Research Paper

Short inter-set rest blunts resistance exercise-induced increases in myofibrillar protein synthesis and intracellular signalling in young males

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New Findings

- **What is the central question of this study?**
  Does shorter rest between sets of resistance exercise promote a superior circulating hormonal and acute muscle anabolic response compared with longer rest periods?
- **What is the main finding and its importance?**
  We demonstrate that short rest (1 min) between sets of moderate-intensity, high-volume resistance exercise blunts the acute muscle anabolic response compared with a longer rest period (5 min), despite a superior circulating hormonal milieu. These data have important implications for the development of training regimens to maximize muscle hypertrophy.

Manipulating the rest-recovery interval between sets of resistance exercise may influence training-induced muscle remodelling. The aim of this study was to determine the acute muscle anabolic response to resistance exercise performed with short or long inter-set rest intervals. In a study with a parallel-group design, 16 males completed four sets of bilateral leg-press and knee-extension exercise at 75% of one-repetition maximum to momentary muscular failure, followed by ingestion of 25 g of whey protein. Resistance exercise sets were interspersed by 1 min ($n = 8$) or 5 min of passive rest ($n = 8$). Muscle biopsies were obtained at rest, 0, 4, 24 and 28 h postexercise during a primed continuous infusion of L-[*ring*-$^{13}C_6$]phenylalanine to determine myofibrillar protein synthesis and intracellular signalling. We found that the rate of myofibrillar protein synthesis increased above resting values from 0 to 4 h postexercise with 1 (76%; $P = 0.047$) and 5 min inter-set rest (152%; $P < 0.001$) and was significantly greater in the 5 min inter-set rest group ($P = 0.001$). Myofibrillar protein synthesis rates at 24–28 h postexercise remained elevated above resting values ($P < 0.05$) and were indistinguishable between groups. Postexercise p70S6K^{Thr389} and rpS6$^{Ser240/244}$ phosphorylation were reduced with 1 compared with 5 min inter-set rest, whereas phosphorylation of eEF2^{Thr56}, TSC2^{Thr1462}, AMPK^{Thr172} and REDD1 protein were greater for 1 compared with 5 min inter-set rest. Serum testosterone was greater at 20–40 min postexercise and plasma lactate greater immediately postexercise for 1 *versus* 5 min inter-set rest. Resistance exercise with short (1 min) inter-set rest

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duration attenuated myofibrillar protein synthesis during the early postexercise recovery period compared with longer (5 min) rest duration, potentially through compromised activation of intracellular signalling.

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Introduction

Contractile loading is a potent stimulus for muscle hypertrophy. It is well established that resistance exercise stimulates myofibrillar protein synthesis (MPS) and that protein/amino acid provision potentiates this response (Moore *et al.* 2009). Thus, muscle hypertrophy occurs as a result of cumulative increases in MPS with each successive bout of resistance training (Phillips, 2000; Brook *et al.* 2015). Much attention has been given to understanding how resistance exercise parameters can be modified to augment the muscle anabolic response. Previously, it has been demonstrated that manipulation of resistance exercise load, intensity and work volume can profoundly influence MPS and hypertrophy (Burd *et al.* 2010*a*,*b*; Mitchell *et al.* 2012).

The mechanisms through which contractile stimuli induce muscle anabolism are complex and poorly defined. A pervasive view is that acute postexercise responses of testosterone (T) and growth hormone (GH) are critical for muscle anabolism (Kraemer & Ratamess, 2005). Indeed, resistance exercise protocols that are high in volume (the product of load x repetitions x sets) activate a large muscle mass and are of sufficient intensity to elicit a high degree of effort, transiently elevate circulating concentrations of T and GH (Goto *et al.* 2003; Smilios *et al.* 2003; McCaulley *et al.* 2009) which, it is assumed, may influence or predict resistance training-induced hypertrophy (Rønnestad *et al.* 2011). However, others have shown that transient increases in the hormonal milieu following resistance exercise are not related to MPS or muscle hypertrophy in healthy young individuals (Wilkinson *et al.* 2006; West *et al.* 2009, 2010*b*; West & Phillips, 2012; Mitchell *et al.* 2013). Alternatively, it is suggested that intracellular mechanisms, activated through a contractile stimulus of sufficient size to achieve maximal motor unit recruitment, may dictate the MPS response irrespective of circulating hormonal changes (West *et al.* 2010*a*; Burd *et al.* 2010*b*; Mitchell *et al.* 2012).

Manipulation of the rest interval between successive sets of resistance exercise is thought to be important for muscle hypertrophy (de Salles *et al.* 2009). This position has been established through studies demonstrating that resistance exercise performed with a short inter-set rest interval ($\leq$1 min) elicits a greater transient rise in circulating GH and T compared with long-duration inter-set rest ($\geq$3 min; Kraemer *et al.* 1990; Goto *et al.* 2004; Bottaro *et al.* 2009; Rahimi *et al.* 2010). However, given the equivocal nature of exercise-induced systemic hormonal alterations on muscle anabolism, the notion that a short inter-set rest duration provides a superior stimulus for muscle hypertrophy is questionable. Furthermore, insufficient rest between resistance exercise sets induces metabolic perturbations (lactate and H^+ accumulation and lowered pH) and facilitates the onset of neural fatigue, which may compromise the ability to sustain repeated high-force contraction and potentially impair muscle anabolic processes (Kleger *et al.* 2001; Willardson & Burkett, 2005). Uncertainty over the superiority of short *versus* long inter-set rest for muscle hypertrophy is reflected in the conflicting findings of chronic resistance training studies, with some demonstrating an advantage of short inter-set rest duration for muscle hypertrophy (Villanueva *et al.* 2015), whereas others report an advantage of long inter-set rest (Buresh *et al.* 2009; Schoenfeld *et al.* 2015) or no clear difference (Ahtiainen *et al.* 2005; de Souza *et al.* 2010).

To provide a clearer insight into the precise mechanisms through which rest-recovery may facilitate resistance exercise-induced muscle remodelling, we investigated the MPS and intracellular signalling response to moderate-intensity, high-volume resistance exercise performed with a short (1 min) or long (5 min) inter-set rest in healthy young males. We hypothesized that the MPS and intracellular signalling response to moderate-intensity high-volume resistance exercise performed to momentary muscular fatigue would be equivalent between short and long inter-set rest, irrespective of any potential difference in the circulating endocrine response between conditions. Given evidence that MPS over 0–6 h of recovery from resistance exercise is not correlated with the magnitude of muscle hypertrophy following chronic resistance training (Mitchell *et al.* 2014) and that MPS rates remain elevated above basal levels for 24–48 h postexercise (Phillips *et al.* 1997; Burd *et al.* 2011*a*), we chose to assess MPS and intracellular signalling during the 'early' (0–4 h) and 'late' (24–28 h) postexercise recovery phase to provide a closer indication of the capacity for muscle remodelling.

Table 1. Participant characteristics

Characteristic	1M	5M	*P* Value
Age (years)	24.5 ± 4.8 (18–34)	23.7 ± 5.4 (18–34)	0.76
Body mass (kg)	79.7 ± 15.2 (66.1–94.5)	82.7 ± 10.4 (66.4–98.6)	0.66
BMI (kg m^{-2})	24.3 ± 3.6 (20–27)	25.1 ± 2.6 (19.8–27.3)	0.72
Whole-body FFM (kg)	63.5 ± 11.2 (51.7–77.9)	64.9 ± 8.5 (51.2–80.1)	0.78
Leg FFM (kg)	20.5 ± 1.3 (17.3–27)	21.3 ± 1.2 (16.1–27.6)	0.64
Whole-body FM (kg)	11.8 ± 5.4 (6.6–22.7)	13.3 ± 4.7 (7.4–22.6)	0.55
Body fat (%)	14.4 ± 3.9 (10.2–22.6)	16.4 ± 4.8 (9.5–25.7)	0.39
Leg-press 1RM (kg)	249 ± 55 (183–359)	269 ± 87 (168–408)	0.45
Leg-press 1RM (kg leg FFM^{-1})	12.3 ± 1.5 (10.2–15.2)	12.4 ± 2.8 (9.8 – 18.5)	0.55
Knee-extension 1RM (kg)	157 ± 33 (122–226)	166 ± 38 (116–242)	0.63
Knee-extension 1RM (kg leg FFM^{-1})	7.7 ± 0.9 (6.6–9.0)	7.8 ± 1.5 (6.4–10.9)	0.84
Training experience (years)	5 ± 5 (1–18)	5 ± 4 (1–10)	0.72
Leg training (days $week^{-1}$)	2 ± 1 (1–3)	2 ± 1 (1–3)	0.94

Abbreviations: BMI, body mass index; FFM, fat-free mass; FM, fat mass; 1M, 1 min inter-set rest; 5M, 5 min inter-set rest; and 1RM: one-repetition maximum. Values are presented as means ± SD (range). $n = 16$ (1M, $n = 8$ and 5M, $n = 8$).

Methods

Participants

Sixteen young males were recruited through local advertisement. Participants habitually performed lower-limb resistance training at least once per week for ≥1 year prior to study enrolment and were deemed 'recreationally trained'. Participant anthropometric, strength and training characteristics are detailed in Table 1. Participants were informed of the purpose and methodology of the study, were deemed healthy by assessment of a general health questionnaire, and provided their written consent. Ethical approval was obtained through the National Health Service Black Country Research Ethics Committee (13/WM/0455). The study conformed to the standards set forward by the *Declaration of Helsinki* (seventh version).

Experimental design

Following initial eligibility screening, participants reported to the School of Sport, Exercise and Rehabilitation Sciences (SportExR) laboratory on three separate occasions. During the initial visit, participants underwent preliminary assessment of body composition and maximal leg strength. Approximately 1 week after preliminary assessments, participants completed two experimental trials on consecutive days (referred to hereafter as experimental trials 1 and 2). Experimental trials consisted of a stable isotope amino-acid infusion, combined with serial muscle biopsy sampling, to determine MPS over the 'early' (0–4 h in experimental trial 1) and 'late' (24–28 h in experimental trial 2) phase of recovery following a bout of bilateral leg resistance exercise. The study had a parallel design (because of the number of biopsies required), with participants matched in pairs based on anthropometric, strength and training characteristics before being randomized to either 1 min (1M; $n = 8$) or 5 min (5M; $n = 8$) rest-recovery between successive exercise sets. Participant matching was used to increase homogeneity between groups.

Preliminary assessments

Body mass and height. Body mass was determined by weighing each participant in loose clothing, without shoes, to the nearest 0.1 kg using a digital balance scale. Measurement of body mass was repeated upon arrival at each visit to ensure that participants maintained a constant weight throughout the duration of the study. Height measurements were made to the nearest 0.1 cm.

Body composition. Following assessment of body mass and height, participants underwent a Dual X-ray Absorptiometry (DXA) scan (Discovery DXA Systems, Hologic Inc., Bedford, MA, USA) to determine whole-body and regional fat and fat-free mass. The DXA scans were performed in a ∼10 h fasted state and lasted 7 min in total. Briefly, participants lay supine on the scanner in light clothing with their feet placed shoulder width apart and held in pace by tape to avoid discomfort. A trained operative analysed all images.

Leg strength. Maximal leg strength was obtained using a standardized, progressive one repetition maximum (1RM) protocol adapted from Mayhew *et al.* (1992) in order to determine the appropriate load of 75% 1RM for the acute exercise protocol in experimental trial 1. Leg 1RM strength was determined on leg-press and knee-extension exercise machines (Cybex VR-3, Medway, MA, USA). The 1RM protocol consisted of a self-selected warm-up, followed

by a 4–5RM set, a 3–4RM set, a 2–3RM set, and finally, a 1RM attempt. Warm-up and lead-in sets were separated by 3 min. Exercise intensity was monitored throughout using a modified Borg category-ratio scale (CR-10; Day *et al.* 2004; Buckley & Borg, 2011). The load for the warm-up and initial 4–5RM set was subjectively chosen based on previous training history. Participants were instructed to lift until momentary muscular failure on the final 1RM attempt only. In the event a 1RM attempt was failed, 5 min of passive rest was taken and the load was progressively lowered by 5 kg until 1RM was achieved. Exercise machine positions were recorded for use during experimental trials 1 and 2.

Dietary control. On the day prior to experimental trial 1, participants consumed a standardized evening meal (917 kcal) composed of ∼18% protein (∼32 g), ∼60% carbohydrate (∼103 g) and ∼22% fat (∼39 g). Following experimental trial 1, prior to reporting to the laboratory the following morning for experimental trial 2, participants consumed a standardized lunch (778 kcal) composed of ∼22% protein (∼34 g), ∼58% carbohydrate (∼88 g) and ∼20% fat (∼32 g), in addition to an evening meal of the same composition consumed on the evening of experimental trial 1 (described above). Participants were asked to consume their evening meal between 19.00 and 21.00 h to ensure a 10–12 h fast prior to each experimental trial. All standardized meals were provided to participants by the research team. Participants were asked to refrain from alcohol and caffeine for 24 h prior to experimental trial 1 until completion of experimental trial 2.

Experimental trials 1 and 2

Participants reported to the SportExR laboratory at 07.00 h having fasted for ∼10–12 h. Upon arrival, body mass was recorded (described above) and a 21-gauge cannula was inserted into a forearm vein of each arm. One cannula was used for frequent arterialized blood sampling, whereas the other was used to infuse the stable isotope tracer. After obtaining a rested-fasted blood sample, participants received a primed continuous infusion of L-[*ring*-$^{13}C_6$]phenylalanine (prime, 2 μmol kg^{-1}; infusion, 0.05 μmol kg^{-1} min^{-1}; Cambridge Isotope Laboratories, Andover, MA, USA) to determine MPS rates. The contralateral arm was warmed in a heated blanket for frequent arterialized blood sampling (Abumarad *et al.* 1981) at −210, −180, −120 and −60 min prior to exercise and 0, 20, 40, 60, 90, 120, 180 and 240 min postexercise. A total of 10 ml of arterialized blood was collected at each sampling time point in EDTA and serum-separator vacutainers (BD, Oxford, UK). Blood samples were centrifuged at 3000*g* for 10 min at 4°C, and serum and plasma aliquots were frozen at −80°C for later analysis. After ∼150 min of stable isotope infusion, a muscle biopsy sample was obtained from the quadriceps vastus lateralis under local anaesthesia (1% lidocaine) using the Bergström needle technique (Bergstrom, 1975). Muscle biopsy tissue was quickly rinsed in ice-cold saline and blotted to remove any visible fat and connective tissue before being frozen in liquid nitrogen at stored at −80°C for later analysis. Immediately after the first biopsy, participants completed a bout of bilateral leg resistance exercise, comprising four sets of leg-press and four sets of knee-extension exercise at 75% of 1RM. Participants performed exercise with a lifting–lowering cadence of ∼1 s in both concentric and eccentric phases, without pause, until momentary muscular failure (i.e. fatigue or level 9–10 on the Borg CR-10 scale). Attempts were made to clamp the exercise load at 75% of 1RM across all exercise sets, with the expectation that the number of repetitions achieved at the point of fatigue would decrease across sets. In exceptional circumstances when participants completed more or fewer than 8–14 repetitions per set, exercise load was adjusted by ±5 kg. Depending on which condition participants were allocated, resistance exercise sets were interspersed with 1 or 5 min of passive rest-recovery, during which participants remained seated on the machine. Following completion of each exercise set, time under tension and Borg CR-10 level were recorded. Immediately after completion of exercise, a second muscle biopsy was obtained ∼3 cm proximal from the first biopsy, followed by the consumption of 25 g of whey protein isolate (MyProtein, Northwich, UK) dissolved in 400 ml of water. This amount of protein has been established to stimulate rates of MPS maximally after resistance exercise in young men (Witard *et al.* 2014). The amino acid content of the whey protein isolate was (as percentage content): Ala, 4.6; Arg, 2.1; Asp, 9.7; Cys, 2.6; Gln, 17.1; Gly, 1.6; His, 1.5; Ile, 5.6; Leu, 9.7; Lys, 9.7; Met, 2.0; Phe, 2.9; Pro, 5.5; Ser, 3.7; Thr, 6.1; Trp, 1.6; Tyr, 2.7; and Val, 4.6. A small amount of L-[*ring*-$^{13}C_6$]phenylalanine tracer (30 mg) was added to the drink (enriched to 4%) to minimize changes in blood phenylalanine enrichment after drink ingestion. Following whey protein ingestion, participants rested supine for 240 min, after which a third muscle biopsy was obtained ∼3 cm proximal to the second biopsy to determine MPS rates over the 'early' phase (0–4 h) of postexercise recovery. Before leaving the laboratory, participants were provided with a standardized lunch and evening meal (described above) and instructed to consume only the food provided throughout the remainder of the day.

The following morning, participants reported to the SportExR laboratory at 07.00 h for experimental trial 2, having fasted for ∼10 h. Cannulas were inserted into both forearm veins for frequent arterialized blood sampling and an identical stable isotope infusion to that described in experimental trial 1. After ∼90 min of stable

isotope infusion, a muscle biopsy was obtained from the quadriceps vastus lateralis of the contralateral leg to that sampled during experimental trial 1. Immediately after the biopsy was obtained, participants ingested an identical 25 g whey protein isolate drink to that consumed in experimental trial 1. After whey protein ingestion, participants rested supine for 240 min before a final muscle biopsy was obtained ∼3 cm proximal to the previous biopsy to determine MPS rates over the 'late' phase (24–28 h) of postexercise recovery. Blood samples were obtained at 0, 20, 40, 60, 90, 120, 180 and 240 min following protein ingestion. A schematic overview of experimental trials 1 and 2 is provided in Fig. 1.

Blood analyses

Plasma amino acid enrichment and concentrations. Plasma [$^{13}C_6$]phenylalanine enrichment was determined by gas chromatography–mass spectrometry (GCMS; model 5973; Hewlett Packard, Palo Alto, CA, USA) by monitoring ions 234/240. Upon thawing, plasma samples were combined with diluted acetic acid and purified on cation-exchange columns (Dowex 50W-X8-200; Sigma-Aldrich, Poole, UK). The amino acids were then converted to their *N-tert*-butyldimethylsilyl-*N*-methyltrifluoracetamide (MTBSTFA) derivative. Simultaneously, concentrations of leucine were determined by GCMS using an internal standard method (Tipton *et al.* 1999; Breen *et al.* 2011), based on the known volume of plasma and internal standard added and the known amino acid concentration of the internal standard. The internal standard was U-[$^{13}C_6$]leucine (ions 302/308) added in a ratio of 100 μl ml^{-1} of blood.

Plasma lactate. Plasma lactate concentrations were determined using BIOSEN C_line Clinic/GP+ Glucose/Lactate Measuring System (EKF Diagnostics, Cardiff, UK). Briefly, 20 μl of plasma was added to a micro test tube provided with the analytical kit and prefilled with a haemolysing solution to dilute the sample to 1:51.

Hormone concentrations. Serum GH, insulin and sex hormone-binding globulin (SHBG) concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (GH and insulin: IBL International, Hamburg, Germany; SHBG: BioVendor, Karasek, Czech Republic) following the manufacturers' instructions. Serum testosterone was measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) using a Waters Xevo mass spectrometer with Acquity uPLC as described previously (Hassan-Smith *et al.* 2015; Oostdijk *et al.* 2015). An electrospray ionization source was used in positive ionization mode. Steroids were extracted from 400 μl of serum via liquid–liquid extraction using 2 ml of *tert*-butyl methyl ether (MTBE).

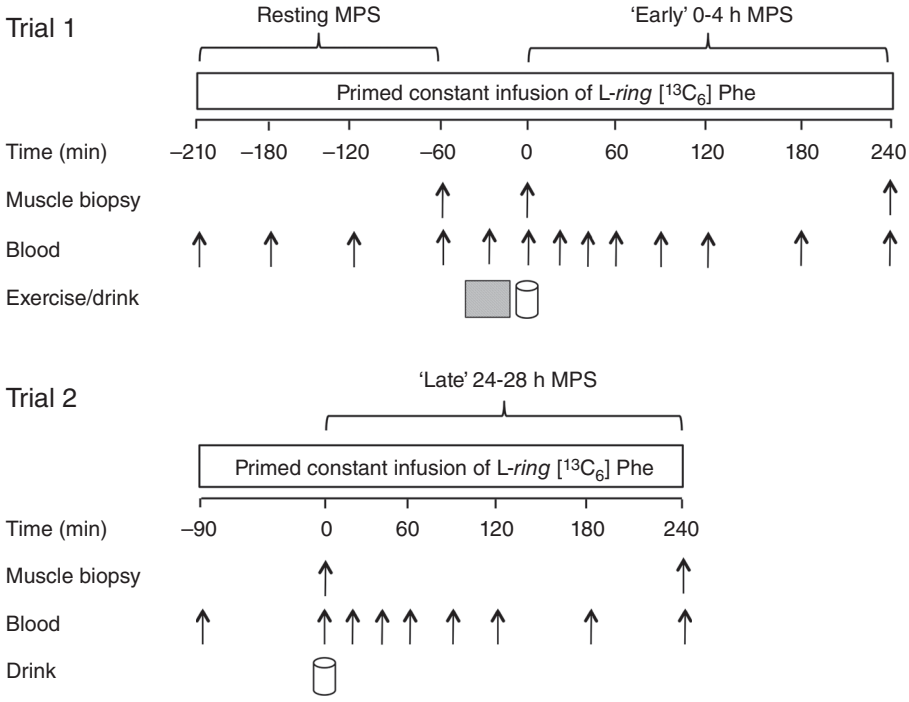

Figure 1. Schematic protocol time line of experimental trials 1 and 2
Abbreviation: MPS, myofibrillar protein synthesis.

The MTBE was subsequently evaporated to dryness, and samples were reconstituted in 125 μl of methanol/water (50/50) prior to LC-MS/MS analysis. An optimized LC method using methanol and water both with 0.1% formic acid was used to separate steroids on a T3, 1.8 μm, 1.2 mm × 50 mm column. Steroids were identified and quantified based on matching retention times and two mass transitions when compared to authentic reference standards (testosterone 289 > 97 and 289 > 109). Steroids were quantified, after addition of appropriate internal standards, relative to a calibration series ranging from 0.25 to 50 ng ml^{-1}. Free testosterone was calculated from total T and SHBG concentrations as described previously (Ly & Handelsman, 2005).

Muscle tissue analyses

Myofibrillar and muscle-free amino acid extraction. From ∼40–50 mg of muscle tissue, the myofibrillar protein fraction and muscle free pool were extracted for analysis of [$^{13}C_6$]phenylalanine enrichment. Muscle tissue was powdered and transferred to an eppendorf tube containing 500 μl of 0.6 M percholoric acid. Muscle tissue was homogenized under liquid nitrogen using small scissors and a Teflon pestle, and centrifuged at 2000 g at 4°C for 5 min. The supernatant containing the intracellular free pool of amino acids was transferred to a 2 ml eppendorf container. A 500 μl volume of doubly distilled water was added to the pellet, which was vortexed and spun at 2000 g at 4°C for 5 min. The sample was then further homogenized with small scissors and a Teflon pestle. A 7.5 μl mg^{-1} volume of homogenization buffer (50 mM Tris–HCl, 1 mM EDTA, 1 M M EGTA, 10 mM β-glycerophosphate and 50 mM sodium fluoride, pH 7.5) was added to the sample and centrifuged at 2000 g at 4°C for 10 min, before the supernatant was discarded. The pellet was washed with 500 μl of doubly distilled water, and the myofibrillar fraction separated from any collagen by adding 1 ml of 0.3 M NaOH and heating for 30 min at 50°C (vortexing every 10 min). The sample was then centrifuged at 9500 g at 4°C for 5 min, and the supernatant was transferred to a 4 ml glass collection tube. Myofibrillar proteins were precipitated by adding 1 ml of 1 M percholoric acid to the supernatant, which was centrifuged at 1500 g at 4°C for 10 min. The pellet was then washed with 1 ml of 70% EtOH. The remaining myofibrillar protein pellet was hydrolysed overnight in 2 ml of 1 M HCl and 1 ml of activated Dowex 50W-X8 100–200 resin (Bio-Rad laboratories Inc., Hercules, California, USA). Constituent amino acids in the myofibrillar fraction and intracellular free pool were purified on cation-exchange columns (Bio-Rad laboratories Inc., USA). Amino acids in the myofibrillar fraction were then converted to their *N*-acetyl-*n*-propyl ester derivative, and phenylalanine labelling was determined by gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS; Delta-plus XP; Thermofinnigan, Hemel Hempstead, UK). Ions 44/45 were monitored for unlabelled and labelled CO_2, respectively. Amino acids in the intracellular free pool were converted to their MTBSTFA derivative, and [$^{13}C_6$]phenylalanine enrichment was determined by monitoring at ions 234/240 using GCMS (as described in '*Blood analyses*').

Western blot. Approximately 25–30 mg of muscle tissue was powdered on dry ice using a Cellcrusher^{TM} tissue pulverizer (Cellcrusher Ltd, Cork, Ireland) and prepared for Western blot analysis as described previously (Philp *et al.* 2015). Equal amounts of protein (30 μg) were boiled for 5 min in 1× Laemmli sample buffer and separated on 7.5–10% gels by SDS-PAGE for 1 h. Following electrophoresis, proteins were transferred to a Protran nitrocellulose membrane (Whatman, Dassel, Germany) at 100 V for 1 h. Membranes were incubated overnight with the following primary antibodies: phospho-S6K1 Thr389 (#9205), total 70 kDa S6 protein kinase (p70S6K1; #9202), phospho-S6 Ser240/244 (#5364), total S6 (#2217), phospho-eukaryotic initiation factor 4E binding protein (14E-BP1) Thr37/46 (#9459), total 4E-BP1 (#9452), phospho-eukaryotic elongation factor 2 (eEF2) Thr56 (#2331), total eEF2 (#2332), phospho-protein kinase B (Akt) Ser473 (#4060), phospho-Akt Thr308 (#4056), total Akt (#9272), phospho-proline-rich Akt substrate 40 kDa (PRAS40) Ser246 (#2640), total PRAS40 (#2610), phospho-tuberous sclerosis 2 (TSC2) Thr1462 (#3611), total TSC2 (#4308), total AMP-activated protein kinase (AMPKα; #2757), phospho-AMPKα Thr172 (#2531) and GAPDH (#5174); each purchased from Cell Signaling Technology [New England Biolabs (UK) Ltd, Hitchin, UK]. In addition, membranes were incubated with regulated in development and DNA damage responses 1 (REDD1) rabbit polyclonal (#10638-1-AP) antibody from Proteintech Ltd (Manchester, UK). Immobilon Western chemiluminescent HRP substrate (Merck Millipore, Watford, UK) was used to quantify protein content following IgG binding, visualized on a G:BOX Chemi XT4 imager using GeneSys capture software (Syngene UK, Cambridge, UK). Imaging and band quantification were carried out using a Chemi Genius Bioimaging Gel Doc System (Syngene).

Calculations

The area under the curve (AUC) was calculated using the trapezium method. Serum hormone concentrations at 0, 20, 40, 60 and 90 min postexercise were used to determine the AUC.

The fractional synthesis rate (FSR) of the myofibrillar protein fraction was calculated from the incorporation

of [$^{13}C_6$]phenylalanine into protein using the standard precursor–product model as follows:

$$\text{FSR}(\%\text{h}^{-1}) = \Delta E_b / E_p \times 1/t \times 100 \quad (1)$$

Where ΔE_b is the change in bound [$^{13}C_6$]phenylalanine enrichment between two biopsy samples, E_p is the precursor enrichment and t is the time between muscle biopsies. We used the mean plasma [$^{13}C_6$]phenylalanine enrichment from arterialized blood to determine an 'estimated' intracellular precursor enrichment as described previously (Breen *et al.* 2011). For the calculation of resting-fasted MPS rates, the recruitment of tracer-'naive' participants allowed for the use of pre-infusion plasma [$^{13}C_6$]phenylalanine enrichment as a proxy for basal muscle protein enrichment, an approach that has been validated previously (Burd *et al.* 2011*b*).

Statistics

Data analysis was performed using SPSS version 22 for Windows (SPSS Inc., Chicago, IL, USA). Anthropometric and training characteristics were analysed using Student's unpaired t test. Myofibrillar protein synthesis rates and Western blot data were analysed using a two-way, mixed-model ANOVA with one within (two levels; 'early' and 'late' recovery) and one between factor (two levels; condition). Exercise and blood parameters were analysed using a two-way, repeated-measures ANOVA (time × condition). Additionally, plasma enrichments were analysed using linear regression. Tukey's HSD *post hoc* analysis was performed whenever a significant F ratio was found to isolate specific differences. Significance for all analyses was set at $P < 0.05$. All values are presented as means ± SEM.

Results

Exercise variables

Anthropometric, strength and training characteristics were similar between 1M and 5M (Table 1). Load-volume (load x repetitions) during leg press exercise decreased significantly during sets 3 and 4 compared with set 1 in 1M (−28 and −33%, respectively; $P < 0.05$), but did not decrease across sets in 5M (Table 2). Load-volume during sets 3 and 4 of leg press exercise were significantly lower in 1M compared with 5M ($P = 0.001$). Total volume for leg press exercise was ∼13% lower in 1M compared with 5M ($P = 0.03$; Table 2). Knee-extension load-volume was not significantly different between groups in each set and did not significantly diminish as sets progressed. However, when taken together the total volume achieved over all four sets of knee-extension exercise was ∼17% lower in 1M compared with 5M ($P = 0.03$; Table 2). The rating

Table 2. Resistance training variables

	1M ($n = 8$)					5M ($n = 8$)				
Variable	Set 1	Set 2	Set 3	Set 4	Total	Set 1	Set 2	Set 3	Set 4	Total
Leg press										
Load (kg)	194 ± 4	196 ± 4	197 ± 4	198 ± 4	785 ± 2	193 ± 4	195 ± 3	192 ± 4	193 ± 3	773 ± 1
Repetitions	14 ± 3	11 ± 2	10 ± 2	9 ± 1	44 ± 2	14 ± 6	12 ± 2	13 ± 2	12 ± 2	51 ± 1
Volume (kg)	2602 ± 550	2177 ± 302	1873 ± 365*	1726 ± 391*	8338 ± 387†	2589 ± 497	2236 ± 282	2467 ± 392	2290 ± 442	9582 ± 163
Time under tension (s)	35.8 ± 10.4	30.8 ± 6.7	28.1 ± 8.3	27.8 ± 6.9	122.5 ± 3.7	40.8 ± 13.8	37.1 ± 8.4	35.4 ± 5.1	35.1 ± 6.3	148 ± 3
Borg CR-10	8 ± 1	9 ± 1	10 ± 1	10 ± 0	10 ± 0	9 ± 1	9 ± 1	10 ± 0	10 ± 1	10 ± 1
Knee extension										
Load (kg)	120 ± 24	118 ± 25	113 ± 21	109 ± 19	460 ± 5	122 ± 17	116 ± 19	116 ± 19	120 ± 17	474 ± 3
Repetitions	9 ± 2	9 ± 1	8 ± 1	8 ± 2	34 ± 2	10 ± 2	10 ± 1	10 ± 1	10 ± 1	40 ± 2
Volume (kg)	1100 ± 261	951 ± 174	982 ± 245	869 ± 158	3902 ± 260†	1052 ± 309	1248 ± 313	1251 ± 349	1166 ± 556	4717 ± 495
Time under tension (s)	19.8 ± 5.7	16.0 ± 3.1	17.1 ± 5.7	16.8 ± 3.9	69.7 ± 1.7	24.1 ± 11.4	19.8 ± 3.4	19.6 ± 3.3	19.9 ± 2.0	83.4 ± 2.2
Borg CR-10	10 ± 1	10.0 ± 0	10.0 ± 0	10.0 ± 0	10 ± 0	10 ± 1	10 ± 1	10 ± 0	10 ± 0	10 ± 0

Leg-press and knee-extension resistance exercise variables for 1M and 5M groups. Abbreviations: Borg CR-10, Borg category-ratio scale; 1M, 1 min inter-set rest; and 5M, 5 min inter-set rest. Data are presented as means ± SD. Significance was set at $P < 0.05$. *Significantly lower than set 1; †significantly lower total volume than 5M.

of perceived exertion and time under tension were not different across exercise sets within or between groups.

Plasma leucine and serum insulin

In experimental trial 1, plasma leucine concentration increased above fasted values 40 min after ingestion of 25 g of whey protein in 1M and 5M ($P < 0.001$ for both; Fig. 2*A*). Plasma leucine concentration peaked 60 min after protein ingestion (~2.2- and 2-fold above fasted values for 1M and 5M, respectively) and returned to fasted values 120 min after protein ingestion for 1M and 5M. In experimental trial 2, plasma leucine concentration displayed a similar temporal response to that in experimental trial 1, increasing above fasted values at 40 min after drink ingestion for 1M and 5M ($P < 0.001$ for both; Fig. 2*B*) and returning to fasted values at 120 min after drink ingestion. Plasma leucine concentration did not differ between 1M and 5M at any time point during experimental trial 1 or 2.

In experimental trial 1, serum insulin concentration increased above fasting values, peaking 40 min after ingestion of 25 g of whey protein in 1M and 5M (~2.8- and 3.1-fold, respectively, $P < 0.001$ for both; Fig. 2*C*) and returning to fasted values thereafter. In experimental trial 2, serum insulin concentration demonstrated the same temporal pattern of response as in experimental trial 1, increasing above fasted values at 40 min after protein ingestion for 1M and 5M (~3.6- and 2.6-fold, respectively, $P < 0.001$ for both; Fig. 2*D*) and returning to fasted values thereafter. Plasma insulin concentration did not differ between 1M and 5M at any time point during experimental trial 1 or 2.

Plasma [$^{13}C_6$]phenylalanine enrichment

In experimental trial 1, plasma [$^{13}C_6$]phenylalanine enrichment increased above basal (pre-infusion) values 60 min after the initiation of the stable isotope tracer infusion ($P < 0.001$) and remained stable for the entire

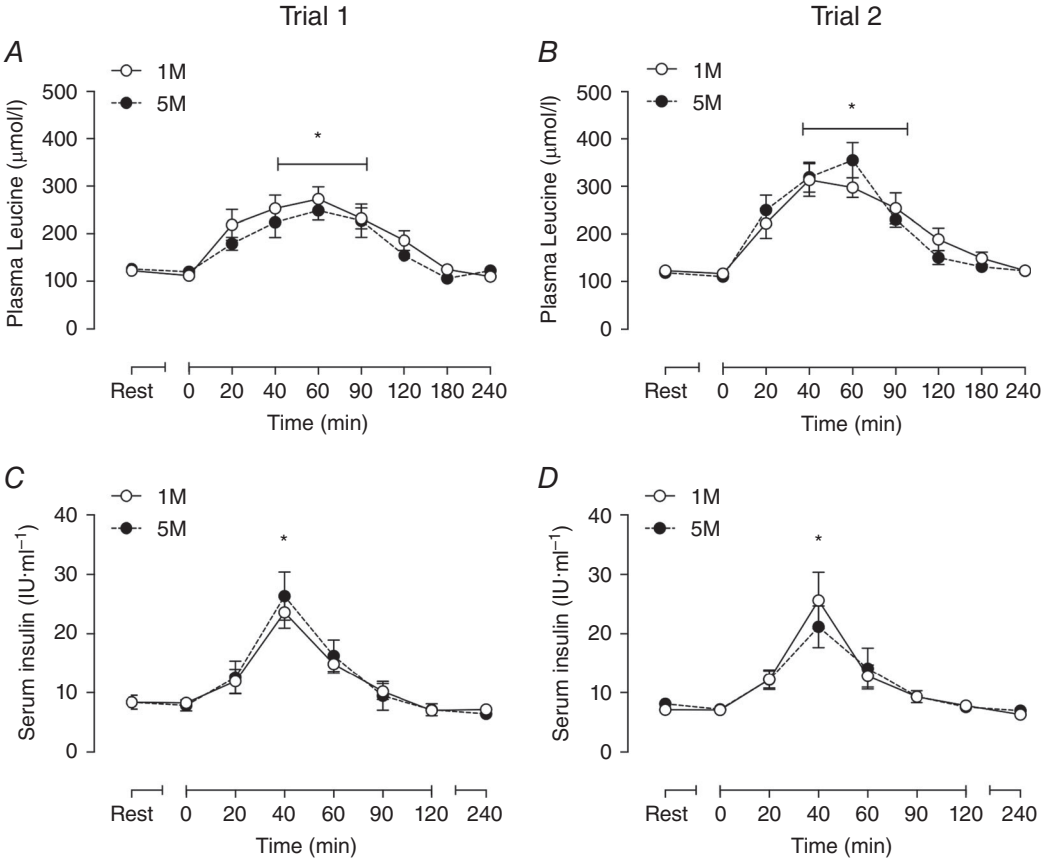

Figure 2. Plasma leucine concentrations in experimental trials 1 (*A*) and 2 (*B*) and serum insulin concentrations in experimental trials 1 (*C*) and 2 (*D*)
At $t = 0$, a 25 g bolus of whey protein isolate was consumed. Values are means ± SEM for 1 min inter-set rest duration (1M; $n = 8$) and 5 min inter-set rest duration (5M; $n = 8$). Significance was set at $P < 0.05$. *Significantly greater than rested fasting values.

trial duration, averaging 6.58 ± 0.96 and 6.32 ± 1.19% for 1M and 5M, respectively (Fig. 3*A*). In experimental trial 2, plasma [$^{13}C_6$]phenylalanine enrichment increased above basal values 120 min after the initiation of stable isotope tracer infusion ($P < 0.001$) and remained stable for the entire trial duration, averaging 6.16 ± 2.56 and 6.04 ± 2.61% for 1M and 5M, respectively (Fig. 3*B*). Linear regression analysis identified that the slopes of the plasma [$^{13}C_6$]phenylalanine enrichments on experimental trials 1 and 2 were not significantly different from zero, indicating the presence of an isotopic steady state ($P = 0.2$).

Myofibrillar protein synthesis

Resting rates of MPS were similar in 1M and 5M. Myofibrillar protein synthesis rates increased significantly above resting values from 0 to 4 h postexercise in 1M (from 0.025 ± 0.002 to 0.042 ± 0.004% h^{-1}; $P = 0.047$) and 5M (from 0.028 ± 0.003 to 0.067 ± 0.003% h^{-1}; $P < 0.001$) and were significantly greater in 5M compared with 1M ($P = 0.001$; Fig. 4; 95% confidence interval 0.034–0.049% h^{-1} for 1M and 0.06–0.074% h^{-1} for 5M). Myofibrillar protein synthesis rates remained elevated above resting values at 24–28 h postexercise in 1M and 5M (0.050 ± 0.007% h^{-1} for both, $P = 0.044 \pm 0.001$, respectively), with no difference between groups (95% confidence interval 0.036–0.065% h^{-1} for 1M and 0.037–0.063% h^{-1} for 5M).

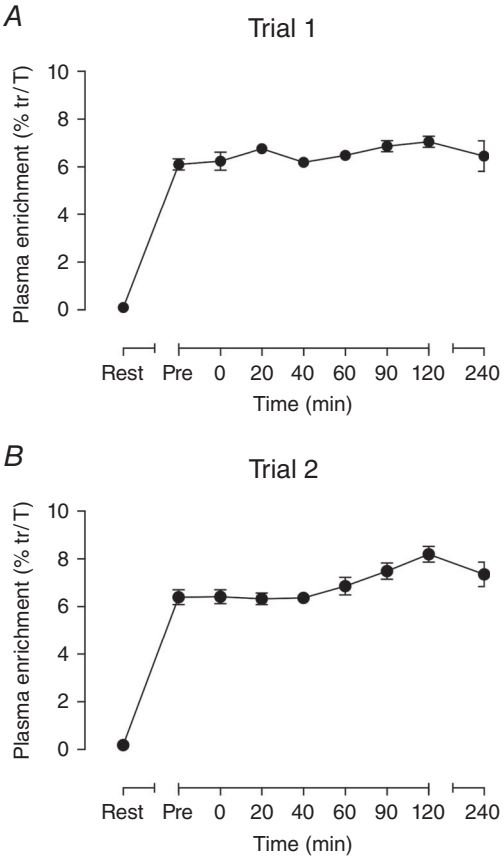

Figure 3. Plasma enrichment of [$^{13}C_6$]phenylalanine in experimental trial 1 (*A*) and 2 (*B*)
Abbreviation: % tr/T, percentage tracer-to-tracee ratio. Values are means ± SEM combined for 1M and 5M ($n = 16$).

Serum hormones and plasma lactate

Serum growth hormone concentration immediately postexercise increased by ∼4.7- and ∼5.3-fold above resting values for 1M ($P = 0.009$) and 5M ($P = 0.001$), respectively, with no difference between groups (Fig. 5*A*). Serum GH AUC over 0–90 min postexercise was not different between 1M and 5M (Fig. 5*B*).

Serum testosterone concentration increased by ∼1.6-fold above resting values at 20 min postexercise for 1M ($P = 0.008$; Fig. 5*C*), returning to resting values by 60 min postexercise. Serum T concentration did not increase above resting values in 5M. Serum T concentration at 20 and 40 min postexercise and the AUC over 0–90 min postexercise were significantly greater in 1M compared with 5M ($P < 0.05$; Fig. 5*D*).

Free testosterone concentrations increased above resting values in 1M at 20 and 40 min postexercise ($P < 0.05$; Fig. 5*E*), returning to resting values at 60 min postexercise. Free T concentration did not increase above resting values in 5M. Free T AUC over 0–90 min postexercise was significantly greater in 1M compared with 5M ($P < 0.05$; Fig. 5*F*).

Plasma lactate concentration was significantly elevated above resting values mid-exercise and immediately

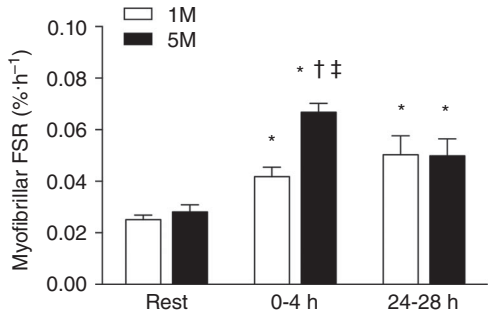

Figure 4. Myofibrillar fractional synthesis rate (FSR) in rested fasting conditions and at 0–4 and 24–28 h postexercise in the postprandial state (25 g of whey protein)
Values are presented as means ± SEM for 1M ($n = 8$) and 5M ($n = 8$). Significance was set at $P < 0.05$. *Significantly greater than rested fasting values; †significantly greater than 1M at 0–4 h; and ‡significantly greater than 5M at 24–28 h.

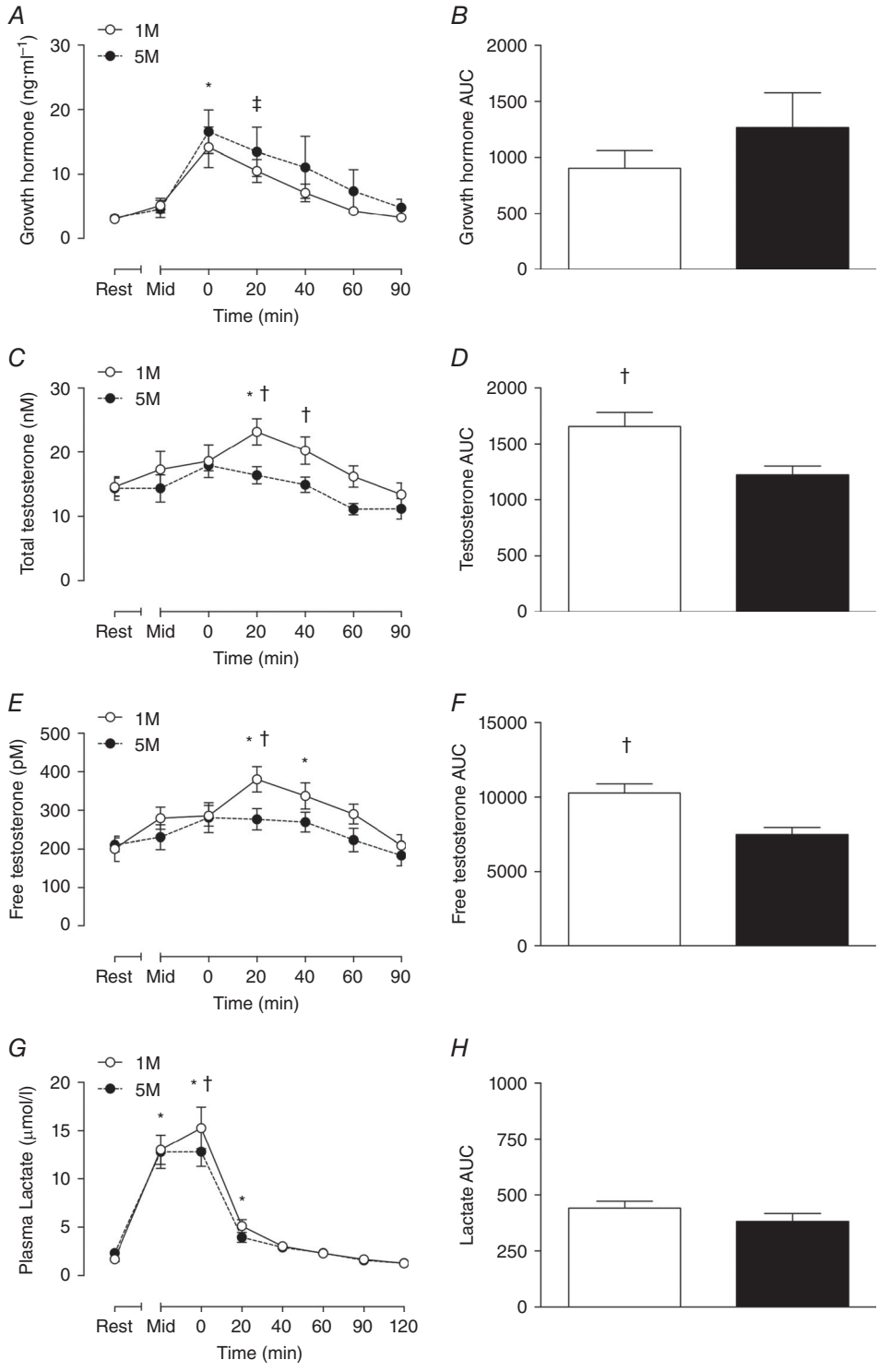

Figure 5. Blood hormone/analyte concentrations
Serum growth hormone concentrations (*A*) and area under the curve (AUC; *B*), serum testosterone concentrations (*C*) and AUC (*D*) and free testosterone concentrations (*E*) and AUC (*F*) over 0–90 min postexercise. Plasma lactate concentrations (*G*) and AUC (*H*) over 0–120 min postexercise. Sample at $t = 0$ indicates immediately postexercise. Values are means ± SEM for 1M ($n = 7$) and 5M ($n = 7$). Significance was set at $P < 0.05$. *Significantly greater than rested fasting values for both groups; †significant difference between groups at specified time point; and ‡significantly greater than rested fasting values for 5M only.

postexercise for 1M and 5M (~9- and 5.5-fold, respectively, $P < 0.001$ for both), returning to resting values at 40 min postexercise. Plasma lactate concentration immediately postexercise was significantly greater in 1M compared with 5M ($P = 0.027$; Fig. 5*G*). Plasma lactate AUC over 0–120 min postexercise was not different between 1M and 5M (Fig. 5*H*).

Intracellular signalling

The phosphorylation of p70S6K^{Thr389} increased ~4.2-fold above rest values at 4 h postexercise in 5M ($P = 0.019$), but was not different from resting values in 1M across the entire postexercise period. Phosphorylation of p70S6K^{Thr389} at 4 h postexercise was significantly greater in 5M compared with 1M ($P = 0.048$; Fig. 6*A*).

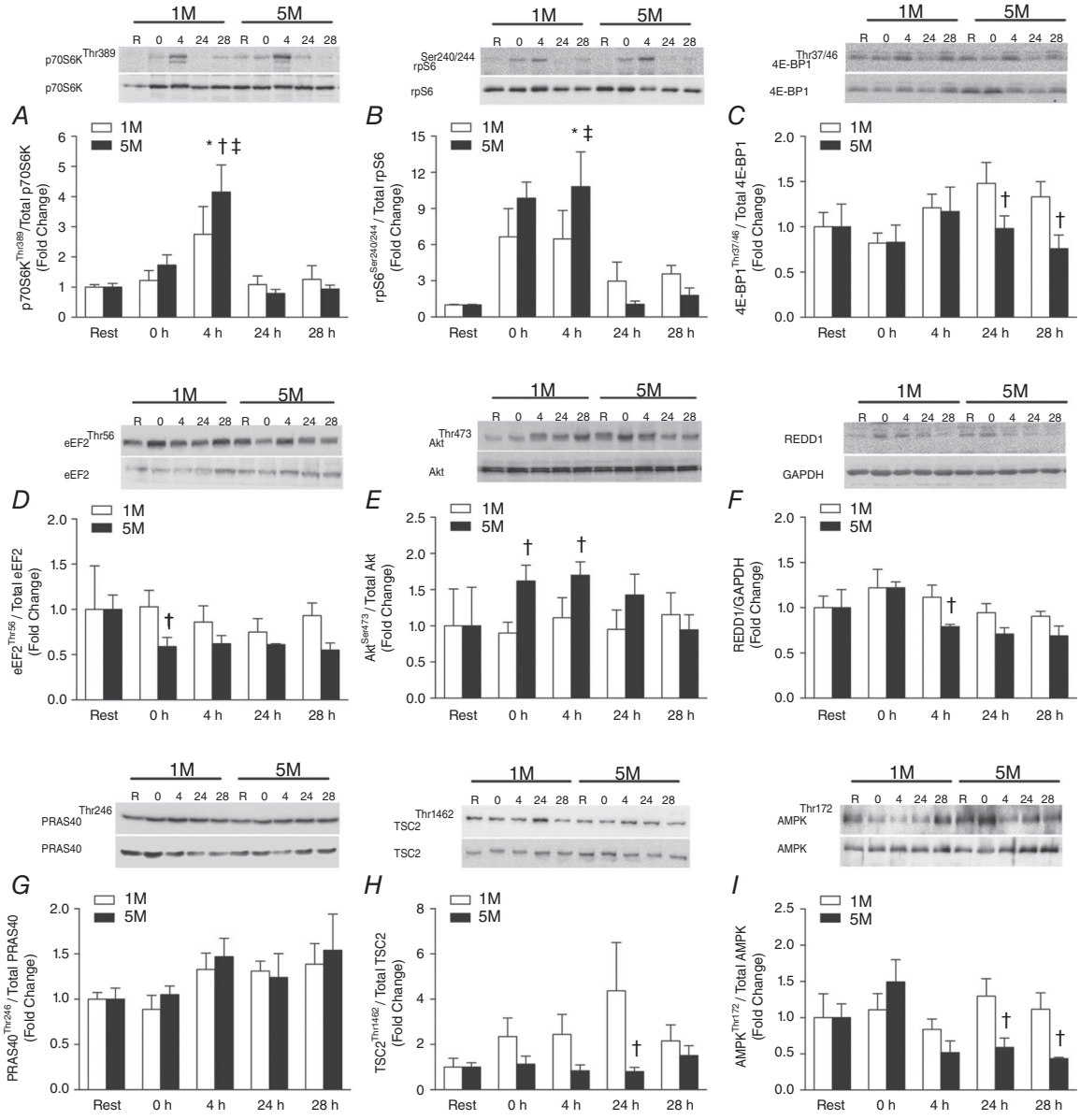

Figure 6. Anabolic signalling phosphorylation of p70S6K^{Thr389} (*A*), rpS6$^{Ser240/244}$ (*B*), 4E-BP1$^{Thr37/46}$ (*C*), eEF2^{Thr56} (*D*), Akt^{Ser473} (*E*), REDD1 (*F*), PRAS40^{Thr246} (*G*), TSC2^{Ser1462} (*H*) and AMPKα^{Thr172} (*I*) in rested fasting conditions and at 0, 4, 24 and 28 h postexercise

Biopsies at 0 and 24 h samples were collected in fasted conditions, and biopsies at 4 and 28 h were collected in a 4 h postprandial state (25 g of whey protein). Data are expressed as the phosphorylated-to-total protein ratio for each signalling target, with the exception of REDD1, which is expressed as the protein-to-GAPDH ratio. Values are means ± SEM normalized to the rest sample for 1M ($n = 8$) and 5M ($n = 8$). Significance was set at $P < 0.05$. *Significantly greater than rested fasting values; †significantly different from 1M; ‡significantly greater than 5M at 28.

The phosphorylation of ribosomal protein kinase S6 (rpS6)$^{Ser240/244}$ increased by ∼11-fold above rest values at 4 h postexercise for 5M ($P = 0.031$; Fig. 6*B*), but was not significantly increased in 1M. The phosphorylation of 4E-BP1$^{Thr37/46}$ was not different from rest values across the entire postexercise period in 1M and 5M. Phosphorylation of 4E-BP1$^{Thr37/46}$ was significantly lower in 5M compared with 1M at 24 and 28 h postexercise ($P < 0.05$; Fig. 6*C*). The phosphorylation of eEF2^{Thr56} was not different from resting values across the postexercise period in 1M and 5M. Phosphorylation of eEF2^{Thr56} was significantly lower in 5M compared with 1M immediately postexercise ($P = 0.022$; Fig. 6*D*). The phosphorylation of Akt^{Ser473} was not different from resting values across the entire postexercise period in 1M and 5M. Phosphorylation of Akt^{Ser473} was significantly lower in 1M compared with 5M at 0 and 4 h postexercise ($P < 0.05$; Fig. 6*E*). The phosphorylation of Akt^{Thr308} was not different from rest values across the entire postexercise period or between groups in 1M and 5M (data not reported). REDD1 was not different from resting values across the postexercise period in 1M and 5M. REDD1 was significantly lower in 5M compared with 1M at 4 h postexercise ($P = 0.027$; Fig. 6*G*). Phosphorylation of PRAS40^{Thr246} was not different from resting values across the entire postexercise period or between groups in 1M and 5M (Fig. 6*H*). Phosphorylation of TSC2^{Thr1462} was not different from resting values across the postexercise period in 1M and 5M. Phosphorylation of TSC2^{Thr1462} was significantly lower in 5M compared with 1M at 24 h postexercise ($P < 0.01$; Fig. 6*I*). Phosphorylation of AMPK^{Thr172} was not different from resting values across the postexercise period in 1M and 5M. Phosphorylation of AMPK^{Thr172} was significantly lower in 5M compared with 1M at 24 and 28 h postexercise ($P < 0.01$; Fig. 6*I*).

Discussion

It is generally well accepted that manipulation of resistance exercise training parameters can influence skeletal muscle remodelling. This is the first study to investigate the acute mechanisms through which resistance exercise inter-set rest duration influences MPS and intracellular signalling. The findings of the present study demonstrate that a short inter-set rest duration (1 min; 1M) during moderate-intensity, high-volume fatigue-inducing resistance exercise blunts MPS rates and intracellular signalling in recreationally trained young men over the 'early' postexercise recovery period (0–4 h), compared with long inter-set rest duration (5 min; 5M). Paradoxically, the blunting of MPS rates and intracellular signalling in the early postexercise recovery period for 1M occurred in parallel with a greater systemic testosterone response compared with 5M. Thus, inter-set rest duration between sets of resistance exercise can profoundly influence acute muscle intracellular signalling and systemic hormonal parameters, with potential implications for resistance training-induced muscle remodelling.

In contrast to our initial hypothesis that the MPS response to moderate-intensity, high-volume fatigue-inducing resistance exercise would be similar between 1M and 5M, we demonstrated that the increase in MPS during the early phase of postexercise recovery was attenuated in 1M compared with 5M (76 *versus* 152% increase above rested-fasted MPS values, respectively). The physiological significance of the acute impairment in MPS in the early postexercise recovery period in 1M was unexpected and, although difficult to interpret, is somewhat contradictory to the belief that a short inter-set rest interval (1–2 min) may provide a superior stimulus for resistance exercise-induced muscle hypertrophy over longer rest intervals (>3 min; American College of Sports Medicine, 2009). Although we did not presently assess muscle hypertrophy in response to long-term training with divergent inter-set rest intervals, others have previously done so. In line with our acute mechanistic findings, resistance training-induced changes in regional muscle thickness and cross-sectional area were greater with longer (2.5–3 min) compared with shorter (1 min) inter-set rest (Buresh *et al.* 2009; Schoenfeld *et al.* 2015). In contrast, others report no difference in the magnitude of muscle hypertrophy between resistance training protocols employing short or long rest periods (Ahtiainen *et al.* 2005; de Souza *et al.* 2010), which may be attributable to the short rest interval exceeding 1 min, thereby avoiding any metabolic perturbations that might blunt adaptive responses. Taken together, the findings of the present study and the paucity of chronic resistance training studies demonstrate that insufficient rest between resistance exercise sets is potentially detrimental, or at least equivocal, for muscle hypertrophy.

The position that resistance exercise inter-set rest duration is an important determinant of muscle hypertrophy is rooted in the premise that short inter-set rest results in greater circulating concentrations of GH and/or T (Kraemer *et al.* 1990; Goto *et al.* 2004; Bottaro *et al.* 2009; Rahimi *et al.* 2010), thought to be important in the stimulation of MPS (American College of Sports Medicine, 2009; de Salles *et al.* 2009). The present findings demonstrated that high-volume, moderate-intensity resistance exercise resulted in a similar elevation in circulating GH in 1M and 5M. Irrespective of inter-set rest interval, all participants exercised the same large area of muscle mass to momentary muscular fatigue to achieve a high total training volume, which we posit may have been sufficient to reach an acute 'ceiling' GH response as previously demonstrated (Smilios *et al.* 2003). Important to note is that our data do not account for the various aggregate and splice variant

isoforms of GH that are reported to be >100 in number (Kraemer *et al.* 2010). Likewise, we chose not measure GH-mediated intramuscular signalling in the Janus kinase/signal transducers and activator of transcription (STAT) pathway, because these proteins are unresponsive to resistance exercise-induced elevations in GH (West *et al.* 2010*b*). Interestingly, the resistance exercise-induced T response was greater for 1M compared with 5M. In line with these data, Buresh *et al.* (2009) reported superior resistance training-induced hypertrophy with 2.5 min of inter-set rest, despite a lower T response compared with a 1 min rest condition. Thus, our data add to a growing body of evidence demonstrating that exercise-induced circulating GH and T concentrations are not related to MPS (West *et al.* 2009; West & Phillips, 2012; Mitchell *et al.* 2013). It is possible that alternative biological functions exist for resistance exercise-induced hormonal increases, including connective tissue remodelling (Doessing *et al.* 2010; West *et al.* 2015). Furthermore, it is important to consider that circulating concentrations of T may be poorly related to intramuscular T concentrations. Exercise-induced intramuscular T or T–androgen receptor complex formation may play an important regulatory role in MPS and hypertrophy (Bamman *et al.* 2001; Mitchell *et al.* 2013) and warrants further investigation.

Given recent evidence that acute resistance exercise-induced MPS rates do not predict chronic training-induced muscle hypertrophy (Mitchell *et al.* 2014), the suggestion that the blunted MPS and intracellular signalling response in the early recovery period for 1M may be indicative of impaired long-term muscle hypertrophy is tenuous. However, exercise-induced elevations in MPS persist for 24–72 h postexercise (Phillips *et al.* 1997; Miller *et al.* 2005; Burd *et al.* 2011*a*), which may explain the discord between acute assessment of MPS over several hours postexercise and long-term hypertrophy. To gain a clearer understanding of the potential for resistance exercise inter-set rest duration to influence muscle remodelling, within the context of our acute mechanistic investigation, we re-assessed MPS in the 'late' 24–28 h postexercise recovery period and found that fed-state MPS rates were elevated to a similar extent in 1M and 5M. Methodological issues associated with prolonged intravenous stable isotope tracer infusions meant that we were unable to investigate MPS continuously over the entire 0–28 h postexercise recovery period. Thus, we cannot rule out the possibility that MPS in 1M was delayed beyond the 0–4 h early postexercise recovery period, potentially 'rebounding' at a later time point prior to MPS reassessment at 24–28 h postexercise. Nonetheless, our observation that MPS was blunted in the early postexercise recovery period in 1M offers a new insight into the acute metabolic implications of insufficient inter-set rest.

The mechanisms underpinning the blunted MPS response in the early postexercise recovery period for 1M are undoubtedly complex and not easy to reconcile. As expected, 1M elicited a greater rate of fatigue than 5M, as evidenced by a higher postexercise plasma lactate response and a marginally lower exercise volume (Richmond & Godard, 2004; Willardson & Burkett, 2005). Previously, others have demonstrated that the volume of resistance exercise affects the acute muscle anabolic response, with three sets increasing MPS to a greater extent than a single set (Burd *et al.* 2010*a*). However, although multiple sets of resistance training elicit greater rates of MPS and hypertrophy compared with a single set, there is a plateau (and potentially a decline) in hypertrophic adaptations beyond certain resistance training volumes (Krieger, 2010). Thus, we ensured that the training volume load completed in 1M would, in theory, be sufficient to exceed the threshold required for maximal MPS stimulation. As such, it is unlikely that the 16% lower exercise volume achieved in 1M can fully explain the 76% lower MPS and attenuated intracellular signalling response compared with 5M. Furthermore, the ∼1.6-fold increase in MPS above resting values from 0 to 4 h postexercise in 1M is notably lower than previously reported in response to resistance exercise protocols of much lower volume (Kumar *et al.* 2009; Burd *et al.* 2010*a*). This comparison further supports the position that the metabolic perturbations associated with insufficient inter-set rest in 1M may have been primarily responsible for the blunting of MPS and intracellular signalling, with the marginal reduction in exercise volume playing a lesser role. Thus, although we are unable to determine the extent to which the marginal reduction in training volume contributed to the inferior muscle anabolic response in 1M, we feel that additional contractions in 1M to match the exercise volume achieved in 5M (which would have required approximately eight more repetitions) would not have completely reversed the blunted MPS and intracellular signalling response in the early postexercise recovery period.

The present data support the position that resistance exercise-induced MPS and hypertrophy are initiated through intramuscular mechanisms activated through contraction-induced calcium influx and mechanical stress/tension, rather than transient systemic hormonal events (West *et al.* 2010*a*; Mitchell *et al.* 2013). The blunted MPS response in the early postexercise recovery period in 1M occurred in parallel with blunted intracellular signalling of targets associated with the mTORC1 pathway, critical for resistance exercise (Drummond *et al.* 2009) and nutrient-induced MPS stimulation (Dickinson *et al.* 2011). Specifically, rPS6$^{Ser240/244}$ and p70S6K^{Thr389} phosphorylation at 4 h postexercise was blunted in 1M compared with 5M. Furthermore, eEF2^{Thr56}

phosphorylation was greater in 1M than 5M immediately postexercise, previously reported to be associated with impairments in ribosome translocation on mRNA, peptide chain elongation and MPS (Carlberg *et al.* 1990). Induction of REDD1 has been closely linked to reduced activity in the mTORC1 pathway (Kimball *et al.* 2008; Murakami *et al.* 2011) and has been linked to adverse physiological conditions, including ATP depletion (Sofer *et al.* 2005) and endoplasmic reticulum stress (Protiva *et al.* 2008). In human skeletal muscle, *REDD1* mRNA expression is repressed following resistance exercise in association with mTORC1 activation (Drummond *et al.* 2008). Herein, we demonstrate that REDD1 protein was greater in 1M compared with 5M at 4 h postexercise, which may partly explain the impaired MPS response over this period. Finally, Akt phosphorylation was greater in 5M immediately and 4 h postexercise compared with 1M. Akt phosphorylates PRAS40, which results in its dissociation from mTORC1, thereby relieving the inhibitory effect (Sancak *et al.* 2007) although, in our hands, PRAS40 phosphorylation was not different over time or between groups. Another mTORC1 inhibitor, TSC2, can be activated by AMPK in response to cellular energy deprivation (Inoki *et al.* 2003) or repressed by Akt (Manning *et al.* 2002). Although AMPK and TSC2 phosphorylation were not different between 1M and 5M in the early recovery phase, a greater response was apparent in the late recovery phase. These surprising findings, alongside a greater 4E-BP1 response for 1M at 24–28 h postexercise, are difficult to explain, because late recovery MPS was equivalent between groups. However, a lack of congruence between the intramuscular signalling phosphorylation and MPS in relatively small cohorts has been well documented (Greenhaff *et al.* 2008). Thus, regardless of any discord in the temporal time course of intramuscular signalling and MPS, the multiple attenuations in anabolic signalling in 1M could collectively explain the blunted MPS response in the early postexercise recovery period.

The practical implications of our acute mechanistic investigation warrant discussion. Short inter-set rest ($\leq$90 s) is typically implemented during high-volume, moderate-intensity resistance training to emphasize muscle hypertrophy, whereas longer rest ($\geq$3 min) is typically implemented in lower volume heavier intensity resistance training to emphasize strength enhancement. Herein, we chose to match the relative intensity and number of exercise sets between 1M and 5M which, although not entirely reflective of 'real-world' practice, was necessary to study the isolated effects of divergent inter-set rest conditions. Incongruent with our acute demonstration of a blunting of MPS and intracellular signalling in 1M are data demonstrating that substantial muscle hypertrophy can be achieved over the course of chronic resistance training in conditions of brief inter-set rest (Mitchell *et al.* 2013). One possible explanation for this inconsistency may be that moderate-intensity resistance exercise with short inter-set rest elicited unfamiliar muscular energetic demands in our recreationally trained participants, who were typically used to resting for 2–3 min between exercise sets. Thus, given evidence that the acute exercise-induced MPS response is modified during long-term resistance training (Tang *et al.* 2008), it is possible that the blunted MPS and intracellular signalling response we observed in the early phase of postexercise recovery in 1M may be abated when individuals routinely undertake resistance exercise with short inter-set rest and adapt to the specific metabolic and neural demands of this contractile stimulus. Therefore, we speculate that the present findings have implications for individuals pursuing specific volume goals for hypertrophy that may need longer rest intervals initially, but may later adapt to shorter rest intervals. In line with this point, de Souza and colleagues (2010) reported similar muscle hypertrophy between individuals who rested for 2 min between sets and those whose inter-set rest gradually declined from 2 min to 30 s over 8 weeks of training.

In conclusion, we have demonstrated that sets of moderate-intensity, high-volume fatiguing resistance exercise interspersed by very brief 1 min rest intervals result in a blunted MPS and intracellular signalling response in the early postexercise recovery period compared with exercise sets interspersed by 5 min of rest in recreationally trained young men. Combined with evidence from chronic resistance training studies, the blunted MPS response in early recovery with short inter-set rest refutes the position that short rest intervals between successive sets of resistance exercise provide a superior stimulus for muscle hypertrophy. However, the blunted MPS response with short inter-set rest may be an acute adaptive response to the metabolic perturbations induced by a novel energetically demanding contractile stimulus. In summary, these findings shed light on the acute muscle anabolic response to resistance exercise performed with divergent inter-set rest conditions, with potentially important implications for those seeking to maximize muscle hypertrophy through resistance training. However, there is a clear need for further, rigorously controlled studies to reconcile the long-term influence of inter-set rest duration on the muscle adaptive response to prolonged resistance exercise training, particularly in well-trained individuals.

References

Abumarad N, Rabin ND & Diamond MP (1981). Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* **30**, 936–941.

Ahtiainen JP, Pakarinen A, Alen M, Kraemer WJ & Hakkinen K (2005). Short vs. long rest period between the sets in hypertrophic resistance training: influence on muscle strength, size, and hormonal adaptations in trained men. *J Strength Cond Res* **19**, 572–582.

American College of Sports Medicine (2009). American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* **41**, 687–708.

Bamman MM, Shipp JR, Jiang J, Gower BA, Hunter GR, Goodman A, McLafferty CL Jr & Urban RJ (2001). Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. *Am J Physiol Endocrinol Metab* **280**, E383–E390.

Bergstrom J (1975). Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* **35**, 609–616.

Bottaro M, Martins B, Gentil P & Wagner D (2009). Effects of rest duration between sets of resistance training on acute hormonal responses in trained women. *J Science Medicine Sport* **12**, 73–78.

Breen L, Philp A, Witard OC, Jackman SR, Selby A, Smith K, Baar K & Tipton KD (2011). The influence of carbohydrate–protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis. *J Physiol* **589**, 4011–4025.

Brook MS, Wilkinson DJ, Mitchell WK, Lund JN, Szewczyk NJ, Greenhaff PL, Smith K & Atherton PJ (2015). Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. *FASEB J* **29**, 4485–4496.

Buckley JP & Borg GA (2011). Borg's scales in strength training; from theory to practice in young and older adults. *Appl Physiol Nutr Metab* **36**, 682–692.

Burd NA, Holwerda AM, Selby KC, West DW, Staples AW, Cain NE, Cashaback JG, Potvin JR, Baker SK & Phillips SM (2010*a*). Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *J Physiol* **588**, 3119–3130.

Burd NA, West DW, Moore DR, Atherton PJ, Staples AW, Prior T, Tang JE, Rennie MJ, Baker SK & Phillips SM (2011*a*). Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *J Nutr* **141**, 568–573.

Burd NA, West DW, Rerecich T, Prior T, Baker SK & Phillips SM (2011*b*). Validation of a single biopsy approach and bolus protein feeding to determine myofibrillar protein synthesis in stable isotope tracer studies in humans. *Nutr Metab* **8**, 15.

Burd NA, West DW, Staples AW, Atherton PJ, Baker JM, Moore DR, Holwerda AM, Parise G, Rennie MJ, Baker SK & Phillips SM (2010*b*). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS ONE* **5**, e12033.

Buresh R, Berg K & French J (2009). The effect of resistive exercise rest interval on hormonal response, strength, and hypertrophy with training. *J Strength Cond Res* **23**, 62–71.

Carlberg U, Nilsson A & Nygård O (1990). Functional properties of phosphorylated elongation factor 2. *Eur J Biochem* **191**, 639–645.

Day ML, McGuigan MR, Brice G & Foster C (2004). Monitoring exercise intensity during resistance training using the session RPE scale. *J Strength Cond Res* **18**, 353–358.

de Salles BF, Simão R, Miranda F, Novaes Jda S, Lemos A & Willardson JM (2009). Rest interval between sets in strength training. *Sports Med* **39**, 765–777.

de Souza TP Jr, Fleck SJ, Simão R, Dubas JP, Pereira B, de Brito Pacheco EM, da Silva AC & de Oliveira PR (2010). Comparison between constant and decreasing rest intervals: influence on maximal strength and hypertrophy. *J Strength Cond Res* **24**, 1843–1850.

Dickinson JM, Fry CS, Drummond MJ, Gundermann DM, Walker DK, Glynn EL, Timmerman KL, Dhanani S, Volpi E & Rasmussen BB (2011). Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. *J Nutr* **141**, 856–862.

Doessing S, Heinemeier KM, Holm L, Mackey AL, Schjerling P, Rennie M, Smith K, Reitelseder S, Kappelgaard AM, Rasmussen MH, Flyvbjerg A & Kjaer M (2010). Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. *J Physiol* **588**, 341–351.

Drummond MJ, Fry CS, Glynn EL, Dreyer HC, Dhanani S, Timmerman KL, Volpi E & Rasmussen BB (2009). Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *J Physiol* **587**, 1535–1546.

Drummond MJ, Fujita S, Abe T, Dreyer HC, Volpi E & Rasmussen BB (2008). Human muscle gene expression following resistance exercise and blood flow restriction. *Med Sci Sports Exerc* **40**, 691–698.

Goto K, Nagasawa M, Yanagisawa O, Kizuka T, Ishii N & Takamatsu K (2004). Muscular adaptations to combinations of high- and low-intensity resistance exercises. *J Strength Cond Res* **18**, 730–737.

Goto K, Sato K & Takamatsu K (2003). A single set of low intensity resistance exercise immediately following high intensity resistance exercise stimulates growth hormone secretion in men. *J Sports Med Phys Fitness* **43**, 243–249.

Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, Wackerhage H, Smith K, Atherton P, Selby A & Rennie MJ (2008). Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* **295**, E595–E604.

Hassan-Smith ZK, Morgan SA, Sherlock M, Hughes B, Taylor AE, Lavery GG, Tomlinson JW & Stewart PM (2015). Gender-specific differences in skeletal muscle 11β-HSD1 expression across healthy aging. *J Clin Endocrinol Metab* **100**, 2673–2681.

Inoki K, Zhu T & Guan KL (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577–590.

Kimball SR, Do AN, Kutzler L, Cavener DR & Jefferson LS (2008). Rapid turnover of the mTOR complex 1 (mTORC1) repressor REDD1 and activation of mTORC1 signaling following inhibition of protein synthesis. *J Biol Chem* **283**, 3465–3475.

Kleger GR, Turgay M, Imoberdorf R, McNurlan MA, Garlick PJ & Ballmer PE (2001). Acute metabolic acidosis decreases muscle protein synthesis but not albumin synthesis in humans. *Am J Kidney Dis* **38**, 1199–1207.

Kraemer WJ, Dunn-Lewis C, Comstock BA, Thomas GA, Clark JE & Nindl BC (2010). Growth hormone, exercise, and athletic performance: a continued evolution of complexity. *Curr Sports Med Rep* **9**, 242–252.

Kraemer WJ, Marchitelli L, Gordon SE, Harman E, Dziados JE, Mello R, Frykman P, McCurry D & Fleck SJ (1990). Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol* **69**, 1442–1450.

Kraemer WJ & Ratamess NA (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Med* **35**, 339–361.

Krieger JW (2010). Single vs. multiple sets of resistance exercise for muscle hypertrophy: a meta-analysis. *J Strength Cond Res* **24**, 1150–1159.

Kumar V, Selby A, Rankin D, Patel R, Atherton P, Hildebrandt W, Williams J, Smith K, Seynnes O, Hiscock N & Rennie MJ (2009). Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol* **587**, 211–217.

Ly LP & Handelsman DJ (2005). Empirical estimation of free testosterone from testosterone and sex hormone-binding globulin immunoassays. *Eur J Endocrinol* **152**, 471–478.

McCaulley GO, McBride JM, Cormie P, Hudson MB, Nuzzo JL, Quindry JC & Travis Triplett N (2009). Acute hormonal and neuromuscular responses to hypertrophy, strength and power type resistance exercise. *Eur J Appl Physiol* **105**, 695–704.

Manning BD, Tee AR, Logsdon MN, Blenis J & Cantley LC (2002). Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/Akt pathway. *Mol Cell* **10**, 151–162.

Mayhew JL, Ball TE, Arnold TE & Bowen JC (1992). Relative muscular endurance as a predictor of bench press in college men and women. *J Appl Sports Sci Res* **6**, 200–206.

Miller BF, Olesen JL, Hansen M, Døssing S, Crameri RM, Welling RJ, Langberg H, Flyvbjerg A, Kjaer M, Babraj JA, Smith K & Rennie MJ (2005). Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J Physiol* **567**, 1021–1033.

Mitchell CJ, Churchward-Venne TA, Bellamy L, Parise G, Baker SK & Phillips SM (2013). Muscular and systemic correlates of resistance training-induced muscle hypertrophy. *PLoS ONE* **8**, e78636.

Mitchell CJ, Churchward-Venne TA, Parise G, Bellamy L, Baker SK, Smith K, Atherton PJ & Phillips SM (2014). Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men. *PLoS ONE* **9**, e89431.

Mitchell CJ, Churchward-Venne TA, West DW, Burd NA, Breen L, Baker SK & Phillips SM (2012). Resistance exercise load does not determine training-mediated hypertrophic gains in young men. *J Appl Physiol* **113**, 71–77.

Moore DR, Tang JE, Burd NA, Rerecich T, Tarnopolsky MA & Phillips SM (2009). Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *J Physiol* **587**, 897–904.

Murakami T, Hasegawa K & Yoshinaga M (2011). Rapid induction of REDD1 expression by endurance exercise in rat skeletal muscle. *Biochem Biophys Res Commun* **405**, 615–619.

Oostdijk W, Idkowiak J, Mueller JW, House PJ, Taylor AE, O'Reilly MW, Hughes BA, de Vries MC, Kant SG, Santen GW, Verkerk AJ, Uitterlinden AG, Wit JM, Losekoot M & Arlt W (2015). PAPSS2 deficiency causes androgen excess via impaired DHEA sulfation—in vitro and in vivo studies in a family harboring two novel PAPSS2 mutations. *J Clin Endocrinol Metab* **100**, E672–E680.

Phillips SM (2000). Short-term training: when do repeated bouts of resistance exercise become training? *Can J Appl Physiol* **25**, 185–193.

Phillips SM, Tipton KD, Aarsland A, Wolf SE & Wolfe RR (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol Endocrinol Metab* **273**, E99–E107.

Philp A, Schenk S, Perez-Schindler J, Hamilton DL, Breen L, Laverone E, Jeromson S, Phillips SM & Baar K (2015). Rapamycin does not prevent increases in myofibrillar or mitochondrial protein synthesis following endurance exercise. *J Physiol* **593**, 4275–4284.

Protiva P, Hopkins ME, Baggett S, Yang H, Lipkin M, Holt PR, Kennelly EJ & Bernard WI (2008). Growth inhibition of colon cancer cells by polyisoprenylated benzophenones is associated with induction of the endoplasmic reticulum response. *Int J Cancer* **123**, 687–694.

Rahimi R, Qaderi M, Faraji H & Boroujerdi SS (2010). Effects of very short rest periods on hormonal responses to resistance exercise in men. *J Strength Cond Res* **24**, 1851–1859.

Richmond SR & Godard MP (2004). The effects of varied rest periods between sets to failure using the bench press in recreationally trained men. *J Strength Cond Res* **18**, 846–849.

Rønnestad BR, Nygaard H & Raastad T (2011). Physiological elevation of endogenous hormones results in superior strength training adaptation. *Eur J Appl Physiol* **111**, 2249–2259.

Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA & Sabatini DM (2007). PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* **25**, 903–915.

Schoenfeld BJ, Pope ZK, Benik FM, Hester GM, Sellers J, Nooner JL, Schnaiter JA, Bond-Williams KE, Carter AS, Ross CL, Just BL, Henselmans M & Krieger JW (2015). Longer inter-set rest periods enhance muscle strength and hypertrophy in resistance-trained men. *J Strength Cond Res* DOI: 10.1519/JSC.0000000000001272.

Smilios I, Pilianidis T, Karamouzis M & Tokmakidis SP (2003). Hormonal responses after various resistance exercise protocols. *Med Sci Sports Exerc* **35**, 644–654.

Sofer A, Lei K, Johannessen CM & Ellisen LW (2005). Regulation of mTOR and cell growth in response to energy stress by REDD1. *Mol Cell Biol* **25**, 5834–5845.

Tang JE, Perco JG, Moore DR, Wilkinson SB & Phillips SM (2008). Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am J Physiol Regul Integr Comp Physiol* **294**, R172–R178.

Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr & Wolfe RR (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol Endocrinol Metab* **276**, E628–E634.

Villanueva MG, Lane CJ & Schroeder ET (2015). Short rest interval lengths between sets optimally enhance body composition and performance with 8 weeks of strength resistance training in older men. *Eur J Appl Physiol* **115**, 295–308.

West DW, Burd NA, Staples AW & Phillips SM (2010*a*). Human exercise-mediated skeletal muscle hypertrophy is an intrinsic process. *Int J Biochem Cell Biol* **42**, 1371–1375.

West DW, Burd NA, Tang JE, Moore DR, Staples AW, Holwerda AM, Baker SK & Phillips SM (2010*b*). Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *J Appl Physiol* **108**, 60–67.

West DW, Kujbida GW, Moore DR, Atherton P, Burd NA, Padzik JP, De Lisio M, Tang JE, Parise G, Rennie MJ, Baker SK & Phillips SM (2009). Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. *J Physiol* **587**, 5239–5247.

West DW, Lee-Barthel A, McIntyre T, Shamim B, Lee CA & Baar K (2015). The exercise-induced biochemical milieu enhances collagen content and tensile strength of engineered ligaments. *J Physiol* **593**, 4665–4675.

West DW & Phillips SM (2012). Associations of exercise-induced hormone profiles and gains in strength and hypertrophy in a large cohort after weight training. *Eur J Appl Physiol* **112**, 2693–2702.

Wilkinson SB, Tarnopolsky MA, Grant EJ, Correia CE & Phillips SM (2006). Hypertrophy with unilateral resistance exercise occurs without increases in endogenous anabolic hormone concentration. *Eur J Appl Physiol* **98**, 546–555.

Willardson JM & Burkett LN (2005). A comparison of 3 different rest intervals on the exercise volume completed during a workout. *J Strength Cond Res* **19**, 23–26.

Witard OC, Jackman SR, Breen L, Smith K, Selby A & Tipton KD (2014). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr* **99**, 86–95.

Additional information

Competing interests

None declared.

Author contributions

J.M., A.P.-L. and L.B. conceived and designed the experiment. J.M., A.P.-L., D.L., B.S. and L.B. collected the data. J.M., A.P.-L., M.M., D.L., B.S., J.R.D., J.Y., A.E.T., A.P. and L.B. analysed and interpreted the data. J.M., L.B. and A.P. drafted the manuscript and prepared all figures. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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