Changes in human muscle protein synthesis after resistance exercise

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The purpose of this study was to investigate the magnitude and time course for changes in muscle protein synthesis (MPS) after a single bout of resistance exercise. Two groups of six male subjects performed heavy resistance exercise with the elbow flexors of one arm while the opposite arm served as a control. MPS from exercised (ex) and control (con) biceps brachii was assessed 4 (group A) and 24 h (group B) postexercise by the increment in L-[1-13C]leucine incorporation into muscle biopsy samples. In addition, RNA capacity and RNA activity were determined to assess whether transcriptional and/or translational processes affected MPS. MPS was significantly elevated in biceps of the ex compared with the con biceps, whereas RNA activity was significantly elevated in the ex biceps of both groups relative to the con biceps, whereas RNA activity was unchanged in the ex biceps of both groups relative to the con biceps, whereas RNA activity was significantly elevated in the ex biceps of both groups (group A, ex 0.1007 ± 0.0330 vs. con 0.067 ± 0.0204 %/h; group B ex 0.0944 ± 0.0363 vs. con 0.0452 ± 0.0126 %/h). RNA capacity was unchanged in the ex biceps of both groups (group A, ex 0.1007 ± 0.0330 vs. con 0.067 ± 0.0204 %/h; group B ex 0.0944 ± 0.0363 vs. con 0.0452 ± 0.0126 %/h). RNA activity may change before RNA capacity (an index of the effectiveness of the ribosomal machinery in translating existing mRNA species into protein molecules (27). There is also indirect evidence suggesting that RNA activity may change before RNA capacity (an index of changes in transcriptional events) during conditions involving acute changes in muscle activity (3).

The mechanisms that mediate acute changes in MPS in response to resistance exercise are not yet known. Wong and Booth (30, 31) found increases in RNA activity after a single bout of concentric or eccentric resistance exercise. RNA activity reflects changes in posttranscriptional events and is used as an index of the effectiveness of the ribosomal machinery in translating existing mRNA species into protein molecules (27). There is also indirect evidence suggesting that RNA activity may change before RNA capacity (an index of changes in transcriptional events) during conditions involving acute changes in muscle activity (3).

The purpose of this study was to examine the magnitude and time course of changes in mixed muscle protein synthetic rates in humans after a single bout of resistance exercise. In addition, RNA capacity and RNA activity (27) were measured to indicate overall changes in transcriptional and/or translational control processes, respectively.

METHODS

Subjects. Twelve healthy males who regularly engaged in resistance training served as subjects. They were advised of the risks associated with the study and provided written informed consent. The study was approved by the University Human Ethics Committee. Subjects were assigned to either a 4 h postexercise group (group A) or a 24 h postexercise group (group B). Subjects were re-

IT IS WELL KNOWN that a program of heavy resistance training can lead to substantial gains in strength and muscle hypertrophy (12, 14, 24). The observed increases in muscle size are due to acute and chronic increases in muscle protein turnover such that protein synthesis exceeds protein degradation (9, 10). Although both acute and chronic increases in protein synthesis have been demonstrated in muscles of animals undergoing hypertrophy in response to tenotomy or stretch (9, 10), such perturbations do not really simulate resistance training as performed by humans (23, 25). Thus there are few data available regarding the time course and magnitude of the acute changes in muscle protein synthesis (MPS) after resistance exercise in humans.

On the basis of a review of animal studies involving muscle stretch, various exercise protocols, or electrical stimulation, Booth et al. (4) postulated that acute increases in muscle protein synthesis occur 1–2 h postexercise and may remain elevated above basal levels for an undefined period thereafter. It also appears that the magnitude of the increase in MPS is dependent on the type, intensity, and duration of exercise (3, 4). Increases in total mixed and myofibrillar protein synthetic rates ranging from 25 to 65% have been documented in rat gastrocnemius and tibialis anterior 17 and 41 h after the completion of a single bout of concentric or eccentric resistance exercise (30, 31). In humans, MPS has been shown to increase by 26% in vastus lateralis 4 h after a single bout of low-intensity treadmill running (6). In addition, whole body protein synthesis (WBPS) was unchanged for up to 2 h after an acute bout of circuit-type resistance exercise performed at 70% of the one-repetition maximum (22), whereas WBPS increased in resistance-trained males 24 h after a similar exercise protocol compared with age-matched sedentary controls (21). MPS has been shown to account for −25–30% of WBPS (15), and thus increases in WBPS are likely to be due in part to increases in MPS.

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crutied so that the two groups could be equated on the basis of resistance training experience and maximal elbow flexor strength. Group characteristics are provided in Table 1.

Preexperimental measurements. Maximum elbow flexor strength [single maximum repetition (1RM)] of the dominant arm was determined for the biceps curl, preacher curl, and concentration curl exercises. Body density was determined by hydrostatic weighing, with residual lung volume measured by helium dilution. Percent body fat and concentation curl exercises. Body density was calculated according to the Brozek method (5).

Table 1. Subject descriptive data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>29±1.5±3.9</td>
<td>23±1.2±2.3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.76±0.09</td>
<td>1.80±0.05</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>79.3±5.1</td>
<td>83.0±10.7</td>
</tr>
<tr>
<td>Lean body weight, kg</td>
<td>67.0±5.1</td>
<td>73.3±8.6</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>15.4±5.1</td>
<td>11.4±5.0</td>
</tr>
<tr>
<td>Years of training</td>
<td>4.9±6.6</td>
<td>4.1±3.5</td>
</tr>
<tr>
<td>Daily energy intake, kcal</td>
<td>3,076±602</td>
<td>3,196±1057</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 in each group.

Experimental protocol. The six subjects in group A performed the resistance exercise protocol on the day of the leucine infusion, whereas the six subjects in group B exercised the day before the leucine infusion. Measurements were made after 3 days of rest during which no resistance training was performed. The exercise protocol consisted of four sets of 6–12 repetitions of the biceps curl, preacher curl, and concentration curl exercises. Body density was determined by hydrostatic weighing, with residual lung volume measured by helium dilution. Percent body fat was then calculated according to the Brozek method (5).

Three day food records (including 1 weekend day) were obtained from each subject for the determination of mean daily energy and protein intakes with use of a computer program for nutrient analysis (Nutritionist III, Silvertown, OR).

The procedure used to isolate and measure $L-[1-^{13}C]$leucine content in muscle tissue was a modification of the technique described by Smith et al. (20). The protein pellet obtained after protein/nucleic acid extraction was hydrolyzed in 6 M HCl and the resulting amino acid mixture was applied to an ion-exchange column as previously described. The samples were then dried in a rotary evaporator and derivatized with 50–75 μl of N-methyl-
butyldimethylsilyl trifluoroacetamide and an equal volume of pyridine in an oven at 85°C for 60–90 min.

Preparative gas chromatography, for the isolation of leucine, was done with a Pye Unicam 304 series chromatograph fitted with a postcolumn splitter (99:1 split ratio) and a wide-bore glass column (6 mm ID × 4.6 m) as previously described (20). The leucine was collected from the postcolumn splitter in a home-made demountable glass U trap cooled in liquid nitrogen. The leucine collected in the U trap was removed by the addition of 0.5 ml of lithium citrate buffer (pH 2.2) to the trap followed by heating at 90°C for 30 min. The liquid was then transferred to a 20-ml Vacutainer tube, and the U trap was rinsed with a further 0.5 ml of buffer. The rubber stoppers from the Vacutainers were degassed overnight in a sealed glass flask in an oven under vacuum at 90°C. The samples were degassed at 140–150°C in a heating block for 30 min and placed on ice. Approximately 25 mg of ninhydrin were added to each tube on ice, and the Vacutainer was evacuated on a vacuum line. The ninhydrin was added to each tube on ice, and the Vacutainer was evacuated on a vacuum line. The ninhydrin reaction was carried out in a 90°C water bath for 30 min. The tubes were then allowed to cool to room temperature and were filled with nitrogen. The 13CO2 enrichment of the samples was determined by isotope-ratio mass spectrometry according to the method of Scrimgeour et al. (18). Plasma α-KIC enrichment was determined by capillary gas chromatography/mass spectrometry according to the method described by Tarnopolsky et al. (22).

Muscle protein synthetic rate was calculated according to the equation

$$\text{FMPS} = \left(\frac{\text{LE}_m \times 100}{K_{Ep} \times t}\right)$$

where FMPS is the fractional muscle protein synthetic rate (%/h), t is the incorporation time (in h) between biopsy samples taken from the same arm (15), LEm is the increment in 13C abundance in muscle protein obtained between t = 2 h and t = 6 h biopsy samples from each arm, and KEp is the mean plasma α-KIC enrichment for t = 2, 4, and 6 h blood samples (corrected for background enrichment from the t = 0 h sample).

Biopsy samples for two subjects in group A were found to be of insufficient size for measurement of protein synthetic rate and RNA activity, and thus data are presented for only four subjects in group A and six subjects in group B. Subject descriptive data, elbow flexor strength, training intensity and volume, and leucine infusion parameters were analyzed with a one-way analysis of variance (ANOVA). Muscle protein, total RNA, and DNA concentrations were analyzed with a two-way ANOVA with repeated measures. Protein synthetic rates and RNA activity were analyzed with a two-way ANOVA with repeated measures for unequal sample sizes. A Tukey post hoc analysis was used when significant differences between means were obtained. P < 0.05 was selected as being indicative of statistical significance. Values are expressed as means ± SD.

**RESULTS**

The two groups did not differ as to age, height, body weight, lean body weight, energy intake, or training history (Table 1). The mean 1 RM for the three biceps exercises was similar between groups as were the mean training intensity and volume (product of the weight lifted and the total number of repetitions) for the experimental day.

The mean plasma α-KIC enrichments over the three separate sampling points during infusion were 4.87 ± 0.95 and 4.63 ± 0.96 atom percent excess for groups A and B, respectively. These values were consistent over time with a coefficient of variation of <8.7%, thus demonstrating isotopic steady state (Fig. 1).

Total muscle protein expressed as a percentage of muscle wet weight was similar between groups and between the exercised and control biceps. In addition, muscle RNA and DNA concentrations were similar between groups and between arms (Table 2). The values of enrichment of 13C in muscle are presented in Table 3. Muscle protein synthetic rates were significantly increased in exercised compared with control biceps of both groups (group A, 0.1007 ± 0.0330 vs. 0.067 ± 0.0204 %/h; group B, 0.094 ± 0.0363 vs. 0.0452 ± 0.0126 %/h; Fig. 2). The observed differences in MPS were apparently due to a significant increase in RNA activity in the exercised vs. control biceps of both groups (group A, 0.19 ± 0.10 vs. 0.12 ± 0.05 μg protein·h⁻¹·μg⁻¹ of RNA; group B, 0.18 ± 0.06 vs. 0.08 ± 0.02 μg protein·h⁻¹·μg⁻¹ of RNA; Fig. 3).

**TABLE 2. Muscle protein content and total RNA (capacity) and DNA concentration**

<table>
<thead>
<tr>
<th></th>
<th>Protein Content, % wet wt</th>
<th>RNA, μg/mg protein</th>
<th>DNA, μg/mg protein</th>
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<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex</td>
<td>14.8 ± 5.4</td>
<td>6.3 ± 1.5</td>
<td>4.7 ± 1.7</td>
</tr>
<tr>
<td>Con</td>
<td>15.0 ± 5.6</td>
<td>5.4 ± 1.5</td>
<td>4.6 ± 1.6</td>
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<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex</td>
<td>17.3 ± 5.8</td>
<td>5.4 ± 1.6</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>Con</td>
<td>16.0 ± 6.6</td>
<td>5.3 ± 0.9</td>
<td>4.9 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 4 for group A and 6 for group B. Ex, exercised biceps; Con, control biceps.
The purpose of this study was to determine the magnitude and time course for acute changes in MPS after an isolated bout of heavy resistance exercise. Because of the number of biopsies required, we considered it necessary to examine two groups, each at a different time point (rather than a single group at two time points). Our interpretation of the time course data is thus based on the assumption that postexercise changes in MPS would have been similar for both groups. In an attempt to ensure this, groups were equated according to body size, strength, and training history and performed identical exercise protocols.

A second assumption is that plasma \( \alpha-[^{13}C] \)KIC labeling is a valid index of the precursor pool available for MPS. Plasma \( \alpha \)-KIC labeling reflects the probable intracellular fate of leucine and as such has been employed in several studies for calculating rates of whole body and MPS (2, 6, 7, 15, 16, 21, 22). Recent data have confirmed agreement with a model proposed by Booth et al. (4), suggesting that MPS increases above basal levels 1–2 h postexercise and remains elevated for an indefinite time thereafter. In the present study the time required to reach isotopic plateau and to allow labeled leucine to accumulate in the biceps precluded the assessment of IMPS sooner than 4 h postexercise. It is possible that protein synthetic rates were elevated before this time point, but studies examining a shorter time course have not been performed in humans. Tarnopolsky et al. (22) found no change in WBPS rates in experienced bodybuilders 2 h after the completion of a circuit-type resistance exercise protocol. Because MPS accounts for 25–30% of WBPS (15), these results suggest that either MPS may have been unaffected at this time or increases in MPS occurred but were masked by larger decreases in protein synthesis in other tissues. Our finding that MPS remained elevated for up to 24 h postexercise is consistent with findings of an elevated WBPS at this time point after a single bout of circuit-type resistance exercise (21). MPS has been shown to be acutely elevated 12–17 and 36–41 h in rat gastrocnemius and tibialis anterior after a single bout of concentric or eccentric resistance exercise, respectively (30, 31).

The duration of increases in MPS in humans after an isolated bout of resistance exercise is not known. Variables that may affect this include the intensity and volume of the exercise, the muscle or muscle groups involved, the type of muscle contractions performed, and the state of training of the subject. There appears to be an optimal training frequency of two or three per times...
week for exercising a muscle group to ensure gains in muscle mass (14, 29). Less or more frequent training may result in little or no muscle growth and suggests that the time course for changes in MPS may be intimately associated with training frequency and subsequent recovery from exercise.

After the resistance exercise, there were wide interindividual differences for leucine enrichment in the control and exercised arms (Table 3). The source of this variation is unknown, but it may be due to differences in training history, differences in muscle fiber composition, and/or the degree of muscle damage and satellite cell activation. There is some evidence suggesting that the basal rate of MPS is higher in type I than in type II muscle fibers (8), although this has not been substantiated by measurements of protein synthesis in humans (15). Another possibility is that muscle damage of the type associated with high-intensity eccentric muscle contractions may have occurred in the exercised biceps. Such damage may result in increased MPS through the possible release of growth factor and subsequent satellite cell activation (28).

To assess whether transcriptional and/or posttranscriptional events were responsible for the increases in MPS, RNA capacity and activity were measured in both exercised and control arms. RNA capacity expressed as the total RNA content relative to noncollagenous protein content (RNA concentration) can be considered an index of changes in transcription (27). RNA activity expressed as the amount of protein synthesized per unit time per unit of RNA can be considered an index of how quickly the ribosomal machinery can decode mRNA molecules into protein (ribosomal efficiency) (27). RNA capacity was unchanged in the exercised biceps of both groups, but RNA activity was significantly elevated compared with that in the unexercised biceps. These findings are similar to those of previous studies that have examined acute changes in RNA capacity and RNA activity after stretch or weight-training protocols (10, 30, 31). It thus appears that posttranscriptional events are important in mediating acute changes in MPS in response to muscle overload. The molecular signals, however, that stimulate acute MPS, RNA capacity and activity were measured in both injured and control biceps. Such damage may result in increased MPS through the possible release of growth factor and subsequent satellite cell activation (28).

In summary, protein synthetic rates were elevated in biceps muscle both at 4 and 24 h after a single unilateral heavy resistance training session. An upregulation of posttranscriptional events may be the mechanism that initiates and maintains an acute increase in MPS after resistance exercise.

The authors thank Stuart Phillips, John Moroz, and Joan Martin for technical assistance.

This study was supported by the Natural Science and Engineering Research Council of Canada. M. A. Tarnopolsky is funded by a National Institute of Nutrition Post Doctoral Fellowship.

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Received 9 December 1991; accepted in final form 17 April 1992.

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