The Time Course for Elevated Muscle Protein Synthesis Following Heavy Resistance Exercise

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Catalogue Data
MacDougall, J.D., Gibala, M.J., Tarnopolsky, M.A., MacDonald, J.R., Interisano, S.A., and Yarasheski, K.E. (1995). The time course for elevated muscle protein synthesis following heavy resistance exercise. **Can. J. Appl. Physiol.** 20(4): 480-486.

***Key words**: L-[^{13}C] leucine, muscle hypertrophy, training frequency, mass spectrometry*
***Mots-clés**: leucine L-[^{13}C], hypertrophie musculaire, fréquence d'entraînement, spectromètre de masse*

Abstract/Résumé

It has been shown that muscle protein synthetic rate (MPS) is elevated in humans by 50% at 4 hrs following a bout of heavy resistance training, and by 109% at 24 hrs following training. This study further examined the time course for elevated muscle protein synthesis by examining its rate at 36 hrs following a training session. Six healthy young men performed 12 sets of 6- to 12-RM elbow flexion exercises with one arm while the opposite arm served as a control. MPS was calculated from the in vivo rate of incorporation of L-[1,2-$^{13}C_2$] leucine into biceps brachii of both arms using the primed constant infusion technique over 11 hrs. At an average time of 36 hrs postexercise, MPS in the exercised arm had returned to within 14% of the control arm value, the difference being nonsignificant. It is concluded that following a bout of heavy resistance training, MPS increases rapidly, is more than double at 24 hrs, and thereafter declines rapidly so that at 36 hrs it has almost returned to baseline.

On a déjà montré que le taux de synthèse de protéine musculaire (SPM) est élevé dans l'homme de 50% à 4 h après une séance d'entraînement et de 109% à 24 h après l'entraînement. La présente étude continue l'examen du cours temporel de la synthèse

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élevée de protéine en examinant le taux à 36 h après une séance d'entraînement. Six jeunes hommes en bonne santé ont effectué un total de 12 séries de 6- à 12-RM d'exercices de flexion du coude avec un seul bras, l'autre agissant comme témoin. Nous avons calculé la SPM à partir de l'enrichissement du leucine L-[1,2-$^{13}C_2$] dans des biopsies du biceps brachii prélevées des deux bras en employant la technique d'infusion constant amorcé pendant 11 h. À 36 h en moyen après l'entraînement, la SPM du bras travaillé s'était rendue jusqu'à moins de 14% de la valeur du bras témoin, la divergence étant non significative. On conclut que la SPM accroît rapidement après une séance d'entraînement de résistance lourde, qu'elle atteint sa valeur à 24 h, et qu'elle décroît rapidement subséquemment, regagnant à peu près sa valeur initiale à 36 h.

Introduction

We have previously shown that the biceps brachii mixed muscle protein synthetic rate (MPS) is elevated in experienced weight trainers by 50% approximately 4 hrs after training, and by 109% approximately 24 hrs after training (Chesley et al., 1992; MacDougall et al., 1992). The present study further investigated the time course for elevated protein synthesis by examining its rate at approximately 36 hrs following a training session.

Methods

As in our previous study (Chesley et al., 1992), MPS was measured in biceps brachii following an acute bout of heavy resistance exercise by the elbow flexors of one arm so that the opposite arm served as a control. Since the duration that protein synthesis might remain elevated in the exercised arm was unknown, our original plan was to examine MPS 36 hrs following training and then at subsequent 12-hr intervals until MPS returned to that of the control arm.

SUBJECTS AND DESIGN

Six healthy young men (23 ± 1.7 yrs) who regularly engaged in heavy resistance (bodybuilding) training served as subjects. They did not differ from our previous subjects as to age, height, body weight, or training experience. They were advised of the risks associated with the study, in accordance with the university's human ethics committee, and provided written informed consent.

Subjects refrained from resistance exercise training for 3 days. At approximately 7 p.m. on the evening of the 4th day, they performed a typical training session for the elbow flexors of one randomly chosen arm. As in our previous study, the session was supervised by a coinvestigator and consisted of 4 sets of single arm biceps, concentration and preacher curls at 80% of maximum (1-RM) so that a total of 12 sets was completed in all. Each set was undertaken to muscular "failure" and a rest period of 3 to 4 min was provided between sets. The young men were instructed to consume their normal breakfast and lunch the following day and to report back to the laboratory at 5 p.m. to consume a standard meal. The energy content of the meal was 1,200 Kcal and consisted of 70% carbohydrate, 16% protein, and 14% fat. Two hours later a 20-Ga catheter was inserted into a hand vein for blood sampling and a second catheter was positioned in a contralateral forearm vein for isotope tracer infusion. Subjects read or watched

movies until their normal bedtime and slept in the laboratory overnight. The rate of tracer incorporation into biceps brachii was measured in both arms between 8 p.m. that night and 7 a.m. the following morning so that the midpoint would be 36 hrs after their previous training session.

MEASUREMENT PROCEDURES

The in vivo rate of MPS was determined from the enrichment of L-[1,2-$^{13}C_2$] leucine in biopsy samples using an 11-hr primed constant intravenous infusion. A priming dose of L-[1,2-$^{13}C_2$] leucine (>99% isotopic purity, Tracer Technologies, Somerville, MA) was administered (7.58 μmol · kg^{-1}), followed by a constant infusion (7.58 μmol · kg^{-1} · h^{-1}) for 12.5 hrs using a Harvard syringe pump. Arterialized blood samples (hot box at 65°C) were taken prior to infusion and at 1.5, 10.5, 11.5, 12, and 12.5 hrs for determination of plasma [1,2-$^{13}C_2$] – α-ketoisocaproic acid (α-KIC). Needle biopsies were taken from biceps brachii of both arms 90 min following the priming dose and 11 hrs later.

Enrichment of plasma [1,2-$^{13}C_2$] – α-KIC (atom % excess) was determined by electron impact ionization gas chromatography–mass spectrometry (Hewlett-Packard 5980A-MSD), as has been previously described (Tarnopolsky et al., 1991). Enrichment of muscle [1,2-$^{13}C_2$] leucine (mole % excess) was determined by capillary gas chromatography/combustion isotope-ratio mass spectroscopy as described by Yarasheski et al. (1992). MPS was calculated for each sample according to the method of Nair et al. (1988) using plasma [1,2-$^{13}C_2$] – α-KIC as the precursor pool enrichment, as we have previously detailed (MacDougall et al., 1992). Data are expressed as % · hr^{-1}, where the incorporation time is the time between biopsies for each subject. Differences in MPS between control and exercised arms and possible changes in plasma [1,2-$^{13}C_2$] – α-KIC during the infusion procedure were assessed by analysis of variance.

Results

Enrichment for plasma [1,2-$^{13}C_2$] – α-KIC at each sampling time is shown in Table 1. Each sample was analyzed in duplicate and the intra-assay coefficient of variation was 0.82%. Although there was a tendency for enrichments to decline slightly toward the end of the infusion period, this was not statistically significant. Plasma enrichments were remarkably consistent between the 1.5- and 10.5-hr

Table 1 Plasma [1,2-$^{13}C_2$] – α-KIC Enrichment (atom % excess)

Sampling time				
1.5 hrs	10.5 hrs	11.5 hrs	12.0 hrs	12.5 hrs
6.15 ±0.60	6.05 ±0.85	5.62 ±0.79	5.88 ±0.86	5.68 ±0.83

Note. $N = 6$. Differences are not statistically significant.

points, which represented the major portion of the time (91%) over which MPS was measured.

MPS for both control and exercised arms are illustrated in Figure 1. Duplicate analysis for enrichment of muscle protein [1,2-$^{13}C_2$] leucine were performed on each sample and the intra-assay coefficient of variation was 2.66%. Although MPS was approximately 14% higher in the exercised arm than in the control arm, this difference was not statistically significant.

MPS in the control arms (0.0408 ±0.0103% · hr^{-2}) was similar to the mean value of 0.0538 (±0.0148% · hr^{-1}) which we found in the control arms in our previous study (Chesley et al., 1992). The results of the present study have been combined with our previous data to illustrate MPS at 4, 24, and 36 hrs following exercise (Figure 2).

Discussion

Combined data from the subjects in the present study and from the two groups in our previous study indicate that biceps brachii MPS is elevated by 50, 109, and 14% at 4, 24, and 36 hrs, respectively, following a bout of heavy resistance training (Figure 2). Although a single within-group design at the three time points would have been preferable, we rejected it on ethical grounds because it would have required a total of six biopsies from biceps of each arm. We are aware of the limitations of a between-group design, and for this reason took care to match our three groups according to age, body size, and training background; we had them perform the identical exercises at the same volume and intensity.

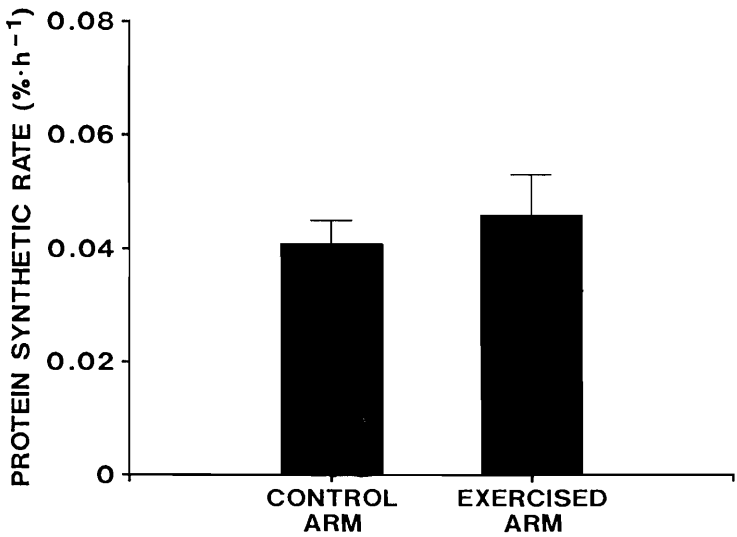

Figure 1. Rate of muscle protein synthesis in biceps brachii for the control and exercised arms 36 hrs after resistance training. $N = 6$, values are means ±*SE*. The difference between both arms was not statistically significant.

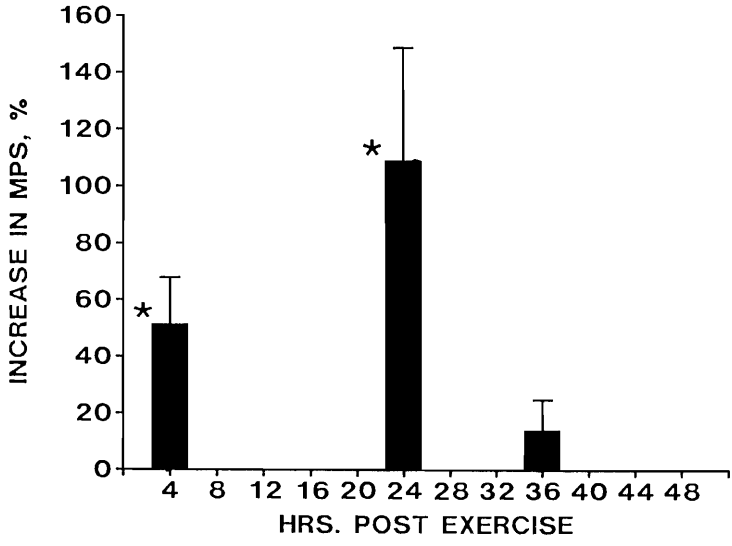

Figure 2. Increase in muscle protein synthetic rate 4, 24, and 36 hrs after a single resistance training bout. Values indicate the difference between MPS in the exercised and control arm. Data at 4 and 24 hrs are from a previous study (see text). *Significant at $p < 0.05$.

The method for measuring MPS was modified somewhat from our previous approach in that we infused doubly labeled (L-[1,2-$^{13}C_2$]) leucine and maintained the constant infusion over 12.5 hrs instead of singly labeled (L-[1-^{13}C]) leucine over 6 hrs. Our finding in the present study, that MPS in the control arm was similar to control arm values in the previous study, indicates that the two methods yield similar results. Moreover, since it is the *difference* in MPS between the exercised and nonexercised arms that indicates an elevated muscle protein synthesis, possible methodological differences have been controlled for.

It should be recognized that the values reported for MPS at 4 and 24 hrs represent the average values over the 4 hrs during which MPS was measured. Similarly, the 36-hr value is the average value over 11 hrs (i.e., between 30.5 and 41.5 hrs after exercise). The assumption in presenting these data at a single time point is that MPS is linear over the assessment period. This may not of course be the case. We interpret our finding—that at 36 hrs, MPS in the exercised arm was not significantly higher than in the control arm (in 4 subjects it was higher but in 2 subjects it was not)—as indicating that, at this point, MPS was already returning to its preexercise rate. The relatively large standard error at this point probably reflects interindividual differences in the rate at which additional protein synthesis is completed after training. In addition, since MPS at this point in time was not significantly elevated, we decided that further investigation at, say, 48 hrs was unnecessary.

Since the elevated MPS following heavy resistance exercise occurs in the absence of an elevation in RNA concentration (Chesley et al., 1992), its up-

regulation appears to be the result of posttranscriptional events (Chesley et al., 1992; Wong and Booth, 1990). The method we have used is a measure of mixed muscle protein synthetic rate. The extent to which muscle hypertrophy occurs is dependent upon *net* protein synthesis, that is, the difference between protein synthesis and degradation. It is apparent that the observed increases in MPS are accompanied by a concomitant increase in muscle protein degradation, or else muscle size would increase to a much greater extent than is known to occur with resistance training (MacDougall et al., 1980).

Degradation of muscle protein may to a large extent be due to the mechanical damage that occurs to contractile protein during heavy resistance exercise. We have recently documented disruption of muscle fine structure in biceps brachii following exercise similar to that in the present study and have found that, 24 hrs after exercise, some degree of myofibrillar disruption could be detected in as many as 80% of the fibres examined with electron microscopy (Gibala et al., 1995).

Knowledge of the time course for elevated MPS following resistance exercise is important to athletes and professionals in muscle rehabilitation who are interested in optimum methods for increasing muscle size and strength. Empirically, one might hypothesize that the most effective training frequency would be such that the subsequent training session should not occur until the protein synthesis stimulated by the previous session has returned to its pretraining rate. If this is true, the present data suggest an optimum recovery time of at least 36 to 48 hrs between training sessions; however, the time course for changes in protein degradation is not known.

In summary, the data from the present and our previous study (Chesley et al., 1992) indicate that when experienced subjects undertake a bout of heavy resistance training, the protein synthetic rate in the exercised muscle increases rapidly, is more than double approximately 24 hrs following exercise, and thereafter declines so that at 36 hrs it is almost back to baseline.

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Acknowledgment

This study was supported by the Natural Sciences and Engineering Research Council of Canada and by a Mass Spectrometry Research Grant (NIH-RR 00954).

The authors acknowledge the technical assistance of Brian Roy and Barbara Wilhelm and the secretarial assistance of Mary Cleland.

Received March 22, 1995; accepted in final form July 31, 1995.