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Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions

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Moore, Daniel R., Stuart M. Phillips, John A. Babraj, Kenneth Smith, and Michael J. Rennie. Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *Am J Physiol Endocrinol Metab* 288: E1153–E1159, 2005. First published November 30, 2005; doi:10.1152/ajpendo.00387.2004.— We aimed to determine whether there were differences in the extent and time course of skeletal muscle myofibrillar protein synthesis (MPS) and muscle collagen protein synthesis (CPS) in human skeletal muscle in an 8.5-h period after bouts of maximal muscle shortening (SC; average peak torque = 225 ± 7 N·m, means ± SE) or lengthening contractions (LC; average peak torque = 299 ± 18 N·m) with equivalent work performed in each mode. Eight healthy young men (21.9 ± 0.6 yr, body mass index 24.9 ± 1.3 kg/m^2) performed 6 sets of 10 maximal unilateral LC of the knee extensors on an isokinetic dynamometer. With the contralateral leg, they then performed 6 sets of maximal unilateral SC with work matched to the total work performed during LC (10.9 ± 0.7 vs. 10.9 ± 0.8 kJ, $P = 0.83$). After exercise, the participants consumed small intermittent meals to provide 0.1 g·kg^{-1}·h^{-1} of protein and carbohydrate. Prior exercise elevated MPS above rest in both conditions, but there was a more rapid rise after LC ($P < 0.01$). The increases ($P < 0.001$) in CPS above rest were identical for both SC and LC and likely represent a remodeling of the myofibrillar basement membrane. Therefore, a more rapid rise in MPS after maximal LC could translate into greater protein accretion and muscle hypertrophy during chronic resistance training utilizing maximal LC.

eccentric; concentric; resistance exercise; z-band streaming

ATTEMPTS BY PREVIOUS WORKERS to determine the influence of contraction types on rates of protein synthesis provided no evidence of any difference in either mixed muscle (6, 19) or myofibrillar (5) protein synthesis after shortening or lengthening contractions. Greater force can be generated during maximal muscle lengthening contractions (LC) than during maximal muscle shortening contractions (SC). Thus less active muscle is required to move a given load with LC than with SC. Whereas this may represent an increase in the force per active muscle fiber, it suggests a reduction in the number of fibers subjected to a potentially hypertrophic (i.e., resistive) stimulus during LC compared with SC. Because high-threshold motor units that innervate fast-twitch muscle fibers are preferentially recruited during submaximal (18) but not maximal LC (2), the conclusion made in previous reports (19) of no difference between the extent or time course of rates of muscle protein synthesis after SC and LC (5, 6, 19) may be due to differences in the amount of active muscle, the muscle tension, and/or the pattern of muscle activity during these studies.

Traditional isotonic resistance training programs require subjects to perform both SC and LC of particular muscles. Training programs exclusively comprising LC are generally agreed to result in greater hypertrophy than those comprising SC alone (9); however, this finding is not exclusive (10–12). LaStayo et al. (14) showed that 8 wk of training on an eccentric (muscle lengthening) but not a concentric (muscle shortening) cycle ergometer were sufficient to increase muscle fiber area by ~52% in healthy young subjects. Because cycling exercise is a stimulus that is not typically associated with muscle fiber hypertrophy (14), LC appear to be unique in their capacity to increase skeletal muscle mass. These results (8–11, 14) suggest that muscle LC, in resulting in a greater change in muscle mass than SC, should induce a greater rise in postexercise muscle protein synthesis, which appears to be the primary regulated variable governing muscle anabolism following both feeding (3) and exercise whether in the fasted (19) or fed (3) states. We hypothesized that the greater hypertrophy seen with training programs of lengthening contractions would be mediated through an enhanced postexercise muscle protein synthetic response.

As well as containing muscle fiber proteins, skeletal muscle also contains a network of extracellular connective tissue. Muscle collagen probably provides external structural support for skeletal muscle cells and should therefore increase as muscle fibers hypertrophy, suggesting that rates of myofibrillar and collagen protein synthesis would both increase after performance of resistance exercise. Previously, marked changes in muscle collagen and myofibrillar protein synthesis have been observed; both have been reported to show the same relative increase and time course (Miller BF and Rennie MJ, unpublished observations). However, the effect of maximal LC or SC on muscle collagen synthesis has yet to be determined.

The purpose of the present investigation was to determine whether there are any differences in the magnitude and/or acute time course of myofibrillar and collagen protein synthesis after maximal SC and LC. In an effort to ensure that muscle activity and active muscle mass were similar between conditions, we utilized an exercise model that required subjects to perform a series of maximal contractions on an isokinetic dynamometer. Maximal LC can generate greater force than maximal SC, so simply matching the number of repetitions should result in the muscle performing more work during the LC. Because it is

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possible that greater muscular work could further stimulate the postexercise muscle protein synthetic response, potentially explaining the greater increases in muscle mass and fiber hypertrophy commonly seen when training with LC (9–11), total work was matched during shortening and lengthening conditions in the present investigation.

METHODS

Subjects. Eight healthy recreationally active males who were not currently weight training participated in the study. The mean age was 22 ± (SE) 1 yr, body weight was 76 ± 3 kg, height was 175 ± 3 cm, and body mass index was 25 ± 1 kg/m^2. Participants were informed about the experimental procedure to be used as well as the purpose of the study and all potential risks. Written consent was obtained from the subjects. The study conformed to all standards for the use of human subjects in research as outlined in the Helsinki declaration and was approved by the local Research Ethics Board of McMaster University and Hamilton Health Sciences.

General design. Participants reported to the laboratory on two separate occasions. During the first visit, subjects received a primed, constant infusion of [1,2-^{13}C]leucine (see *Infusion protocol*) to measure rates of myofibrillar and collagen protein synthesis at rest in the fed state. At least 3 wk later, participants performed a bout of unilateral knee extension exercise with equivalent amounts of total work by one leg performing maximal LC and the other performing maximal SC. A second primed, constant infusion of [1,2-^{13}C]leucine was then initiated to measure protein synthesis 4.5 and 8.5 h after participants had performed maximal LC and SC, in the fed state. We chose to measure muscle protein synthesis without making measurements of muscle breakdown because alterations in muscle protein breakdown influence the anabolic responses to feeding (3) and exercise (3, 19) to a more limited extent and because measurement of muscle protein breakdown would have been technically challenging within our protocols.

Exercise protocol. Participants performed the exercise protocol described below on two occasions at least 2 wk apart, the purpose of the first exercise bout being a familiarization session. Having refrained from any strenuous exercise for at least 3 days, subjects reported to the laboratory at 0700 after an overnight fast to begin the exercise protocol. Participants were seated in a Biodex isokinetic dynamometer (Shirley, NY) with the knee visually aligned with the axis of rotation of the measurement arm; thigh, waist, and chest straps were applied to reduce extraneous movements, thereby isolating the action of the quadriceps group. Exercise modes were randomly assigned to participants' legs according to limb dominance, with half of the individuals (n = 4) performing maximal LC with their dominant leg and the others with their nondominant leg. The contralateral leg was used to perform maximal SC. Subjects first performed three 5-s maximal isometric unilateral knee extensions at 70° knee flexion (0° being full knee extension) separated by a 30-s rest to determine maximal voluntary isometric strength (MVC), taken as the average peak torque of the two strongest contractions. The exercise protocol then began with participants performing 6 sets of 10 maximal LC of the knee extensors at 0.79 rad/s. To do this, subjects resisted the motion of the dynamometer as their muscle was forcibly lengthened throughout the entire range of motion, which progressed from 10° to 90° of knee flexion. The interset rest period was 2.5 min, and the set-by-set work was recorded and summed to yield the total for the six sets. After an isometric MVC measurement, as described above, the contralateral leg was used to perform six sets of maximal SC. However, because maximal LC can generate greater force, subjects were required to perform a number of extra repetitions per set during SC so that total work was equivalent between the LC and SC conditions. Because the ability to produce force during maximal LC varies considerably between individuals (range: 101–170% of maximal shortening in the present study), participants always performed the maximal LC first to ensure that the muscle was receiving an adequate exercise stimulus. Upon completion of the exercise, stable isotope tracer leucine infusion was initiated.

Infusion protocol. Muscle protein synthesis was assessed in the fed state at rest and after exercise on separate days. In each case, subjects reported to the laboratory at 0700 after an overnight fast, having refrained from any strenuous physical activity for at least 3 days. After exercise, catheters were inserted in the medial vein of both arms, one for venous blood sampling and the other for tracer infusion. Baseline blood samples were drawn, and then subjects received a priming dose of [1,2-^{13}C]leucine (99 atom%, 1.0 mg/kg) and a constant tracer infusion (1.0 mg·kg^{-1}·h^{-1}; Cambridge Isotopes, Andover, MA) (Fig. 1). Participants rested on a bed throughout the infusion. Blood samples (~10 ml) were taken every 30–60 min using evacuated heparinized tubes and were chilled on ice until further analysis. After

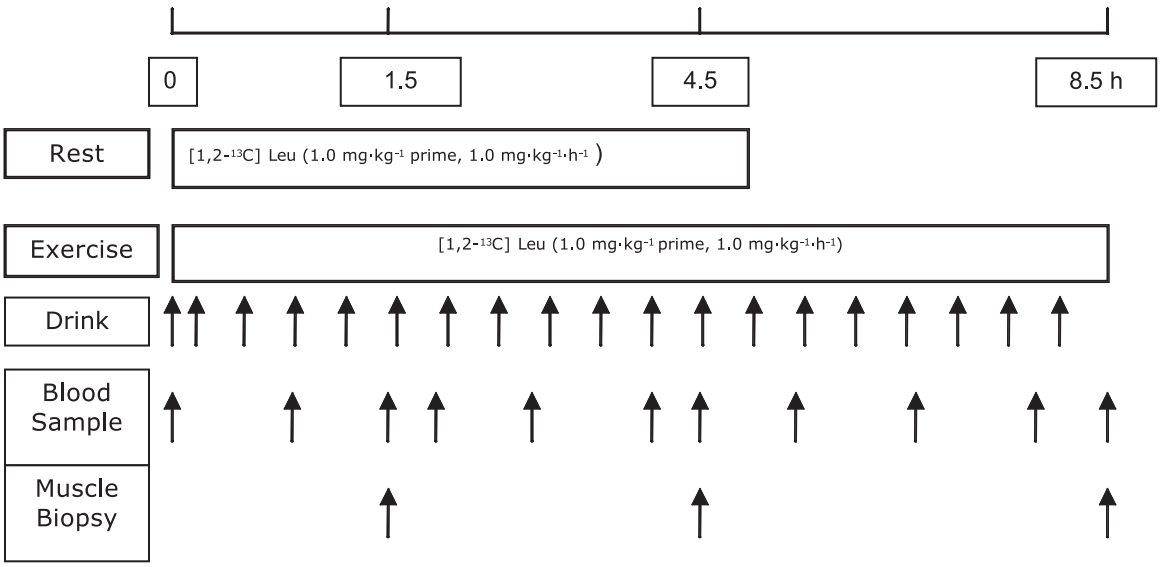

Fig. 1. Experimental protocols.

90 min, a muscle biopsy was taken from the vastus lateralis using the Bergström needle (modified for manual suction) technique under 2% xylocaine local anesthesia. Muscle biopsy samples were freed from any blood and visible connective and fat tissue; a small (~10 mg) longitudinal section of the 1.5-h muscle biopsy was immediately placed into 2% glutaraldehyde for later histological examination and stored at 4°C until processed. The remaining portion of the 1.5-h biopsy and all subsequent biopsies were rapidly frozen in liquid nitrogen and stored at −80°C until further analysis. The timing of the muscle biopsies for each leg was adjusted to account for when exercise ended. During the resting infusion, muscle biopsies were taken at least 3 h apart to allow adequate time for tracer incorporation into protein. Muscle biopsies were obtained from separate incisions at least 3 cm proximal from the previous one. Throughout the tracer infusions, participants received small aliquots every 30 min of a liquid protein supplement (Myoplex Lite; EAS, Golden, CO) with a double priming dose to provide 0.1 $g \cdot kg^{-1} \cdot h^{-1}$ of both carbohydrate and protein to provide sustained hyperaminoacidemia and mild hyperinsulinemia, which should support maximal rates of protein synthesis. Tracer was added to the drink to ensure stable isotopic enrichment. Total energy intake during the resting and the exercise infusions was one-sixth and one-third, respectively, of the subject's estimated 24-h energy requirements, as determined using the Harris-Benedict equation. Total resting and postexercise macronutrient consumption are provided (Table 1).

Analyses. All blood samples were collected into heparinized, evacuated containers (Vacutainer). A perchloric acid (PCA) extract was collected by adding 100 μl of whole blood to 500 μl of cold 0.6 M PCA, mixed, and allowed to sit on ice for 10 min. This mixture was then centrifuged at 15,000 rpm for 2 min at 4°C. The PCA was neutralized with 250 μl of 1.25 M potassium bicarbonate ($KHCO_3$), and the reaction was allowed to proceed on ice for 10 min. Samples were then centrifuged at 15,000 rpm for 2 min at 4°C. The supernatant was stored at −50°C until further analysis. Unfortunately, because of technical failure, we were unable to measure the enrichment of intracellular leucine. Therefore, plasma was separated from venous blood samples for measurement of isotopic enrichment of plasma α-[1,2-^{13}C]ketoisocaproate acid (α-KIC), determined by gas chromatography-mass spectrometry as a surrogate for leucyl-tRNA labeling (22, 23). Plasma insulin concentration was determined using Coat-a-Count insulin kits (Diagnostic Products, Los Angeles, CA).

To determine whole blood amino acid concentrations, the PCA extract was derivatized before injection, using an AccQ·Fluor reagent kit (Waters, Millford, MA), by heating for 30 min at 55°C to form the 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate derivative of all physiological amino acids. Samples and standards (Sigma, St. Louis, MO) were run on a Waters 2695 HPLC separations module through a Water Nova-Pak C_{18}, 4-μm column to separate the amino acids. The amino acids were detected using a Waters 474 scanning fluorescence detector with an excitation wavelength of 250 nm and emission at 395 nm. Amino acid peak areas were integrated, compared with known standards, and analyzed using the Waters Millenium^{32} software package. This method achieved separation of 18 of the 20 physiological amino acids, with the exception of cysteine and tryptophan, which were not included in the analysis.

Table 1. *Macronutrient consumption at rest and after exercise*

	Carbohydrate, g	Protein, g	Fat, g	Energy, kcal
Rest	3.6±0.2	35.8±1.5	35.8±1.5	320±14
Exercise	6.6±0.3	66.6±2.7	66.6±2.7	592±24

Values are means ± SE for total macronutrient consumption during the resting (rest) and postexercise (exercise) infusions; $n = 8$ subjects. Macronutrients were consumed over 4.5 h at rest and over 8.5 h after exercise.

Aliquots of frozen muscle sample (40–50 mg) were ground in liquid nitrogen, and the frozen powder was then added to homogenization buffer containing protease and phosphatase inhibitors (0.15 M NaCl, 0.1% Triton, 0.02 M Tris, 50 μM DTT, 0.1 M EDTA, and 1 mM PMSF). The resulting homogenate was subjected to centrifugation at 1,600 *g* for 20 min to precipitate the myofibrillar and collagen fractions. The supernatant was subjected to high-speed centrifugation (7,000 *g*, 15 min), and the sarcoplasmic fraction was precipitated by bringing the supernatant to 70% with ethanol and collected by centrifugation (1,600 *g*). The pellet containing myofibrillar and collagen protein fractions was washed twice with 0.7 M KCl and centrifuged at 1,600 *g* for 20 min. Myofibrillar protein was precipitated by bringing the supernatant to 70% with ethanol and collected by centrifugation. The remaining pellet was washed with 0.5 M acetic acid and spun at 1,600 *g* for 20 min, and the collagen protein fraction was extracted overnight with 0.1% pepsin and 0.5 M acetic acid at 4°C and collected by centrifugation. Protein-bound amino acids were released by acid hydrolysis (0.05 M HCl) at 110°C overnight. The HCl was evaporated under nitrogen, and the amino acids were purified by ion exchange chromatography on Dowex H^+ resin. Incorporation of [1,2-^{13}C]leucine into protein was determined by gas chromatography combustion-isotope ratio mass spectrometry as previously described (16).

Small sections of muscle stored in glutaraldehyde at 4°C were post fixed in 1% osmium tetroxide, dehydrated in graded alcohol, and embedded in Spurr's plastic resin. Longitudinal sections (~1 μm in thickness) were cut with a glass knife and stained with toluidine blue for light microscopic evaluation. Areas of Z-band streaming of 3–10 adjacent sarcomeres were quantified using light microscopy at ×1,000 power. Results are expressed as the number of areas of moderate Z-band streaming per square millimeter of muscle tissue. The entire data set was counted by two blinded, independent investigators, and the resultant scores were then averaged.

Breath-by-breath pulmonary oxygen consumption at rest and during exercise was assessed using an online gas collection system (Moxus modular oxygen uptake system; AEI Technologies, Pittsburgh, PA).

Calculations. The rates of myofibrillar and collagen protein synthesis were calculated using the standard precursor-product method: fractional protein synthesis (k_s, $\% \cdot h^{-1}$) = $\Delta E_m/E_p \times 1/t \times 100$, where ΔE_m is the change in protein enrichment between two biopsy samples, E_p is the mean enrichment over time of venous α-KIC enrichment, and t is the time between biopsies. Net area under the fractional synthetic rate vs. time curve (AUC) was calculated as $AUC = t_1(FSR_R + FSR_1)/[2 + t_2(FSR_1 + FSR_2)]/[2 - FSR_R(t_1 + t_2)]$, where FSR_R is the resting fractional synthetic rate, FSR_1 is the fractional synthetic rate at 4.5 h, FSR_2 is the fractional synthetic rate at 8.5 h, and t_1 and t_2 are 4.5 and 4 h, respectively. The AUC was expressed as net area to illustrate the effect of the exercise alone on protein accretion independently of the effect of feeding.

Total oxygen consumption ($\dot{V}O_2$, in liters) during exercise was calculated as $\dot{V}O_2 = (\dot{V}O_{2E} \times t)$, where $\dot{V}O_{2E}$ is the average rate of oxygen uptake from the beginning of *set 1* to 2 min after the completion of *set 6* and t is the time from the beginning of *set 1* to 2 min after the completion of *set 6*. The rate of oxygen consumption during exercise was taken as the average rate of oxygen uptake from the beginning of a given exercise set to 1 min after the completion of the set (i.e., the 1-min of rest immediately following the set).

Statistics. Strength, total work, and total oxygen consumption data were analyzed using a two-tailed paired Student's *t*-test to compare differences between conditions. Because we used a within-subject study design, myofibrillar and collagen protein synthesis data were compared using one-factor (1 × 5; time) repeated-measures analysis of variance (ANOVA). One-factor repeated-measures ANOVA was also used to analyze both plasma glucose and insulin concentrations at

rest (1 × 4; time) and after exercise (1 × 5; time). Rate of oxygen consumption during exercise was analyzed using two-factor (2 × 6; condition, time) repeated-measures ANOVA. Tukey's post hoc test was performed to compare differences between means for all significant interactions and main effects. Muscle damage data were analyzed using Kruskal-Wallis ANOVA, and differences in median scores were assessed using Dunn's multiple comparison post hoc. For all analyses, statistical significance was accepted as $P \leq 0.05$. Values are expressed as means ± SE.

RESULTS

Strength. There was no difference in maximal isometric tension (MVC) developed before the maximal SC and LC were performed (SC, 276 ± 12 N·m; LC, 259 ± 19 N·m). However, average peak torque during the exercise was ~25% greater for LC (260 ± 17 N·m) than for SC (209 ± 7 N·m; $P < 0.01$). During the LC, subjects also exercised at a greater percentage of their preexercise isometric MVC (SC, 76 ± 4%; LC, 100 ± 6%, $P < 0.05$).

Work. Total work performed during SC and LC conditions was identical (SC, 10.9 ± 0.7 kJ; LC, 10.9 ± 0.8 kJ; $P = 0.83$) with a significant linear correlation between the two exercise modes (Fig. 2). Subjects had to perform 80 ± 7 repetitions during SC to match total LC work (60 repetitions).

Plasma glucose and insulin. Plasma glucose concentration was constant at ~5 mM during the resting infusion and was only significantly ($P < 0.05$) reduced at 1 h during the postexercise infusion (Table 2). Plasma insulin increased from a value of ~6 μU/ml to a peak at 2.5 h of ~15 μU/ml at rest but thereafter returned to basal values. Postexercise insulin concentration was relatively stable and was only elevated above basal at 5.5 h.

Plasma amino acid concentration. Plasma nonessential amino acid concentration was relatively unchanged at rest and after exercise (Table 3). However, feeding significantly ($P < 0.05$) elevated essential (~21–42%) and especially branched-chain (~27–54%) amino acid concentration above basal at rest. There was a trend ($P = 0.08$) toward a greater essential amino acid concentration at 2 h after exercise at compared with basal, and by 5.5 and 8.5 h postexercise, both essential and branched-chain amino acid concentration was significantly increased ($P < 0.01$) above basal values.

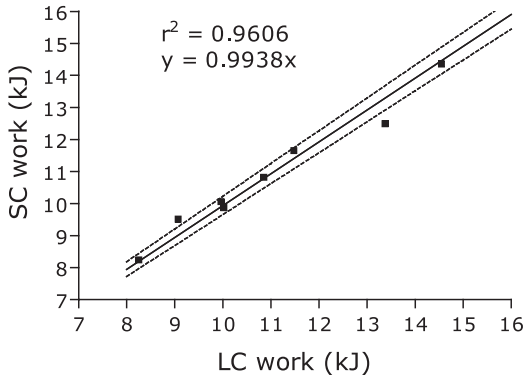

Fig. 2. Correlation of maximal shortening (SC) and lengthening contraction (LC) total work. Each point represents the total work performed by a subject during the SC and LC exercise trials. The solid line represents the regression, and the dashed lines represent the 95% confidence intervals.

Table 2. *Plasma glucose and insulin concentrations at rest and after exercise*

	Time, h	Glucose, mM	Insulin, μU/ml
Rest	0	4.3±0.1	6.3±0.6
	1	4.6±0.2	13.7±3.3
	2.5	4.8±0.1	14.9±3.9*
	4.5	4.3±0.1	8.3±0.8
Exercise	0	4.5±0.2	5.0±0.4
	1	3.6±0.2†	7.7±1.1
	3	4.0±0.2	6.3±0.6
	5.5	4.6±0.2	9.5±1.5‡
	8.5	4.5±0.2	8.7±1.5

Values are means ± SE for glucose and insulin; $n = 8$ subjects. *$P < 0.05$ vs. 0 h. †$P < 0.05$ vs. 0, 5.5, and 8.5 h. ‡$P < 0.05$ vs. 0 h.

Plasma precursor labeling. Plasma α-KIC enrichment was at plateau throughout with the exception of a small (~4%) fall immediately postexercise (which was incorporated into the calculation of FSR anyway). Otherwise, plasma α-KIC enrichment was stable at rest [4.8 ± 0.1 atom percent excess (APE)] and after exercise (5.1 ± 0.1 APE).

Muscle protein synthesis. The rates of incorporation of labeled leucine into myofibrillar and collagen proteins were ~0.07 and ~0.015%·h^{-1} in the fed state, respectively. Resistance exercise, independent of contraction mode, significantly elevated myofibrillar protein synthesis above rest at 4.5 (by ~48%) and 8.5 h (by ~67%) after exercise (Fig. 3*A*). However, there were significantly greater rates of myofibrillar fractional synthesis at 4.5 h after LC than after SC ($P < 0.01$). By 8.5 h, there was no difference between conditions. There was a greater area under the myofibrillar protein synthesis vs. time curve after 8.5 h in the LC than in the SC conditions (Fig. 3*B*). Collagen protein synthesis was increased ~300% above rest after both SC and LC at both the early and later acute time points (Fig. 4), without effective mode of exercise.

Oxygen consumption. Total whole body oxygen consumption was significantly greater for SC than LC ($P < 0.01$; Table 4). The rate of whole body total oxygen consumption at rest was not different between conditions (SC, 0.35 ± 0.04 l/min; LC, 0.34 ± 0.05 l/min, *P = 0.99*) but increased during exercise more for SC than for LC with a main effect for repetition set number. There was also a significant interaction between mode and oxygen consumption in that the rate of oxygen consump-

Table 3. *Blood amino acid concentration at rest and after exercise*

	Time, h	NEAA, μM	EAA, μM	BCAA, μM
Rest	0	1953±116	836±50	362±23
	2	1932±122	1008±63*	460±34
	4	2283±142*	1192±53†‡	557±27†‡
Exercise	0	2168±107	771±29	310±17
	2	2241±157	978±31	430±22
	5.5	2165±184	1123±79†	526±36†
	8.5	2105±148	1106±106†	544±80†

Values are means ± SE; $n = 8$. NEAA, nonessential amino acids (sum of Ala, Arg, Asn, Asp, Gln, Glu, Gly, Pro, Ser, Tyr; note Cys not measured); EAA, essential amino acids (sum of His, Ile, Leu, Lys, Met, Phe, Thr, Val: note Trp not measured); BCAA, branched-chain amino acids (sum of Ile, Leu, Val). *$P < 0.05$ vs. 0 h. †$P < 0.01$ vs. 0 h. ‡$P < 0.05$ vs. 2 h.

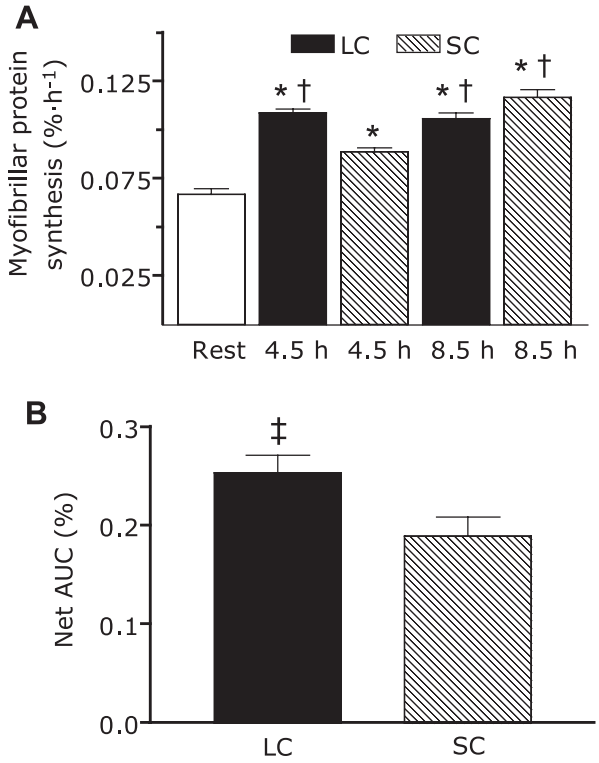

Fig. 3. *A*: rates of myofibrillar protein synthesis in the fed state at rest and at 4.5 and 8.5 h after maximal SC and LC. *$P < 0.001$, different from values at rest. †$P < 0.01$, different from SC values at 4.5 h. *B*: net (exercise − rest) area under the myofibrillar protein synthetic rate vs. time curve (AUC) after SC and LC. ‡$P < 0.01$, different from SC values.

tion was greater for SC than for LC in all but the first set of repetitions.

Z-band streaming. The number of areas of moderate Z-band streaming per area of tissue was significantly greater for LC than for SC both after exercise and at rest (Fig. 5). There was no difference between the resting and SC conditions.

DISCUSSION

In this report, we present values for resting and postexercise myofibrillar (15) and collagen protein synthesis (1) similar to those we have reported previously. As expected, resistance exercise in conjunction with postexercise feeding significantly elevated myofibrillar protein synthesis above rest in both conditions (20). However, despite muscles doing an equivalent amount of mechanical work, we observed a more rapid rise in myofibrillar protein synthesis after maximal LC than after SC, which would, assuming similar rates of proteolysis, have resulted in greater myofibrillar protein accretion over the 8.5 h studied. We also found that collagen protein synthesis, unlike myofibrillar protein synthesis, was stimulated to a similar extent at 4.5 h after both SC and LC.

Our finding of a more rapid rise in myofibrillar protein synthesis after maximal LC is in contrast to previous reports of a lack of differences in myofibrillar (5) or mixed muscle protein synthesis (6, 7, 19) between the period after submaximal SC and LC. However, the results of previous studies are not directly comparable to our own for a number of reasons. First, subjects in the present investigation performed maximal contractions and were therefore compared with subjects in previous studies (19) exercising at a similar intensity to that used previously during SC (~75% of MVC) but at a greater intensity during LC (~100% of MVC) (5–7). Maximal LC displayed no evidence of the preferential recruitment of high-threshold motor units (2) that can occur during submaximal LC (18), suggesting a similar pattern of muscle fiber recruitment between conditions in the present study. Furthermore, although maximal LC are associated with a slightly lower muscle activation compared with maximal SC, all muscle fiber populations are recruited, albeit at a lower rate, during maximal LC (2). This suggests that during contractions in each condition in the present investigation, a greater amount of active muscle would have been recruited than in previous studies (6, 7, 19), providing a stimulus to increase protein synthesis throughout the whole muscle. Second, in previous studies the response of mixed muscle protein synthesis was measured, whereas in the present study we assessed myofibrillar protein synthesis specifically (6, 7, 19). Because resistance exercise can increase global muscle rates of protein synthesis, measuring mixed muscle protein synthesis could mask the response of the myofibrillar protein fraction. Finally, total exercise time was much greater (~12 min) in one previous study (5) than in our present study (~3 min/condition), so total ATP use would have been greater. This marked difference in energy use could be responsible for the different postexercise protein synthetic responses between the current and previous studies (5). Hence, our finding of a more rapid rise in the rate of myofibrillar protein synthesis after maximal LC compared with SC appears to represent a true difference between the two contraction types and is not the result of methodological artifact.

There was a greater fuel use associated with performing a given volume of work with maximal SC than with LC, indicated by both a higher total oxygen consumption and a higher rate of oxygen consumption (Table 4). The greater energy cost of performing an equivalent volume of work with maximal SC compared with LC may have somehow delayed the rise in protein synthesis. Whereas the extent of the reduction in the rate of protein synthesis within perfused rat skeletal muscle during maximal contractions is correlated with falls in phosphocreatine and the ATP:ADP ratio (indexes of intense muscle activity) (4), it is unclear whether myofibrillar protein synthesis in human skeletal muscle would be influenced by the greater exercise intensity during SC at 4.5 h in the present investigation. Louis et al. (15) recently found that creatine supplementation, which should maintain the PCr:Cr ratio during intense

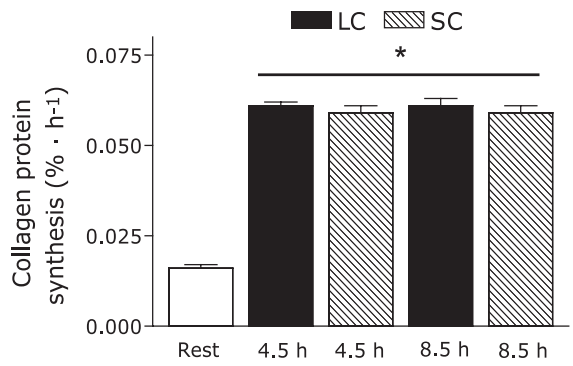

Fig. 4. Rates of collagen protein synthesis at 4.5 and 8.5 h after maximal SC and LC. *$P < 0.001$, different from values at rest.

Table 4. *Oxygen consumption during exercise*

	Total	*Set 1*	*Set 2*	*Set 3*	*Set 4*	*Set 5*	*Set 6*
SC	14.0±1.4*	0.87±0.19	1.00±0.28†	1.05±0.27†	1.12±0.30†	1.13±0.34†	1.15±0.30†
LC	10.2±0.6	0.74±0.11	0.79±0.11	0.85±0.16	0.89±0.23	0.86±0.12	0.94±0.19

Values are means ± SE; $n = 8$ subjects. SC, shortening contraction condition; LC, lengthening contraction condition. *$P < 0.01$ vs. LC. †$P < 0.01$ vs. LC. Main effect for set, $P < 0.01$.

exercise (13) and possibly attenuate an exercise-induced fall in the ATP-to-ADP ratio, had no effect on myofibrillar protein synthesis 3 h after an acute bout of exercise. The authors speculated that the change in protein synthesis after exercise was in part dependent on the extent of the reduction in the energy status of the muscle during exercise (15). If this were true, we could expect to find a greater myofibrillar protein synthesis after maximal SC compared with LC, which was not the case.

We found that, because of differences in protein synthesis at 4.5 h, there was a greater area under the myofibrillar protein fractional synthetic rate vs. time curve after maximal LC compared with SC. These data suggest there would have been a greater net protein accretion, assuming equivalent rates of proteolysis, occurring at least 8.5 h after maximal LC. It is possible that this early acute difference in myofibrillar protein accretion could explain the greater increases in muscle mass and fiber hypertrophy commonly seen after training with LC (9–11). However, protein accretion is the net difference between synthesis and breakdown, which we did not assess. Considering that these two processes are elevated for at least 24 h after a bout of intense resistance exercise (19), any difference in the rates of synthesis and/or breakdown at later time points (i.e., 24–48 h postexercise) may markedly influence muscle protein balance and thus the accretion of new protein. Therefore, future work should focus on determining any differences in myofibrillar protein synthesis and degradation up to 48 h after maximal SC and LC, when total work is equivalent.

This study is, to our knowledge, the first to directly measure changes in human muscle collagen synthesis after maximal muscle contractions. Recent work by Babraj et al. (1) demonstrated that collagen synthesis measured 3 h after exercise was greater after submaximal LC (stepping down from a knee-high box step while subjects carried 25% of bodyweight) compared with SC (stepping up onto the same step). The authors concluded that muscle fibroblasts, which synthesize muscle collagen, are stimulated more rapidly after submaximal LC, possibly because of a greater response to stretch (1). In the present investigation, we found no difference in the time course of muscle collagen synthesis after maximal SC and LC. This may be related to the high muscle forces associated with maximal contractions, which could have elicited a similar stretch response in the fibroblasts in both conditions; however, it is impossible to discount the possibility that the discrepancies are caused by differences in the timing of the muscle biopsies. Recently, Mackey et al. (17) reported an increase in type IV collagen content as well as evidence of enhanced myofiber basement membrane remodeling in the days after maximal LC. Therefore, the increase in collagen synthesis after both maximal SC and LC in the present study likely represents an elevated muscle collagen turnover, which would be a beneficial adaptive response to resistance exercise that would provide a more extensive external support lattice for muscle fibers.

We also demonstrated significantly greater Z-band streaming at only 1.5 h after a bout of maximal LC compared with SC, which is congruent with previous results of submaximal contractions (6, 7). Previous authors have examined Z-band streaming via electron microscopy and have concluded that this represents exercise-induced sarcomere disruption (7, 21). However, Yu et al. (24) recently suggested that these areas of myofibrillar and cytoskeletal alterations, identified as Z-band streaming, actually reflect an adaptive remodeling of myofibrillar structure without previous damage. Considering that immunohistochemical signs of myofibrillar remodeling are more pronounced at 2–7 days than at 1 h after LC exercise (24), we interpret our results to suggest that this short-term (i.e., within hours after exercise) Z-band streaming indicates physically altered muscle ultrastructure. These areas of acute myofibrillar disruption may later be the foci of enhanced tissue remodeling in the days after exercise (24).

We have demonstrated that human muscle protein synthesis is elevated above rest after both maximal SC and LC. However, despite equivalent external work being performed, there was a more rapid rise in myofibrillar protein synthesis after maximal LC. We also are the first, to our knowledge, to demonstrate that muscle collagen synthesis is elevated after maximal contractions, suggesting that muscle fibroblasts are stimulated to a similar extent 4.5 h after maximal SC and LC. Future work should determine whether differences exist in both myofibrillar and collagen protein synthesis and degradation at later acute time points (i.e., 24–48 h) after maximal muscle SC and LC.

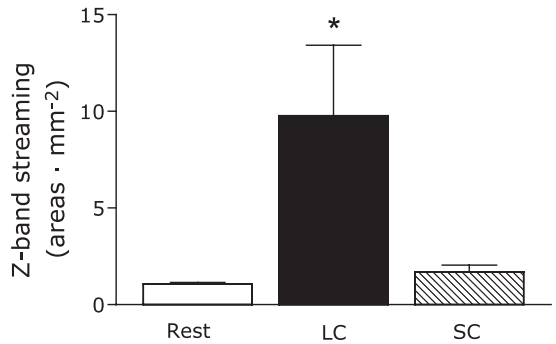

Fig. 5. Incidence of Z-band streaming in muscle at rest and after maximal SC and LC, expressed as the number of areas of moderate Z-band streaming (3–10 adjacent sarcomeres) per mm^2. *$P < 0.05$, different from rest and SC values.

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