td>
</tr>
<tr>
<td>Free-testosterone</td>
<td>0.532</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DHT</td>
<td>0.617</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Italics denote significance at $P < 0.05$. 

Figure 3. Effect of exercise on 5α-reductase type 1 and androgen receptor protein expression. A) Representative immunoblotting results and histogram of 5α-reductase type 1 protein expression in the muscle. B) Representative immunoblotting results and histograms of androgen receptor protein expression. Young pre, young subjects at baseline; old pre, older subjects at baseline; old post, older subjects after training. Data represent means ± SE. *$P < 0.01$ vs. old pre.
In the previous study, chronic resistance training induced to elevate serum testosterone and DHEA levels (8). A recent study reported that gene expression of steroidogenesis-related enzymes in human muscle reduced with aging (2). In addition, serum DHEA-S level was significantly correlated with muscle force per CSA in female subjects (2). In the present study, significant correlations were seen between training effects of muscle sex steroid hormone levels, muscle CSA and maximum isokinetic strength. In addition, steroidogenic enzyme especially 5α-reductase protein expression significantly correlated with muscle CSA and isokinetic strength. However, no significant correlation was seen between serum DHEA level and muscle force per CSA in men (r=0.318, P=0.082). The mechanism underlying the relationship between androgen levels in muscle and muscle mass and strength remains to be elucidated. In several previous studies, androgen replacement enhanced skeletal muscle mass and strength in older adults (18, 19, 20, 21). Testosterone supplementation induces increases in satellite cell replication and inhibition of satellite cell apoptosis and may cause muscle hypertrophy (18, 19). In addition, testosterone deficiency lowers levels of mammalian target of rapamycin (mTOR) phosphorylation and impairs Akt activation, which may contribute to reduced skeletal muscle mass through down-regulation of protein synthesis (21). In our previous study, 6 wk of DHEA administration in rats with sucrose-induced obesity increased muscle IGF-1 gene expression in greater muscle mass and strength (6, 22, 23). On the other hand, DHT induces IGF-1 gene expression and isokinetic strength. There was also a significant correlation between muscle Akt phosphorylation and muscle DHT levels in rats in the aerobic exercise and DHEA groups (11). Thus, enhancement of Akt-mTOR signaling may underlie increases in intramuscular androgen levels by sex steroid hormone administration or exercise, resulting in greater muscle mass and strength (6, 22, 23). On the other hand, DHT induces IGF-1 gene expression in humans (24). In the present study, serum IGF-1 levels did not increase significantly with 12 wk of resistance exercise. In fact, no significant association between serum IGF-1 levels and muscle function has been reported (25). However, increased muscular sex steroid hormone levels might potentiate the increase in the IGF-1 isofrom mechano-growth factor (MGF) that is induced by muscle contractions and has a powerful anabolic effect on muscle (26).

Both exercise and DHEA administration induce elevations in serum sex steroid hormone levels and increase muscle sex steroid hormone levels through increased muscle sex steroidogenesis. In fact, muscular DHEA significantly correlated with muscular DHT level, and the expression of 5α-reductase and muscular DHT level was significantly correlated in the present study. However, the origin of sex steroid hormones measured in skeletal muscle remains unclear because muscle levels could reflect a combination of systemic endocrine and local intracrine processes.

In summary, muscle levels of steroidogenic enzymes and sex steroid hormones were significantly lower in older men as compared with young men. However, progressive resistance training significantly restored age-related decline of steroidogenic enzyme expressions and sex steroid hormone levels, and these enzymes and hormone levels significantly correlated with muscle size and strength (21). Therefore, resistance training-induced increase muscular sex steroid hormone may positively affect age-related concerns such as accidental falls, diabetes, sarcopenia, and osteoporosis and may improve the quality of life for older individuals.

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