



STIMULATION OF MYOFIBRILLAR PROTEIN SYNTHESIS IN HINDLIMB SUSPENDED RATS BY RESISTANCE EXERCISE AND GROWTH HORMONE

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Summary

The objective of this study was to determine the ability of a single bout of resistance exercise alone or in combination with recombinant human growth hormone (rhGH) to stimulate myofibrillar protein synthesis (Ks) in hindlimb suspended (HLS) adult female rats. Plantar flexor muscles were stimulated with resistance exercise, consisting of 10 repetitions of ladder climbing on a 1m grid (85°), carrying an additional 50% of their body weight attached to their tails. Saline or rhGH (1 mg/kg) was administered 30' prior to exercise, and Ks was determined with a constant infusion of ³H-Leucine at 15', 60', 180', and 360' following exercise. Three days of HLS depressed Ks ~65% and 30-40% in the soleus and gastrocnemius muscles, respectively (p<0.05). Exercise increased soleus Ks in saline-treated rats 149% 60' following exercise (p<0.05), decaying to that of non-exercised animals during the next 5 hours. Relative to suspended, non-exercised rats rhGH+exercise increased soleus Ks 84%, 108%, and 72% at 15', 60' and 360' following exercise (p<0.05). Gastrocnemius Ks was not significantly increased by exercise or the combination of rhGH and exercise up to 360' post-exercise. Results from this study indicate that resistance exercise stimulated Ks 60' post-exercise in the soleus of HLS rats, with no apparent effect of rhGH to enhance or prolong exercise-induced stimulation. Results suggests that exercise frequency may be important to maintenance of the slow-twitch soleus during non-weightbearing, but that the ability of resistance exercise to maintain myofibrillar protein content in the gastrocnemius of hindlimb suspended rats cannot be explained by acute stimulation of synthesis.

Key Words: hindlimb suspension, resistance exercise, growth hormone

Skeletal muscle atrophies in response to space flight or simulated microgravity (hindlimb suspension), an effect that is much greater in postural slow-twitch muscles such as the soleus than in predominantly fast-twitch muscles such as the gastrocnemius or plantaris (1,2). Previously, it has been demonstrated that the biological activity of growth hormone is decreased following 14 days of spaceflight (3) or 28 days of hindlimb suspension (4). Growth hormone restores growth and increases skeletal muscle protein synthesis in hypophysectomized rats (5). Thus, it may be that the decrease in the biological activity of growth hormone is linked to skeletal muscle atrophy resulting from non-weightbearing. However, the use of growth hormone to prevent atrophy of rat skeletal muscle during spaceflight has not been successful (6). Recently, we reported that daily resistance exercise (ladder climbing) in conjunction with recombinant human growth hormone (rhGH; 1 mg/kg/day) increased myofibrillar protein synthesis, and

spared loss of myofibrillar protein in the predominantly fast-twitch gastrocnemius of hindlimb suspended rats, with no such sparing effect observed in the predominantly slow-twitch soleus (7).

Exercise countermeasures have been partially successful in attenuating loss of muscle mass during hindlimb suspension (7,8,9,10,11). Current literature suggests that frequent bouts of daily exercise may be more effective in attenuating loss of soleus muscle protein during hindlimb suspension than a longer single bout of exercise (8,9,11). Furthermore, the rate of myofibrillar protein synthesis appears to be a principal determinant of myofibrillar protein content (7). The synthesis of myofibrillar protein is increased in response to single and chronic muscle contractions (12), and occurs prior to a detectable change in muscle wet weight or protein content. Furthermore, myofibrillar protein synthesis is reportedly decreased within hours of hindlimb suspension (13,14), prior to a detectable change in muscle wet weight. Thus, it is plausible that the more frequently myofibrillar protein synthesis is stimulated by exercise, the more effectively myofibrillar protein would be maintained during exposure to microgravity.

Muscle stretch or tension reportedly potentiates the effect of anabolic adjuvants, such as rhGH, insulin-like growth factor I, and anabolic steroids, to stimulate myofibrillar protein synthesis and muscle hypertrophy (7,15,16,17). However, this interactive effect is limited to muscles composed predominantly of fast-twitch fibers.

In the present investigation we hypothesized that a single bout of resistance exercise (ladder climbing) would stimulate myofibrillar protein synthesis in rats exposed to 3 days of hindlimb suspension, but that this stimulatory effect would be relatively short lived. In addition, it was hypothesized that in the predominantly fast-twitch gastrocnemius that rhGH would be additive to resistance exercise in stimulating myofibrillar protein synthesis, and would slow the decay in myofibrillar protein synthesis following an single bout of exercise.

Methods

Animal care. An animal care protocol was approved by the Institutional Animal Care and Use Committee of Ames Research Center in accord with the Ames Research Center Animal User's Guide (AHB 7180) and the Guidelines of the National Institute of Health. Female albino rats (Simonsen; Gilroy, CA) weighing ~250 g were housed singly in Plexiglass suspension cages as described previously (7,10), maintained on a 12h reverse dark (0700h to 1900h) and light (1900h to 0700h) cycle in a room maintained at $24 \pm 1^\circ \text{C}$, and given food and water *ad libitum*. Animals were acclimated to ladder climbing with twice daily bouts of 5 repetitions for 1 week up to the date of surgery, carrying no more than 20% of the animal's body weight attached to their tails.

Surgical procedures. Rats were anesthetized with an intraperitoneal (ip) injection of Ketamine (55 mg/kg body wt), Xylazine (4 mg/kg), and Acepromazine (0.75 mg/kg) and jugular cannulas were implanted, as described previously (18).

Experimental procedures. On the third day of recovery from surgery rats were matched according to pre-surgical body weight and assigned to ambulatory or suspended groups, such that initial bodyweights were not different (Table 1). Animals were suspended for 3 days using a modification of the model of Wronski and Morey-Holton (19), as previously described (10). On the morning following the third day of hindlimb suspension suspended rats were given an ip injection of either saline or recombinant human growth hormone (rhGH; 1 mg/kg/body weight; Genentech; South San Francisco, CA) and returned to their cages. This dose has been shown to restore musculoskeletal growth in hypophysectomized rats (10).

Thirty minutes following injection of either saline or rhGH the rats climbed a 1m grid (85° incline) 10-15 times with an additional 50% of their body weight attached to their tails (7,10). It has been our experience that this intensity and duration (~10 minutes) of exercise is nearly the maximum that untrained rats exposed to simulated microgravity will perform reliably (unpublished observation). An unprimed constant infusion of ^3H -Leucine (Amersham Corp., Arlington Heights, IL) was initiated 15, 60, 180, and 360 minutes following exercise for the measurement of myofibrillar protein synthesis.

Measurement of protein synthesis. Hindlimb suspended rats were infused in a suspended position with ^3H -Leucine at a rate of 1 mCi/hour in a Plexiglass cage similar to their suspension cage, while ambulatory animals were infused unrestrained (7). Following 2.5 hours of infusion rats were anesthetized with pentobarbital sodium (50 mg/kg body weight) and hindlimb muscles were dissected rapidly, trimmed of visible connective tissue, weighed, frozen in liquid nitrogen, and stored at -70°C . Myofibrillar protein was isolated according to the procedures of Wong and Booth (12). Intracellular free amino acids were isolated from trichloroacetic acid supernatants by ion exchange chromatography (Dowex-50 X8 (200-400 mesh, H^+ form); BIO-RAD Richmond, CA) (7). Myofibrillar protein concentration was determined with Pierce BCA reagent kits (Pierce; Rockford, IL) compared to bovine serum albumin standards, and protein content determined from the product of protein concentration and muscle wet weight.

Calculation of protein synthesis. Rates of protein synthesis were estimated from the equation described by Garlick et al. (20) and programmed for use on a Macintosh computer (Think Pascal; Symantec Corp) (7). Protein synthesis ($\mu\text{g}/\text{day}$) was the product of the fractional rate of protein synthesis and protein content (7).

Statistical analysis. Data are presented as mean \pm SEM. Data were compared using a one-way analysis of variance (Super Anova; Abacus, Berkeley, CA). Where appropriate differences between means were assessed with a Fisher Protected Least-Squared Difference post-hoc test. The 0.05 level of significance was chosen for all analyses.

Results

Body weight. As has been noted previously (7,10,19), hindlimb suspension (HLS) decreases growth rate as determined by body weight (Table 1). Initial body weights of Ambulatory and HLS rats, exercised or not, were not different. Body weights of Ambulatory rats did not change during the 3-day experimental period. In contrast, following 3 days of HLS body weight of HLS rats was slightly (5%), but significantly less than Ambulatory rats ($p \leq 0.05$).

TABLE I
The Effect of Hindlimb Suspension on Body Weight and Plantar Flexor Muscle Weights

	Body Weight		Soleus		Gastrocnemius		Plantaris	
	Pre-	Post-	mg/pair	mg/100 g BW	mg/pair	mg/100 g BW	mg/pair	mg/100 g BW
AMB	247 \pm 4	249 \pm 4	230 \pm 8	93 \pm 3	3142 \pm 60	1265 \pm 20	637 \pm 13	256 \pm 3
HLS	242 \pm 2	237 \pm 3*	225 \pm 9	95 \pm 3	2930 \pm 65*	1237 \pm 19	644 \pm 37	272 \pm 15

All values are mean \pm SEM, n=9. AMB; Ambulatory, HLS; hindlimb suspension, BW; body weight. *; significantly different vs. Ambulatory ($p \leq 0.05$).

Muscle wet weight. Absolute (mg/pair) and relative (mg/100g body weight) weights of the soleus and plantaris muscles were not affected by HLS (Table 1). The absolute weight of the gastrocnemius was $\sim 7\%$ less in HLS rats ($p \leq 0.05$), but the relative mass of the gastrocnemius was unchanged. Thus, the difference in absolute gastrocnemius wet weight was similar in magnitude to the difference in body weight.

TABLE II
The Effect of 3 Days of Hindlimb Suspension on Myofibrillar Protein Synthesis

	fraction/day	Soleus		Gastrocnemius		fraction/day	fraction/day	fraction/day	% Δ
		% Δ	$\mu\text{g}/\text{day}$	% Δ	% Δ				
AMB	0.133 \pm 0.016		1170 \pm 111 (n=7)		0.053 \pm 0.003		5020 \pm 331		
HLS	0.045 \pm 0.008*	-66	410 \pm 91* (n=7)	-65	0.036 \pm 0.004*	-32	2970 \pm 519*		-40

See TABLE I for explanation of abbreviations and symbols.

Protein synthetic activity. Hindlimb suspension decreased soleus and gastrocnemius myofibrillar protein synthesis (fraction/day) or ($\mu\text{g}/\text{day}$). Furthermore, consistent with previous observations (17) the effect of HLS on myofibrillar protein synthesis (Ks) was greater in the predominantly slow-twitch soleus than the predominantly fast-twitch gastrocnemius (Table 2). In suspended saline-treated rats soleus Ks (fraction/day) and ($\mu\text{g}/\text{day}$) was decreased over 60% ($p \leq 0.05$). Relative to suspended non-exercised rats soleus Ks was increased $\sim 150\%$ 60' following exercise in saline-treated animals, ($p \leq 0.05$), and soleus Ks was not different from Ambulatory animals (Figure 1). However, the 13% difference between Ambulatory rats and HLS saline-treated rats 60 minutes following exercise is likely of physiological significance. Although somewhat variable, Ks in saline-treated exercised rats decayed to the level of saline-treated non-exercised animals over the next 5 hours. Administration of rhGH in conjunction with exercise significantly increased soleus Ks at 15, 60, and 360 minutes post-exercise. Relative to saline-treated non-exercised rats soleus Ks was increased 84%, 108%, and 72% in rhGH-treated exercise animals at 15, 60, and 360 minutes following exercise, respectively ($p \leq 0.05$).

Stimulation of soleus myofibrillar protein synthesis (Ks) with exercise and rhGH

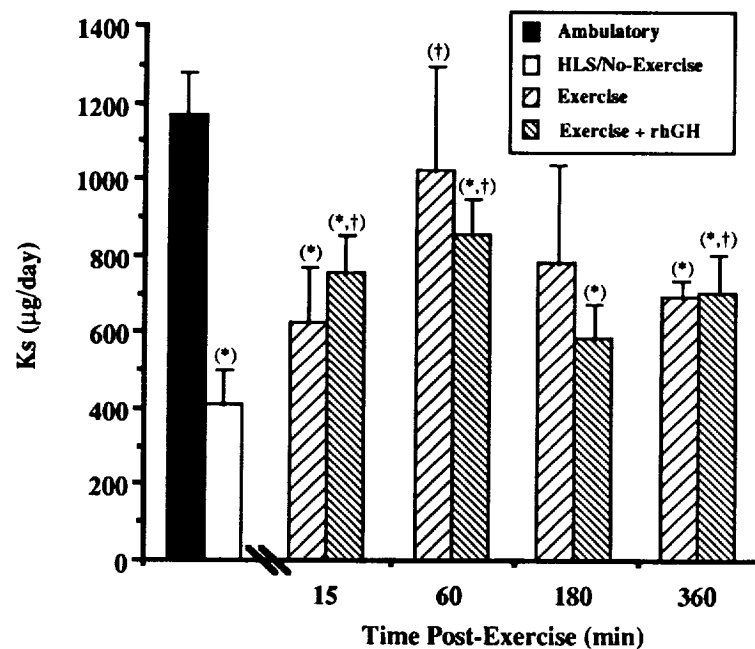


Fig. 1

The effect of hindlimb suspension (HLS), ladder climbing (Exercise), and recombinant human growth hormone (rhGH), on soleus myofibrillar protein synthesis (Ks). All values are mean \pm SEM, $n=9$. *, significantly different vs. Ambulatory rats ($p \leq 0.05$), †; significantly different vs. HLS/No-Exercise ($p \leq 0.05$).

Consistent with previously published results (7,15), HLS decreased gastrocnemius Ks 30-40% ($p \leq 0.05$), substantially less than the effect of unweighting on the slow-twitch soleus muscle (Table 2). A single bout of resistance exercise did not significantly increase gastrocnemius Ks in saline- or rhGH-treated animals (Figure 2). Similar to the soleus muscle (Figure 1) gastrocnemius Ks appeared to be stimulated $\sim 25\%$ 60' post-exercise in saline-treated animals, but this difference was not significant ($p > 0.05$).

Stimulation of gastrocnemius myofibrillar protein synthesis (Ks) with exercise and rhGH

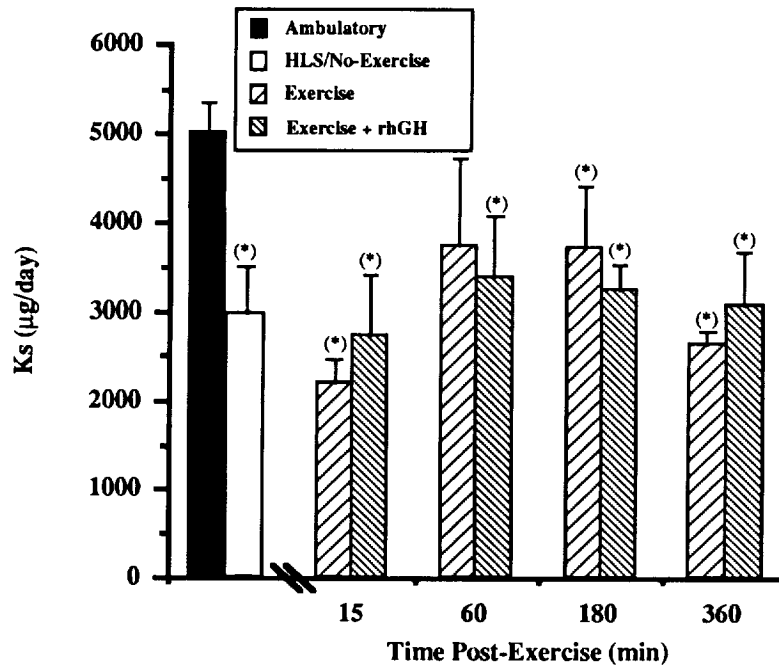


Fig. 2

The effect of hindlimb suspension (HLS), ladder climbing (Exercise), and recombinant human growth hormone (rhGH), on gastrocnemius myofibrillar protein synthesis (Ks). All values are mean \pm SEM, n=9. *: significantly different vs. Ambulatory rats ($p \leq 0.05$).

Discussion

Results of the present investigation indicate that a single bout of resistance exercise has differential effects on myofibrillar protein synthesis in predominantly slow- and fast-twitch plantar flexors undergoing non-weightbearing. Furthermore, results from the present investigation suggest that other mechanisms such as protein degradation, the additive effect of multiple bouts of exercise, or delayed effects not seen in the present study account for the sparing effect of daily resistance exercise and rhGH on non-weightbearing gastrocnemius myofibrillar protein content previously reported (7).

Effects of hindlimb suspension on protein metabolism. Consistent with previously published results from our laboratory (7) and others (13), results from the present investigation indicate that myofibrillar protein synthesis (Ks) in predominantly slow- and fast-twitch plantar flexors decreases within 3 days of non-weightbearing. Thomason and co-workers (13) reported that 7 days of hindlimb suspension (HLS) decreased soleus and medial gastrocnemius Ks (fraction/day) ~60% and 50%, respectively in 250-300 g female rats. In the present investigation 3 days of HLS decreased soleus and whole gastrocnemius Ks (fraction/day) 66% and 32%, respectively, in female rats weighing ~250 g (Table 2). Therefore, it is clear from past and present investigations the effect of non-weightbearing to decrease Ks is greater in skeletal muscles composed predominantly of slow-twitch fibers.

Effects of resistance exercise on myofibrillar protein synthesis. Current literature suggests that stimulation of protein synthesis following exercise may be related to the mode and intensity of loading (21,22,23), as well as the fiber type of the muscle in which protein synthesis is measured (23). To our knowledge, our measurements of soleus and gastrocnemius myofibrillar protein synthesis (Ks) 15-360' post-exercise, represents the first attempt to measure *in vivo* contractile protein synthesis immediately following resistance exercise, and are consistent with results indicating that muscle stretch increases protein synthesis (21,24). Our results indicate that resistance exercise stimulated soleus Ks in hindlimb suspended (HLS) rats 60' post-exercise (Figure 1), and Ks decayed towards the level seen in non-exercised HLS rats over the next 5 hours. In contrast, no exercise-induced stimulation of Ks was seen in the fast-twitch gastrocnemius for the 360' that Ks was measured post-exercise (Figure 2). Therefore, results of the present investigation suggest that not only is Ks more sensitive to unweighting in soleus than gastrocnemius, but that the soleus was more sensitive, at least initially, to a single increase in loading in HLS rats.

Several investigations have indicated that exercise initially suppresses protein synthesis, specifically in predominantly fast-twitch skeletal muscles (21,22,23). An exhaustive bout of treadmill running suppressed the *in vivo* rate of gastrocnemius mixed protein synthesis 71% (23). Additionally, a 10 minute isometric contraction of the soleus, gastrocnemius, and plantaris muscles markedly suppressed the *in vitro* rate of protein synthesis in the fast-twitch gastrocnemius and plantaris muscles immediately post-exercise, but did not suppress soleus protein synthesis (22). Consistent with these latter results, in the present investigation gastrocnemius Ks was ~25% lower 15' post-exercise in saline-treated rats, when compared to suspended non-exercised animals ($p>0.05$). This finding was very consistent with 7 of 8 rats having a value for gastrocnemius Ks that was less than the mean value for suspended non-exercised animals. Thus, present data appear to support the contention that a single bout of exercise initially suppresses protein synthesis in predominantly fast-twitch muscles.

We were somewhat surprised that gastrocnemius Ks was not stimulated by exercise, with or without rhGH. Previously, we reported that resistance exercise is more effective in attenuating loss of gastrocnemius than soleus muscle wet weight in non-hypophysectomized rats suspended for 5 days (7). One possible interpretation of this finding is that the gastrocnemius muscle was recruited more by resistance exercise than was the soleus. Data from the present investigation do not support this contention (Figures 1, 2). In this study soleus Ks was increased within 60' following exercise in saline-treated rats, and within 15' in rhGH-treated animals. In contrast, no initial or delayed stimulation of gastrocnemius Ks was observed in saline- or rhGH-treated rats following exercise. Based upon this observation it could be concluded that: 1) the gastrocnemius was only minimally recruited; 2) the protein synthetic machinery of the gastrocnemius is far less sensitive to an single increase in loading than is the soleus; or 3) other factors such as decreased protein degradation account for the sparing effect of resistance exercise previously reported in the non-weightbearing gastrocnemius (7).

Based upon EMG data it is known that the soleus is nearly maximally recruited at rest, or during slow walking in the guinea pig (25). As grade, speed, or load are increased, other plantar flexors such as the medial gastrocnemius are recruited to accomplish the increase in intensity. In our experience the load imposed upon the rats in the present investigation, an additional 50% of body weight, was nearly the maximum that untrained hindlimb suspended female rats will maintain reliably for 10 repetitions. Thus, it is unlikely that the gastrocnemius was not recruited during the exercise bout imposed in the present investigation.

In support of the contention that gastrocnemius protein synthetic machinery is less sensitive to acute changes in load, it is known that while soleus Ks is decreased 16-22% within 5 hours of non-weightbearing, medial gastrocnemius Ks is not initially (hours) affected by non-weightbearing (13), but is significantly affected within 5-7 days (7,15). In addition, data from past (12) and present investigations (Figure 2) indicate that the gastrocnemius protein synthesis is not affected by about of resistance exercise, but is affected hours to days later. Wong and Booth (12) reported that gastrocnemius mixed and myofibrillar Ks was increased 12-17 following a single bout of concentric exercise, and remained elevated up to 41 hours post-exercise. Previously we reported that gastrocnemius Ks was increased ~18 hours following the last bout of

daily exercise in hindlimb suspended rats (7). Thus, results from previous investigations indicate that the contractile protein synthetic machinery of the gastrocnemius is affected by resistance exercise, but this effect is apparently delayed more than 6 hours post-exercise.

The design of the present investigation did not allow for measurement of protein degradation. However, by choosing to suspend animals for 3 days we attempted to maximize the initial effect (hours) of unweighting on suppressing Ks, while avoiding the delayed increase (days) reported for protein degradation (13). As mentioned previously, when compared to the results of others who suspended female rats of a similar size for 7 days (15), the effect of hindlimb suspension on soleus and gastrocnemius Ks was similar to results of the present study following 3 days of hindlimb suspension, suggesting that an increase in the duration of hindlimb suspension would have had little or no additional effect on Ks, particularly in the soleus. The possibility that exercise with or without rhGH suppresses protein degradation and contributes to the sparing effect of exercise on loss of contractile protein in the non-weightbearing gastrocnemius cannot be excluded.

Our finding that soleus Ks was increased following an single bout of exercise was somewhat surprising. Previous results in our lab suggested that resistance exercise had no effect on loss of soleus mass in intact rats during 5 days of hindlimb suspension (7). The stimulatory effect of exercise on soleus Ks lasted less than 180'. Thus, it is possible that more frequent bouts of exercise would be necessary to maintain soleus Ks at or near that of Ambulatory animals, and thus, more effectively spare protein loss (7,8,11).

Interactive effect of resistance exercise and rhGH. We are aware of no evidence indicating that rhGH and exercise are capable of stimulating the *in vivo* rate of soleus Ks in intact (non-hypophysectomized) rats. Consistent with this later observation no significant difference existed between soleus Ks measured in saline- and rhGH injected rats, though soleus Ks appeared to be less variable in rhGH treated rats. Thus, results of present investigation (Figure 1) appear to confirm the contention that exogenous anabolic adjuvants have no additive effect to resistance exercise in stimulating soleus Ks, or attenuating atrophy of the non-weightbearing soleus in intact rats. Furthermore, other factors such as the cumulative effect of daily exercise and rhGH, or decreased protein degradation, likely account for the interactive effect of resistance exercise and rhGH to stimulate gastrocnemius Ks and spare loss of myofibrillar protein previously reported in the non-weightbearing gastrocnemius (7).

Summary. Results of the present investigation indicate that the soleus Ks is highly sensitive to changes in loading status, decreasing in response to 3 days of unweighting, and increasing within 60' in response to an single bout of resistance exercise.

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