

Human soleus muscle protein synthesis following resistance exercise

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Abstract

Aim: It is generally believed the calf muscles in humans are relatively unresponsive to resistance training when compared with other muscles of the body. The purpose of this investigation was to determine the muscle protein synthesis response of the soleus muscle following a standard high intensity bout of resistance exercise.

Methods: Eight recreationally active males (27 ± 4 years) completed three unilateral calf muscle exercises: standing calf press/heel raise, bent-knee calf press/heel raise, and seated calf press/heel raise. Each exercise consisted of four sets of 15 repetitions (~ 15 repetition maximum, RM, or $\sim 70\%$ 1RM). Fractional rate of muscle protein synthesis (FSR) was determined with a primed constant infusion of [$^2\text{H}_5$]phenylalanine coupled with muscle biopsies immediately and 3 h following the exercise in both the exercise and non-exercise (resting control) leg.

Results: FSR was elevated ($P < 0.05$) in the exercise (0.069 ± 0.010) vs. the control (0.051 ± 0.012) leg. Muscle glycogen concentration was lower ($P < 0.05$) in the exercise compared with the control leg (Decrease from control; immediate post-exercise: 54 ± 5 ; 3 h post-exercise: 36 ± 4 mmol kg^{-1} wet wt.). This relatively high amount of glycogen use is comparable with previous studies of resistance exercise of the thigh (i.e. vastus lateralis; ~ 41 – 49 mmol kg^{-1} wet wt.). However, the exercise-induced increase in FSR that has been consistently reported for the vastus lateralis (~ 0.045 – 0.060% h^{-1}) is on average $\sim 200\%$ higher than reported here for the soleus ($0.019 \pm 0.003\%$ h^{-1}).

Conclusions: These results suggest the relatively poor response of soleus muscle protein synthesis to an acute bout of resistance exercise may be the basis for the relative inability of the calf muscles to respond to resistance training programs.

Keywords amino acid metabolism, glycogen utilization, soleus muscle, stable isotopes, weight lifting.

Anecdotal evidence from weightlifting men and women suggests that the calf (i.e. triceps surae) muscles in humans are relatively difficult to hypertrophy. This suggestion is supported by the research studies that have examined muscle size or strength before and after resistance training of the triceps surae in young and old individuals (Young *et al.* 1985, Weiss *et al.* 1988, Alway

et al. 1989, Porter & Vandervoort 1997, Connelly & Vandervoort 2000, Krishnathasan & Vandervoort 2002, Ferri *et al.* 2003). That is, these studies have reported muscle strength and size responses that are much less than are typically seen following resistance training of the thigh or upper arm. In fact, some of these studies failed to show an increase in calf muscle size (Weiss *et al.* 1988) or

strength (Porter & Vandervoort 1997) following training. These observations are important since the calf muscles play a major role in walking, balance, and everyday ambulatory activities (Ericson *et al.* 1986, Winter & Yack 1987) in both young and old women and men.

A large component of the increase in muscle strength following chronic resistance exercise training is a result of the increase in muscle size (Moritani & de Vries 1979, Cooke 1997). In turn, the increase in muscle size is a result of a net accumulation of muscle protein throughout the resistance training period. Measurements of muscle protein synthesis have been used in the last 15 years to better understand the metabolic response of muscle to resistance training (Chesley *et al.* 1992, Yarasheski *et al.* 1993, Biolo *et al.* 1995b, Welle *et al.* 1995, Balagopal *et al.* 2001). Resistance training studies of the thigh and upper arm muscles show that muscle protein synthesis is significantly elevated within the first hours and remains elevated for up to 48 h following a single exercise session (Chesley *et al.* 1992, Biolo *et al.* 1995b, 1997, 1999, MacDougall *et al.* 1995, Phillips *et al.* 1997, 1999, Gibala *et al.* 2000, Trappe *et al.* 2002). However, only one study has examined muscle protein synthesis of any of the calf muscles (Fowles *et al.* 2000). These authors showed that passive stretch of the calf muscles for ~30 min was unable to increase soleus muscle protein synthesis, yet active contraction of the calf muscles at the same force level (~40% of maximal voluntary contraction) did result in an increase in soleus muscle protein synthesis. The absolute increase in muscle protein synthesis was only slightly above that induced by the passive stretch protocol and relatively small when compared with the post-exercise responses from studies of other muscles (Chesley *et al.* 1992, Biolo *et al.* 1995b, 1997, 1999, MacDougall *et al.* 1995, Phillips *et al.* 1997, 1999, Gibala *et al.* 2000, Trappe *et al.* 2002). The relatively small increase in soleus muscle protein synthesis might be expected given the relatively low forces generated during the exercise.

The purpose of the current study was to determine if the apparent reduced ability to adapt to strength training might be, at least partially, explained by the acute protein metabolic response of the calf muscles to resistance exercise. Specifically, we measured the rate of soleus muscle protein synthesis following a standard bout of resistance exercise. Muscle glycogen utilization and electromyographic activity were also monitored as indices of muscular activity.

Materials and methods

Subjects

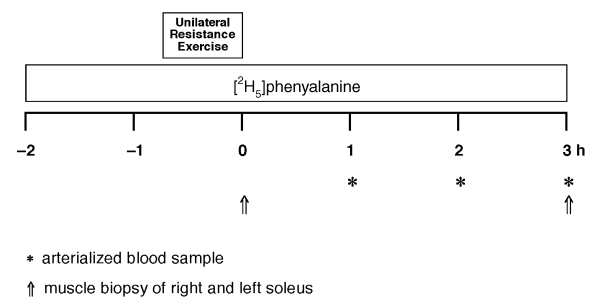
Eight male subjects were recruited from the greater Stockholm area (27 ± 4 years, 182 ± 8 cm, and

80.5 ± 8.4 kg; mean $\pm$ SD). All of the subjects were moderately active and none of them were involved in a regular aerobic or resistance training regimen. All procedures, risks and benefits associated with the experimental testing were explained to the subjects prior to providing consent according to the guidelines of the Institutional Review Board of the Karolinska Institute. The study was conducted in accordance with the Declaration of Helsinki.

Experimental protocol

Each subject was asked to refrain from any physical training and to maintain their normal dietary habits for 3 days prior to the experimental exercise bout and measurement of muscle protein synthesis (see below). On the third day of the self-monitored diet and activity control period, each subject reported to the laboratory to spend the night and to consume his evening meal. The meal was provided in liquid form and contained 40% of their estimated caloric requirement [estimated from Harris–Benedict (Harris & Benedict 1919) times an activity factor of 1.5] and 54% carbohydrate, 29% fat, and 17% protein (Ensure Plus). The meal was consumed at a time that would standardize the morning post-absorptive muscle protein synthesis measurements (~12 h fast).

Muscle protein synthesis protocol. The following morning each subject was awoken, allowed to void, and underwent the measurement of mixed muscle protein synthesis of the soleus following a bout of resistance exercise of one leg (Fig. 1) (Chesley *et al.* 1992, Phillips *et al.* 1999, Fowles *et al.* 2000, Trappe *et al.* 2002). The non-exercise leg served as a resting control. Each subject had an 18-gauge catheter placed in an antecubital vein for the infusion of a stable isotopically labelled amino acid ($[^2\text{H}_5]$ phenylalanine; Cambridge Isotope Laboratories, Andover, MA, USA). A 20-gauge catheter was placed in retrograde fashion in a dorsal vein of the hand, which was heated to provide an arterialized blood



* arterialized blood sample

↑ muscle biopsy of right and left soleus

Figure 1 Infusion and exercise schematic.

sample. [$^2\text{H}_5$]phenylalanine was dissolved in sterile 0.9% saline, filtered through a 0.2 μm filter before infusion, and infused with a calibrated infusion pump (Harvard Apparatus, Natick, MA, USA) at a rate of 0.05 $\mu\text{mol kg}^{-1} \text{min}^{-1}$ following a priming dose of 2.0 $\mu\text{mol kg}^{-1}$. This protocol has been shown to result in steady state [$^2\text{H}_5$]phenylalanine enrichments in the blood and muscle intracellular free amino acid pool within 2 h (Phillips *et al.* 1997, Trappe *et al.* 2002). Blood samples were drawn ($t = 1, 2$ and 3 h post-exercise) for the measurement of plasma enrichment of [$^2\text{H}_5$]phenylalanine. Muscle biopsies were taken from the soleus of both legs immediately after the exercise protocol ($t = 0$ h) and 3 h later ($t = 3$ h) for the measurement of muscle intracellular free amino acid and protein-bound enrichment of [$^2\text{H}_5$]phenylalanine, as well as muscle glycogen. Each muscle biopsy was taken through a new incision, proximal to the previous biopsy. Tissue was obtained following local anaesthetic (Carbocaine HCl 1%) using a 5 mm Bergstrom needle with suction (Bergstrom 1962). The muscle was cleansed of excess blood, connective tissue, and fat and immediately frozen in liquid nitrogen. The tissue was stored at -80°C or lower until analysis.

Exercise protocol. Each subject completed three separate unilateral calf exercises: (1) standing calf press/heel raise, (2) bent-knee calf press/heel raise, and (3) seated calf press/heel raise. Each exercise consisted of four sets of 15 repetitions with a load that resulted in volitional fatigue and loss of form within each set (i.e. ~ 15 repetition maximum, RM or $\sim 70\%$ 1RM). Two minutes of rest were given between sets and 5 min between exercises. The standing calf press/heel raise was completed with a hip and knee angle of $\sim 180^\circ$ on a platform that allowed for a full range of motion through plantar and dorsiflexion. The weight lifted was the subject's body weight and up to 10 additional kilograms. The additional load was adjusted between sets, if needed, to assure good form and a full range of motion. The bent-knee calf press/heel raise was performed with a hip angle of $\sim 90^\circ$ and a knee angle of $\sim 150^\circ$ and the seated calf press/heel raise was performed with a hip and knee angle of $\sim 90^\circ$, both completed in a similar fashion as the standing calf press/heel raise.

Electromyography studies. Separate electromyography (EMG) studies were completed on eight individuals (5M, 3F; 27 ± 4 years, 177 ± 9 cm, and 78 ± 8 kg; mean $\pm$ SD) to determine the relative activities of the soleus and gastrocnemius muscles during each of the three calf exercises. During the first visit to the laboratory, maximal surface EMG activity was assessed for the soleus muscle and the medial and lateral aspects of the gastrocnemius muscle in either

the standing or seated position. During the second visit, subjects performed the same protocol that was used for the muscle protein synthesis studies (i.e. four sets of 15 repetitions at 15RM for each exercise). The order of the three exercises was random and at least 60 min of recovery was allowed between exercises. EMG activity was recorded using disposable bi-polar Ag-Ag/Cl surface EMG electrodes (Multi Bio Sensors, El Paso, TX, USA) affixed over the soleus and the lateral and medial gastrocnemius muscles. A reference electrode was placed over the proximal tibial bone. Electrode position was retained throughout the series of experiments. The amplified raw EMG signal was filtered through a band-pass filter with low and high cut-off frequencies of 6 and 1500 Hz, respectively. The filtered signal was converted to a root mean square (RMS) signal using an AD536 circuit (Analog Devices, Norwood, MA, USA) with an averaging constant of 100 ms. The converted signal, RMS of the raw signal, was then sampled at 100 Hz using the MuscleLabTM system (Ergotest AS, Langesund, Norway) as described elsewhere (Bosco *et al.* 1999). Maximal EMG of any muscle was defined as the RMS of the EMG during a 1-s window that showed the highest activity during several maximal isometric actions performed at various joint angles. The EMG activity for each muscle was averaged across all repetitions and sets of each exercise and normalized to the maximal EMG activity of that muscle.

Laboratory analysis

Measurement of isotope enrichment. Blood samples were analysed for plasma [$^2\text{H}_5$]phenylalanine enrichment and muscle samples were analysed for free intracellular and protein-bound [$^2\text{H}_5$]phenylalanine enrichment by mass spectrometry as previously described (Biolo *et al.* 1995a, Phillips *et al.* 1997), using the tert-butyldimethylsilyl (t-BDMS) derivative of phenylalanine. Briefly, each blood sample was deproteinized with 15% sulfosalicylic acid. Each muscle sample (~ 30 mg) was weighed on a precision microbalance (Cahn 35; Orion Research, Beverly, MA, USA) at -45°C . Each sample was then homogenized with 500 μL of ice-cold 14% perchloric acid, centrifuged, and the supernatant was collected. This process was completed two more times and the supernatants for each sample were pooled for the muscle intracellular sample. The remaining pellet was washed in purified and deionized H_2O , washed three times in ethanol, and then dried overnight at 50°C . The dried pellet was placed in 6 N HCl and hydrolysed for 24 h at 120°C . The deproteinized blood samples, the muscle intracellular samples, and the protein hydrolysates (i.e. the protein-bound samples) were deionized using

an acid-washed cation exchange column (Dowex AG 50W-8X, 100–200 mesh, H⁺ form; Bio-Rad, Hercules, CA, USA). Amino acids were eluted from the column with 4 N NH₄OH, and the eluate was collected and dried under vacuum with a Speed-Vac rotary drying apparatus (Savant Instruments, Farmingdale, NY, USA). The samples were then processed to produce the t-BDMS derivative of phenylalanine. Derivatives were analysed by gas chromatography–mass spectrometry (GC–MS) (GC-6890 and MS-5973N; Agilent Technologies, Wilmington, DE, USA) using electron impact ionization and selected ion monitoring of *m/z* 336 (*m* + 0), 337 (*m* + 1), and 341 (*m* + 5) for the blood samples and 234 (*m* + 0), 235 (*m* + 1), 237 (*m* + 3), and 239 (*m* + 5) for the muscle intracellular and protein-bound samples, with *m* + 0 representing the lowest molecular weight of the ion. Plasma and muscle intracellular [²H₅]phenylalanine enrichments were measured from the *m* + 5 to *m* + 0 ratio. Enrichment of the protein-bound samples was determined using the *m* + 5 to *m* + 3 ratio and a linear standard curve from mixtures of known *m* + 5 to *m* + 0 ratios, as previously described (Calder *et al.* 1992, Patterson *et al.* 1997).

Measurement of muscle glycogen. Prior to determination of muscle glycogen concentrations, a piece of muscle (~10 mg) was weighed on a precision microbalance (Cahn 35; Orion Research) at –45 °C. Each muscle sample was freeze-dried for 72 h and re-weighed to determine the dry weight and water content of the muscle. Total muscle glycogen was determined in triplicate after hydrolysis of the muscle in hydrochloric acid (Passonneau & Lauderdale 1974). The resultant glucose residues from the hydrolysis were measured fluorometrically with the hexokinase-glucose-6-phosphate dehydrogenase reaction (Passonneau & Lowry 1993).

Calculations

Fractional rate of muscle protein synthesis (FSR) was calculated as the rate of [²H₅]phenylalanine tracer incorporation into muscle protein using the muscle intracellular free [²H₅]phenylalanine enrichment as the precursor and the following equation:

$$\text{FSR}(\% \text{ h}^{-1}) = \{(Et_3 - Et_0)/[E_p(t_3 - t_0)]\} \times 60 \times 100$$

where *Et*₀ and *Et*₃ are the protein-bound [²H₅]phenylalanine enrichments from the *t* = 0 h and *t* = 3 h biopsies, respectively, (*t*₃ – *t*₀) is the phenylalanine tracer incorporation time (in minutes), and *E*_p is the mean intracellular free [²H₅]phenylalanine enrichment from both biopsies (*t* = 0 and 3 h) (Phillips *et al.* 1997, Trappe *et al.* 2002).

Statistics

FSR between the exercise and control leg was compared with a paired *t*-test (*n* = 7). Muscle glycogen concentrations and muscle water content were compared with a two-way ANOVA with repeated measures (leg and time) (*n* = 8). Significance was accepted at a level of *P* < 0.05. All data are presented as mean ± SE, except where were noted.

Results

Plasma [²H₅]phenylalanine enrichment (expressed as the tracer to tracee ratio) was similar over the 3-h period used to calculate muscle protein synthesis (1 h: 0.078 ± 0.005, 2 h 0.079 ± 0.005, and 3 h: 0.075 ± 0.005). Muscle intracellular [²H₅]phenylalanine enrichment was also similar between the first and second biopsy in the control (0 h: 0.039 ± 0.003 and 3 h 0.043 ± 0.004) and the exercise (0 h: 0.047 ± 0.003 and 3 h 0.043 ± 0.003) leg. Soleus mixed muscle protein synthesis was elevated (~35%, *P* < 0.05) in the exercise (0.069 ± 0.010) compared with control (0.051 ± 0.012) leg (Fig. 2).

Muscle glycogen concentration was lower (*P* < 0.05) in the exercise compared with the control leg immediately after exercise (–54 ± 5 mmol kg^{–1} wet wt.) and three hours later (–36 ± 4 mmol kg^{–1} wet wt.) (Fig. 3). Water content of the muscle was elevated (*P* < 0.05, main effect for exercise vs. control leg) in the exercise leg (0 h: 79.4 ± 0.7 and 3 h 78.9 ± 0.6; mean: 79.2 ± 0.6%) compared with the control leg (0 h: 76.9 ± 0.7 and 3 h 78.0 ± 0.9; mean: 77.5 ± 0.6%). However, this did not influence the interpretation of the muscle glycogen results and for the purpose of comparing to previously published values, muscle glycogen concentrations are presented based on the wet weight of the muscle.

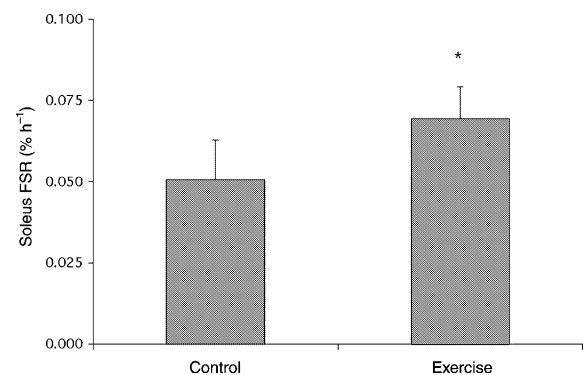


Figure 2 Soleus muscle protein synthesis in the control and exercise leg. **P* < 0.05 from the control leg.

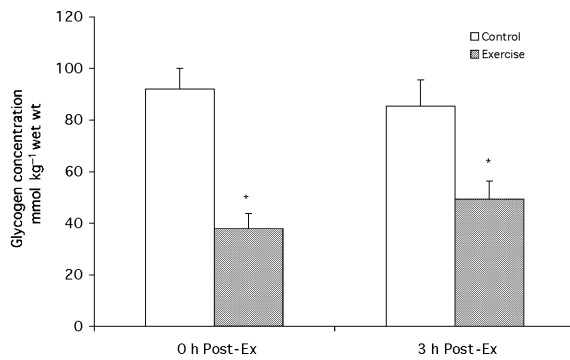


Figure 3 Soleus muscle glycogen concentration in the control and exercise leg. * $P < 0.05$ from the control leg.

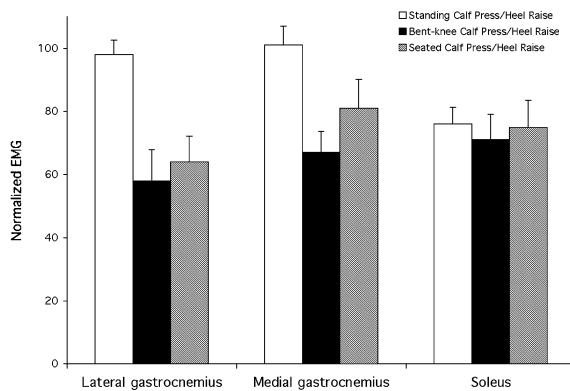


Figure 4 Normalized EMG activity of the lateral and medial gastrocnemius muscles and the soleus muscle for each of the three exercises used during the soleus muscle protein synthesis measurements.

The average normalized EMG activity varied across muscles and exercises (Fig. 4). The lateral and medial gastrocnemius muscles showed very similar responses to each of the three exercises (~60–100%) and the soleus muscle showed very similar magnitude of use across the three exercises (~70–80%).

Discussion

The practical and available scientific information suggests the calf muscles are relatively resistant to strength training adaptations when compared with other muscles. This information coincides with the findings from the present study of a relatively small increase in mixed muscle protein synthesis of the soleus ($0.019 \pm 0.003\% \text{ h}^{-1}$) following an acute bout of resistance exercise compared with consistently reported values for the vastus lateralis (~0.045–0.060% h^{-1}) (Fig. 5).

The resistance exercise regimen used in the current study was designed to provide a resistance exercise stimulus that when performed chronically would induce

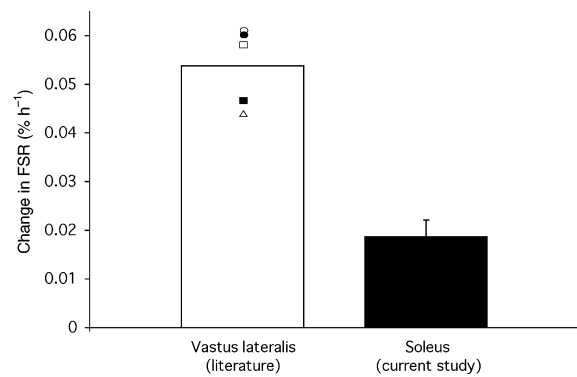


Figure 5 Resistance exercise-induced change in soleus (current study) and vastus lateralis [open circle (Biolo *et al.* 1995b), filled circle (Phillips *et al.* 1997), open square (Hastén *et al.* 2000), filled square (Biolo *et al.* 1999), open triangle (Phillips *et al.* 1999)] muscle protein synthesis.

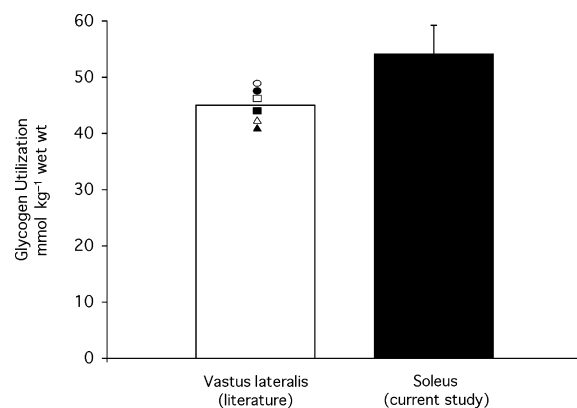


Figure 6 Resistance exercise-induced change in soleus (current study) and vastus lateralis [open circle (Essen-Gustavsson & Tesch 1990), filled circle (Robergs *et al.* 1991), open square (Robergs *et al.* 1991), filled square (Pascoe *et al.* 1993), open triangle (Tesch *et al.* 1986), filled triangle (Pascoe *et al.* 1993)] glycogen content.

muscle hypertrophy of the calf muscles. The repetitions performed for the calf muscle exercises for each set (15 reps) was slightly higher than is typically used (6–12 reps) for resistance training of other muscles. However, we based the design of the exercise regimen on the recommendation of weight training authorities for different levels of basic training and what is common practice among weightlifters. The large amount of glycogen use (Figs 3 and 6) and EMG activity (Fig. 4) of the soleus show this muscle was highly active during the exercise regimen. When compared with studies that have measured glycogen use during an acute bout of resistance exercise of the thigh (vastus lateralis), the amount of glycogen use in the current study was equal to or greater than has been reported (Fig. 6). Further, it

is well known that the resistance exercise paradigms used in those studies (Tesch *et al.* 1986, Essen-Gustavsson & Tesch 1990, Robergs *et al.* 1991, Pascoe *et al.* 1993), when performed chronically, will result in large increases in muscle mass and strength.

When discussing muscle-specific metabolic responses, the fibre type composition of the muscle must also be considered, as muscle fibres of different type (i.e. different myosin heavy chain profile) have different metabolic profiles (Saltin & Gollnick 1983). In humans the soleus is comprised primarily of slow (type I) muscle fibres (>80–90%), while the vastus lateralis is considered a 'mixed' muscle since it contains proportions of slow and fast (IIa and IIx) muscle fibres, and usually none of which alone make up more than 50% of the fibre population (Gollnick *et al.* 1974, Saltin & Gollnick 1983, Pette & Staron 2000). The calf or triceps surae, in addition to the soleus, also contains the medial and lateral gastrocnemius muscles, which are generally considered mixed muscles. Thus, the response of the gastrocnemius muscles may be unlike the soleus and more like the vastus lateralis. We specifically examined the soleus in the current study because it is a significant percentage of the triceps surae muscle mass and because it is highly involved in normal daily ambulatory activities. Studies of individuals that undergo chronic resistance training of the thigh show that within the vastus lateralis muscle, the type I fibres are less responsive (i.e. hypertrophy less) than the type II fibres (Tesch & Karlsson 1985, Hather *et al.* 1991). Thus, differences in fibre type between muscles may be an underlying basis for the muscle specific protein synthesis responses that are discussed here. However, factors that may influence or give rise to the fibre type of a muscle, such as the general daily activity pattern, may also influence the specific metabolic response of a muscle.

Data from studies that have examined muscle protein synthesis of the vastus lateralis (Phillips *et al.* 1999, 2002) and biceps brachii (Chesley *et al.* 1992, MacDougall *et al.* 1995) in chronically resistance trained individuals show that the muscle protein synthesis response to a single bout of resistance exercise is attenuated when compared with moderately trained or untrained individuals. These findings suggest that chronically trained muscle has adapted in some way that reduces the protein synthetic response to an acute bout of resistance exercise. Further, the results from these studies show an acute increase in muscle protein synthesis following resistance exercise ($0.02\text{--}0.03\% \text{ h}^{-1}$) that is closer to the response of the soleus in the current study. Given the relatively high level of soleus muscle activity during normal activities of daily living (Ericson *et al.* 1986, Winter & Yack 1987), this muscle could be considered to be chronically trained, even in individuals that do not regularly exercise their

calf muscles or possibly even in sedentary men and women.

The rate of resting soleus muscle protein synthesis in the current study ($0.051 \pm 0.012\% \text{ h}^{-1}$) is comparable with the only other study that has examined human soleus muscle protein synthesis (0.049 ± 0.018 and $0.067 \pm 0.025\% \text{ h}^{-1}$) (Fowles *et al.* 2000). These data are also comparable with rates of muscle protein synthesis that have been reported for the vastus lateralis (Yarasheski *et al.* 1993, Biolo *et al.* 1995a,b, Phillips *et al.* 1997, Balagopal *et al.* 2001, Trappe *et al.* 2002), biceps brachii (Chesley *et al.* 1992, MacDougall *et al.* 1995), and posterior deltoid (Tipton *et al.* 1996). Thus, it appears that resting muscle protein synthesis is not different in human muscles with different fibre type composition or general function. This is in contrast to the animal literature, which shows a much larger rate of muscle protein synthesis in muscles comprised primarily of the slow fibre type compared with muscles that are primarily of the fast fibre type (Fluckey *et al.* 1996, Hernandez *et al.* 2000).

The results from this study suggest that the relative inability of the soleus muscle to increase protein synthesis following an acute bout of resistance exercise may partially explain the general observation that the calf muscles are relatively difficult to hypertrophy with chronic resistance training. Further studies are needed to examine the time course of the increase in muscle protein synthesis following an acute bout of resistance exercise of the soleus and other calf muscles as compared with other muscles of the body. Studies are also needed to determine the underlying factors that regulate the muscle specific protein synthesis response following acute and chronic resistance exercise training.

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References

- Alway, S.E., MacDougall, J.D. & Sale, D.G. 1989. Contractile adaptations in the human triceps surae after isometric exercise. *J Appl Physiol* **66**, 2725–2732.
- Balogopal, P., Schimke, J.C., Ades, P., Adey, D. & Nair, K.S. 2001. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. *Am J Physiol Endocrinol Metab* **280**, E203–E208.
- Bergstrom, J. 1962. Muscle electrolytes in man. *Scand J Clin Lab Invest* **14**(Suppl. 68), 7–110.

- Biolo, G., Fleming, D., Maggi, S.P. & Wolfe, R.R. 1995a. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am J Physiol* 268, E75–E84.
- Biolo, G., Maggi, S.P., Williams, B.D., Tipton, K.D. & Wolfe, R.R. 1995b. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol* 268, E514–E520.
- Biolo, G., Tipton, K.D., Klein, S. & Wolfe, R.R. 1997. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 273(1 Pt 1), E122–E129.
- Biolo, G., Williams, B.D., Fleming, R.Y. & Wolfe, R.R. 1999. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes* 48, 949–957.
- Bosco, C., Cardinale, M. & Tsarpela, O. 1999. Influence of vibration on mechanical power and electromyogram activity in human arm flexor muscles. *Eur J Appl Physiol Occup Physiol* 79, 306–311.
- Calder, A.G., Anderson, S.E., Grant, I., McNurlan, M.A. & Garlick, P.J. 1992. The determination of low d5-phenylalanine enrichment (0.002–0.09 atom percent excess), after conversion to phenylethylamine, in relation to protein turnover studies by gas chromatography/electron ionization mass spectrometry. *Rapid Commun Mass Spectrom* 6, 421–424.
- Chesley, A., MacDougall, J.D., Tarnopolsky, M.A., Atkinson, S.A. & Smith, K. 1992. Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol* 73, 1383–1388.
- Connelly, D.M. & Vandervoort, A.A. 2000. Effects of isokinetic strength training on concentric and eccentric torque development in the ankle dorsiflexors of older adults. *J Gerontol A Biol Sci Med Sci* 55, B465–B472.
- Cooke, R. 1997. Actomyosin interaction in striated muscle. *Physiol Rev* 77, 671–697.
- Ericson, M.O., Nisell, R. & Ekholm, J. 1986. Quantified electromyography of lower-limb muscles during level walking. *Scand J Rehabil Med* 18, 159–163.
- Essen-Gustavsson, B. & Tesch, P.A. 1990. Glycogen and triglyceride utilization in relation to muscle metabolic characteristics in men performing heavy-resistance exercise. *Eur J Appl Physiol Occup Physiol* 61, 5–10.
- Ferri, A., Scaglioni, G., Pousson, M., Capodaglio, P., Van Hoecke, J. & Narici, M.V. 2003. Strength and power changes of the human plantar flexors and knee extensors in response to resistance training in old age. *Acta Physiol Scand* 177, 69–78.
- Fluckey, J.D., Vary, T.C., Jefferson, L.S., Evans, W.J. & Farrell, P.A. 1996. Insulin stimulation of protein synthesis in rat skeletal muscle following resistance exercise is maintained with advancing age. *J Gerontol: Biol Sci* 51A, B323–B330.
- Fowles, J.R., MacDougall, J.D., Tarnopolsky, M.A., Sale, D.G., Roy, B.D. & Yarasheski, K.E. 2000. The effects of acute passive stretch on muscle protein synthesis in humans. *Can J Appl Physiol* 25, 165–180.
- Gibala, M.J., Interisano, S.A., Tarnopolsky, M.A., Roy, B.D., MacDonald, J.R., Yarasheski, K.E. et al. 2000. Myofibrillar disruption following acute concentric and eccentric resistance exercise in strength-trained men. *Can J Physiol Pharmacol* 78, 656–661.
- Gollnick, P.D., Sjodin, B., Karlsson, J., Jansson, E. & Saltin, B. 1974. Human soleus muscle: a comparison of fiber composition and enzyme activities with other leg muscles. *Pflugers Arch* 348, 247–255.
- Harris, J.A. & Benedict, F.G. 1919. *A Biometric Study of Basal Metabolism in Man*. Carnegie Institution of Washington, Washington, DC.
- Hasten, D.L., Pak-Loduca, J., Obert, K.A. & Yarasheski, K.E. 2000. Resistance exercise acutely increases MHC and mixed muscle protein synthesis rates in 78–84 and 23–32 yr olds. *Am J Physiol* 278, E620–E626.
- Hather, B.M., Tesch, P.A., Buchanan, P. & Dudley, G.A. 1991. Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiol Scand* 143, 177–185.
- Hernandez, J.M., Fedele, M.J. & Farrell, P.A. 2000. Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats. *J Appl Physiol* 88, 1142–1149.
- Krishnathasan, D. & Vandervoort, A.A. 2002. Ankle plantar flexion strength in resistance and endurance trained middle-aged adults. *Can J Appl Physiol* 27, 479–490.
- MacDougall, J.D., Gibala, M.J., Tarnopolsky, M.A., MacDonald, J.R., Interisano, S.A. & Yarasheski, K.E. 1995. The time course for elevated muscle protein synthesis following heavy resistance exercise. *Can J Appl Physiol* 20, 480–486.
- Moritani, T. & de Vries, H.A. 1979. Neural factors versus hypertrophy in the time course of muscle strength gain. *Am J Phys Med* 58, 115–130.
- Pascoe, D.D., Costill, D.L., Fink, W.J., Robergs, R.A. & Zachwieja, J.J. 1993. Glycogen resynthesis in skeletal muscle following resistive exercise. *Med Sci Sports Exerc* 25, 349–354.
- Passonneau, J.V. & Lauderdale, V.R. 1974. A comparison of three methods of glycogen measurement in tissues. *Anal Biochem* 60, 405–412.
- Passonneau, J.V. & Lowry, O.H. 1993. A collection of metabolite assays. In: *Enzymatic Analysis. A Practical Guide*. pp. 111–128. Humana, Totowa, NJ.
- Patterson, B.W., Zhang, X.J., Chen, Y., Klein, S. & Wolfe, R.R. 1997. Measurement of very low stable isotope enrichments by gas chromatography/mass spectrometry: application to measurement of muscle protein synthesis. *Metabolism* 46, 943–948.
- Pette, D. & Staron, R.S. 2000. Myosin isoforms, muscle fiber types, and transitions. *Microsc Res Tech* 50, 500–509.
- Phillips, S.M., Tipton, K.D., Aarsland, A., Wolf, S.E. & Wolfe, R.R. 1997. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol* 273(1 Pt 1), E99–E107.
- Phillips, S.M., Tipton, K.D., Ferrando, A.A. & Wolfe, R.R. 1999. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *Am J Physiol* 276(1 Pt 1), E118–E124.
- Phillips, S.M., Parise, G., Roy, B.D., Tipton, K.D., Wolfe, R.R. & Tarnopolsky, M.A. 2002. Resistance-training-induced adaptations in skeletal muscle protein turnover in the fed state. *Can J Physiol Pharmacol* 80, 1045–1053.

- Porter, M.M. & Vandervoort, A.A. 1997. Standing strength training of the ankle plantar and dorsiflexors in older women, using concentric and eccentric contractions. *Eur J Appl Physiol Occup Physiol* 76, 62–68.
- Robergs, R.A., Pearson, D.R., Costill, D.L., Fink, W.J., Pascoe, D.D., Benedict, M.A. et al. 1991. Muscle glycogenolysis during differing intensities of weight-resistance exercise. *J Appl Physiol* 70, 1700–1706.
- Saltin, B. & Gollnick, P.D. 1983. Skeletal muscle adaptability: significance for metabolism and performance. In: L.D. Peachey, R.H. Adrian & S.R. Geiger (eds) *Handbook of Physiology – Skeletal Muscle*, pp. 555–631. American Physiological Society, Bethesda, MD.
- Tesch, P.A. & Karlsson, J. 1985. Muscle fiber types and size in trained and untrained muscles of elite athletes. *J Appl Physiol* 59, 1716–1720.
- Tesch, P.A., Colliander, E.B. & Kaiser, P. 1986. Muscle metabolism during intense, heavy-resistance exercise. *Eur J Appl Physiol Occup Physiol* 55, 362–366.
- Tipton, K.D., Ferrando, A.A., Williams, B.D. & Wolfe, R.R. 1996. Muscle protein metabolism in female swimmers after a combination of resistance and endurance exercise. *J Appl Physiol* 81, 2034–2038.
- Trappe, T.A., White, F., Lambert, C.P., Cesar, D., Hellerstein, M. & Evans, W.J. 2002. Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. *Am J Physiol Endocrinol Metab* 282, E551–E556.
- Weiss, L.W., Clark, F.C. & Howard, D.G. 1988. Effects of heavy-resistance triceps surae muscle training on strength and muscularity of men and women. *Phys Ther* 68, 208–213.
- Welle, S., Thornton, C. & Statt, M. 1995. Myofibrillar protein synthesis in young and old human subjects after three months of resistance training. *Am J Physiol* 268, E422–E427.
- Winter, D.A. & Yack, H.J. 1987. EMG profiles during normal human walking: stride-to-stride and inter-subject variability. *Electroencephalogr Clin Neurophysiol* 67, 402–411.
- Yarasheski, K.E., Zachwieja, J.J. & Bier, D.M. 1993. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am J Physiol* 265(2 Pt 1), E210–E214.
- Young, K., McDonagh, M.J. & Davies, C.T. 1985. The effects of two forms of isometric training on the mechanical properties of the triceps surae in man. *Pflugers Arch* 405, 384–388.