RESISTANCE EXERCISE-INDUCED MUSCLE HYPERTROPHY

ENDOGENOUS AND EXOGENOUS FACTORS AND THEIR INFLUENCE ON RESISTANCE EXERCISE TRAINING-INDUCED MUSCLE HYPERTROPHY

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LAY ABSTRACT

Resistance exercise training (RET) increases muscle size (hypertrophy); however, the relative influence that protein supplementation, specific training variables, and individual (genetic) variation have on the RET-induced hypertrophy is controversial and largely unknown. Broadly, data in this thesis show that protein supplementation slightly augments RET-induced hypertrophy, and that the magnitude of RET-induced hypertrophy may be related to the number of androgen (e.g., testosterone) receptors inside an individual's muscle. In contrast, we found that neither load nor hormones affect RET-induced hypertrophy. Interestingly, data in this thesis also show that RET-induced hypertrophy is consistent within an individual but varies considerably between people, which illustrates the greater influence that individual variation has on RET-induced hypertrophy. We conclude that when RET is performed with a high degree of effort, protein supplementation and specific training variables confer a relatively small benefit on RET-induced hypertrophy compared to the influence of biological variability between people.

ABSTRACT

Resistance exercise training (RET) can lead to muscle hypertrophy; however, the relative contribution that exogenous (protein supplementation and specific training variables) versus endogenous (biology inherent to the individual) factors have on RET-induced muscle hypertrophy is controversial. In Study 1, we provided an evidence-based conclusion that protein supplementation during periods of RET results in a small but statistically significant increase in RET-induced muscle hypertrophy. In Study 2, we corroborate previous research and observed that the amount of mass lifted per repetition (load) did not determine RET-induced muscle hypertrophy in resistance-trained men when RET was performed to volitional fatigue. In Study 4, we observed similar muscle fibre activation following resistance exercise with lighter versus heavier loads when both were lifted until volitional fatigue. In Studies 2 and 3, we observed no relationship between circulating anabolic hormones (e.g., testosterone) and RET-induced muscle hypertrophy. Nonetheless, in Study 3, we found significantly greater muscle androgen receptor content in the top versus the bottom quintile of respondents for muscle hypertrophy following 12 weeks of RET indicating that androgen receptor content, and not circulating androgen concentration, may be an important determinant of hypertrophy. Finally, in Study 5, we observed that RET-induced muscle hypertrophy was an consistent within an individual (independent of load and limb) but considerably different between participants. Together, these data suggest that the exogenous factors we studied – protein supplementation and load (when RET was performed to volitional fatigue) – had a relatively small influence on RET-induced muscle hypertrophy. In contrast, we found that endogenous variables, such as intramuscular androgen receptor content and likely other genetic influences, appear to contribute more to the significant heterogeneity seen in RET-induced muscle hypertrophy. Future research in this area should prioritize understanding the biology that underpins the individual variability in RET-induced muscle hypertrophy.

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1RM – one repetition maximum
30R – 30%1RM and regular contraction cadence
30S – 30%1RM and slow contraction cadence
4E-BP1 - eukaryotic initiation factor 4E binding protein 1
80R – 80%1RM and regular contraction cadence
80S – 80%1RM and slow contraction cadence
AAS - androgenic-anabolic steroid
AIC – akaike information criterion
Akt – protein kinase B
ALM – appendicular lean mass
ACSM – American College of Sports Medicine
ANOVA – analysis of variance
AR – androgen receptor
AUC – area under the curve
BB – biceps brachii
BP – bench press
CSA – cross sectional area
D_2O – deuterium oxide (^2H_2O)
DHEA – dehydroepiandrosterone
DHT – dihydrotestosterone
DXA – dual energy x-ray absorptiometry
EMG – electromyography
EMG_{amp} – electromyography amplitude
ERK1/2 – extracellular signal-regulated kinases 1/2
FAK – focal adhesion kinase
FBFM – fat- and bone-free mass
FFM – fat-free mass
fIGF-1 – free insulin-like growth factor 1
FM – fat mass
FSR – fractional synthesis rate
fT – free testosterone
GH – growth hormone
HIQR – highest quartile of responders
HIR – higher (top quintile) responders
HL – heavier load (~75-90 % 1RM)
HR – higher repetition (20-25 repetitions per set; lower load)
iEMG – integrated (total) electromyography
IGF-1 – insulin-like growth factor 1
KE – knee extension
LBM – lean body mass
LH – luteinizing hormone
LL – lighter load (~30-50 %1RM; higher-repetition)

LLM – leg lean mass
LOQR – lower quartile of responders
LOR – lower (bottom quintile) responders
LP – leg press
LR – lower repetition (8-12 repetitions per set; higher load)
MAPK – mitogen-activated protein kinase
MHC – myosin heavy chain
MnPF – mean power frequency
MPB – muscle protein breakdown
MPS – muscle protein synthesis
mRNA – messenger ribonucleic acid
MT – muscle thickness
mTORc1 – mechanistic target of rapamycin complex 1
MVC – maximum voluntary contraction
MVE – maximum voluntary excitation
NSCA – National Strength and Conditioning Association
p70S6K - p70 s6 kinase
PAS – periodic acid Schiff
PCA – principal component analysis
PLS-SEM – partial least squares structural equation modeling
RCT – randomized controlled trial
RDA – recommended dietary allowance
RE – resistance exercise
RET – resistance exercise training
rpS6 - ribosomal protein s6
RT – resistance training
SP – shoulder press
ST - semitendinosus
T – testosterone
TBM – total body mass
US – ultrasound
VL – vastus lateralis
VM – vastus medialis

DECLARATION OF ACADEMIC ACHIEVEMENT

FORMAT AND ORGANIZATION OF THESIS

This thesis is prepared in the "sandwich" format as outlined in the School of Graduate Studies' Guide for the Preparation of Theses. It includes a general introduction, five independent studies prepared in journal article format, and an overall discussion. The candidate is the first author on all of the manuscripts. At the time of thesis preparation, Chapters 2, 3, 4, and 5 were published in peer-reviewed journals and Chapter 6 was in preparation for submission.

CONTRIBUTION TO PAPERS WITH MULTIPLE AUTHORSHIP

Chapter 2 (Study 1)

Morton RW, Murphy KT, McKellar SR, Schoenfield BJ, Henselmans M, Helms E, Aragon AA, Devries MC, Banfield L, Krieger JW, and Phillips SM. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. Br J Sports Med. 52(6): 376-384. (2018)

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RWM, BJS., MH, EH, AAA, MCD, JWK and SMP. contributed to the conception and design of the study. **RWM**, BJS, MH, EH, AAA, MCD, LB, JWK, and SMP. contributed to the development of the search strategy. LB conducted the systematic search. **RWM**, KTM, and SRM. completed the acquisition of data. **RWM** and SMP performed the data analysis. **RWM** and SMP were the principal writers of the manuscript. All authors contributed to the drafting and revision of the final article. All authors approved the final submitted version of the manuscript.

Chapter 3 (Study 2)

Morton RW, Oikawa SY, Wavell C, Mazara N, McGlory C, Quadrilatero J, Baechler B, Baker S, and Phillips SM. Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men. J App Physiol. 121(1): 129-138. (2016).

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RWM, SYO, CM, and SMP. conception and design of research; **RWM**, SYO, CGW, NM, CM, JQ, SKB, and SMP performed experiments; **RWM**, SYO, CGW, NM, CM, BLB, and SMP analyzed data; **RWM**, SYO, CGW, NM, CM, SKB, and SMP interpreted results of experiments; **RWM**, SYO, CM, JQ, and SMP prepared figures; **RWM**, SYO, CM, SKB, and SMP drafted manuscript; **RWM**, SYO, CGW, NM, CM, JQ, SKB, and SMP edited and revised manuscript; **RWM**, SYO, CGW, NM, CM, JQ, BLB, SKB, and SMP approved final version of manuscript.

Chapter 4 (Study 3)

Morton RW, Sato K, Gallaugher M, Oikawa SY, McNicholas P, Fujita S, and Phillips SM. Muscle and not systemic hormonal variables influence skeletal muscle hypertrophy. Front Physiol. 9:1373. (2018).

Author contributions:

RWM, SYO, and SMP conception of research design; **RWM**, SYO, and SMP study conduction; **RWM** and MPBG statistical analyses; PDM and SMP statistical advice; **RWM**, KS, SF, and SMP data analysis; **RWM** and SMP drafted the manuscript; **RWM**, KS, MPBG, SYO, PDM, SF, and SMP revised and approved the final draft of the manuscript.

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RWM, CM, JRP, and SMP designed the study, **RWM**, MWS, AFZ, AJ, and SMP performed the data collection, **RWM**, MWS, AFZ, IYZM, and PJK performed the data analysis, **RWM** and SMP drafted the manuscript, and all authors critically revised the manuscript and approved the final version.

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CHAPTER 1:

INTRODUCTION

7.1 The role of skeletal muscle mass and strength in human health

Skeletal muscle is responsible for human locomotion, its function is associated with improved athletic performance (1), and its mass and function are inversely related to risk of sarcopenia (2), loss of independence (3), obesity/insulin resistance (4), cardiovascular disease (5), and mortality (5-8). Therefore, elucidating the various factors that augment and/or maintain skeletal muscle are of considerable interest for sport performance and public health.

7.2 Increasing skeletal muscle mass and strength in humans

To date, there are two reliable ways to increase skeletal muscle size and strength in humans: provision of exogenous androgenic-anabolic steroid (AAS) provision (9) and resistance exercise training (RET) (10). Combining RET with exogenous AAS provision augments the RET-induced increases in muscle size and strength (9, 11-13); however, AAS provision is associated with negative health outcomes such as increased cardiovascular disease risk (14) and mood disorders (15), so there is no public health recommendation that includes AAS provision. In contrast, Canadian (16) and international (17) physical activity guidelines include recommendation to perform strength training at least twice per week.

7.3 Introduction to muscle protein turnover

Muscle protein turnover describes the dynamic processes of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) that are in constant operation in skeletal

muscle. Stimuli such as exogenous AAS, resistance exercise, and amino acid provision stimulate and/or suppress either or both MPS and MPB and lead to changes in the size of the protein pool within a muscle fibre. An increase in the size of muscle fibres, hypertrophy, is chiefly determined by the net balance between MPS and MPB (18, 19). Indeed, following administration of AAS (20, 21) or performance of a bout of resistance exercise (18, 22-25) there is an increase in the rate of MPS (with less change in the rate of MPB), and both chronic AAS (9) and RET (10) result in muscular hypertrophy.

7.4 Amino acid provision and muscle protein turnover

Hyperaminoacidemia following protein provision results in the activation of a number of anabolic signalling proteins and a rise in MPS (26, 27). Indeed, it is well-known that hyperaminoacidemia following a bout of unilateral (28-34) or whole-body (35) resistance exercise results in a synergistic stimulation of MPS; however, only recently have a number of variables related to protein consumption been shown to modulate the post-prandial rise in MPS.

7.4.1. Factors that influence amino acid-induced muscle protein turnover

At rest, there is a dose-response relationship between MPS and protein dose such that the stimulation by high quality protein of MPS plateaus at approximately 0.24 g of protein/kg of body mass in young adults (22±4 y) and ~0.4 g/kg in older adults (71±1 y) (36). In addition, MPS is greatest when protein is consumed in moderate doses distributed evenly throughout the day (e.g., 20-25 g/meal for four meals) as opposed to larger (e.g., 40-60

g/meal for two meals) or smaller (e.g., 10-15 g/meal for eight meals) doses distributed more or less times throughout a day, respectively (37-39). Moreover, most data show that protein sources higher in leucine (e.g., whey protein versus soy or wheat) confer greater postprandial stimulation of MPS (40-44), though some do not (45-47). Further, others have found that protein ingestion before sleep augments MPS overnight in both young (47, 48) and older individuals (49). Finally, some data show that co-ingesting protein as a whole food bolus with other macronutrients, for example whole eggs versus egg whites, augments the post-resistance exercise increase in MPS (50); however, casein protein with or without milk fats does not result in any additional stimulation of MPS (51). Indeed, whey with or without carbohydrate (24, 52), whey with or without insulin infusion (53), and whey with or without branched-chain amino acids (41) all result in similar post-resistance exercise increases in MPS. In summary, it appears that proximity to a bout of resistance exercise (28-35), protein dose (36), protein distribution (37-39), protein source (40-44), and protein ingestion pre-sleep (47-49) are factors that modulate the post-prandial rise in MPS.

7.5 Protein consumption and changes in muscle size and strength

There have been numerous randomized controlled trials on protein supplementation with RET to date, but disparate inclusion criteria have resulted in conflicting conclusions from recent narrative reviews (54-58) and meta-analyses (59-66). For example, previous meta-analyses have included only trained participants (61), only older adults (62, 64, 66), supplements containing more than just protein (61, 63, 66), only one source of protein

(61, 65), short (<4 weeks) RET interventions (63, 65), frail/sarcopenic participants (60, 62, 64, 66), and/or participants who were energy-restricted (59, 60, 65). Therefore, the efficacy of increased protein intake during chronic RET, let alone the effectiveness of manipulating protein dose, distribution, or source, on changes in muscle size and strength in healthy persons is uncertain.

7.6 Resistance exercise load and changes in muscle size and strength

Canadian (16) and international (17) exercise guidelines include recommendations to practice strengthening exercises two or more times per week. Consequently, many look to the American College of Sports Medicine (ACSM) (67) and National Strength and Conditioning Association (NSCA) (68) for specific RET suggestions, which endorse that RET with heavier loads (HL; 75-90 % one repetition maximum [1RM]) results in increased muscle size and strength whereas RET with lighter loads (LL; 30-50 %1RM) results in increased muscle endurance (i.e., the strength-endurance continuum). As discussed below, there is now substantial evidence that challenges the disparities in RET-induced increases in muscle size and strength between HL and LL RET, which has led to extensive academic debate (69, 70).

7.6.1. The influence of load on muscle protein turnover and changes in muscle size and strength

Performing a bout of RET with HL results in greater resistance exercise-induced MPS than a work-matched bout of RET with LL (71, 72); however, when a bout of RET with

LL is performed to volitional fatigue (i.e., until the participant cannot generate enough force to lift the load), there is similar stimulation of resistance exercise-induced MPS (71). Furthermore, data from our laboratory showed that performing unilateral RET with HL versus LL for 10 weeks resulted in similar increases in RET-induced muscular hypertrophy when the RET was performed to volitional fatigue (73). These data (73) are in contrast to an earlier study (on which current exercise guidelines are based on (67, 68)) that observed a significant increase in the cross sectional area (CSA) of all muscle fibre types following moderate-load (~70-80 %1RM) and high-load (~90-95 %1RM) RET but no increase in the CSA of any muscle fibre type following low-load (~30-50 %1RM; similar to our definition of LL) RET (74). However, an important distinction is that the LL group was volume-matched to the HL group, which meant that the LL group did not perform RET to volitional fatigue (74). Nonetheless, the studies from our laboratory that found similar increases in MPS (71) and hypertrophy (73) following LL and HL RET were criticized on the basis that the participants were naïve to resistance training, the RET was only unilateral knee extension, and each participant performed both the HL and LL conditions concurrently (within-subject design) (69).

The impact of load on RET-induced increases in 1RM strength are less contentious than hypertrophy. For example, our work (73) and that of others (74-79) have observed that RET-induced changes in 1RM are greater when participants perform RET with HL. However, both ourselves (73) and others (76, 77, 79-82) have also observed that RET-induced changes in an unpracticed strength test (e.g., peak torque of the knee extensors during an isometric maximum voluntary contraction) are similar between HL

and LL RET. Indeed, simply practicing a 1RM test five times twice per week for eight weeks resulted in similar RET-increases in 1RM strength as performing a typical high-volume RET regime (four sets of 8-12 repetitions) twice per week for 8 weeks (81). In summary, it appears that RET-induced muscle hypertrophy is independent of load when RET is performed to volitional fatigue and that RET-induced changes in muscle strength are dependent on the specificity of training to the method of assessment (56, 81, 83).

7.6.2. The size principle and motor unit recruitment

In 1965, Dr. Elwood Henneman published a series of papers in the *Journal of Neurophysiology* that described the coordinated pattern of motor neuron firing that progressed from small motor units (lower depolarization threshold) to, in the presence of fatigue or increased force of contraction, larger motor units (higher depolarization threshold; reviewed elsewhere (84)). Since, a number of studies in human exercise physiology have demonstrated that fatiguing contractions in both aerobic (85-90) and resistance (91-93) exercise results in the recruitment of type II muscle fibres with increased force demand and/or progression toward fatigue. Nonetheless, the thesis that HL are *required* for muscle hypertrophy of type II muscle fibres has been ostensibly supported by the observation of greater surface electromyography (EMG) amplitude following HL versus LL resistance exercise (94-96). Indeed, the misinterpretation that surface EMG amplitude is indicative of motor unit activation (particularly during fatiguing contractions) (97-101) has sustained the thesis that lifting HL is a requirement for the recruitment of larger motor units (67, 102). In contrast, data from our laboratory

(73) and others (76) have shown that performing RET to volitional fatigue results in hypertrophy of type II muscle fibres (innervated by larger motor units) independent of load, which implies recurrent recruitment of higher threshold motor units.

7.7 Systemic hormones and changes in muscle size and strength

In eugonodal men, an acute bout of resistance exercise stimulates a small, transient increase in a number of circulating hormones (including growth hormone [GH], insulin-like growth factor [IGF-1], and testosterone [T]) that subsides within 15 to 60 minutes and is largely dependent on the amount of muscle mass engaged and the volume/intensity of the work performed (103-110). Indeed, many exercise scientists have hypothesized that circulating or salivary, as a proxy of circulating, concentrations of the aforementioned hormones are indicative, related, and/or are predictive of RET-induced muscular hypertrophy (herein referred to as the 'hormone hypothesis') (111-114).

7.7.1. Exogenous hormones, muscle protein turnover, and changes in muscle size

The hormone hypothesis has part of its notional support in the observation that administration of AAS increases muscle size and strength. Indeed, in healthy young men, exogenous provision of exogenous synthetic forms of T increases basal MPS (with little [if any] effect on MPB) (20, 21, 115, 116) and skeletal muscle size both with (11-13) and without (9, 117) RET. Similarly, in hypogonodal men (a result of aging or drug treatment for a clinical prognosis of, for example, prostate cancer), exogenous AAS provision increases both basal MPS (118-120) and skeletal muscle size independent of RET (119-

122). Nevertheless, administration of exogenous T (e.g., 600 mg of T enanthate) results in four- to 10-fold higher T concentrations (e.g., 3000 ng/dl) (9) than post-exercise T concentrations in eugonoadal men (e.g., T: 500 ng/dl) (105, 108). In addition, the post-resistance exercise rise in circulating hormones is transient (~15-60 minutes; if detectable at all), as opposed to the 24-hour and 15-fold increase in resting T when receiving exogenous T (9). Thus, we hypothesize that the small and transient post-exercise rise in systemic hormones is not a comparable scenario to the magnitude and duration of increases in hormones, most notably T, seen with prolonged exogenous AAS provision (123).

7.7.2. Endogenous hormones, muscle protein turnover, and changes in muscle size

There is mounting evidence that the post-exercise rise in systemic hormones (e.g., T, GH, and IGF-1) are not associated with resistance exercise-induced MPS (105, 108) or RET-induced muscular hypertrophy (124-126). Indeed, non-hypertrophic exercise (e.g., cycling) that is comparable in duration to a bout of resistance exercise produces similar increases in circulating T (127-130), GH (128, 130, 131), and IGF-1 (130-132). Further, women have 10-15-fold lower circulating T at rest and 45-fold lower circulating T post-resistance exercise but have similar rates of MPS at rest (133), similar increases in MPS following a bout of resistance exercise (108), and similar RET-induced muscle hypertrophy (134) compared to men. Nonetheless, some studies have found correlations on ≤11 participants between RET-induced muscle hypertrophy and the post-exercise rise in circulating GH (135) and T (136, 137), and others have used what we propose are

incorrectly applied statistical models on small data sets (26 participants) to conclude that a composite 'score' of circulating hormones is associated with a composite 'score' of RET-induced muscular hypertrophy (111). Consequently, the hormone hypothesis has persisted amongst exercise scientists despite a growing body of conflicting evidence (114).

7.7.3. Androgen receptors and changes in muscle size

The canonical action of an androgenic hormone (e.g., T) is, when bound with an androgen receptor (AR), to co-translate to the nucleus to bind to upstream elements and modify downstream gene transcription (138). Interestingly, both a bout of resistance exercise (107, 108, 139, 140) and exogenous T administration (21) can increase intramuscular AR mRNA, and both weeks of RET (141, 142) and weeks of exogenous T administration (121) result in an increase intramuscular AR protein content. Further, others have observed an association between the increase in AR content with RET-induced muscle hypertrophy (126, 142). Therefore, it plausible that AR content (and other intramuscular hormone-related variables), as opposed to circulating androgens, mediates RET-induced increases in muscle size and strength.

7.8 Methodological considerations for quantifying resistance exercise training-induced changes in muscle size and strength

There are a number of modalities that can be used to quantify RET-induced changes in muscle mass and muscle size. Commonly, fat-free mass (air plethysmography or

hydrostatic weighing), fat- and bone-free mass (FBFM; dual x-ray absorptiometry [DXA]), muscle fibre CSA (muscle biopsy and histochemistry), whole-muscle CSA (ultrasound, computed tomography, or magnetic resonance imaging), and muscle thickness (ultrasound) are used to assess RET-induced muscle hypertrophy. However, these methods quantify muscle via divergent methods that are likely unrelated to each other (143). Similarly, as discussed above, muscle strength is commonly measured with a 1RM test or a peak torque test using dynamometry, but many laboratories have demonstrated that RET-induced changes in skeletal muscle strength are contingent on the method of strength assessment (73, 78, 81). Thus, it would seem prudent that multiple methods of assessment of strength (i.e., practiced and not practiced) be used to elucidate the true contribution of various factors on RET-induced changes in muscle size and strength.

7.9 Study objectives and hypotheses

Broadly, the purpose of this thesis was to evaluate the efficacy of exogenous and endogenous factors on RET-induced increases in muscle size and strength. We recognized that common exogenous factors manipulated during periods of RET are either nutritional or a specific RET parameter; therefore, we chose to study protein supplementation, load, and contraction cadence (i.e., time under tension; all of which are common and highly disputed RET-related exogenous factors). In addition, we recognized that many laboratories and exercise scientists are still convinced that circulating hormones are indicative of RET-induced muscle adaptation; therefore, we chose to study

a spectrum of circulating biomarkers (including the most canonical in resistance exercise science: GH, T, and IGF-1) and incorporate intramuscular hormone analyses to explore the mechanism that may be underpinned by exogenous AAS-induced anabolism.

7.9.1. Study 1: A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults.

Study 1 was a systematic review that had the aim of evaluating the efficacy of protein supplementation on RET-induced increases in muscle size and strength. Given the discordant message around the efficacy of protein supplementation to enhance hypertrophy that is evident from a number of narrative reviews (54-58) and meta-analyses (59-65), we designed our literature search for Study 1 so as to provide as broad and comprehensive appraisal of protein supplementation during RET as was reasonably possible without including diseased populations or individuals in caloric restriction. In addition, because variables such as protein dose (35), protein distribution (37, 38), protein source (40-43), age (36), and training status (144, 145) affect acute rates of MPS (and thus potentially hypertrophy), we also explored the contribution of these covariates, via meta-regressions, on the efficacy of protein supplementation during RET. Further, because there is a dose-response relationship between MPS and the amount of protein ingested (36), we performed an unadjusted segmented regression to reveal if there was a dose-response relationship between daily protein intake and RET-induced muscle hypertrophy. Our hypotheses were that protein supplementation would provide a

significant benefit on RET-induced adaptations, that training status, protein dose, baseline protein intake, and age would mediate the efficacy of protein supplementation on RET-induced adaptations, and that there would be a dose-response relationship between daily protein intake and RET-induced muscle hypertrophy.

7.9.2. Study 2: Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men.

In contrast to RET guidelines (67, 68), data from our laboratory has shown that when RET is performed to volitional fatigue, post-resistance exercise rates of MPS (71) and RET-induced muscle hypertrophy (73) are independent of load. However, the aforementioned studies have been criticized because they were in a small sample size, the resistance exercise was unilateral, the study design was within-subject, and both studies used training naïve participants (69). In addition, data from our laboratory (73) and others (74-79) suggests that RET-induced changes in muscle strength are influenced substantially by practice, or at least close replication, of the strength test. However, the efficacy of periodic practice on RET-induced changes in muscle strength between HL and LL was unknown. Therefore, the primary purpose of Study 2 was to determine the effect of performing RET with HL versus LL on RET-induced changes in muscle size and strength using a large sample size, previously resistance-trained participants, whole-body RET, and with periodic practice of 1RM testing. We hypothesized that periodic practice of the 1RM tests would reduce the post-testing differences between HL and LL on RET-

induced changes in 1RM and that load would not determine RET-induced changes in muscle size.

7.9.3. Study 3: Muscle androgen receptor content but not systemic hormones is associated with resistance training-induced skeletal muscle hypertrophy in healthy, young men.

In Study 2 we observed no correlations between baseline or post-exercise increases in circulating hormones before or after the RET regime with any index of RET-induced hypertrophy (146), which is consistent with previous data from our laboratory (124-126). Nonetheless, the hormone hypothesis has been ostensibly supported by what we view as incorrectly applied statistical modeling (partial least squares structural equation modelling on only 26 participants) (111) and correlations in ≤11 participants (135, 137). Therefore, the primary purpose of Study 3 was to further explore the relationship between hormones and RET-induced hypertrophy using more sophisticated statistical modeling (backwards elimination regression and principal component regression on 49 participants and 10 circulating biomarkers). In addition, because the RET-induced increase in AR may be correlated with an increase in RET-induced muscle hypertrophy (126, 142), the second purpose of Study 3 was to evaluate the association between intramuscular hormone-related variables (including AR content but also intramuscular T, dihydrotestosterone, and 5a-reductase expression) and RET-induced muscle hypertrophy. We hypothesized that backwards elimination regression and principal component regression would not show significant associations between systemic hormones and RET-induced changes in muscle

size or strength but that AR content would be associated with RET-induced muscle hypertrophy.

7.9.4. Study 4: Muscle fibre activation is unaffected by load and repetition duration when resistance exercise is performed to task failure.

There is mounting evidence that the load used during RET, particularly when the RET is performed to volitional fatigue, does not determine RET-induced muscle hypertrophy (75, 76, 79, 82, 147-151). Moreover, there are similar RET-induced increases in type II muscle fibre CSA (73, 76, 146), which would seemingly require type II fibre recruitment (56, 146). Nonetheless, on the basis of greater muscle surface EMG amplitude (94-96) or decomposition of the EMG signal (152), there is a belief that HL are *necessary* (or at least more efficacious) for the activation and ensuing hypertrophy of type II muscle fibres (102). Therefore, the purpose of Study 4 was to evaluate the effect of manipulating load and contraction cadence on EMG amplitude, muscle fibre activation, and anabolic signaling related to MPS and RET-induced muscle hypertrophy (126). Our hypothesis was that EMG amplitude would be higher in HL versus LL RET but that muscle fibre activation and anabolic signaling would be independent of load or contraction cadence.

7.9.5. Study 5: Variability in resistance training-induced hypertrophy and strength are independent of load and limb location in healthy young men.

To date, data show that changing exogenous variables such as protein intake (153), exercise volume (154, 155), training frequency (156), training velocity (157), and

external load (76) have small (if any) effects on RET-induced adaptations. The purpose of Study 5 was to further discern the relative influence of load (HL versus LL, an exogenous factor) compared to an endogenous factor (limb location: upper versus lower body) on RET-induced increases in muscle size and strength. We hypothesized that there would be considerable variability in RET-induced adaptations between participants but that the relative increases in muscle size and strength would be consistent within an individual (i.e., independent of load and limb).

7.10 References

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CHAPTER 2:

A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults

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Review

A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults

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ABSTRACT

Objective We performed a systematic review, meta-analysis and meta-regression to determine if dietary protein supplementation augments resistance exercise training (RET)-induced gains in muscle mass and strength.

Data sources A systematic search of Medline, Embase, CINAHL and SportDiscus.

Eligibility criteria Only randomised controlled trials with RET ≥6 weeks in duration and dietary protein supplementation.

Design Random-effects meta-analyses and meta-regressions with four a priori determined covariates. Two-phase break point analysis was used to determine the relationship between total protein intake and changes in fat-free mass (FFM).

Results Data from 49 studies with 1863 participants showed that dietary protein supplementation significantly (all $p<0.05$) increased changes (means (95% CI)) in: strength—one-repetition-maximum (2.49 kg (0.64, 4.33)), FFM (0.30 kg (0.09, 0.52)) and muscle size—muscle fibre cross-sectional area (CSA; 310 μm^2 (51, 570)) and mid-femur CSA (7.2 mm^2 (0.20, 14.30)) during periods of prolonged RET. The impact of protein supplementation on gains in FFM was reduced with increasing age (−0.01 kg (−0.02,−0.00), p=0.002) and was more effective in resistance-trained individuals (0.75 kg (0.09, 1.40), p=0.03). Protein supplementation beyond total protein intakes of 1.62 g/kg/day resulted in no further RET-induced gains in FFM.

Summary/conclusion Dietary protein supplementation significantly enhanced changes in muscle strength and size during prolonged RET in healthy adults. Increasing age reduces and training experience increases the efficacy of protein supplementation during RET. With protein supplementation, protein intakes at amounts greater than ~1.6 g/kg/day do not further contribute RET-induced gains in FFM.

INTRODUCTION

Resistance exercise training (RET) in combination with dietary protein supplementation is a common practice, in athletes and recreational exercisers alike, with the aim of enhancing RET-induced gains in muscle mass and strength. Recognised as a potent antisarcopenic stimulus, protein supplementation has also been advocated for ageing persons participating in RET. Despite a large volume of work in this area, narrative reviews[1–5] and even meta-analyses[6–12] yield conflicting results as to the actual effectiveness of protein supplementation to enhance RET-mediated gains in muscle mass and strength. This lack of agreement on the efficacy of protein supplementation[6–12] is likely due to the use of divergent study inclusion criteria and inclusion of subjects with differing: ages, training statuses, total protein intakes, protein sources and protein doses. Thus, an evidence-based answer to the main question of the efficacy of protein supplementation, while previously reported,[7] now appears to be controversial.[4]

We conducted a meta-analysis that was more inclusive in nature than previous meta-analyses[6–12] to provide a broad, systematic and evidence-based assessment on whether protein supplementation can augment changes in relevant RET outcomes. We used meta-regression to evaluate the impact of important potentially mediating covariates that were decided a priori to the meta-analysis. The present meta-analysis includes more than double the number of studies and participants than the largest published comprehensive meta-analysis on protein supplementation during RET to date.[7ST1]

We also undertook an additional rational, mechanism-based analysis that had the aim of answering the following question: is there a protein intake beyond which protein supplementation ceases to provide a measurable benefit in increasing muscle mass during RET? To answer this question, we recognised that the process of muscle protein synthesis (MPS), as the primary determinant of muscle hypertrophy,[13] shows a saturable dose-response relationship with increasing protein intake.[14] Since measures of MPS show good agreement with hypertrophy[13] we theorised that the effect of daily protein intake on RET-induced changes in muscle mass would show a dose-responsive relationship but that this would ultimately plateau.

METHODS

Inclusion criteria

Any randomised controlled trials (RCTs) that combined a RET and protein supplement intervention were considered for this meta-analysis. Trials had to be at least six weeks in duration,

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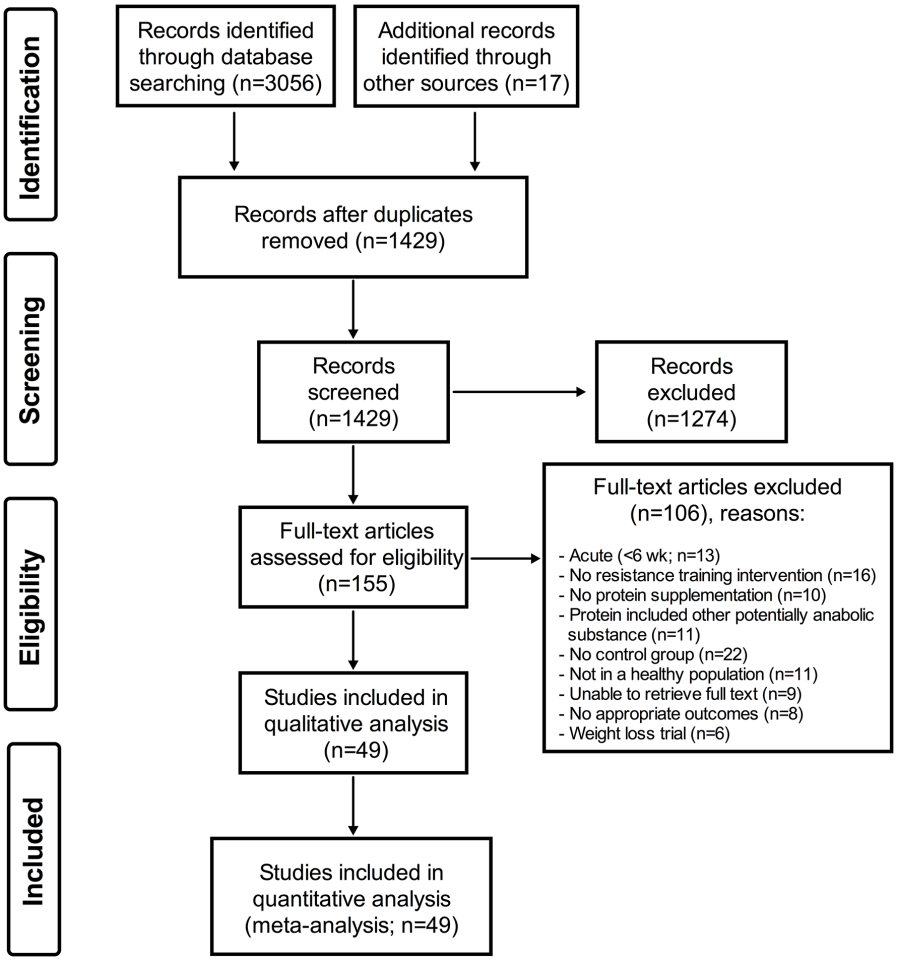

Figure 1 PRISMA flow chart.

participants had to be performing RET at least twice per week, and at least one group had to be given a protein supplement that was not co-ingested with other potentially hypertrophic agents (eg, creatine, β-HMB, or testosterone-enhancing compounds). Only trials with humans who were healthy and not energy-restricted were accepted. Manuscripts had to be original research (not a review or conference abstract) and be written in English.

Search strategy

A systematic search of the literature was conducted (LB) in Medline, Embase, CINAHL and SportDiscus, current to January 2017 (see online supplementary appendix 1). As appropriate, a combination of keywords and subject headings was used for the following concepts: protein supplementation and resistance training or muscle strength. The original search yielded 3056 studies. Any overlooked trials were identified by consulting other reviews and meta-analyses on the subject and were added in manually (17 studies). After deduplication and screening for inclusion criteria, 155 articles were independently read/reviewed by three authors (RWM, KTM and SRM). A total of 49 RCTs were selected for inclusion in this meta-analysis (figure 1).

Data extraction

Predetermined relevant variables from each included study were gathered independently by three investigators (RWM, KTM and SRM). Relevant variables included those regarding the study design, details of the RET intervention, participant characteristics, protein supplement information, placebo/control information, performance outcomes, body composition outcomes and any other notable information (eg, sources of bias/conflict of interest). Where data were not presented in table or text and authors could not be reached, data were extracted using WebPlotDigitizer (Web Plot Digitizer, V.3.11. Texas, USA: Ankit Rohatgi, 2017) or calculated from baseline values and/or percentage change. Where there were any discrepancies between the three reviewers the manuscripts were revisited by all reviewers (RWM, KTM and SRM) and agreed on by discussion. We also conducted a post hoc reassessment of 10 randomly selected studies and

compared the extracted results.[15] Coder drift was <10% in all cases for each investigator and inter-rater (RWM, KTM and SRM) reliability was excellent (>95%).

A total of 58 different body composition and 66 performance outcomes were extracted from the final 49 studies.[16–64] Primary outcomes were limited and amalgamated to include two different performance outcomes and four different body composition outcomes based on those most commonly reported in the 49 RCTs. Performance outcomes were: one-repetition-maximum strength (1RM; measured by any 1RM strength test) and maximum voluntary contraction (MVC; measured by both isokinetic and/or isometric contractions using a dynamometer with any muscle group/action). Body anthropometric and composition outcomes included: total body mass (TBM; measured by any scale); fat-free mass (FFM) and bone-free mass (or lean mass if FFM was not available; FFM; measured by dual-energy X-ray absorptiometry (DXA), hydrodensitometry, or whole-body air plethysmography (BodPod)); fat mass (FM; measured by DXA, hydrodensitometry and/or BodPod); muscle fibre cross-sectional area (CSA; measured in any fibre subtype (I, IIa, and/or IIx) obtained from either vastus lateralis and/or latissimus dorsi biopsies using microscopy); and mid-femur whole muscle CSA (mid-femur CSA, measured by MRI and/or CT).

Data syntheses

When data were reported in different units (eg, pounds vs kilograms) the data were converted to metric units. In all analyses the comparator group received an identical RET intervention but was non-supplemented or placebo-supplemented. If a study included a protein-supplemented group, a non-supplemented control group and a placebo-supplemented control group that were all part of the RET intervention, the protein-supplemented and placebo-supplemented groups were retrieved. If a study had multiple time points, only the preintervention and postintervention outcomes were retrieved. Where the change in SD (ΔSD) was available it was collected alongside the preintervention and postintervention SD. Where ΔSD was not reported, the correlation coefficient (corr) for each primary outcome was calculated according to the *Cochrane Handbook for Systematic Reviews of Interventions*:[65]

$$\text{corr} = (\text{SD}_{\text{pre}}^2 + \text{SD}_{\text{post}}^2 - \text{SD}_{\text{change}}^2)/(2 \times \text{SD}_{\text{pre}} \times \text{SD}_{\text{post}})$$

and the ΔSD was then calculated as:

$$\Delta\text{SD} = \sqrt{(\text{SD}_{\text{pre}}^2 + \text{SD}_{\text{post}}^2 - 2 \times \text{corr} \times \text{SD}_{\text{pre}} \times \text{SD}_{\text{post}})}.$$

The change in mean (ΔMean) and ΔSD were calculated for each condition and uploaded to RevMan (Review Manager (RevMan), V.5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Where studies had more than one protein-supplemented group (eg, soy and whey), measure of MVC (eg, isokinetic and isometric) or measure of 1RM (eg, bench press and leg press) the ΔMean and ΔSD were independently calculated and later combined, unless otherwise stated, using the RevMan calculator (Review Manager (RevMan), V.5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

Meta-analyses

Random-effects meta-analyses were performed in RevMan (Review Manager (RevMan), V.5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) on the change in each outcome. Effect sizes are presented as mean difference (MD) with means±SD and 95% CIs for 1RM, TBM, FFM, FM, fibre CSA and mid-femur CSA and as standardised mean difference (SMD) and 95% CIs for MVC because it had multiple outcomes presented on non-comparable scales (eg, N and Nm).

Heterogeneity and risk of bias

Heterogeneity was assessed by χ^2 and I^2 and significance was set at $p<0.05$. The internal validity of each study was determined by domain-based evaluation to quantify risk of bias for each study[65] and was independently performed by three investigators (RWM, KTM and SRM). The data included in the meta-analyses were restricted to studies with less than three reported high or unclear risk domains (predominately due to reported conflicts of interest and lack of blinding investigators and/or participants; (see online supplementary appendix 2)). Funnel plots were visually inspected to determine publication bias. Multiple sensitivity analyses were performed to determine if any of the results were influenced by the studies that were removed.

Meta-regression

In an effort to understand the sources of heterogeneity meta-regressions were performed on 1RM, FFM and fibre CSA because they were statistically significant, had considerable unexplained heterogeneity (I^2) and had a sufficient number of studies (≥10). Meta-regression was used instead of subgroup analyses to allow for the use of continuous covariates and to allow for the inclusion of more than one covariate at a time. Four covariates were chosen a priori to be included in our meta-regression: baseline protein intake (g/kg/day), postexercise protein dose (g), chronological age and training status because there is evidence that baseline protein intake,[66] protein dose,[14] age[67] and training status[68] could influence the efficacy of protein supplementation; summarised here.[4 5] These covariates were meta-regressed individually and together in a random-effects meta-regression model using Stata (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, Texas, USA). The random-effects meta-regression used residual restricted maximum likelihood to measure between-study variance (τ^2) with a Knapp-Hartung modification as recommended.[69] When all four covariates were analysed together permutation tests were performed (n=1000) to address the issue of multiple testing by calculating adjusted p values.[70] Additional covariates were identified and individually analysed post hoc to further explore the unexplained variance of the effect of protein supplementation during RET on changes in 1RM and FFM. Continuous covariates were: MD in the change in protein intake (g/day), MD in the total relative protein intake (g/kg/day), number of repetitions/set, number of sets/exercise, number of exercises/session, number of sessions/week, number of weeks and total RET volume in kg: repetitions/set × sets/exercise × exercises/session × sessions/week × intervention duration in weeks. Categorical variables were: protein supplement source (whey vs soy), sex (male vs female), type (dietary-supplement vs RET-supplement), whole-body RET (whole-body RET vs not whole-body RET) and RET supervision (supervised vs not supervised). Protein supplement source was limited to soy and whey because there were few study groups that were provided either a casein (n=3[21 59 60]) or pea (n=1[22]) protein supplement exclusively.

Subgroup analyses

Subgroup analyses were performed in RevMan (Review Manager (RevMan), V.5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Subgroup analyses were

performed on changes in FFM and 1RM with training status (untrained vs trained) as the subgroup to generate forest plots and neatly present training status as a categorical variable. Subgroup analyses were also performed on changes in FFM with age categorised into subgroups (old (>45 years) and young (<45 years)) to be presented below for the interested reader.

Break point analysis

To investigate the influence of protein intake as a continuous variable on individual study arms (as opposed being limited to MDs between groups in a meta-regression) linear and segmental regressions on the change in FFM (measured by DXA) were plotted against daily and baseline protein intake. Linear and segmental regressions were performed using GraphPad Prism (V.6, GraphPad Software, La Jolla, California, USA) to determine models of best fit as has been previously done in acute tracer trials measuring MPS.[14] Where segmental regression was the preferred model the slope of the second line was set to zero to determine the break point (biphasic regression). Each group from each study that presented daily or baseline protein intake with changes in FFM from DXA was included. Significance was set at $p<0.05$ and data for the break point is presented as mean (95% CI).

RESULTS

Participant characteristics

Participant details and outcomes are presented elsewhere (see online supplementary table 1. A total of 49 studies from 17 countries met the inclusion criteria (figure 1). There were 10 studies in resistance-trained participants and 14 study groups in exclusively female participants. Publications ranged from 1962 to 2016. There was a total of 1863 participants (mean±SD; 35±20 years).

RET characteristics

The RET characteristics are also presented elsewhere (see online supplementary table 1). The RET interventions lasted from 6 weeks to 52 weeks (13±8 weeks) performing RET between 2 days and 5 days per week (3±1 days/week) with between 1 to 14 exercises per session (7±3 exercises/session), 1 to 12 sets per exercise (4±2 sets/exercise) and anywhere between 3 to 25 repetitions per set (9±4 repetitions/set). Four studies used just lower-body RET, two studies used just knee extensor RET, one study used elbow flexor RET only, and two studies used one lower-body and one upper-body exercise only.

Protein supplementation

Details regarding the experimental (protein supplementation) and control (placebo- or no-supplement) groups are presented elsewhere (see online supplementary table 2). A range of 4 g to 106 g of protein was supplemented per day to the protein group (36±30 g/day; young: 42±32 g/day; old: 20±18 g/day) with a range of 5 g to 44 g of protein supplemented postexercise on training days (24±11 g; young: 24±12 g; old: 23±10 g). Twenty-three conditions supplemented with whey protein, 3 with casein protein, 6 with soy protein, 1 with pea protein, 10 with milk or milk protein, 7 with whole food (eg, beef, yogurt, between-meal snack) and 13 with non-specific protein blends or blends containing multiple protein sources (eg, whey, casein, soy and egg). In 40 studies the participants consumed part or all of their daily protein supplement after their RET sessions. In 36 studies with 48 different conditions authors reported either total (g/day) or relative (g/kg/day or %kcal/day) daily protein intake preintervention and/or postintervention. There was an increase in daily protein intake in the protein group (mean±SD; range: 23±41 g/day; −25 g/day to 158 g/day; p=0.004) and no change in the control group (1±14 g/day; −17 g/day to 40 g/day; p=0.83) such that the change in daily protein intake was significantly greater in the protein group (p=0.01). Relative daily protein intake (g/kg/day) increased in the protein group (pre: 1.4±0.4, post: 1.8±0.7, Δ: 0.3±0.5 g/kg/day, p=0.002) and did not change in the control group (pre: 1.4±0.3, post: 1.3±0.3, Δ: −0.02±0.1 g/kg/day, p=0.48) such that there was a greater change in the protein group (p<0.001). Daily energy intake (kcal/day) was gathered from 23 studies with 29 conditions and did not change with the prolonged RET and protein supplementation nor was it significantly different between the protein or control groups (Δ protein group: 50±293 kcal/day, Δ control group: 70±231 kcal/day, p=0.71).

Heterogeneity and risk of bias

Significant heterogeneity was found for changes in 1RM (χ^2=53.49, I^2=33%, p=0.003) and fibre CSA (χ^2=30.97, I^2=68%, p=0.0006). Nine studies were removed based on risk of bias[17 18 25 26 50 63] (see online supplementary appendix 2) or publication bias assessment[24 32 64] (see online supplementary figure 1). In particular, four studies were removed from 1RM,[17 26 32 50] four from TBM,[17 18 63 64] three from FM,[17 18 63] five from FFM,[17 18 24 63 64] three from MVC[25 26 50] and one from fibre CSA.[50]

Sensitivity analyses

Sensitivity analysis was performed with the nine high-risk studies mentioned above included in the outcomes they were removed from to determine if their removal changed any of the results. The inclusion of those studies did not influence the difference in means or significance in 1RM, TBM, FFM or mid-femur CSA; however, when Mitchell *et al*[50] was included in the fibre CSA assessment the effect of protein supplementation (310 μm^2 (51, 570), p=0.02) was eliminated (153 μm^2 (−137, 443), p=0.30). This is likely due to the small number of studies that included muscle biopsies but may warrant caution when interpreting the effect of protein supplementation on changes fibre CSA during RET. In no instance did fixed-effect meta-analysis deliver a different magnitude of effect or significance compared with random-effect meta-analysis.

Meta-analyses

Protein supplementation during prolonged RET significantly improved gains in 1RM strength (MD: 2.49 kg (0.64, 4.33), p=0.01; figure 2) but had no effect on MVC (SMD: 0.04 (-0.09, 0.16), p=0.54). Protein supplementation did not have a significant effect on changes in TBM (MD: 0.11 kg (−0.23, 0.46), p=0.52) but improved changes in FFM (MD: 0.30 kg (0.09, 0.52), p=0.007; figure 3), FM (MD: −0.41 kg (−0.70,−0.13), p=0.005), fibre CSA (MD: 310 μm^2 (51, 570), p=0.02; see online supplementary figure 2: panel A) and mid-femur CSA (MD: 7.2 mm^2 (0.20, 14.30), p=0.04; see online supplementary figure 2: panel B) during prolonged RET.

Meta-regression.

The results from the full model meta-regressions are presented in table 1. When combined, baseline protein intake, protein dose, age and training status did not explain any of the variance in the changes in 1RM (15 studies, 1216 subjects, p=0.77) or FFM (15 studies, 642 participants, p=0.12). There were insufficient observations (<10) when all covariates were compared with the changes in fibre CSA.

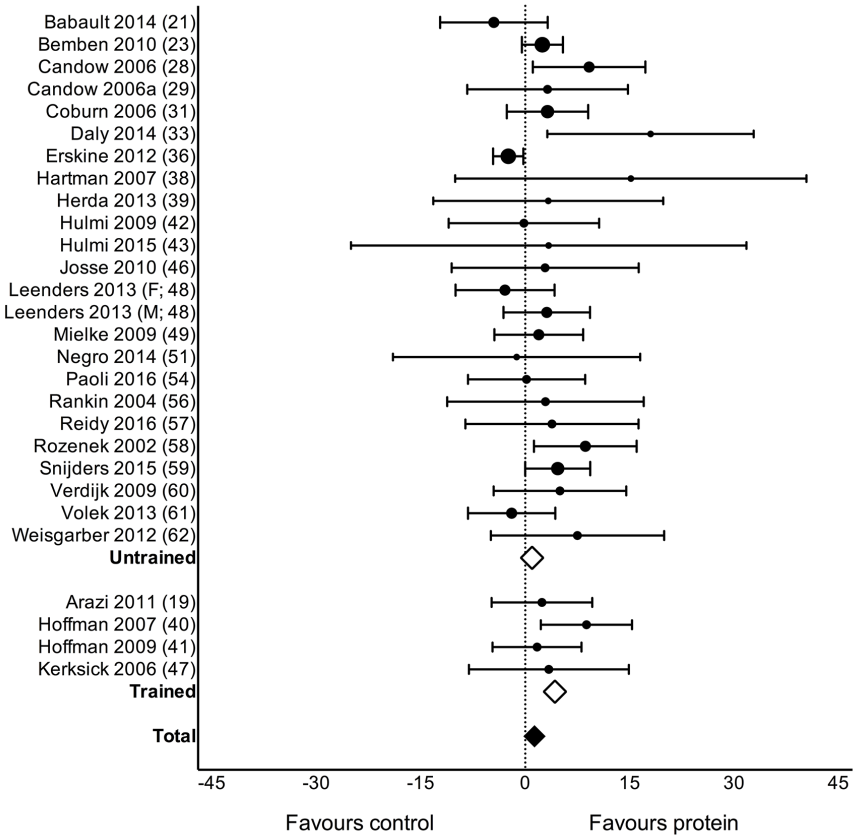

Figure 2 Forest plot of the results from a random-effects meta-analysis shown as mean difference with 95% CIs on one-repetition-maximum (1 RM; kg) in untrained and trained participants. For each study, the circle represents the mean difference of the intervention effect with the horizontal line intersecting it as the lower and upper limits of the 95% CI. The size of each circle is indicative of the relative weight that study carried in the meta-analysis. The rhombi represent the weighted untrained, trained and total group's mean difference. Total: 2.49 kg (0.64, 4.33), p=0.01, untrained: 0.99 kg (–0.27, 2.25), p=0.12 and trained: 4.27 kg (0.61, 7.94), p=0.02.

Univariate meta-regressions on changes in 1RM and FFM following prolonged RET are also presented in table 1. None of our covariates explained any of the heterogeneity of protein supplementation's effect on changes in 1RM: baseline protein intake (21 studies, 814 participants, p=0.59), age (27 studies, 802 participants, p=0.78), training status (28 studies, 858 participants, p=0.40) and post-exercise protein dose (23 studies, 589 participants, p=0.13). In contrast, when the ability of protein supplementation to affect changes in FFM was evaluated with univariate meta-regressions, the postexercise protein dose was the only covariate that did not influence the efficacy of protein supplementation on changes in FFM (20 studies, 793 participants, p=0.25) whereas baseline protein intake (22 studies, 988 participants, p=0.045; see online supplementary figure 3: panel A), age (25 studies, 1033 participants, p=0.02; figure 4) and training status (26 studies, 1089 participants, p=0.03) all influenced the effect of protein supplementation. When the effect of protein supplementation on changes in FFM was evaluated with age stratified into two subgroups the difference between old (>45; 67±7 years; MD: 0.06 (-0.14, 0.26)) and young (<45; 24±4 years; MD: 0.55 (0.30, 0.81)) participants remained significant (χ^2=8.71, I^2=89%, p=0.003). There were no covariates that explained any of the variance in the change in fibre CSA following RET: age (10 studies, 474 participants, I^2=65%, Adj. R^2=-3%, p=0.50), baseline protein intake (8studies, 384 participants, I^2=43%, Adj. R^2=-44%, p=0.84), postexercise protein dose (10 studies, 270 participants, I^2=77%, Adj. R^2=-38%, p=0.92) and training status (11 studies, 586 participants, I^2=71%, Adj. R^2=-24%, p=0.94).

Additional univariate meta-regressions are presented in elsewhere (see online supplementary table 3). Only whether the RET was whole-body (27 studies, including only 4 studies that were not whole-body RET, I^2=2%, Adj. R^2=76%, p=0.01) or supervised (28 studies, I^2=5%, Adj. R^2=58%, p=0.047) explained part of the variance in the effectiveness of protein supplementation on changes in 1RM. No other covariates explained any of the variance associated with the efficacy of protein supplementation on changes in 1RM or FFM.

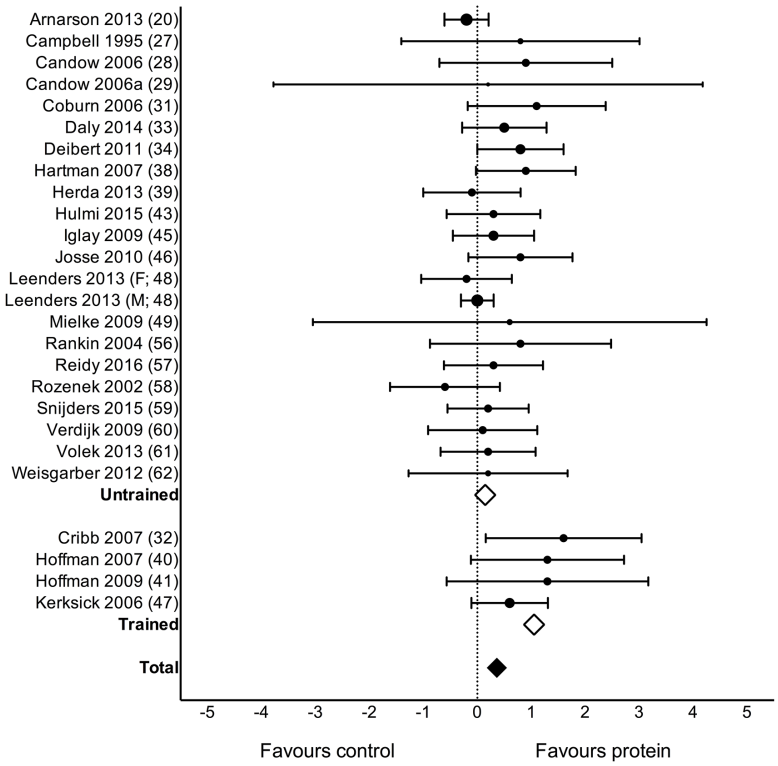

Figure 3 Forest plot of the results from a random-effects meta-analysis shown as mean difference with 95% CIs on lean or fat-free mass (FFM; kg) in untrained and trained participants. For each study, the circle represents the mean difference of the intervention effect with the horizontal line intersecting it as the lower and upper limits of the 95% CI. The size of each circle represents the relative weight that study carried in the meta-analysis. The rhombi represent the weighted untrained, trained and total group's mean difference. Total: 0.30 kg (0.09, 0.52) p=0.007, untrained: 0.15 kg (–0.02, 0.31), p=0.08 and trained: 1.05 kg (0.61, 1.50), p<0.0001.

Break point analysis

Biphasic regression (42 study arms, 723 participants) explained more variation than a linear regression between the change in FFM and daily protein intake (break point=1.62 (1.03, 2.20) g/kg/day, slope=1.75, R^2=0.19, df=36) and is presented as a segmental regression despite not being statistically significant (p=0.079;figure 5) When plotting the change in FFM against baseline protein intake, linear regressions explained significantly more variance than biphasic regressions in both young (slope=−1.54 g/kg/day, R^2=0.17, df=34) and old (slope=0.16 g/kg/day, R^2=0.04, df=14) participants with a statistically significant difference between

Table 1 Meta-regression output

		1RM (kg)					Fat-free mass (kg)					
Model	N	Coeff. (95% CI)	τ^2	Adj. R^2	I^2	p Value	N	Coeff. (95% CI)	τ^2	Adj. R^2	I^2	p Value
No covariates	28	2.49 (0.64 to 4.33)	6.05		33%	0.01	27	0.30 (0.09 to 0.52)	0.05		7%	<0.01
Univariate												
Baseline protein intake	21	2.85 (-8.15 to 13.84)	7.82	1%	37%	0.59	22	0.64 (0.02 to 1.27)	0	100%	0%	0.045
Protein dose	23	0.13 (-0.04 to 0.31)	3.16	40%	0%	0.13	20	0.02 (-0.01 to 0.04)	0.09	0%	0%	0.25
Age	27	0.01 (-0.09 to 0.11)	6.51	–9%	34%	0.78	25	–0.01 (-0.02 to 0.00)	0	100%	0%	0.02
Training status	28	5.77 (-2.96 to 7.13)	5.77	5%	31%	0.40	26	0.75 (0.09 to 1.40)	0.03	49%	0%	0.03
All covariates	15		5.36	10%	0%	0.77	15		0	100%	0%	0.12
Baseline protein intake	15	6.40 (-11.62 to 24.42)				0.43	15	–0.57 (-2.50 to 1.37)				0.95
Protein dose	15	0.05 (-0.78 to 0.88)				0.70	15	–0.01 (-0.07 to 0.06)				0.99
Age	15	0.07 (-0.18 to 0.33)				0.23	15	–0.01 (-0.02 to 0.00)				0.19
Training status	15	–2.81 (-20.80 to 15.17)				0.63	15	1.19 (-1.34 to 2.19)				0.48

age groups (p=0.042; see online supplementary figure 3: panel D).

DISCUSSION

This is the largest meta-analysis on interventions including dietary protein supplementation with muscle and strength-related outcomes during prolonged RET to date. Our main finding was that dietary protein supplementation augmented RET-induced increases in 1RM strength (figure 2) and FFM (figure 3). For changes in FFM, dietary protein supplementation was more effective in resistance-trained individuals (table 1 and figure 3), less effective with increasing chronological age (table 1 and figure 4) and did not increase beyond total protein intakes of ~1.6 g/kg/day (figure 5). Our data show dietary protein supplementation is both sufficient and necessary to optimise RET adaptations in muscle mass and strength.

Previous meta-analyses[6–12] have reached varying conclusions when examining the impact of protein supplementation on changes in lean mass or FFM and 1RM strength during RET. The discrepancies are likely a consequence of differing study inclusion criteria. For example, previous meta-analyses have included only trained participants,[8] only older adults,[9 11] supplements containing more than just protein,[8 10] only one source of protein,[8 12] shorter RET interventions,[10 12] frail/sarcopenic participants[7 9 11] and/or participants who were energy-restricted.[6 7 12] Previously, the largest comprehensive meta-analysis to date on protein supplementation during RET included 22 studies and 680 participants[7] and did show a significant effect of protein supplementation on RET-stimulated gains in strength and FFM. In agreement with this previous report,[7] and strengthening the conclusion of that same report by including 49 studies and 1863 participants, we show that protein supplementation augmented gains in FFM and strength with RET.

Strength

The average RET-induced increase, with all measures of 1RM included, was 27 kg (mean±SD; 27±22 kg[22 32]). Notably, dietary protein supplementation augmented the increase in 1RM strength by 2.49 kg (9%; figure 2; see online supplementary figure 4), which strongly suggests that the practice of RET is a far more potent stimulus for increasing muscle strength than the addition of dietary protein supplementation. None of our covariates (age, training status, postexercise protein dose or baseline protein intake) influenced the efficacy of protein supplementation on changes in 1RM strength. Improving performance of a specific task (eg, the 1RM of an exercise) is predominately determined by the practice of that task.[71] Though protein supplementation may slightly augment changes in 1RM (~9%), which may be important for those competing in powerlifting or weightlifting, it is pragmatic to advocate that if an increase in 1RM is the objective of an RET programme, a sufficient amount of work and practice at or around the 1RM is far more influential than protein supplementation.

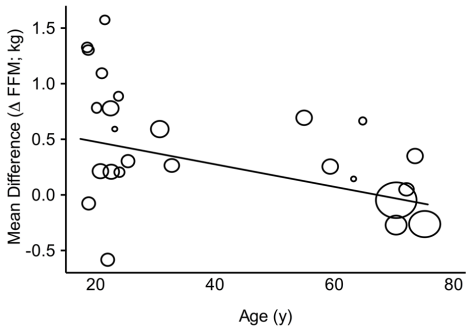

Figure 4 Random-effects univariate meta-regression between age and the mean difference in fat-free mass (FFM) between groups. Each circle represents a study and the size of the circle reflects the influence of that study on the model (inversely proportionate to the SE of that study). The regression prediction is represented by the solid line (–0.01 kg (–0.02,–0.00), p=0.02).

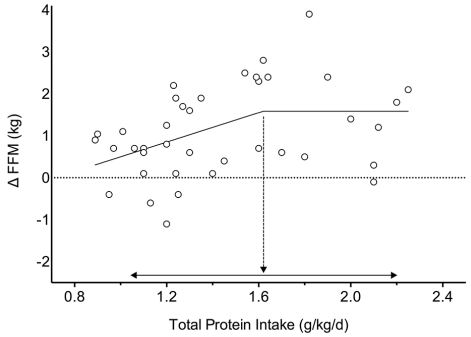

Figure 5 Segmental linear regression between relative total protein intake (g/kg body mass/day) and the change in fat-free mass (ΔFFM) measured by dual energy X-ray absorptiometry. Each circle represents a single group from a study. Dashed arrow indicates the break point=1.62 g protein/kg/day, p=0.079. Solid arrow indicates 95% CI, (1.03 to 2.20).

Muscle mass

In addition to increasing changes in muscle strength, RET alone (≥6; 13±8 weeks) resulted in an increase in FFM (1.1±1.2 kg), an increase in fibre CSA (808±) and an increase in mid-femur CSA (52±30 mm^2). Dietary protein supplementation augmented the increase in FFM by 0.30 kg (27%; figure 3; see online supplementary figure 4), fibre CSA by 310 μm^2 (38%; see online supplementary figure 2: panel A) and mid-femur CSA by 7.2 mm^2 (14%; see online supplementary figure 2: panel B). The postexercise protein dose did not affect the efficacy of protein supplementation on RET-induced changes in FFM whereas training status (positive), age (negative) and baseline protein intake (positive) did. Relative to untrained participants, resistance-trained participants have a smaller potential for muscle growth[72] and an attenuated postexercise muscle protein turnover.[73] As a result, we speculate that trained persons may have less 'degrees of freedom' to change with RET and therefore have a greater need for protein supplementation to see increases in muscle mass. Our thesis is supported by the observation of a more consistent impact of protein supplementation on gains in FFM in resistance-trained individuals than in novice trainees (figure 3).

Older individuals are anabolically resistant[74] and require higher per-meal protein doses to achieve similar rates of MPS, the primary variable regulating changes in skeletal muscle mass,[75] compared with younger participants.[14] The average supplemental daily protein dose given to older participants was surprisingly low (20±18 g/day); thus, it is perhaps not surprising that we did not find that older individuals were responsive to protein supplementation (figure 4). Though age did not affect

the RET-induced change in fibre CSA, the negative effect age had on changes in FFM leads us to speculate that even though exercise sensitises muscle to the effect of protein ingestion,[3] older persons have an increased need for higher protein intakes to optimally respond to this effect and see gains in FFM.[76]

It has been theorised that the increased deviation from normal protein intake (g/kg/day) will positively affect the RET-induced gains in FFM.[77] Contrary to this thesis, we found that a higher prestudy protein intake actually resulted in a greater effect of protein supplementation on changes in FFM (table 1); however, this was likely driven by the lower mean baseline protein intake (old: 1.2±0.2 g/kg/day, young: 1.5±0.4 g/kg/day) and daily protein dose (old: 20±18 g/day, young: 42±32 g/day) in the studies that included older participants (see online supplementary figure 3: panel B and D). Indeed, a sensitivity analysis that did not include older (>45; 65±14 years) versus younger (<45; 24±4 years) individuals found that baseline protein intake had no effect on the efficacy of protein supplementation in young individuals (see online supplementary figure 3, panel C). In an unadjusted meta-regression analysis, a higher baseline protein intake in young individuals actually attenuated the change in FFM (see online supplementary figure 3, panel D).

A goal of this meta-analysis was to deliver evidence-based recommendations that could be readily translated. A crucial point is that even though the mean baseline protein intake for the 1863 participants was ~1.4 g protein/kg/day, which is 75% greater than the current US/Canadian recommended dietary allowance (RDA),[78] an average supplementation of ~35 g protein/day still augmented RET-stimulated gain in FFM (figure 3) and 1RM strength (figure 2). Thus, consuming protein at the RDA of 0.8 g protein/kg/day appears insufficient for those who have the goal of gaining greater strength and FFM with RET. This conclusion is emphasised for older men[79] and women[80 81] wishing to obtain strength and gain lean mass with RET and protein supplementation.

A recent retrospective analysis showed a 'breakpoint' for the stimulation of MPS when ingesting an isolated protein source at 0.24 g protein/kg and 0.40 g protein/kg in younger and older participants, respectively.[14] Given the observation of a dose-responsive relationship between protein intake and MPS[82–85] and the fact that MPS is aligned with muscle hypertrophy,[13] we elected to use an identical two-segment regression approach between total daily protein intake and changes in FFM (figure 5) as has been done for changes in protein dose and MPS.[14] Here we provide significant insight (using 42 study arms including 723 young and old participants with protein intakes ranging from 0.9 g protein/kg/day to 2.4 g protein/kg/day) by reporting an unadjusted plateau in RET-induced gains in FFM at 1.62 g protein/kg/day (95% CI: 1.03 to 2.20). These results are largely in congruence with previous narrative reviews that comment on the optimal nutritional strategies to augment skeletal muscle adaptation during RET.[3 86] Given that the CI of this estimate spanned from 1.03 to 2.20, it may be prudent to recommend ~2.2 g protein/kg/d for those seeking to maximise resistance training-induced gains in FFM. Though we acknowledge that there are limitations to this approach, we propose that these findings are based on reasonable evidence and theory and provide a pragmatic estimate with an incumbent error that the reader could take into consideration.

Although the present analysis provides important and novel data, there are limitations that we acknowledge. First, the lack of RET research in older individuals has led to inconclusive recommendations from previous meta-analyses specifically focusing on older individuals.[9 11] Indeed, in this manuscript there were only 13 studies that met our inclusion criteria in older (>45 years) individuals and only six of those studies reported baseline protein intakes with changes in FFM. In addition, only four studies[27 29 33 45] in older individuals had participants that consumed what we consider to be close to optimal total protein intake (~1.2 g/kg/day to 1.6 g/kg/day) in non-exercising adults[5]. Furthermore, only two studies[23 30] in older individuals provided a postexercise supplemental protein dose that we consider to be close to optimal (~35–40 g) to stimulate FFM accretion in elderly individuals.[76] Given that older adults require more protein per day,[79–81] consume less protein per day[87] and that dietary protein ingestion and RET are effective strategies to maintain muscle mass and function with age,[67] future RET research should focus on using higher protein doses (or potentially higher leucine), larger sample sizes and longer interventions in ageing populations. Second, we included a variety of additional covariates into univariate meta-regressions to elucidate the variables that may modify whether protein supplementation affects RET-induced changes in muscle mass and strength. Such an approach is generally considered to be hypothesis generating. The only significant findings we found were that if the RET sessions were whole-body (adjusted R^2=76%, p=0.01) or supervised (adjusted R^2=58%, p=0.047), protein supplementation was more effective at augmenting changes in 1RM. No variable affected changes in FFM (see online supplementary table 3). Given the relatively small effect that protein supplementation has on changes in FFM and 1RM, clearly other variables as a component of RET programmes are of much greater importance. Our meta-analyses also only included studies with participants that were at or above their energy requirements, which may have omitted the significant impact protein has during periods of weight loss with RET.[88] Lastly, we found that the postexercise protein dose did not affect the efficacy of protein supplementation on RET-induced changes in FFM. Our analysis, and those from others,[6] leads us to conclude that the specifics of protein supplementation (eg, timing, postexercise protein dose or protein source) play a minor, if any, role in determining RET-induced gains in FFM and strength over a period of weeks. Instead, our results indicate that a daily protein intake of ~1.6 g/kg/day, separated into ~0.25 g/kg doses,[14] is more influential on adaptive changes with RET, at least for younger individuals.

CONCLUSION

Dietary protein supplementation augments changes in muscle mass and strength during prolonged RET. Protein supplementation is more effective at improving FFM in young or resistance-trained individuals than in older or untrained individuals. Protein supplementation is sufficient at ~1.6 g/kg/day in healthy adults during RET. Based on limited data we observed no overtly apparent sex-based differences but acknowledge that far less work has been done in women than men. This analysis shows that dietary protein supplementation can be, if protein intake is less than 1.6 g protein/kg/day, both sufficient and necessary to optimise RET-induced changes in FFM and 1RM strength. However, performance of RET alone is the much more potent stimulus, accounting, at least according to this meta-analysis, for a substantially greater portion of the variance in RET-induced gains in muscle mass and strength.

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Contributors RWM, BJS, MH, EH, AAA, MCD, JWK and SMP contributed to the conception and design of the study. RWM, BJS, MH, EH, AAA, MCD, LB, JWK and SMP contributed to the development of the search strategy. LB conducted the systematic search. RWM, KTM and SRM completed the acquisition of data. RWM and SMP performed the data analysis. All authors assisted with the interpretation.

Summary box

Background

- There is no consensus on the efficacy of protein supplementation during prolonged resistance exercise training (RET).

Novel findings

- Dietary protein supplementation augments changes in fat-free mass (FFM, (0.30 kg (0.09, 0.52), p=0.007) and one-repetition-maximum strength (2.49 kg (0.64, 4.33), p=0.01) during prolonged RET.
- Dietary protein supplementation during RET is more effective at increasing changes in FFM in resistance-trained individuals (0.75 kg (0.09, 1.40), p=0.03) and less effective in older individuals (−0.01 kg (−0.02,−0.00), p=0.02).
- Protein supplementation beyond a total daily protein intake of ~1.6 g/kg/day during RET provided no further benefit on gains in muscle mass or strength.

RWM and SMP were the principal writers of the manuscript. All authors contributed to the drafting and revision of the final article. All authors approved the final submitted version of the manuscript.

Competing interests SMP has received grant support, travel expenses, and honoraria for presentations from the US National Dairy Council. This agency has supported trials reviewed in this analysis.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All data are available in the submitted manuscript or as supplementary files.

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CHAPTER 3:

Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men

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Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men

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Morton RW, Oikawa SY, Wavell CG, Mazara N, McGlory C, Quadrilatero J, Baechler BL, Baker SK, Phillips SM. Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men. *J Appl Physiol* 121: 129–138, 2016. First published May 12, 2016; doi:10.1152/japplphysiol.00154.2016.—We reported, using a unilateral resistance training (RT) model, that training with high or low loads (mass per repetition) resulted in similar muscle hypertrophy and strength improvements in RT-naïve subjects. Here we aimed to determine whether the same was true in men with previous RT experience using a whole-body RT program and whether postexercise systemic hormone concentrations were related to changes in hypertrophy and strength. Forty-nine resistance-trained men (23 ± 1 yr, mean ± SE) performed 12 wk of whole-body RT. Subjects were randomly allocated into a higher-repetition (HR) group who lifted loads of ~30-50% of their maximal strength (1RM) for 20–25 repetitions/set ($n = 24$) or a lower-repetition (LR) group (~75–90% 1RM, 8–12 repetitions/set, $n = 25$), with all sets being performed to volitional failure. Skeletal muscle biopsies, strength testing, dual-energy X-ray absorptiometry scans, and acute changes in systemic hormone concentrations were examined pretraining and posttraining. In response to RT, 1RM strength increased for all exercises in both groups ($P < 0.01$), with only the change in bench press being significantly different between groups (HR, 9 ± 1, vs. LR, 14 ± 1 kg, $P = 0.012$). Fat- and bone-free (lean) body mass and type I and type II muscle fiber cross-sectional area increased following training ($P < 0.01$) with no significant differences between groups. No significant correlations between the acute postexercise rise in any purported anabolic hormone and the change in strength or hypertrophy were found. In congruence with our previous work, acute postexercise systemic hormonal rises are not related to or in any way indicative of RT-mediated gains in muscle mass or strength. Our data show that in resistance-trained individuals, load, when exercises are performed to volitional failure, does not dictate hypertrophy or, for the most part, strength gains.

load; testosterone; growth hormone; anabolism; strength training

NEW & NOTEWORTHY

We provide novel evidence of the effect of lifting markedly different (lighter vs. heavier) loads (mass per repetition) during whole-body resistance training on the development of muscle strength and hypertrophy in previously trained persons. Using a large sample size ($n = 49$), and contradicting dogma, we report that the relative load lifted per repetition does not determine skeletal muscle hypertrophy or, for the most part, strength development. In line with our previous work, acute postexercise systemic hormonal changes were unrelated to strength and hypertrophic gains.

RESISTANCE TRAINING (RT) is a potent stimulus for increasing skeletal muscle mass and strength (9, 30); however, the exact RT variables that determine skeletal muscle hypertrophy and strength remain a topic of continued investigation (3, 36). Current recommendations are that RT with relatively heavy [i.e., at ~70–85% one-repetition maximum (1RM)] loads ("load" herein referring to the amount of mass used per repetition) is a prerequisite for maximizing RT-induced hypertrophy (12, 31). It has even been suggested, on the basis of only acute electromyography (EMG) data [despite caution on use of EMG in this manner (10)], that greater motor unit recruitment occurs when lifting heavier loads even if heavier and lighter loads are performed to volitional failure (16, 21). Notably, this conclusion is at odds with existing data determined from long-term training studies (28, 33). We reported that load from as low as 30% and up to 90% of 1RM played a minimal role in stimulating muscle protein synthesis (4). Similar loading strategies also did not affect hypertrophy in a small sample of trained (33) or untrained (28) men following RT when the participants performed their RT to volitional failure. In addition, and in contrast to what others have proposed (18, 19, 31), we have also demonstrated that resistance exercise-induced increases in circulating hormones play little role in regulating muscle protein synthesis after an acute bout of resistance exercise (51) or skeletal muscle hypertrophy following RT (50). Taken together, our data suggest that factors regulating skeletal muscle hypertrophy in response to RT include neither load nor systemic hormonal concentrations (4, 28, 33, 50, 51).

While there is growing evidence that neither load (28, 33) nor acute postexercise increases in circulating hormones (50) affect RT-induced skeletal muscle hypertrophy, it is important to acknowledge that many of the aforementioned studies were conducted in healthy, but untrained participants (4, 28, 50, 51). Given that resistance-trained individuals exhibit an attenuated muscle protein synthetic response to resistance exercise (17, 53), they are likely less "adaptable" than untrained persons in terms of phenotypic adaptations of skeletal muscle in response to RT. In addition, the model used in previous trials (4, 28) was unilateral in nature, which is not a training model used in practice, and limb cross-education may have obscured a true estimate of strength development with the comparison of lighter vs. heavier loads (6).

The primary aim of this study was to determine the effects of a 12-wk higher-repetition (lower load) vs. a lower-repetition (higher load) RT intervention on skeletal muscle hypertrophy

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and strength development in resistance-trained young men. The secondary aim was to examine whether the acute postexercise increase in systemic hormones was correlated with changes in skeletal muscle mass or strength. Our hypothesis was that neither load nor the acute postexercise increase in systemic hormones would determine RT-induced adaptations.

METHODS

Participants. Forty-nine healthy young men (23 ± 1 yr, 86 ± 2 kg, 181 ± 1 cm, means ± SE) who had been engaging in RT for at least the past 2 yr [4 ± 2 yr, training >2 sessions per week (range 3–6 days/wk), including at least one weekly dedicated lower body session] volunteered to participate in this study. Recognizing the high interindividual response variability in hypertrophy and strength gain that occurs with RT (13, 27, 28, 48), we conducted the study with a large enough number of participants to allow detection of a 15% difference in hypertrophy via muscle fiber cross-sectional area (CSA) change and a 10% difference in fat- and bone-free (lean) body mass change measured by dual-energy X-ray absorptiometry (DXA) with 90% power based on previous work in trained men (33).

Ethics statement. All participants were informed of the purpose of the study, experimental procedures, and associated risks prior to participation and exercise testing. All participants gave verbal and written informed consent, which was approved by the Hamilton Integrated Research Ethics Board and conformed to the most recent Tri-Council policy statement on the use of human participants in research (http://www.pre.ethics.gc.ca/pdf/eng/tcps2-2014/TCPS_2_FINAL_Web.pdf). The trial was registered at https://clinicaltrials.gov as NCT02139865.

Familiarization and strength testing. Two weeks prior to the start of the RT protocol, participants completed a familiarization session to assess each participant's 10RM for each exercise. At least 72 h after any exercise, participants returned to the laboratory to complete 1RM (strength) testing on the inclined leg press (LP; Maxam Fitness, Hamilton, ON, Canada), barbell bench press (BP), machine-guided knee extension (KE; Atlantis, Laval, QC, Canada), and machine-guided shoulder press (SP; Life Fitness, Rosemont, IL). The same investigators administered all strength testing. In short, after a brief general warm-up, a specific warm-up of the given exercise was then performed at ~50% of the participant's estimated 1RM based on the 10RM testing. Load was progressively increased by ~10–20% for each repetition until a true 1RM was reached as previously described (5, 40). Three to five minutes of rest was given between each attempt. A successful attempt required the participant to move the load throughout the full range of motion with correct form.

Experimental design. A schematic illustration of the experimental design can be seen in Fig. 1*A*. A between-group, repeated measures design in which participants were randomly allocated to one of two possible conditions, high repetition (HR; $n = 29$) or low repetition (LR; $n = 27$; Fig. 2), was employed. For the training program the HR group performed 3 sets of 20–25 repetitions per set such that the load varied between ~30 and 50% of 1RM with each set being performed to volitional failure. The LR group performed 3 sets of 8–12 repetitions per set that corresponded to ~75–90% of 1RM with each set being performed to volitional failure (38). The loads were adjusted in between each set to ensure that the correct repetition range was maintained. Each participant underwent 12 wk of full-body RT 4 days per week. Session attendance was 97 ± 2% for the HR group and 96 ± 2% for the LR group with no difference between groups. Both groups performed 1RM testing at baseline and retested at 3, 6, 9, and 12 wk on what would be the participants' first session of the week. Participants consumed 30 g of whey protein (BioPRO; Davisco Foods International, Le Sueur, MN) twice per day: immediately following RT on training days (8) and the other prior to sleep (39). On nontraining days, participants consumed the first dose in the morning and the second dose 1–2 h prior to sleep, similar to training days.

Acute protocol. A schematic illustration of the acute blood sampling protocol can be seen in Fig. 1*B*. At least 72 h following the familiarization and strength testing, each participant came in after an overnight fast and received a muscle biopsy from the vastus lateralis and a resting blood sample via an intravenous antecubital cannula. Following the resting blood draw, a bout of resistance exercise was performed that consisted of a "superset" (exercises conducted in succession with no rest in between) including an incline leg press, hamstring curl, and knee extension. Participants were given 1 min of rest following each superset with three supersets performed in total. Each exercise was performed until volitional failure in their respective group repetition ranges (HR or LR). Following the bout of resistance exercise, the participant was given 30 g of pure whey protein (BioPRO; Davisco Foods International) mixed with 500 ml of water. Blood samples were collected at 0 (immediately post), 15, 30, and 60 min following the consumption of the protein beverage.

Hormone concentrations. Blood samples were obtained via a cannula that was inserted into an antecubital vein kept patent by periodic flushes of 0.9% saline. Tubes containing whole blood were allowed to clot for 30 min at room temperature before serum (4 ml) was isolated.

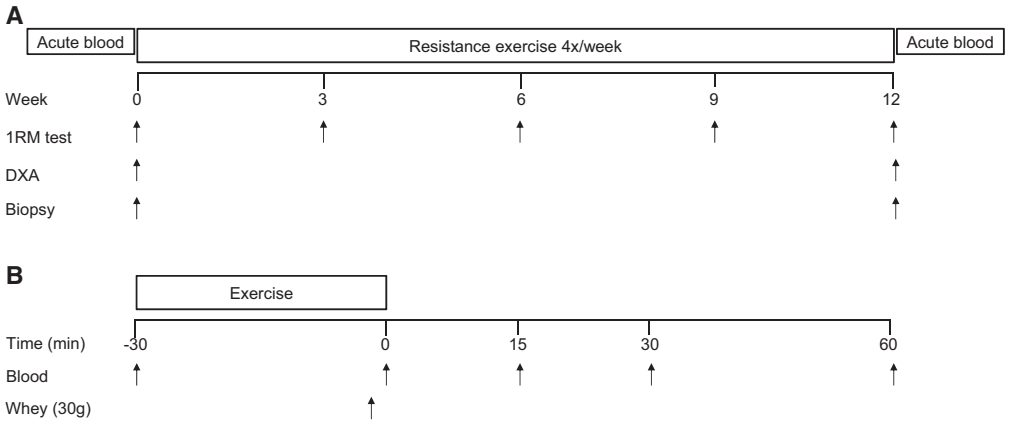

Fig. 1. Schematic representation of study protocol (*A*) and acute blood sampling protocol (*B*).

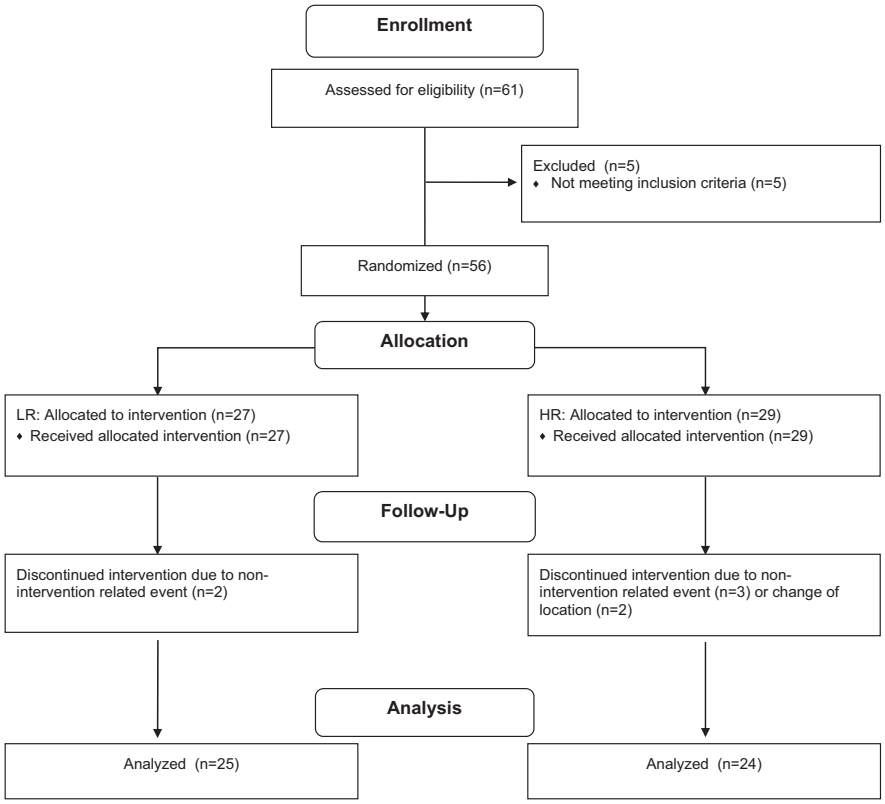

Fig. 2. Group allocation.

Heparinized tubes were used to isolate plasma (4 ml). All blood tubes were centrifuged at 4,000 *g* for 10 min at 4°C prior to serum and plasma being separated into cryotubes and frozen at −80°C until further analysis. Blood samples were analyzed for serum total testosterone (T; ng/dl), free T (fT; pg/ml), cortisol (nM), dihydrotestosterone (DHT; ng/ml), dehydroepiandrosterone (DHEA; ng/ml), luteinizing hormone (LH; IU/l), insulin-like growth factor 1 (IGF-1; μg/dl), free IGF-1 (fIGF-1; ng/ml), lactate (mM), and growth hormone (GH; ng/ml) using solid-phase, two-site chemiluminescence immunometric assays (Immulite; Intermedico, Holliston, MA) or radio-immunoassay (Diagnostics Products, Los Angeles, CA). All analyses resulted in interassay coefficients of variation (CV; $n = 245$) of less than 6% and intraassay CV ($n = 2{,}450$) on replicates of less than 4%.

Body composition. Body composition was assessed following an overnight fast (12 h) and >72 h following their last exercise bout both preintervention and postintervention. DXA measurements were conducted using a GE Lunar iDXA total body scanner (GE Medical Systems Lunar, Madison, WI) and analyzed with software (Lunar enCORE version 14.1; GE Medical Systems Lunar) in the medium scan mode. The machine was calibrated each testing day by using a three-compartment Universal Whole Body DXA Phantom: Oscar, Jr (Orthometrix, Naples, FL). The analysis regions used were standard regions where the head, torso, arms, and legs were subdivided by the software, but were subsequently checked manually, in a blinded manner, by a single investigator. Intrascan (without repositioning) and interscan (on different occasions) variability using the phantom was <1.6% for all tissues.

Dietary records. Dietary intake records were collected at 0, 3, 6, 9, and 12 wk and analyzed using the NutriBase dietary analysis software (Nutribase11 Professional Edition, version 11.5; Cybersoft, Phoenix, AZ).

Resistance-training intervention. The full-body RT was performed 4 days/wk (Monday, Tuesday, Thursday, and Friday). Each day included five exercises, consisting of two separate supersets and one additional exercise. Exercises were performed for three sets, with each set executed until volitional failure. One minute of rest was given between each set or superset. Each workout was repeated twice per week [Monday/Thursday: inclined leg press with seated row (superset 1), barbell bench press with cable hamstring curl (superset 2), and front planks (set 3). Tuesday/Friday: machine-guided shoulder press with bicep curls (superset 1), triceps extension with wide-grip pull downs (superset 2), and machine-guided knee extension (set 3)]. If necessary, loads were decreased (~5–10%) between sets to ensure repetitions were performed within the participant's assigned repetition range. Each participant was individually supervised by a trainer for each session to ensure each set was performed to volitional failure with correct technique. Participants' load was increased with subsequent training sessions when they could perform more repetitions than their designated repetition range. Weeks during the training intervention that included 1RM testing (*weeks 4*, *7*, and *10*) involved only

three prescribed sessions with 1RM testing to serve as the fourth session. Participants were asked to refrain from any additional exercise outside of the study.

Volume. The volume, sometimes referred to as "volume-load," of each set was calculated by multiplying the number of repetitions with the load. Total volume was calculated as the sum of each set's volume throughout the 12-wk RT intervention. Average session volume was calculated by dividing the total volume by the number of sessions that participant attended.

Muscle fiber type and cross-sectional area. Muscle biopsies were obtained from the vastus lateralis preintervention and postintervention. Biopsies were taken using a 5-mm Bergström needle custom modified for manual suction under local anesthesia (1% lidocaine). Participants had not participated in any physical activity for 72 h prior to each biopsy. Upon excision, the muscle samples were immediately cleared of visible connective tissue and fat and were oriented vertically by visual inspection before being embedded in optimal cutting temperature medium. The mounted muscle was frozen in isopentane, cooled by liquid nitrogen, and stored at −80°C until further analysis. Cross sections (7-μm thick) were cut on a Microm HM550 Cyrostat (Thermo Fisher Scientific, Waltham, MA), mounted on glass slides, and stained. Fiber type and CSA were assessed via immunofluorescent staining of myosin heavy chain (MHC) isoforms and dystrophin as previously described (2, 37). Primary antibodies against dystrophin (MANDYs), MHCI (BA-F8), MHCIIA (SC-71), and MHCIIX (6H1; Developmental Studies Hybridoma Bank, Iowa City, IA) followed by isotope-specific fluorescent secondary antibodies allowed for the identification of type I, type IIA, and type IIX fibers. Slides were mounted with Prolong Diamond Antifade Reagent (Life Technologies, Burlington, ON, Canada) and imaged the following day. Images were taken with a Nikon Eclipse 90i microscope at a magnification of 20X and captured with a Photometrics Cool SNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY). Analysis was completed using the Nikon NIS elements AR software (Nikon Instruments) on a large-scale image. All data reported in this manuscript, unless otherwise stated, have type IIA and type IIX fiber types pooled together and reported as type II fibers because of the number necessary to individually analyze type IIA and IIX fibers (~50–60) per sample (24, 25). Fiber CSA was determined by counting at least 100 individual fibers, and fiber type was assessed using the whole cross section of fibers (367 ± 18 fibers). All fibers selected for analysis were free of freezing artifact, and care was taken so that obliquely or longitudinally oriented fibers were not used in the analysis. Muscle fibers on the periphery of muscle cross sections were not used in the analysis. The same investigator, who was blinded to the time and group of each sample, conducted all immunofluorescent analyses. All mention of CSA refers to the muscle fiber CSA determined by muscle biopsy.

Statistical analysis. All analyses were performed using SPSS (version 22.0; Chicago, IL). Baseline characteristics were compared between groups using an independent *t*-test. The postexercise hormonal area under the curve (AUC) was calculated by subtracting the baseline concentration from the postexercise AUC of each hormone (60 min). Bivariate correlations were run for the two-tailed Pearson correlation coefficient between the postexercise hormone AUC and the change in strength and muscle mass. Muscle strength, lean body mass, muscle fiber CSA, muscle fiber type, and postexercise hormonal AUC were all analyzed using a two-factor (group × time) repeated measures analysis of variance (ANOVA) with group (between) and time (within) as the experimental variables. In addition, independent *t*-tests were performed with the independent variable as condition and the dependent variable as the absolute change for each measure of strength and muscle mass, all reported with their mean and 95% confidence intervals (CI). Statistical significance was accepted when $P \leq 0.05$. Results are presented as means ± SE in text and tables unless otherwise specified. To show the variability in response, graphs are presented as box-and-whisker plots including the median (lines), mean (crosses), interquartile range (boxes), and 95% CI (tails).

RESULTS

Descriptive characteristics. Forty-nine participants completed this study (Table 1). Participants were similar at baseline for all descriptive characteristics with no differences between groups ($P > 0.05$) with the exception of fat mass ($P < 0.05$; Table 1). Seven participants did not complete the study protocol because of non-intervention-related injuries ($n = 5$) or relocation ($n = 2$; Fig. 2). There was no significant difference in dietary intake of macronutrients or energy between groups at 0, 3, 6, 9, or 12 wk ($P > 0.05$; data not shown).

Body composition and muscle fiber CSA. Kolmogorov-Smirnov and Levene's tests were run for normality and homogeneity of variance, respectively, and all assumptions were met ($P > 0.05$). Following the intervention (using pooled means), there was an increase in type I [5,448 ± 152 to 6,113 ± 150 μm^2; $F(1,47) = 19.45$, $P < 0.001$; Fig. 3*B*] and type II [6,193 ± 176 to 7,171 ± 158 μm^2; $F(1,47) = 26.11$, $P < 0.001$; Fig. 3*D*] CSA with no significant difference between groups. Independent *t*-tests on the absolute change also revealed no difference between groups for muscle fiber CSA in either type I [$t(47) = -0.29$, $P = 0.77$, mean (M) = −88, 95% CI (−693, 518)] or type II [$t(47) = -0.52$, $P = 0.61$, M = −198, 95% CI (−967, 569)].

There were no group, time, or group by time interactions for type I and type II fiber type distributions with the intervention; however, with means pooled and all fiber types included (type I, IIA, and IIX), there was a shift from type IIX [10.3 ± 1.1 to 6.5 ± 0.72%; $F(1,47) = 8.95$, $P = 0.004$] to type IIA fibers [45 ± 1.7 to 49.7 ± 1.2%; $F(1,47) = 5.11$, $P = 0.03$].

Following the intervention (using pooled means), there was a significant increase in total fat- and bone-free mass [FBFM; 64.6 ± 1.1 to 65.8 ± 1.1 kg; $F(1,47) = 40.50$, $P < 0.01$; Fig. 3*F*] with no significant difference between groups indicated by ANOVA and by an independent *t*-test [$t(47) = -1.91$, $P = 0.091$, M = −0.73, 95% CI (−1.49, 0.04)]. There was also a significant increase in appendicular lean mass [ALM; 33.1 ± 0.6 to 34.0 ± 0.6 kg; $F(1,47) = 30.19$, $P < 0.001$] and leg lean mass [LLM; 24.4 ± 0.5 to 25.0 ± 0.5 kg; $F(1,47) = 16.97$, $P < 0.001$] with no significant differences between groups.

Strength. All exercises passed normality assessed by the Kolmogorov-Smirnov test ($P > 0.05$) with the exception of preintervention LP ($P = 0.03$) and BP ($P = 0.01$); however, assessment of histogram and probability-probability (P-P) plots revealed no kurtosis or skewness. Levene's test revealed no significance for any variable ($P > 0.05$). Maximum isotonic

Table 1. *Participants' baseline characteristics*

	HR ($n = 24$)	LR ($n = 25$)	*P*
Age, yr	23 ± 2	23 ± 2	0.73
Training age, yr	4.2 ± 2	4.6 ± 3	0.54
Total body mass, kg	88 ± 4	85 ± 2	0.57
Height, m	1.81 ± 1	1.80 ± 1	0.81
BMI, kg/m^2	26.9 ± 2	26.0 ± 2	0.41
Lean mass, kg	65.7 ± 2	65.7 ± 1	0.99
Total fat mass, kg	19.4 ± 2	16.9 ± 1	0.03
Leg press 1RM, kg	357 ± 21	353 ± 13	0.87
Bench press 1RM, kg	98 ± 4	97 ± 4	0.88
Knee extension 1RM, kg	76 ± 3	76 ± 3	0.92
Shoulder press 1RM, kg	91 ± 5	92 ± 4	0.87

Values are means ± SE. BMI, body mass index.

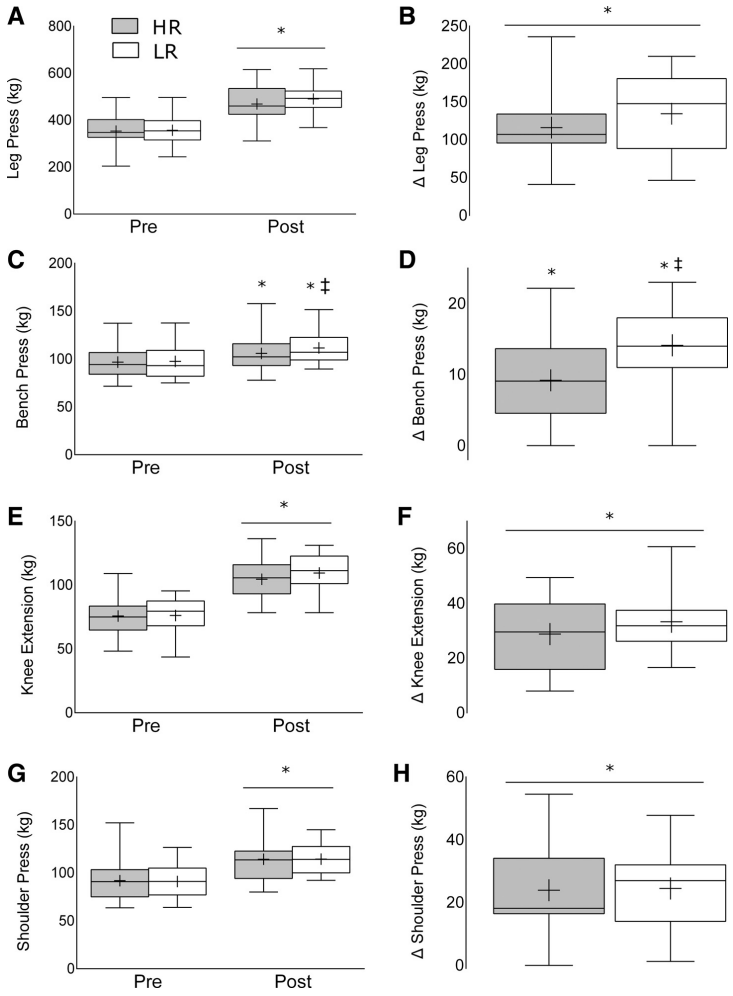

Fig. 4. Strength changes in the high-repetition (HR) and low-repetition (LR) groups following 12 wk of resistance training for the leg press absolute values (*A*) and change following training (*B*), bench press absolute values (*C*) and change following training (*D*), knee extension absolute values (*E*) and change following training (*F*), and shoulder press absolute values (*G*) and change following training (*H*). Values are presented as median (lines) with interquartile range (boxes) ± range (minimum and maximum), where + indicates mean. *Significantly different ($P < 0.05$) from baseline. ‡Significantly different ($P < 0.05$) between HR and LR.

DISCUSSION

Twelve weeks of supervised, higher- and lower-load per repetition RT programs were similarly effective at inducing skeletal muscle hypertrophy in resistance-trained participants when RT was performed to volitional failure. Additionally, when participants were tested periodically for maximal strength (i.e., essentially being allowed to practice their 1RM), the increases in muscular strength were not significantly different between groups. The exception was bench press 1RM, which increased to a greater extent in the LR group. Additionally, postexercise levels of circulating hormones did not change as a result of the RT intervention and were unrelated to changes in muscle mass and strength.

The amount of mass lifted per repetition (referred to here as load) is not a primary determinant of changes in muscle protein synthesis (4) or hypertrophy (28) when resistance exercise is performed until volitional failure in untrained participants. Mitchell et al. (28) demonstrated greater gains in muscle mass than in the present study following 10 wk of RT in untrained participants who performed only knee extension thrice weekly [i.e., Mitchell et al. (28) vs. present study: type I CSA, ~23 vs. ~12%; type II CSA, ~19 vs. 16%]. The attenuated gains in muscle size in the present study vs. those seen by Mitchell et al. (28) are congruent with previous literature showing a blunted training response in resistance-trained individuals, who would presumably have less capacity for adaptation since they are regularly exposed to the stimulus of RT (17, 42). Taken together with previous data (4, 28), the findings of the present study, along with a recent metaanalysis (35), do not support the assertion that higher-load RT is a prerequisite to maximize

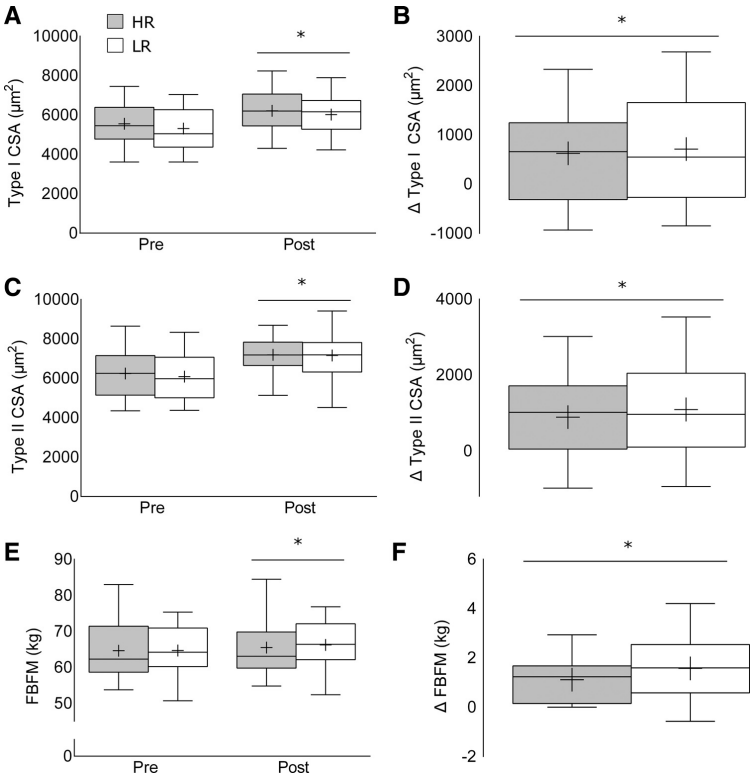

Fig. 3. Fiber cross-sectional area (CSA) and body composition changes in the high-repetition (HR) and low-repetition (LR) groups following 12 wk of resistance training including type I CSA absolute values (*A*) and change following training (*B*), type II fiber CSA absolute values (*C*) and change following training (*D*), and fat- and bone-free (lean) body mass (FBFM) absolute values (*E*) and change following training (*F*). Values are presented as median (lines) with interquartile range (boxes) ± range (minimum and maximum), where + indicates mean. *Significantly different ($P < 0.05$) from baseline.

strength (using pooled means) increased for LP [355 ± 10 to 480 ± 11 kg; $F(1,48) = 249.77$, $P < 0.001$], KE [76 ± 2 to 107 ± 2 kg; $F(1,47) = 216.91$, $P < 0.001$], SP [91 ± 3 to 112 ± 12 kg; $F(1,46) = 113.83$, $P < 0.001$], and BP [97 ± 3 to 109 ± 3 kg; $F(1,47) = 152.07$, $P < 0.001$; Fig. 4] following the intervention. There were no group by time differences for LP, KE, or SP; however, the change in BP was greater in the LR group (14 ± 1 kg) than in the HR group [9 ± 1 kg; $F(1,47) = 6.75$, $P = 0.012$; Fig. 4, *C* and *D*]. Independent *t*-tests on the absolute change also revealed no significant difference between groups for LP [$t(47) = -0.1$, $P > 0.05$, M = −2.55, 95% CI (−53, 48)], KE [$t(47) = -1.47$, $P > 0.05$, M = −6.03, 95% CI (−14, 2)], and SP [$t(47) = 0.55$, $P > 0.05$, M = 4.3, 95% CI (−11, 19)]; however, as the ANOVA results showed, there was a significant difference between group difference for BP [$t(47) = -2.6$, $P < 0.05$, M = −4.9, 95% CI (−8.7, −1.1)].

Resistance-training volume. Average volume per session was significantly lower in the LR group (14,805 ± 592 kg) than in the HR group (23,969 ± 901 kg; $P < 0.001$).

Hormone concentrations. Kolmogorov-Smirnov tests showed normality for all postexercise hormone AUCs ($P > 0.05$) with the exception of preintervention and postintervention cortisol ($P < 0.001$); however, assessment of histogram and P-P plots revealed little to no kurtosis or skewness. Levene's test revealed that preintervention lactate ($P = 0.03$), preintervention cortisol ($P = 0.03$), and postintervention lactate ($P = 0.01$) were significant. The hormone concentrations were not "corrected" for blood volume shifts, which have a negligible impact on the results, as we propose that the "uncorrected" concentrations are what the target tissues (i.e., muscle) would be exposed to in vivo. Every blood outcome (T, fT, DHT, DHEA, cortisol, IGF-1, fIGF-1, GH, LH, and lactate) increased as a result of the acute exercise bout ($P < 0.001$). There was a group difference preintervention for the postexercise AUC of DHT [HR, 13.6 ± 0.7; LR, 17.7 ± 0.7 ng·ml^{-1}·min^{-1}] with a group by time effect [HR, 1.2 ± 1; LR, −2.9 ± 0.8 ng·ml^{-1}·min^{-1}, $P = 0.003$] such that the postexercise AUC for DHT was similar between groups postintervention (Fig. 5). There were no other group, time, or group by time differences for any postexercise hormonal AUC.

Correlations. There were weak to moderate correlations for a variety of hormones though the change in type II CSA with preintervention ($r = -0.34$, $P = 0.02$) and postintervention ($r = -0.31$, $P = 0.04$) cortisol, the change in LP with preintervention fIGF-1 ($r = 0.40$, $P = 0.01$), the change in SP with postintervention lactate ($r = -0.36$, $P = 0.01$), and the change in BP with preintervention LH ($r = 0.43$, $P = 0.003$) AUC were all significant (Table 2). No other hormone at any time point was significantly correlated with the change in hypertrophy or strength.

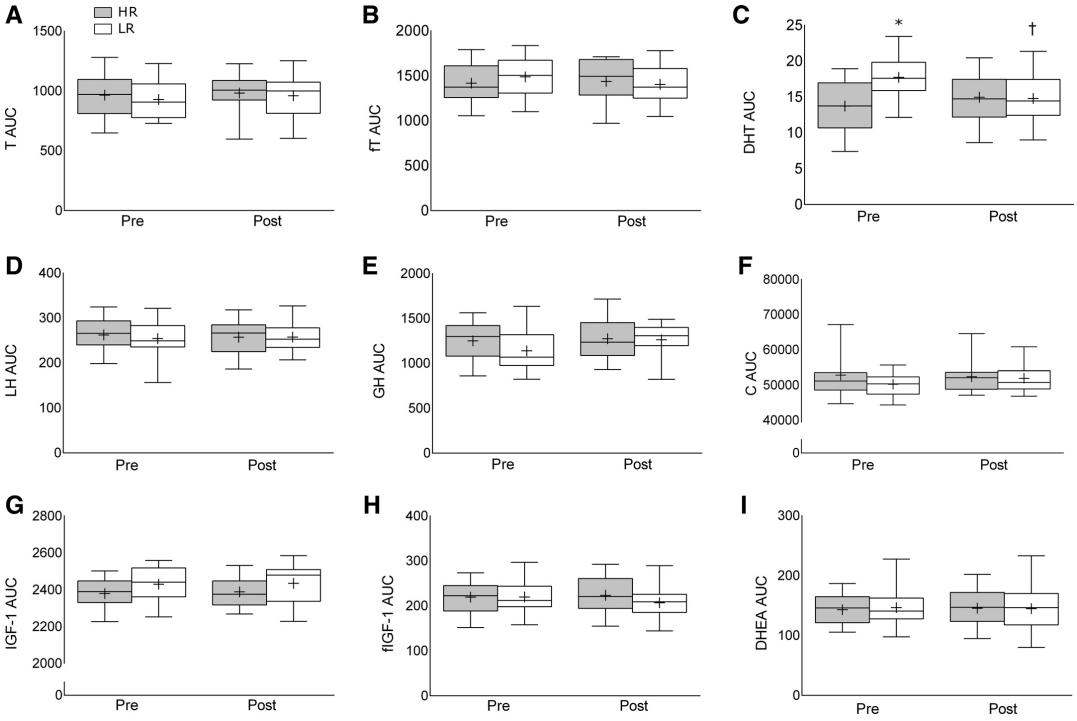

Fig. 5. Acute postexercise area under the curve (AUC) preintervention and postintervention for testosterone (T; *A*), free testosterone (fT; *B*), dihydrotestosterone (DHT; *C*), luteinizing hormone (LH; *D*), growth hormone (GH; *E*), cortisol (C; *F*), insulin-like growth factor 1 (IGF-1; *G*), free IGF-1 (fIGF-1; *H*), and dehydroepiandrosterone (DHEA; *I*). Values are presented as median (lines) with interquartile range (boxes) ± range (minimum and maximum), where + indicates mean. HR, high-repetition group (20–25 repetitions per set); LR, low-repetition group (8–12 repetitions per set). *Significantly different ($P < 0.05$) from HR. †Significant group by time effect ($P < 0.05$).

RT-induced muscle hypertrophy especially when lower-load exercises are performed to volitional failure.

Few studies have addressed the effect of load with hypertrophy and strength as main outcomes when the exercise sessions are not volume-matched (20, 28). Indeed, in a volume-matched situation, low-repetition (high load) RT appears to provide a greater stimulus for hypertrophy and strength gains (5, 15, 41); however, it is obvious that when performing RT with lighter loads, a greater lifting volume (repetitions × load) is needed to reach volitional failure. In the present study, which had participants perform RT until volitional failure, average session volume performed in the LR group was only ~62% of that performed by the HR group. We hypothesize that the increased volume performed by the HR group allowed them to reach volitional failure, which led to the similar adaptations seen in the LR group, a finding consistent with previous studies

Table 2. *Pearson correlation coefficients for the postexercise hormonal area under the curve preintervention and postintervention and measures of muscle hypertrophy and strength*

	Postexercise AUC													
	T		fT		DHT		IGF-1		fIGF-1		GH		Cortisol	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Δ type I CSA	0.26	0.1	0.29	0.07	−0.1	0.13	0.06	0.17	−0.16	−0.03	−0.1	−0.28	−0.06	−0.07
Δ type II CSA	0.13	0.02	0.18	0.20	0.02	0.06	0.16	−0.02	0.02	−0.05	−0.2	−0.21	−0.34*	−0.3*
Δ LBM	−0.01	−0.02	0.08	−0.12	0.22	−0.26	0.15	0.11	0.25	−0.04	0.19	−0.01	0.05	0.26
Δ LP	0.26	0.1	0.02	0.10	0.06	−0.06	−0.2	−0.05	0.4*	−0.23	0.04	−0.12	−0.16	0.07
Δ BP	−0.12	0.23	−0.1	−0.11	0.14	0.1	−0.1	0.01	0.12	−0.09	−0.3	−0.15	−0.22	0.01

Change (Δ) in type I muscle fiber cross-sectional area (CSA), type II muscle fiber CSA, lean body mass (LBM), leg press (LP), and bench press (BP). The preexercise and postexercise hormone areas under the curve (AUCs, 60 min, see METHODS for details) are reported. Pre, preintervention; post, postintervention; T, total testosterone; fT, free testosterone; DHT, dihydrotestosterone; IGF-1, insulin growth-like factor 1; fIGF-1, free IGF-1; GH, growth hormone. *Significantly correlated ($P < 0.05$).

(4, 28, 33). Alternatively viewed, performance of a LR set at 80% of 1RM and a HR set performed at 40% of 1RM would result, at volitional failure, in the LR set having lost only ~20% of their force-generating capacity and the HR group having lost ~60% of their force-generating capacity. To be clear, it is apparent that a HR group would have to perform more repetitions (thus more volume) and lose more of their force-generating capacity (fatigue) to reach volitional failure on any given set. While the mechanisms underlying fatigue may be different between groups (11, 22), at volitional failure the size principle would dictate that larger motor units have been recruited in an attempt to sustain the required force (14, 26). There have been recent claims that greater EMG amplitude seen with a higher- vs. a lower-load condition is equivalent to greater motor unit drive and thus greater potential for hypertrophy (21); however, such a premise is fundamentally incorrect as has been pointed out (45, 46). The current data, along with previous work (28, 35), are direct proof that hypertrophy and strength gains are not a function of the load lifted and directly contradict the assertion that acute EMG recordings predict hypertrophic potential (21). Instead, we propose that exercising until volitional failure with adequate volume and load (between 30–90% 1RM) will sufficiently activate muscle motor units, which drives skeletal muscle hypertrophy.

Studies that have used volume-matched groups often have participants lift in a lower-repetition (higher load) condition to volitional failure to determine the volume that the higher-repetition (lower load) group will match (15, 41). This scenario would, we argue, not allow the high-repetition group to perform their RT to volitional failure and would result in an inferior stimulus. For example, Holm et al. (15) examined untrained young men performing volume-matched unilateral RT and found that low-repetition RT resulted in a significantly greater increase in muscle CSA (measured via magnetic resonance imaging) compared with the high-repetition RT (7.6 vs. 2.6%, respectively). Indeed, work from our group using a similar model indicates that a higher-repetition, lower-load group volume-matched to a lower-repetition, higher-load group produces a substantially inferior muscle protein synthesis response (4). In contrast, however, lower loads, when lifted to volitional failure (i.e., using a greater volume than the higher-load condition), results in a similar stimulation of muscle protein synthesis (4) and equivalent hypertrophy (28). Even if different RT programs are manipulated to have participants exercise until volitional failure and be volume-matched (e.g., more sets) (34), it remains apparent that the similar adaptations are a result of the resistance exercise being performed until volitional failure. Thus, in the current protocol, our participants performed their RT, regardless of group assignment, to volitional failure. As mentioned previously, allowing the HR group to perform more volume, resulting in volitional failure, there was fatigue that would have driven motor unit recruitment (4, 28) and therefore hypertrophy of the muscle fibers innervated by both large and small motor units (28, 29).

Following the 12-wk intervention, there were similar increases in muscular strength between groups. Specifically, both HR and LR increased LP, KE, and SP 1RM with no differences between groups. However, while both groups increased BP 1RM, the increase was greater in the LR group compared with the HR group (15 vs. 9%; Fig. 4, *C* and *D*). Notably, others have also found similar increases in 1RM in healthy untrained (15) and trained (33) men performing either low- or high-load RT. It is evident that current literature supports the use of both low-repetition (high load) (1, 20, 41) and high-repetition (low load) (5, 28, 44) RT to induce increases in maximal strength. Our results support the concept that maximal strength increases can be achieved with the use of either low or high loads, so long as there is periodic practice of lifting with heavier loads, whereas the disparity in BP 1RM changes remain in agreement with literature supporting the use of high loads with a low repetition range. We have previously reported greater increases in isotonic 1RM when performing RT with high loads (80% 1RM) than low loads (30% 1RM); however, when strength was evaluated with an unpracticed test, a 5-s isometric maximum voluntary contraction using a dynamometer, there was no difference between groups (28). Indeed, strength is a product of muscle mass (23), neural adaptation (7, 32), and "practice" of the desired outcome. Though there is no apparent advantage of lifting with different loads on changes in muscle mass, there is undoubtedly a neuromuscular advantage to lifting heavier loads if the primary outcome is performing a 1RM test (28). Conversely, it appears that periodic practice of the chosen strength outcome (e.g., 1RM) is effective at eliminating the majority of any posttraining difference.

A further purpose of the current study was to investigate the effects of novel (DHT, DHEA, and LH) and canonical (IGF-1, GH, and T) postexercise, circulating hormones that have been hypothesized to provide an anabolic stimulus [for reviews, see Kraemer and Ratamess (19) and Vingren et al. (47)]. An acute bout of exercise induces a significant but transient systemic rise in a variety of hormones and metabolites (19). It has been previously reported that the postexercise hormonal environment does not contribute to the resistance exercise-induced muscle protein synthetic response (51) or hypertrophy following RT (50). Despite women having ~15- and 45-fold lower resting and postexercise systemic T concentrations, respectively, men and women experience similar magnitudes of myofibrillar protein synthesis in response to the same RT stimulus (49). West and Phillips (52) concluded that anabolic hormones such as GH, IGF-1, and T have little to no correlation with changes in hypertrophy and strength as a result of a 12-wk RT intervention. The present study adds to these results by comparing the hormonal response to different (high and low load) RT regimens in resistance-trained persons. We observed no correlations, at any time point, between the postexercise AUC for T, GH, and IGF-1 and changes in muscle mass and strength. Last, the postexercise concentrations of any of the aforementioned hormones are not even moderately ($r > 0.45$) relevant indicators of RT-induced changes in muscle mass and strength in resistance-trained men (Table 2) and do not change as a result of RT (Fig. 5). We acknowledge that the acute exercise trial was conducted in the fasted state, which may limit the direct applicability of these data to the applied setting; however, when subjects were fed, we have also not observed relationships between hormones and hypertrophy (52).

It is important to acknowledge that our repetition ranges and loads were chosen to match previous study "intensities" (4, 5, 15, 28, 43, 44) and replicate those of current guidelines set forth by the American College of Sports Medicine (31) and National Strength and Conditioning Association (12). As mentioned before (28) and in a recent review (29), we propose that

muscle hypertrophy is fundamentally driven by motor unit activation. The current data demonstrate that performing RT with high and low repetitions (using low and high loads, respectively) to volitional failure provides a similar and sufficient stimulus, though neither are necessary, for hypertrophy or strength. In conjunction with previous data (28), it appears that if 1RM strength is the primary goal, performing the to-be-tested exercise with heavier loads, either consistently and/or periodically, may be required for optimal improvement. Thus lifting heavier and lighter loads should not be mutually exclusive in terms of promoting RT adaptations, but as training "zones" that could easily be used in RT programs without the expectation that strength or muscle mass gains would be significantly compromised, though we acknowledge that training paradigms should be tailored to the individual's goals and preferences.

In conclusion, high- and low-repetition (low and high load, respectively) training paradigms elicit a comparable stimulus for the accretion of skeletal muscle mass when resistance exercise is performed until volitional failure. The current findings taken together with previous reports (1, 20, 28) show that these effects are not contingent upon training status or study design. Increases in lean body mass, as an indirect measure of muscle mass, and muscle fiber CSA, a direct measure of muscle area, occurred in both LR and HR groups with no differences between groups. There was a significant increase in 1RM strength for the leg press, knee extension, and shoulder press exercises, again with no differences between groups. While 1RM bench press increased in both groups, it increased to a greater extent in the LR group. We speculate that because the participants in the HR group performed greater volume, they were able to exercise until volitional failure, which allowed for maximal activation of their motor units and ultimately led to the similar increases in muscle strength and hypertrophy seen in the LR group. In agreement with previous studies (50–52) it is clear that the postexercise increases in systemic hormone concentrations are unrelated to changes in muscle hypertrophy or strength.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.W.M., S.Y.O., C.M., and S.M.P. conception and design of research; R.W.M., S.Y.O., C.G.W., N.M., C.M., J.Q., S.K.B., and S.M.P. performed experiments; R.W.M., S.Y.O., C.G.W., N.M., C.M., B.L.B., and S.M.P. analyzed data; R.W.M., S.Y.O., C.G.W., N.M., C.M., S.K.B., and S.M.P. interpreted results of experiments; R.W.M., S.Y.O., C.M., J.Q., and S.M.P. prepared figures; R.W.M., S.Y.O., C.M., S.K.B., and S.M.P. drafted manuscript; R.W.M., S.Y.O., C.G.W., N.M., C.M., J.Q., S.K.B., and S.M.P. edited and revised manuscript; R.W.M., S.Y.O., C.G.W., N.M., C.M., J.Q., B.L.B., S.K.B., and S.M.P. approved final version of manuscript.

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CHAPTER 4:

Muscle androgen receptor content but not systemic hormones is associated with resistance training-induced skeletal muscle hypertrophy in healthy, young men

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Muscle Androgen Receptor Content but Not Systemic Hormones Is Associated With Resistance Training-Induced Skeletal Muscle Hypertrophy in Healthy, Young Men

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The factors that underpin heterogeneity in muscle hypertrophy following resistance exercise training (RET) remain largely unknown. We examined circulating hormones, intramuscular hormones, and intramuscular hormone-related variables in resistance-trained men before and after 12 weeks of RET. Backward elimination and principal component regression evaluated the statistical significance of proposed circulating anabolic hormones (e.g., testosterone, free testosterone, dehydroepiandrosterone, dihydrotestosterone, insulin-like growth factor-1, free insulin-like growth factor-1, luteinizing hormone, and growth hormone) and RET-induced changes in muscle mass (n = 49). Immunoblots and immunoassays were used to evaluate intramuscular free testosterone levels, dihydrotestosterone levels, 5α-reductase expression, and androgen receptor content in the highest- (HIR; n = 10) and lowest- (LOR; n = 10) responders to the 12 weeks of RET. No hormone measured before exercise, after exercise, pre-intervention, or post-intervention was consistently significant or consistently selected in the final model for the change in: type 1 cross sectional area (CSA), type 2 CSA, or fat- and bone-free mass (LBM). Principal component analysis did not result in large dimension reduction and principal component regression was no more effective than unadjusted regression analyses. No hormone measured in the blood or muscle was different between HIR and LOR. The steroidogenic enzyme 5α-reductase increased following RET in the HIR (P < 0.01) but not the LOR (P = 0.32). Androgen receptor content was unchanged with RET but was higher at all times in HIR. Unlike intramuscular free testosterone, dihydrotestosterone, or 5α-reductase, there was a linear relationship between androgen receptor content and change in LBM (P < 0.01), type 1 CSA (P < 0.05), and type 2 CSA (P < 0.01) both pre- and post-intervention. These results indicate that intramuscular androgen receptor content, but neither circulating nor intramuscular hormones (or the enzymes regulating their intramuscular production), influence skeletal muscle hypertrophy following RET in previously trained young men.

Keywords: resistance exercise, testosterone, intramuscular, androgen receptor, hypertrophy

INTRODUCTION

There is substantial individual variability in RET-induced skeletal muscle hypertrophy (Hubal et al., 2005; Davidsen et al., 2011). The post-exercise rise in circulating, presumably anabolic, hormones (e.g., T, GH, and IGF-1) are believed to be causative in determining RET-induced skeletal muscle hypertrophy (Kraemer et al., 2017; Mangine et al., 2017). However, there is substantial contrary evidence for a causal, or even related (i.e., sharing common variance) role of such hormones in both RET-induced increases in muscle protein synthesis (West et al., 2009) and hypertrophy (West et al., 2010; West and Phillips, 2012; Mitchell et al., 2013; Morton et al., 2016; Mobley et al., 2018).

It is plausible that, as opposed to systemic circulating hormones, local intramuscular androgenesis could mediate RET-induced muscle hypertrophy as has been proposed for older men (Sato et al., 2014). In addition, the RET-induced increase in intramuscular androgen receptor content has been significantly correlated with RET-induced muscle hypertrophy (Ahtiainen et al., 2011; Mitchell et al., 2013). Thus, it may be that an increase in intramuscular androgens and/or their receptors, via an autocrine mechanism, are important in determining RET-induced hypertrophy.

The purpose of this study was to determine if the heterogeneity in RET-induced skeletal muscle hypertrophy, measured using multiple indices, was associated with circulating hormones, intramuscular hormones, intramuscular steroidogenic enzyme content, or androgen receptor content. We performed additional statistical and intramuscular analyses on data from a previous study in healthy, resistance-trained men (n = 49; Morton et al., 2016). To further explore the relationship between systemic hormones and hypertrophy we used backward elimination and principal component regression on systemic hormone concentrations both at rest and post-resistance exercise with indices of hypertrophy as separate outcome variables in all participants. To evaluate the significance of intramuscular androgenesis we completed an analysis on only our highest- (HIR – top quintile) and lowest- (LOR – bottom quintile) responders that included evaluation of intramuscular T, DHT, 5α-reductase expression, and androgen receptor content. Consistent with our previous work (West et al., 2010; West and Phillips, 2012; Mitchell et al., 2013; Morton et al., 2016), we hypothesized that circulating systemic hormones would not be related to any measure of hypertrophy; however, we hypothesized, given previous findings (Ahtiainen et al., 2011; Mitchell et al., 2013), that androgen receptor content would be associated with RET-induced hypertrophy.

Abbreviations: AUC, area under the curve; CSA, cross sectional area; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; fIGF-1, free insulin-like growth factor 1; fT, free testosterone; GH, growth hormone; HIR, high responders; HR, high repetition; IGF-1, insulin-like growth factor 1; LBM, lean body mass; LH, luteinizing hormone; LOR, low responders; LR, low repetition; RET, resistance exercise training; T, testosterone.

MATERIALS AND METHODS

Participants and Resistance Exercise Training Intervention

Forty-nine resistance-trained (performing RET at least 2 days/week [range 3–6 days/week] for 4 ± 6 years) young men volunteered for this study. Each participant was informed of associated risks with the RET intervention and testing and the study was carried out in accordance with the recommendations of the most recent Tri-Council statement on research in human participants[1]. The protocol was approved by the Hamilton Integrated Research Ethics Board and all subjects gave written informed consent in accordance with the Declaration of Helsinki. The trial was registered at https://clinicaltrials.gov/ as NCT02139865. An overview of the RET intervention can be read in detail in the original manuscript (Morton et al., 2016). Briefly, participants were randomly allocated to either a high repetition (HR) or low repetition (LR) group. The HR group performed all exercises with relatively light resistance [∼30–50% of their repetition maximum (RM)] until volitional failure (20–25 repetitions) and the LR group performed all exercises with relatively heavy resistance (∼75–90% RM), also until volitional failure (8–12 repetitions). Each participant underwent a 12-week RET intervention where they performed whole-body RET 4 days/week and received 30 g of whey protein isolate twice per day (BioPRO; Davisco Foods International, Le Sueur, MN, United States).

Blood Collection and Hormone Analysis

The pre- and post-intervention testing day was performed after an overnight fast at the same time of day for each participant. Each participant performed an acute bout of resistance exercise within their designated group assignment (HR or LR) and blood was drawn from an intravenous catheter inserted in an antecubital vein. Two 4 mL vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, United States) were collected pre-exercise and 0-, 15-, 30-, and 60-min post-exercise. One 4 mL tube was allowed to clot for 30 min at room temperature to later isolate serum and the other was heparinized to later isolate plasma. Blood sample analysis was done blinded for: cortisol (nM), LH (IU/L), lactate (mM), DHEA (ng/mL), T (ng/mL), free T (fT; ng/dL; i.e., testosterone that is not bound to sex hormone-binding globulin or albumin in the blood), DHT (ng/mL), and GH (ng/mL) using solid-phase, two site chemiluminescence immunometric assays (Immulite 2000 Immunoassay System; Siemens Healthineers, Erlangen, Germany) and IGF-1 (μg/dL) and free IGF-1 (fIGF-1; ng/mL) using radio-immunoassays (Diagnostics Products Corporation, Los Angeles, CA, United States). The 60-min post-resistance exercise AUC was calculated for each hormone, using the trapezoidal rule, with time points at 0, 15, 30, and 60 min.

[1]http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf

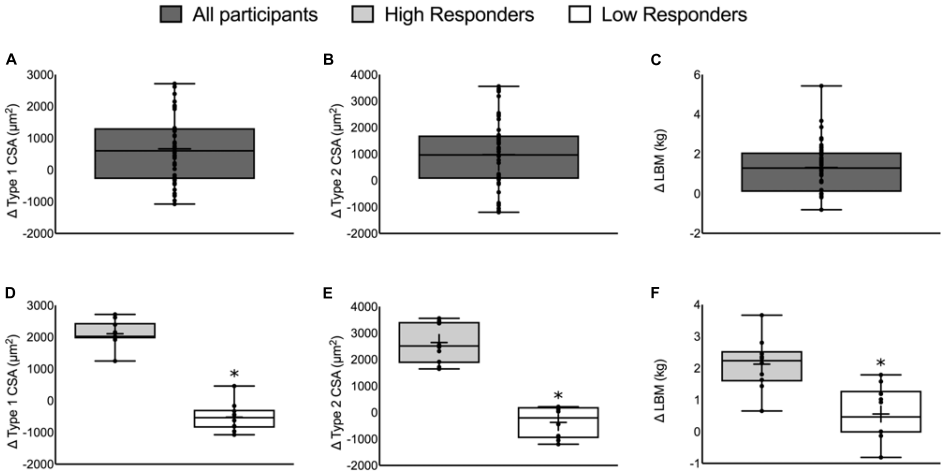

FIGURE 1 | The change in muscle mass in all participants (top) and HIR and LOR (bottom). Top panels: The change in **(A)** type 1 CSA, **(B)** type 2 CSA, and **(C)** LBM from all 49 participants. Bottom panels: The change in **(D)** type 1 CSA, **(E)** type 2 CSA, and **(F)** LBM categorized into HIR and LOR. Values are presented as median (lines) with interquartile range (boxes), range (minimum and maximum), and mean (cross). *Significant difference between high- and low-responders ($P < 0.01$). Panels **A–C** adapted from Morton et al. (2016).

Stepwise Regressions

HR and LR data were collapsed due to a lack of difference in both circulating hormones and outcomes between-groups (Morton et al., 2016). The outcomes considered were type 1 fiber CSA, type 2 fiber CSA, and fat- and bone-free (lean) body mass (LBM). Each outcome at each time of measurement (i.e., the change, absolute pre-, and absolute post-intervention values) were regressed against hormones from each time point: pre-intervention resting, pre-intervention post-exercise AUC, post-intervention resting, and post-intervention post-exercise AUC. Backward elimination, with the Akaike Information Criterion (AIC) as the elimination criterion, was used to choose the final model. The post-exercise AUC values used in the analysis did not subtract out the resting concentrations. We did, however, run the analysis with the resting concentrations subtracted from the AUC raw values and there were no major differences in our results.

Immunoblot Analysis

As previously described (Aizawa et al., 2010), after homogenization, the protein concentration of resulting supernatant was determined by a Bradford protein assay, and muscle proteins (both cytoplasmic and nuclear, 20 μg protein) were separated on 10% SDS-polyacrylamide gels and then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, United States). The membranes were blocked for 1 h with blocking buffer (5% skim milk in phosphate-buffered saline with 0.1% Tween 20) and then incubated for 12 h at 4°C with primary antibodies against androgen receptor (#3202, Cell Signaling Technology, Beverly, MA, United States) and 5α-reductase (H00006715, Abnova, Taipei, Taiwan) diluted to 1:1000 in blocking buffer. The membranes were washed three times with PBST before being incubated for 1 h with a horseradish peroxidase-conjugated secondary antibody and anti-rabbit immunoglobulin (#7074, Cell Signaling Technology, Beverly, MA, United States) diluted to 1:3000 in the blocking buffer. The membranes were then washed with PBST three times. The proteins were detected using an enhanced chemiluminescence plus system (GE Healthcare Biosciences) and visualized on an LAS4000 imager (GE Healthcare Biosciences). Band intensities were quantified using ImageJ version 1.46 (National Institutes of Health, Bethesda, MD, United States).

Enzyme Immunoassays for Intramuscular Hormones

Muscle sample was homogenized using the same method as the immunoblot analysis. The levels of T and DHT in skeletal muscle were determined using an enzyme-linked immunosorbent assay kit, after being diluted 200 times with each assay buffer as previously described (Horii et al., 2016). The immobilized polyclonal antibodies were raised against T (Cayman Chemical, Ann Arbor, MI, United States) and DHT (IBL Hamburg, Germany) before secondary horseradish peroxidase antibodies were added. Optical density at 450 nm was qualified on a microplate reader (BioLumin 960; Molecular Dynamics, Tokyo, Japan) and were assayed in duplicate. The coefficient of variation value was 3.0 and $r^2 = 0.974$ in the present study. The researchers that performed the intramuscular analyses (KS and SF) were not blinded to which samples were HIR and LOR.

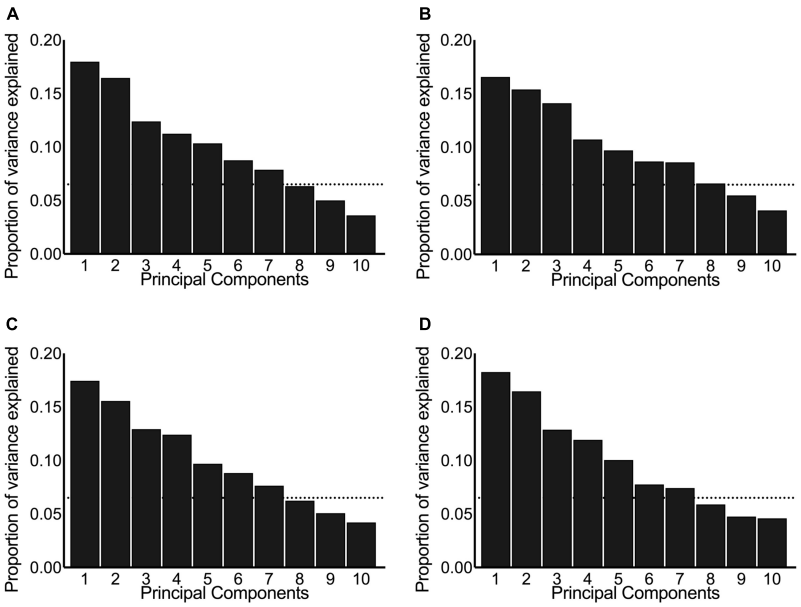

FIGURE 2 | Scree plots illustrating the proportion of variance in the original hormones explained by each principal component. The panels include the principal components derived from systemic hormones measured: **(A)** pre-intervention post-exercise, **(B)** post-intervention post-exercise, **(C)** pre-intervention resting, and **(D)** post-intervention resting. The dotted horizontal line indicates the cut-off point where the principal components above explained ≥80% variance between the original data set of hormones.

Principal Component Analysis and Regression

The data were centered and scaled before principal component analysis (PCA) was performed on the hormones from each time of measurement (pre-intervention resting, pre-intervention post-exercise AUC, post-intervention resting, and post-intervention post-exercise AUC). The purpose of PCA is to use orthogonal transformation to create a set of new linear, uncorrelated variables (principal components), a subset of which is taken that effectively accounts for most of the variability seen in the original data. Ultimately, these principal components are linear combinations of the original variables (e.g., hormones) that are later used as covariates in regression analyses herein. We present the PCA here in scree plots. Backward elimination was performed on the principal components (i.e., principal component regression) using AIC as the model fit criterion. PCA and principal component regression were performed in R (R Core Team, 2017).

High- vs. Low-Responders

Skeletal muscle biopsies from each participant's *vastus lateralis* and DXA were used to assess the change in fiber CSA (both type 1 and type 2) and LBM, respectively, as described in detail elsewhere (Morton et al., 2016). The determination of HIR and LOR was done by individually ranking (from 1 to 49) the change in each outcome for each participant and then averaging each participant's rank across all three outcomes (type 1 CSA, type 2 CSA, and LBM). With a probability of type II error (*alpha*) of 0.05, a type I error probability (*beta*) of 0.20, and a relatively moderate expected difference in RET-induced changes in muscle mass between HIR and LOR (effect size, f = 0.60), *a priori* sample size calculations required 18 participants (nine in each group). Thus, the top quintile (n = 10) of ranked participants were categorized as the HIR and the bottom quintile (n = 10) of ranked participants were categorized as the LOR. Statistical analyses between HIR and LOR was performed using SPSS (version 22.0, Chicago, IL, United States). Type 1 CSA, type 2 CSA, LBM, and all intramuscular hormone-related data were analyzed using a two-factor (group × time) repeated measures analysis of variance (ANOVA) with group (HIR vs. LOR) and time (pre- vs. post-intervention) as the experimental variables. If indicated, independent two-tailed t-tests were run to evaluate any differences between-groups at a specific time point (e.g., pre-intervention intramuscular T). Correlations between intramuscular outcomes and the change in type 1 CSA, type 2 CSA, and LBM were performed in SPSS (version 22.0, Chicago, IL, United States). Statistical significance was accepted when $P < 0.05$. Data are presented as box and

whisker plots (including the median [line], mean [cross], interquartile range [box], and minimum and maximum values [whiskers]) in **Figures 1**, **3** and mean ± SD in text and tables.

RESULTS

Changes in Muscle Mass With Resistance Exercise Training

Fifty-six participants were recruited and 49 participants completed the whole intervention (HR: n = 24, LR: n = 25; 23 ± 2 years, 86 ± 5 kg, 181 ± 6 cm). Two individuals dropped out from the LR group due to work relocation and a non-intervention related injury and five individuals dropped out of the HR group due to either a change in location or a non-intervention related injury. Twelve weeks of RET resulted in an increase in type 1 CSA (665 ± 149 μm^2), type 2 CSA (978 ± 189 μm^2), and LBM (1.22 ± 1.37 kg, $P < 0.01$; **Figures 1A–C**, respectively; Morton et al., 2016). There were no differences between repetition groups (HR versus LR – see Morton et al., 2016) for any of the outcomes.

Stepwise Regressions

For each outcome (change in type 1 CSA, type 2 CSA, and LBM) none of the post-exercise AUC (**Table 1**) or the resting concentrations (**Table 2**) of any hormone measured either pre- or post-intervention were consistently significant (i.e., significant with multiple outcomes or at more than one time of measurement) in the final models. Furthermore, the coefficients of determination (i.e., R^2) values were low (<0.25) for all outcomes at each time of measurement indicating that little of the variation seen in the hypertrophic response can be explained by any model fitted here. Similar results were found when evaluating the pre- and post-intervention type 1 CSA, type 2 CSA, and LBM against resting hormone concentrations (**Supplementary Table 1**).

Principal Component Analysis

Principal component analysis was performed on centered and scaled predictors and is presented here as scree plots for the pre-intervention post-exercise AUC (**Figure 2A**), post-intervention post-exercise AUC (**Figure 2B**), pre-intervention resting concentrations (**Figure 2C**), and post-intervention resting concentrations (**Figure 2D**). As illustrated by the shallow-sloped

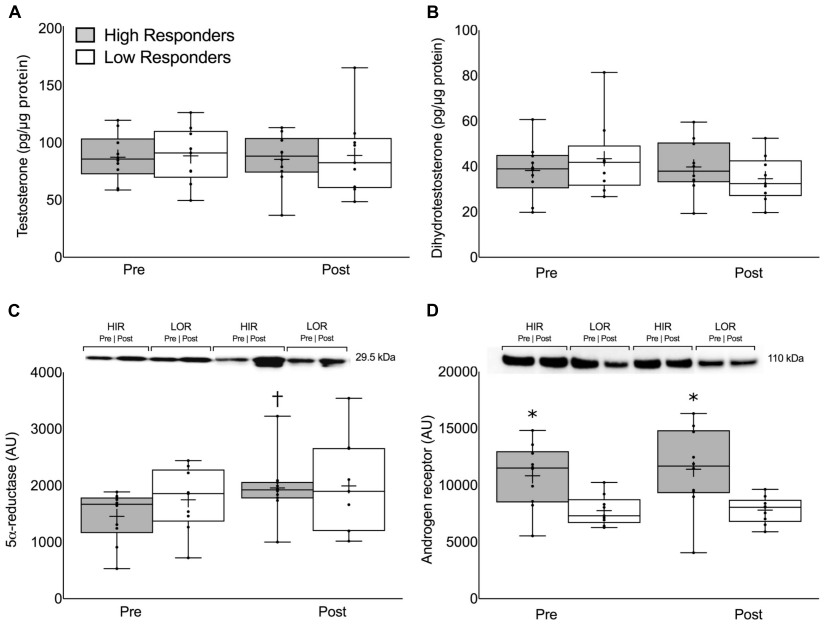

FIGURE 3 | Intramuscular **(A)** free testosterone concentration, **(B)** dihydrotestosterone concentration, **(C)** 5α-reductase expression, and **(D)** androgen receptor content presented pre- and post-intervention in both high- and low-responders. Representative immunoblots for 5α-reductase expression **(C)** and androgen receptor content **(D)** are shown between HIR and LOR both pre- and post-intervention with their respective weights. Values are presented as median (lines) with interquartile range (boxes), range (minimum and maximum), and mean (cross). †Significant difference between pre- and post-intervention ($P < 0.01$). *Significant difference between high- and low-responders ($P < 0.05$).

scree plots, no principal component was particularly effective at explaining variance in the original data set.

We chose to keep the number of principal components that explain ≥80% of the variance in the original predictors, which resulted in seven principal components included in each of our principal component stepwise regressions. Running principal component stepwise regression (regardless of whether the hormones were evaluated at rest, post-exercise, pre-intervention, or post-intervention) revealed that no principal component was consistently significant or consistently included in any of the final models and that the final R^2 never exceeded 0.25 and was as low as 0.05 (**Supplementary Tables 2–4**). These results indicate that very little of the variation seen in the hypertrophic response to RET can be explained by any of the fitted models.

High- vs. Low-Responders

There was a significant difference between HIR and LOR in the change in type 1 CSA (HIR: 2106 ± 412, LOR: −520 ± 450 μm^2), type 2 CSA (HIR: 2642 ± 756, LOR: −373 ± 593 μm^2), and LBM (HIR: 2.1 ± 0.8, LOR: 0.6 ± 0.8 kg, $P \leq 0.001$; **Figures 1D–F**). There was no difference in the number of participants from each training group (HIR: four and six and LOR: six and four from HR and LR, respectively).

There was no difference in any resting hormone concentration between HIR and LOR with the exception of the post-intervention resting concentration of LH (HIR: 3.67 ± 0.63; LOR 4.59 ± 1.15 IU/L, $P < 0.01$) and lactate (HIR: 0.52 ± 0.05; LOR: 0.55 ± 0.07 mM, $P = 0.02$), which were greater in the LOR. There was no difference in the post-exercise AUC for any hormone between HIR and LOR with the exception of pre-intervention post-exercise cortisol, which was higher in the HIR (HIR: 576 ± 100; LOR: 508 ± 199 nM; $P < 0.001$).

Intramuscular Hormones

There were no differences in the pre-intervention, post-intervention, or change in intramuscular T or DHT between HIR and LOR (**Figures 3A,B**, respectively). The change in 5α-reductase expression was significant in HIR (pre: 1457 ± 450, post: 1957 ± 543 AU, $P < 0.01$) but not in LOR (pre: 1748 ± 559, post: 1994 ± 840 AU, $P = 0.32$; **Figure 3C**). The pre-intervention (HIR: 10827 ± 2789, LOR: 7759 ± 1323 AU, $P < 0.01$) and post-intervention (HIR: 11406 ± 2789, LOR: 7801 ± 1189 AU, $P = 0.01$; **Figure 3D**) intramuscular androgen receptor content was significantly greater in HIR versus LOR. There was no change in intramuscular androgen receptor content pre- to post-intervention (Δ319 ± 1314 AU, $P = 0.75$) and there was a linear relationship between the participants' pre- and post-intervention androgen receptor content ($r = 0.92$).

There were no significant correlations between the pre-intervention, post-intervention, or change in intramuscular T, DHT, or 5α-reductase with the change in type 1 CSA, type 2 CSA, or LBM ($P > 0.05$; **Supplementary Table 5**). In contrast, pre-intervention, post-intervention, and the average between pre- and post-intervention androgen receptor content was significantly correlated with the change in LBM (pre: $r = 0.76$, $P < 0.01$; post: $r = 0.75$, $P < 0.01$; average: $r = 0.77$, $P < 0.01$), type 1 CSA (pre: $r = 0.51$, $P = 0.03$; post: $r = 0.49$, $P = 0.04$; average: $r = 0.51$, $P = 0.03$), and type 2 CSA (pre: $r = 0.61$, $P < 0.01$; post: $r = 0.65$, $P < 0.01$; average: $r = 0.64$, $P < 0.01$; **Supplementary Table 5** and **Figure 4**). One participant's data was removed from the regression analyses that included the change in LBM because it was identified as a statistical outlier via the robust regression and outlier removal method at a coefficient of 1% (Motulsky and Brown, 2006). We have indicated the location of this participant in **Figure 4** for illustrative purposes.

TABLE 1 | Backward elimination regression final output between post-exercise systemic hormone AUC and the change in type 1 CSA, type 2 CSA, and LBM.

	Pre-intervention post-exercise AUC					Post-intervention post-exercise AUC			
	Estimate	**SEM**	***t*-Value**	***p*-Value**		**Estimate**	**SEM**	***t*-Value**	***p*-Value**
Δ Type 1 CSA					**Δ Type 1 CSA**				
Intercept	636	160	4.0	0.01	Intercept	669	145	4.6	0.01
DHEA	−230	162	−1.4	0.16	DHT	−239	147	−1.6	0.11
					fIGF-1	305	147	2.1	0.04
	F = 2.03	*df = 42*	$R^2 = 0.05$	*pv = 0.16*		*F = 3.27*	*df = 45*	$R^2 = 0.13$	*pv = 0.05*
Δ Type 2 CSA					**Δ Type 2 CSA**				
Intercept	949	184	5.2	0.01	Intercept	982	190	5.2	0.01
LH	−508	197	−2.6	0.01	fT	−337	200	−1.7	0.10
GH	371	199	1.9	0.07	DHEA	−287	200	−1.4	0.16
DHEA	−287	188	−1.5	0.14					
	F = 3.63	*df = 40*	$R^2 = 0.21$	*pv = 0.02*		*F = 1.93*	*df = 45*	$R^2 = 0.08$	*pv = 0.16*
Δ LBM					**Δ LBM**				
Intercept	1.2	0.2	6.0	0.01	Intercept	1.2	0.2	6.3	0.01
fIGF-1	0.3	0.2	1.6	0.12	DHT	−0.3	0.2	−1.4	0.17
					Lactate	−0.4	0.2	−2.0	0.05
	F = 2.54	*df = 42*	$R^2 = 0.06$	*pv = 0.12*		*F = 2.67*	*df = 45*	$R^2 = 0.11$	*pv = 0.08*

TABLE 2 | Backward elimination regression final output between resting hormones and the change in type 1 CSA, type 2 CSA, and LBM.

	Pre-intervention resting					Post-intervention resting			
	Estimate	**SEM**	***t*-Value**	***p*-Value**		**Estimate**	**SEM**	***t*-Value**	***p*-Value**
Δ Type 1 CSA					**Δ Type 1 CSA**				
Intercept	667	147	4.6	0.01	Intercept	667	140	4.8	0.01
IGF-1	232	148	1.6	0.12	T	−207	143	−1.4	0.16
	F = 2.45	*df = 47*	*R^2 = 0.03*	*pv = 0.12*	LH	−258	143	1.8	0.08
					Cortisol	−218	143	−1.5	0.13
						F = 2.93	*df = 45*	*R^2 = 0.16*	*pv = 0.04*
Δ Type 2 CSA					**Δ Type 2 CSA**				
Intercept	978	182	5.4	0.01	Intercept	978	183	5.4	0.01
LH	−403	186	−2.2	0.04	LH	−327	185	−1.8	0.08
GH	293	186	1.6	0.12	Cortisol	−283	185	−1.5	0.13
	F = 3.10	*df = 46*	*R^2 = 0.12*	*pv = 0.06*		*F = 2.76*	*df = 46*	*R^2 = 0.11*	*pv = 0.07*
Δ LBM					**Δ LBM**				
Intercept	1.2	0.2	6.8	0.01	Intercept	1.2	0.2	6.8	0.01
DHT	−0.4	0.2	−2.2	0.03	fT	0.3	0.2	1.7	0.11
Lactate	−0.3	0.2	−1.7	0.09	DHT	−0.3	0.2	−1.8	0.09
Cortisol	0.4	0.2	2.0	0.06	GH	0.4	0.2	1.9	0.06
	F = 3.84	*df = 45*	*R^2 = 0.20*	*pv = 0.02*		*F = 4.26*	*df = 45*	*R^2 = 0.22*	*pv = 0.01*

DISCUSSION

The main finding of the present study, consistent with our previous work, was that no systemic hormone shared significant variance with RET-induced changes in skeletal muscle fiber CSA or skeletal muscle mass in resistance-trained men (**Tables 1, 2**). We extend these findings to include local muscle-measured hormonal concentrations, which also did not show a significant association with any index of hypertrophy. We found that HIR had increased 5α-reductase content following 12 weeks of RET and had significantly higher androgen receptor content, which did not change with RET, than LOR both prior to- and after-RET (**Figure 3**). We conclude that neither systemic nor local muscular hormone availability influence RET-induced skeletal muscle hypertrophy in healthy young men. Consistent with previous work, we propose instead that the magnitude of RET-induced skeletal muscle hypertrophy is modulated in part by intramuscular androgen receptor content (**Figure 4**) and likely other intramuscular variables.

Circulating Hormones and Resistance Exercise Training

Recent publications (Kraemer et al., 2017; Mangine et al., 2017) and guidelines (Ratamess et al., 2009) claim that circulating hormones are mechanistically and directly related to, and predictive of, RET-induced changes in skeletal muscle mass despite contrary evidence that they are not (West et al., 2010; West and Phillips, 2012; Mitchell et al., 2013; Morton et al., 2016; Mobley et al., 2018). In a previous study, we ran 120 correlations, each on 49 participants, between 10 different hormones and various measures of changes in muscle mass and strength. We found that only the post-exercise rise in cortisol was correlated with changes in type 2 CSA (pre-intervention: $r = -0.34$, $P = 0.02$; post-intervention: $r = -0.31$, $P = 0.04$) (Morton et al., 2016). Others have found significant correlations between the post-exercise rise in circulating GH (McCall et al., 1999) and T (Ahtiainen et al., 2003; Brook et al., 2016) with changes in muscle mass but those correlations were run on samples consisting of less than 11 participants, which could give rise to spurious correlations. Here, we ran an additional 48 stepwise regressions from 49 participants, 10 hormones, and three separate hypertrophy-related outcomes including muscle fiber size. We found that no hormone was consistently significant, nor did any final model have a high coefficient of determination, i.e., all R^2 values were below 0.25. Moreover, PCA was not effective at reducing the total variance amongst the original hormone data (**Figure 2**) and there was no regression model with the principal components used as covariates that explained a meaningful proportion of the variability in any outcome (**Supplementary Tables 2–4**). There is now substantial evidence to suggest that circulating systemic hormones measured at rest (McCall et al., 1999; Morton et al., 2016; Mobley et al., 2018) and/or post-exercise (Ahtiainen et al., 2003; West et al., 2010; West and Phillips, 2012; Mitchell et al., 2013; Morton et al., 2016) share no common variance and are thus neither related to nor predictive of RET-induced changes in muscle mass in healthy young participants.

A recent study (Mangine et al., 2017) used partial least squares-structural equation modeling (PLS-SEM) and reported that a model with composite hormonal scores (T, GH, IGF-1, insulin, and cortisol) and a composite measure of hypertrophy (muscle CSA and thickness from the vastus lateralis and rectus femoris) resulted in a significant coefficient of determination ($R^2 = 0.73$). The interpretation of this finding was that the composite hormonal score was related to a composite score of

hypertrophy. What is troubling with this interpretation is that the model without T (the model's best hormonal predictor) still had a substantial coefficient of determination (R^2 = 0.43) with the hypertrophy composite score and was statistically significant. In fact, individual removal of the other hormones (GH, IGF-1, insulin, and cortisol) showed negligible effect on the shared variance of the model and yet the model *without* its 'best' predictive hormone, T, accounted for almost 60% of the variance seen *with* that hormone present in the model. While the authors argued for unexplained interactions between hormones as being a reason for the model variance without T, we suggest it is more likely that PLS weights capitalize on chance to exaggerate the correlations (Goodhue et al., 2012). While we see value in PLS-SEM for examination of large datasets, there are substantial limitations to interpretation when small sample sizes (n = 26) are used (Goodhue et al., 2012). Defining PLS as an appropriate SEM method has also been called into question for estimation and inference (Rönkkö and Evermann, 2013) and the coefficient of determination (e.g., R^2) is a poor yardstick for assessing PLS-SEM model fit because inconsistent estimators can produce models with high R^2. Consequently, not all well-fit models are predictive (Henseler et al., 2014) and not all predictive models are well-fit (McIntosh et al., 2014).

High- vs. Low-Responders to Resistance Exercise Training

To investigate potential determinants of the heterogeneity in RET-induced skeletal muscle hypertrophy (Hubal et al., 2005; Davidsen et al., 2011; Morton et al., 2016), we stratified 49 participants into HIR (n = 10) and LOR (n = 10) based on their change in three indicators of skeletal muscle mass (type 1 CSA, type 2 CSA, and LBM; **Figure 1**). Despite large between-group differences in each outcome there were no meaningful differences in any circulating pre- or post-exercise hormone measured either pre- or post-intervention. Considering steroid hormones are lipid-soluble (e.g., they diffuse across the sarcolemma according to their concentration gradient) it is not surprising that intramuscular T and DHT measured pre- and post-intervention were also not different between HIR and LOR (**Figure 3**). The lack of difference in circulating and intramuscular hormones between HIR and LOR provides evidence that neither hormone delivery to the muscle nor the transfer of steroid hormones inside the muscle are rate-limiting steps in healthy, young individuals.

Androgen receptor content was significantly higher both pre- and post-intervention in the HIR compared to the LOR (**Figure 3**) and was correlated with changes in muscle mass (**Figure 4**). Though another group has found no difference in androgen receptor content between HIR and LOR to RET (Mobley et al., 2018), it is important to acknowledge the differences in study design (e.g., untrained vs. trained participants) and outcome measurements (i.e., cluster analysis based on muscle thickness vs. an aggregate score of DXA and fiber CSA) between them and our work, respectively. The function of an androgen receptor is, when bound with an androgen, to translocate to the nucleus and modify expression of target genes [reviewed elsewhere (Beato and Klug, 2000)], many of which are known targets involved in skeletal muscle growth and development (Wyce et al., 2010). Indeed, when androgen receptors are knocked out in male mice there is a significant reduction in muscle mass and strength (MacLean et al., 2008). Importantly, most steroid hormones have a high affinity with

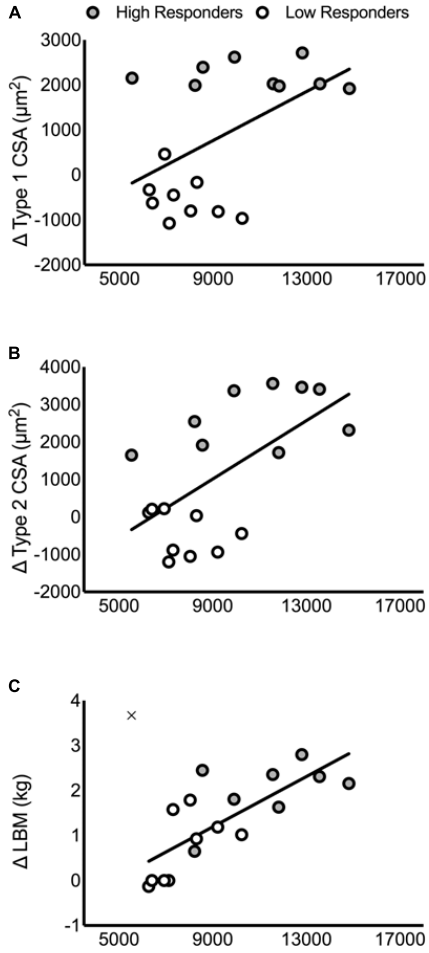

FIGURE 4 | Correlations between the pre-intervention intramuscular androgen receptor content and changes in muscle mass. Correlations are presented in panels for: **(A)** type 1 CSA (r = 0.51, P = 0.03), **(B)** type 2 CSA (r = 0.61, $P < 0.01$), and **(C)** LBM (r = 0.76, $P < 0.01$). In **(C)**, the outlier that was removed from the correlational analysis between pre-intervention androgen receptor content and LBM is included on the figure as an '×.'

their steroid receptors. For example, the dissociation constant of the androgen receptor to T and DHT is only ∼0.2 to 0.5 nM (Wilson and French, 1976). In the present study, at rest, the molarity of serum T (HIR: 28 ± 7; LOR: 31 ± 7 nM), serum fT (HIR: 0.5 ± 0.01; LOR: 0.5 ± 0.01 nM) and serum DHT (HIR and LOR: 0.7 ± 0.2 nM) all exceeded 0.2–0.5 nM. Given there was no difference in circulating or intramuscular hormones between HIR and LOR, along with high androgen-androgen receptor binding affinity, it seems likely that both at rest and post-exercise existing androgen receptors would have been saturated in skeletal muscle. We hypothesize that though androgen delivery may be a rate-limiting step for RET-induced muscle hypertrophy in hypogonadal men (Bhasin et al., 1997; Kvorning et al., 2013), androgen receptor content is the more important variable in RET-induced androgen-mediated skeletal muscle protein accretion in healthy men (Diver et al., 2003).

Limitations

We performed 120 correlations in a previous study (Morton et al., 2016) and 48 stepwise regressions here (24 on original data and 24 on the principal components). Applying multiple analyses on the same data was intentional data mining to demonstrate the lack of ability of resting or post-exercise circulating and intramuscular hormones to predict baseline or RET-induced changes in skeletal muscle mass. We could have performed additional statistics to account for multiple testing but this would be uninformative because none of our models explained much variance (as assessed by R^2 values, which did not exceed 0.25). We also acknowledge that although we included a large sample size (n = 49) for our systemic hormone analysis we limited ourselves to a relatively smaller sample size (n = 20) for our HIR and LOR comparison. We fully admit that in the case of the androgen receptor correlation what we present is an inflated estimate due to the choice of measuring only higher and lower responders to our training protocol. We did our analysis this way to illustrate the difference in RET-induced muscle hypertrophy and investigate the influence of circulating and intramuscular hormone-variables on two distinct groups. Though we were limited by the amount of tissue collected, it is a fair critique that our correlational analysis would be more telling if we included all participants and if we performed additional analyses [e.g., nuclear and cytoplasmic fractions of androgen receptor content as well as multiple gene expressions (Cheung et al., 2017)]. Hence, there is an opportunity for future work to focus on the specific biology that governs androgen receptor regulation and function. Others have postulated that mass spectrometry analysis (as opposed to immunoassays) is necessary to detect small, intramuscular concentrations of steroid hormones (Handelsman and Wartofsky, 2013); however, our intent was to analyze our samples using methods similar to those that others have used in exercise science, which may be dissimilar to those in clinical endocrinology. We recognize that using DXA to measure changes in LBM is not the gold standard, which is why we elected to also include change in type 1 and type 2 fiber CSA to determine our HIR and LOR (Buckinx et al., 2018). In regards to our interpretation, it is naïve to suggest that androgen signaling is exclusively operational via its tendency to bind to an androgen receptor [reviewed elsewhere (Herbst and Bhasin, 2004; Dubois et al., 2012)]. Though transcriptional regulation (e.g., androgen–androgen receptor signaling) is evidenced here as a potent modulator of RET-induced changes in muscle mass, it is also clear that post-transcriptional regulation is at least equally as important for protein synthesis (Schwanhausser et al., 2011) as has been highlighted by recent findings (Figueiredo et al., 2015; Robinson et al., 2017; Mobley et al., 2018) and reviews (Chaillou et al., 2014; McGlory et al., 2017). Lastly, though there is genetic influence that underpins RET-induced skeletal muscle hypertrophy, there are still many environmental considerations, for example consuming adequate dietary protein (Morton et al., 2017), that modulate RET-induced muscle hypertrophy.

CONCLUSION

We performed backward elimination and principal component regression on a relatively large cohort (n = 49) of resistance-trained men and conclude that the post-exercise AUC (i.e., acute transient net hormonal exposure) and resting hormone concentrations measured in the blood do not share common variance with RET-induced changes in muscle mass. That is, systemic hormone concentrations are not related to, or in any way predictive of, RET-induced changes in muscle mass. Performing subset analysis on the highest- and lowest-responders revealed that androgen receptor content, not intramuscular androgen levels, does not change with RET in trained participants but is significantly higher in HIR than LOR to RET. This study, in conjunction with others (Bamman et al., 2007; Petrella et al., 2008; Davidsen et al., 2011; Eynon et al., 2013), provides evidence that the relative increase in skeletal muscle mass following RET is underpinned by local intramuscular factors and not systemic hormonal concentrations.

AUTHOR CONTRIBUTIONS

RM, SO, and SP conceived the research design and conducted the study. RM and MG performed the statistical analyses. PM and SP provided statistical advice. RM, KS, SF, and SP performed data analysis. RM and SP drafted the manuscript. RM, KS, MG, SO, PM, SF, and SP revised and approved the final draft of the manuscript.

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Institutes for Health Research, and the Natural Science and Engineering Research Council of Canada for their support during the completion of this work. The authors declare that the results of the study above are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01373/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CHAPTER 5:

Muscle fibre activation is unaffected by load and repetition duration when resistance exercise is performed to task failure

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Muscle fibre activation is unaffected by load and repetition duration when resistance exercise is performed to task failure

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Key points

- Performing resistance exercise with heavier loads is often proposed to be necessary for the recruitment of larger motor units and activation of type II muscle fibres, leading to type II fibre hypertrophy. Indirect measures [surface electromyography (EMG)] have been used to support this thesis, although we propose that lighter loads lifted to task failure (i.e. volitional fatigue) result in the similar activation of type II fibres.
- In the present study, participants performed resistance exercise to task failure with heavier and lighter loads with both a normal and longer repetition duration (i.e. time under tension).
- Type I and type II muscle fibre glycogen depletion was determined by neither load, nor repetition duration during resistance exercise performed to task failure.
- Surface EMG amplitude was not related to muscle fibre glycogen depletion or anabolic signalling; however, muscle fibre glycogen depletion and anabolic signalling were related.
- Performing resistance exercise to task failure, regardless of load lifted or repetition duration, necessitates the activation of type II muscle fibres.

Abstract Heavier loads (>60% of maximal strength) are considered to be necessary during resistance exercise (RE) to activate and stimulate hypertrophy of type II fibres. Support for this proposition comes from observation of higher surface electromyography (EMG) amplitudes during RE when lifting heavier *vs.* lighter loads. We aimed to determine the effect of RE, to task failure, with heavier *vs.* lighter loads and shorter or longer repetition durations on: EMG-derived variables, muscle fibre activation, and anabolic signalling. Ten recreationally-trained young men performed four unilateral RE conditions randomly on two occasions (two conditions, one per leg per visit). Muscle biopsies were taken from the vastus lateralis before and one hour after RE. Broadly, total time under load, number of repetitions, exercise volume, EMG amplitude (at the beginning and end of each set) and total EMG activity were significantly different between

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conditions ($P < 0.05$); however, neither glycogen depletion (in both type I and type II fibres), nor phosphorylation of relevant signalling proteins showed any difference between conditions. We conclude that muscle fibre activation and subsequent anabolic signalling are independent of load, repetition duration and surface EMG amplitude when RE is performed to task failure. The results of the present study provide evidence indicating that type I and type II fibres are activated when heavier and lighter loads are lifted to task failure. We propose that our results explain why RE training with higher or lower loads, when loads are lifted to task failure, leads to equivalent muscle hypertrophy and occurs in both type I and type II fibres.

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Introduction

It has been proposed that performing resistance exercise (RE) with heavier loads [greater than 60% one repetition maximum (1RM) strength] is required to elicit muscle hypertrophy and to recruit and result in hypertrophy of type II muscle fibres (Ratamess *et al.* 2009; Grgic & Schoenfeld, 2018). By contrast, studies show that performing RE training with relatively lighter loads to task failure (i.e. volitional fatigue) results in hypertrophy of both type I and type II muscle fibres (Mitchell *et al.* 2012; Morton *et al.* 2016; Schoenfeld *et al.* 2017). Indeed, type II muscle fibre hypertrophy, even when lighter loads are lifted to task failure, is indicative of recurrent type II fibre activation (Mitchell *et al.* 2012; Morton et al. 2015, 2016). However, on the basis of greater surface electromyography (EMG) amplitude (Jenkins *et al.* 2015; Looney *et al.* 2016; Haun *et al.* 2017) or decomposition of the EMG signal (Muddle *et al.* 2018), other studies have reported that heavier loads are superior to lighter loads in terms of recruiting higher threshold motor units and thus the eventual hypertrophy of type II fibres (Grgic & Schoenfeld, 2018).

According to the size principle of motor unit recruitment, performing submaximal contractions results predominantly in the recruitment of smaller (i.e. lower threshold) motor units that innervate type I fibres, although increasing fatigue necessitates the recruitment of larger (i.e. higher threshold) motor units that innervate type II muscle fibres (Mendell, 2005). Accordingly, several acute aerobic (Gollnick et al. 1973, 1974*b*; Vollestad *et al.* 1984; Vollestad & Blom, 1985; Prats *et al.* 2013; Kristensen *et al.* 2015) and resistance (Bell & Jacobs, 1989; Robergs *et al.* 1991; Koopman *et al.* 2006) exercise studies have shown that sustained submaximal contractions result in the substrate depletion (which is indicative of preceding depolarization or 'activation') of type II muscle fibres as fatigue ensues. Nonetheless, despite considerable debate on the ability of surface EMG to provide insight into motor unit recruitment during fatiguing contractions (Dideriksen et al. 2010, 2011; Enoka & Duchateau, 2015; Vigotsky *et al.* 2016), the thesis that type II fibre activation is confined to or superior with the lifting of heavier loads has been asserted.

The primary purpose of the present study was to evaluate the effect of manipulating load and repetition duration (i.e. time under tension) during RE performed to task failure on muscle fibre activation, which we quantified via fibre type-specific glycogen depletion (Bell & Jacobs, 1989; Robergs *et al.* 1991; Koopman *et al.* 2006). In addition, we measured surface EMG to determine how well EMG amplitude aligned with muscle fibre type-specific glycogen depletion. Additionally, to obtain mechanistic insight into how muscle fibre activation would be translated, we examined the phosphorylation of select signalling proteins prominent in contraction-related anabolism. We hypothesized that performing RE to task failure, independent of any specific RE variable, would result in the activation of type I and type II muscle fibres to an equivalent extent and show comparable increases in anabolic signalling. In addition, we hypothesized that surface EMG would be a poor indicator of muscle fibre type-specific glycogen depletion (i.e. fibre activation) and that muscle fibre glycogen depletion and anabolic signalling would be related.

Methods

Ethical approval

All participants were informed of the purpose, methodology, and potential risks of the study before giving verbal and written informed consent. The study conformed to the standards set by the latest revision of the *Declaration of Helsinki* and to the most recent Canadian Tri-Council policy statement on the use of human participants in research (http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default). The study was approved by the Hamilton Integrated Research Ethics Board (Project Number 0802) and was registered at clinicaltrials.gov (NCT03991117).

Study participants

Ten recreationally-trained young men (mean ± SD: 22 ± 3 years, 81.6 ± 8.9 kg, 178 ± 6 cm) volunteered to participate in the present study. We defined 'recreationally-trained' as engaging in at least one to three RE sessions per week for at least 2 years.

Resistance exercise training conditions

Participants' legs were assigned in randomized cross-over fashion to perform one of four unilateral RE protocols. The four RE conditions varied in the repetition duration and load (percentage of single maximal voluntary isotonic strength: %1RM). The conditions were: 80 %1RM Regular [80R; 1s:1s:1s (eccentric:pause:concentric)], 80 %1RM Slow (80S; 3:1:3), 30 %1RM Regular (30R; 1:1:1) and 30 %1RM Slow (30S; 3:1:3). Three sets were performed for each condition and each set was separated by 180 s rest. Repetition cadence was maintained by an in-ear metronome at 60 beats min^{-1}; however, for greater accuracy, repetition duration was quantified with the rise and fall of vastus lateralis (VL) EMG activity. RE volume (kg) was calculated by multiplying the number of repetitions in all three sets by the load lifted per repetition. Total time under load (TUL; s) was calculated by multiplying repetition duration by the number of repetitions in all three sets determined from signal from the VL EMG. Finally, impulse (kg·s) was calculated by multiplying the load lifted per repetition by the repetition duration and by the number of repetitions in all three sets.

Study design

Each participant came in for a familiarization session before the RE trials began, which was used to obtain an independent assessment of 1RM for each leg during knee extension (Atlantis, Laval, QC, Canada) and to familiarize them with performing isometric maximum voluntary contractions (MVC; leg curl and knee extension; Biodex dynamometer, System 3; Biodex Medical Systems Inc., Shirley, NY, USA). Using a unilateral within-subject cross-over design, participants came in on two separate occasions (separated by at least 72 h) to perform two of the four RE conditions each day (one on each leg) in a randomized order (Fig. 1). Briefly, on each of the two trial days, participants arrived following an overnight fast and a muscle biopsy was taken from their VL under local anaesthesia (2% xylocaine) to serve as the baseline for both conditions performed that day. After the muscle biopsy, dry reusable electrodes (Biometrics SX230; Biometrics Ltd., Newport, UK) were placed on each participant's VL, vastus medialis (VM) and semi-tendinosus (ST; in line with the direction of muscle action) along with a reference electrode and electronic joint goniometer (SG 150, Biometrics Ltd.) on the head of the fibula and about their knee joint, respectively. When the electrodes were in place, each participant performed three isometric knee extensions with their leg positioned at 60° and isometric leg curls at 45° to record peak torque and maximum voluntary excitation (MVE; i.e. the highest EMG signal: knee extension and leg curls, respectively) (Mathiassen *et al.* 1995) of the quadriceps and hamstrings, respectively. Afterwards, each participant performed two of the four conditions consecutively (one on each leg), which involved three sets to task failure (i.e. the participant was unable to complete another concentric muscle action) with three isometric knee extension MVCs between each condition's set (∼15 s delay between the knee extension machine and their first MVC). One h following the last MVC in each condition, a muscle biopsy was taken from the VL (one each leg).

Reduction in peak torque and EMG analyses

Muscle fatigue was quantified in the knee extensors as the reduction in isometric peak torque relative to the pre-testing peak toque. Surface EMG was recorded on a Biometrics data logger (DataLOG MWX8, Biometrics Ltd.; band-pass 20–450 Hz, input impedance ∼10^{15} Ω,

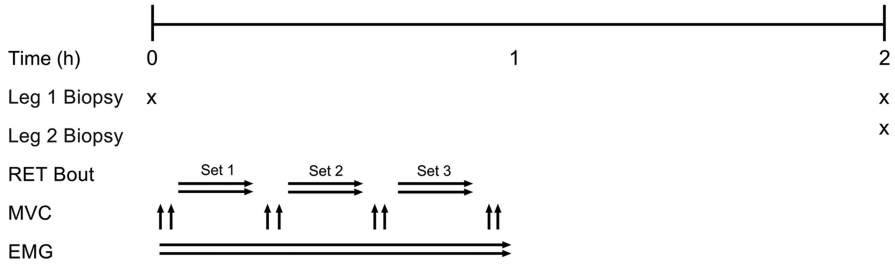

Figure 1. Study schematic representing one of the two trial days
The two arrows represent each of the participant's legs.

common mode rejection ratio >96 dB) and analysed with LabVIEW, version 8.2 (National Instruments, Austin, TX, USA). The raw EMG signals were sampled at 2048 Hz, full-wave rectified and smoothed with a 6 Hz low pass filter. The skin was shaved and marked (with a dot from a permanent marker) prior to bipolar integral dry reusable electrode (Biometrics SX230; Biometrics Ltd.) placement with a fixed inter-electrode distance of 2 cm. Care was taken not to place electrodes directly over a biopsy site in the case that the biopsy-induced oedema impaired motor unit recruitment or EMG signal. The average for each phase of each repetition was modelled with a second order polynomial regression equation, and a fast Fourier transformation was performed on each 250 ms window to calculate mean power frequency (MnPF).

The peak EMG amplitude (EMG_{amp}) of the second repetition of each set is referred to as the 'initial EMG_{amp}'. Similarly, the peak EMG_{amp} of the last repetition of each set is referred to as 'final EMG_{amp}'. The integrated (or total) EMG is the area under the curve throughout each set. The initial EMG_{amp}, final EMG_{amp} and integrated EMG were calculated as %MVE for each muscle (VL, VM and ST). MVE was measured each trial day during the initial isometric knee extension (VL and VM) and leg curl (ST) MVCs. MnPF and average EMG of the second repetition of each set (initial MnPF and initial average EMG) and the last repetition of each set (final MnPF and final average EMG) were also calculated.

Muscle glycogen and fibre-type histochemistry

Muscle tissue from each biopsy was mounted in OCT media, frozen in liquid nitrogen-cooled isopentane and stored in a −80 °C freezer until analysis. Cross-sections were cut 5 μm thick using a Microm HM550 Cyrostat (Thermo Fisher Scientific, Waltham, MA, USA) with particular care taken not to expose samples to any freeze-thaw cycles (Fairchild & Fournier, 2004). Fibre type-specific glycogen depletion was quantified by combining a brightfield periodic acid-Schiff stain (PAS), as described previously (McManus, 1948; Gollnick *et al.* 1973; Gollnick et al. 1974*a*, 1974*b*; Vollestad *et al.* 1984; Vollestad & Blom, 1985; Robergs *et al.* 1991; Koopman *et al.* 2006; Cumming *et al.* 2014), with a immunofluorescent myosin heavy chain (MHC) stain (Bloemberg & Quadrilatero, 2012; Morton *et al.* 2016; Jakubowski *et al.* 2019) on single cross-sections similar to the methodology described elsewhere (Schaart *et al.* 2004). Briefly, cross-sections were fixed using 3.7% formaldehyde in PBS for 60 min, treated with 1% periodic acid in distilled water for 5 min (#3951; Sigma-Aldrich, Toronto, ON, Canada), rinsed in tap water, stained with Schiff's reagent for 15 min (#3952016; Sigma-Aldrich), rinsed with distilled water and then rinsed in PBS prior to fluorescence staining. For fluorescence immunohistochemistry, antibodies raised against dystrophin [MANDYS1 (3B7)], MHC I (BA-F8), MHC IIA/X (SC-71) and MHC IIX (6H1) (Developmental Studies Hybridoma Bank, Iowa City, IA, USA) were combined with secondary isotype-specific antibodies [488 (A-21131), 594 (A-21125) and 647 (A-21238)] (Alexa Fluor, Thermo Fisher Scientific) before they were mounted with Prolong Diamond Antifade Reagant (Life Technologies, Toronto, ON, Canada) (Bloemberg & Quadrilatero, 2012). Each slide included muscle sections from a single participant within a single day (e.g. slide 1: pre, 80R and 30R; slide 2: pre, 30S and 80S) and all staining was performed within a period of 2 weeks in batches of three to five slides per day. One day after each stain cross-sections were imaged (brightfield before fluorescence, similar to a previous study; Schaart *et al.* 2004) with a CoolSNAP HQ2 fluorescence camera (Nikon Instruments, Melville, NY, USA) at 20× magnification with the exposure times: 400 ms (FITC), 100 ms (TRITC) and 200 ms (Cy5).

Muscle glycogen and fibre type analyses

Fibre type, cross-sectional area and glycogen content were determined by tracing the fibre dystrophin border in ImageJ, version 2 (NIH, Bethesda, MD, USA). Each trace was converted to a region of interest (ROI) and saved before being superimposed to another image of interest (i.e. brightfield or another fluorescence channel). Quantification of PAS intensity was determined by first converting the image to a greyscale image and then calibrating the stain to 0.68 μm $pixel^{-1}$. In addition, by setting thresholds for background *vs.* stain intensity, we excluded the quantification of freezing-induced artefact from each ROI on every channel. To quantify fibre type, the intensity of each colour within each ROI was exported alongside the brightfield data for objective quantification of type I and type II fibres. Only fibres with a circularity >0.85 were used for analyses and care was taken not to circle any fibres along the outside of the cross-section. An average of 275 ± 167 and 191 ± 126 fibres per section (1322 ± 400 and 896 ± 350 fibres per participant) were used for the fibre type/PAS and cross-sectional area analysis, respectively. The tracer was blinded to both the participant and conditions during the image analysis.

Western blot analysis

Muscle samples were homogenized using RIPA buffer (#R0278; Sigma-Aldrich) and a bead homogenizer with protease and phosphatase inhibitors (#05892970001 and 04906837001; Sigma-Aldrich). A bicinchoninic acid assay (#23227; Thermo Fisher Scientific) was performed on the whole muscle homogenate to quantify the protein

Table 1. Resistance exercise training variables

	80R	80S	30R	30S
Load per repetition (kg)	66 ± 8[a]	65 ± 8[a]	25 ± 4[b]	25 ± 3[b]
Repetition duration (s)	2.9 ± 0.4[a]	5.3 ± 0.6[b]	2.7 ± 0.6[a]	5.4 ± 0.5[b]
Repetitions per set	9 ± 2[a]	6 ± 1[b]	20 ± 4[c]	14 ± 4[d]
Volume (kg)	1788 ± 574[a]	1242 ± 348[b]	1532 ± 400[a]	1071 ± 354[b]
Total TUL (s)	76 ± 20[a]	99 ± 17[b]	158 ± 19[c]	225 ± 52[d]
Impulse (kg·s)	5055 ± 1680[a]	6518 ± 1590[b]	3938 ± 603[a]	5723 ± 1639[b]

Impulse (kg·s) is calculated by by multiplying the load lifted per repetition, by the repetition duration and by the number of repetitions in all sets. Significant differences identified via *post hoc* analyses are indicated by a superscript lowercase letter where means with different letters are significantly different (all $P < 0.05$). Values are the mean ± SD.

content of each sample. Samples were prepared in Laemmli buffer (#1610747; Bio-Rad, Hercules, CA, USA) with beta-mercaptoethanol (M6250; Sigma-Aldrich) and brought to equal concentrations of 20 μg μL^{-1}. SDS-PAGE was performed on 7.5 μL per sample along with two 7.5 μL prestained protein standards (#1610375; Bio-Rad) and a calibration curve (2.5, 5, 7.5 and 10 μL of all post-training samples pooled) on 26-well gels (4-15% Criterion TGX Stain-Free, #5678085; Bio-Rad). As a quality check for protein separation along the gel, the gel was imaged by ultraviolet activation with the Chemidoc MP StainFree Imager (Bio-Rad) before it was transferred to a nitrocellulose membrane via a Trans-Blot Turbo Transfer System (Bio-Rad) at 100 V for 30 min in 4°C transfer buffer (25 mM Tris, 192 mM glycine, 0.1% SDS and 20% methanol, pH 8.3). Transfer success was visualized with ultraviolet activation of both the gel and membrane via a Chemidoc MP StainFree Imager (Bio-Rad).

Nitrocellulose membranes were blocked in BSA for 2 h, washed three times for five minutes with Tris-buffered saline-Tween 20 (TBST), cut into specific sections according to the molecular weights of our protein targets, and incubated in primary antibodies at 4°C with the 5% BSA block at concentrations between 1:500 and 1:1500 (depending on the affinity of the primary antibody). The primary antibodies we used were total mTOR (#2972), phosphorylated mTOR (Ser2448; #5536), total p70 S6k (#9202), phosphorylated p70 S6k (Thr389; #9205), total 4E-BP1 (#9452), phosphorylated 4E-BP1 (Thr37 and Thr46; #2855), total S6 ribosomal protein (#2217), phosphorylated S6 ribosomal protein (Ser240 and Ser244; #2211), total AkT (#4691), phosphorylated AkT (Ser473; #9271), total FAK (#13009), phosphorylated FAK (Tyr397; #8556), total p44/42 MAPK ERK1/2 (#9102) and phosphorylated p44/42 MAPK ERK1/2 (Thr202 and Tyr204; #9101), which were all obtained from Cell Signaling Technologies (Danvers, MA, USA). After an overnight incubation, membranes were washed again three times for 5 min in TBST, incubated in secondary antibody (dilution 1:20,000; anti-rabbit, HRP-linked; #7074; Cell Signaling Technologies) for 1 h at room temperature, washed another three times in TBST, rocked for 5 min in ECL substrate (Clarity Max; #1705062; Bio-Rad) and then imaged on the ChemiDoc MP (Bio-Rad). The ladder was imaged in colourmetric mode and the proteins of interest were measured in chemilumescence mode. All image analysis was performed in ImageLab, version 5.2.1 (Bio-Rad). Each gel lane was calibrated to the gel lanes of our calibration curve and each protein band was calibrated to the protein bands of our calibration curve as described elsewhere (Murphy & Lamb, 2013; MacInnis *et al.* 2017). Afterwards, the calibrated protein band was divided by the calibrated gel lane to quantify absolute protein band intensity.

Statistical analysis

When there was only one within-subject independent variable of interest (e.g. fibre type), one-way repeated measures ANOVA was used. When there were two within-subject independent variables (e.g. time and condition) a two-way repeated measures ANOVA was used. When there were three within-subject independent variables (e.g. repetition duration, load, and initial *vs.* final repetitions), a three-way repeated measures ANOVA was used. Whenever statistical significance was found with an ANOVA test, a Bonferroni *post hoc* test was used. Lastly, bivariate, two-tailed Pearson's correlations were run to assess relatedness on select variables. All statistical analyses were performed in SPSS, version 20 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant. Values are reported as the mean ± SD unless indicated otherwise.

Results

Resistance exercise training variables

All RE variables are presented in Table 1. There were significant differences between conditions for every RE variable ($P < 0.01$). Specifically, *post hoc* analyses revealed a

significant difference between the load lifted per repetition (80R and 80S > 30R and 30S), repetition duration (80S and 30S > 80R and 30R), number of repetitions per set (30R > 30S > 80R > 80S), volume per session (80R and 30R > 80S and 30S), total TUL per session (30S > 30R > 80R > 80S) and impulse per session (80S and 30S > 80R and 30R; $P < 0.05$) (Table 1).

EMG and decline in peak torque

EMG results are presented for the VL because that was the muscle from which the biopsies were taken. Our analyses and conclusions would not change if we were to report VL and VM instead of only VL (data not shown).

Initial peak EMG_{amp} was greater in higher-load conditions (80R: 66±15 and 80S: 65±15 %MVE) compared to lower-load conditions (30R: 46±21 and 30S: 45±15 %MVE; $P < 0.01$) (Fig. 2*A*). Following an increase in peak EMG_{amp} in each condition ($P < 0.01$), final peak EMG_{amp} remained higher in high-load conditions (80R: 90±27 and 80S: 88±26 %MVE) compared to lower-load conditions (30R:79±38 and 30S: 64±25 %MVE; $P = 0.04$) (Fig. 2*B*) with no time by condition interaction ($P = 0.34$). Similarly, the initial average EMG (80R: 66±15; 80S: 64±15; 30R: 34±13; 30S: 39±12 %MVE; $P < 0.01$) and final average EMG (80R: 76±18; 80S: 68±18; 30R: 48±13; 30S: 54±14 %MVE; $P < 0.01$) were significantly greater in the higher load conditions (Fig. 3); however, there was a greater increase in average EMG in the lighter-load conditions (30R: 14±4 and 30S: 14±8 %MVE) compared to the higher-load conditions (80R: 10±7 and 80S: 4±6 %MVE; $P < 0.01$) and integrated EMG was significantly higher in lower-load conditions (30R: 29±13 and 30S: 24±13 %MVE·s) compared to higher-load conditions (80R: 16±7 and 80S: 17±5 %MVE·s; $P < 0.01$) (Fig. 2*C*). In addition, there was a trend for higher initial MnPF in regular repetition duration conditions (80R: 90±10 and 30R: 91±15 Hz) compared to slower repetition duration conditions (80S: 83±11 and 30S: 88±8 Hz; $P = 0.06$) and, after a significant decrease in each condition ($P < 0.01$), a similar trend for higher MnPF in regular repetition duration conditions (80R: 81±7 and 30R: 78±12 Hz) compared to slower repetition duration conditions (80S: 77±13 and 30S: 73±5 Hz; $P = 0.07$). Finally, the decrease in MnPF was greater in lighter-load conditions (30R: 14±11 and 30S: 15±10 Hz) compared to higher-load conditions (80R: 9±5 and 80S: 6±3 Hz, $P = 0.03$).

Muscle peak torque data are presented in Fig. 2*D*. The data are presented collapsed across conditions because there was a main effect for time ($P < 0.01$) but no differences between conditions ($P = 0.83$) or time by condition interaction ($P = 0.73$). *Post hoc* analyses revealed significant differences between each set ($P < 0.05$) with the exception of peak torque measured after the second and third sets, which was not different ($P = 0.53$).

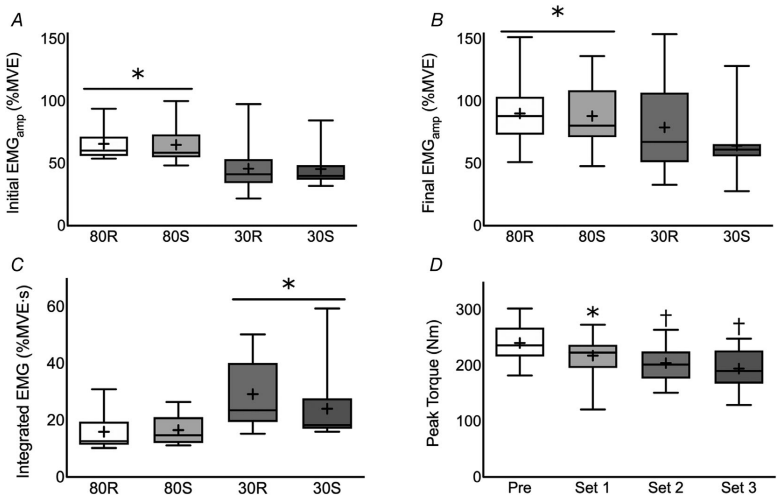

Figure 2. Surface EMG outcomes and reductions in peak torque
EMG amplitude during the second (*A*) and last (*B*) repetitions of each set, integrated (or total) EMG throughout each set (*C*) and peak torque before and after each set (*D*). * and † indicate significant differences between the other conditions or times of measurement (all $P < 0.05$). The data are presented as box and whisker plots with the median (line), mean (cross), inter-quartile range (box), and minimum and maximum values (tails).

Fibre size and distribution

There was no difference between conditions for fibre distribution or cross-sectional area (type I: 44 ± 10 %, 5622 ± 1291 μm^2 and type II: 57 ± 9 %, 7460 ± 1503 μm^2, respectively)

Fibre-specific glycogen depletion

Muscle glycogen data are presented in Fig. 4. There was significantly greater glycogen content in type II *vs.* type I muscle fibres types at rest ($P < 0.01$) (Fig. 4*A*). Glycogen content decreased in each condition ($P < 0.01$) with a significant time by fibre type interaction such that there was a greater decrease in glycogen in type II fibres (−0.06 ± 0.05 AU) (Fig. 4*B*) than type I fibres (−0.04 ± 0.05 AU, $P = 0.02$) (Fig. 4*C*). However, there were neither main, nor interaction effects for condition, indicating that there was no influence of load or repetition duration on muscle fibre activation (all $P > 0.20$).

Western blot analysis

The ratio of phosphorylated/total protein expression data are presented in Fig. 5. Phosphorylated corrected for total expression of S6 ribosomal protein, FAK, ERK1 and ERK2 changed following RE ($P < 0.05$), although there were no main effects for condition, with the exception

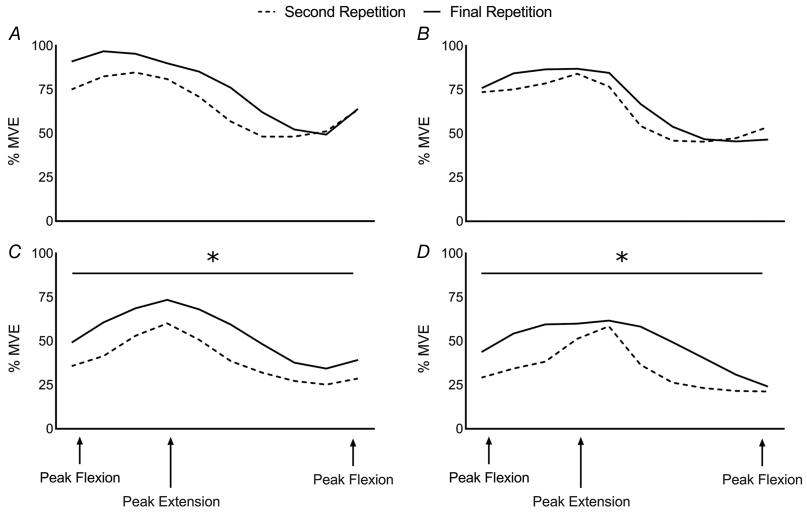

Figure 3. Surface EMG amplitude during repetitions with varying load and repetition duration
The average EMG in the VL relative to knee angle during the second (dotted line) and final (continuous line) repetition in each condition: 80R (*A*), 80S (*B*), 30R (*C*) and 30S (*D*). *Significant difference between 80R and 80S conditions ($P < 0.01$).

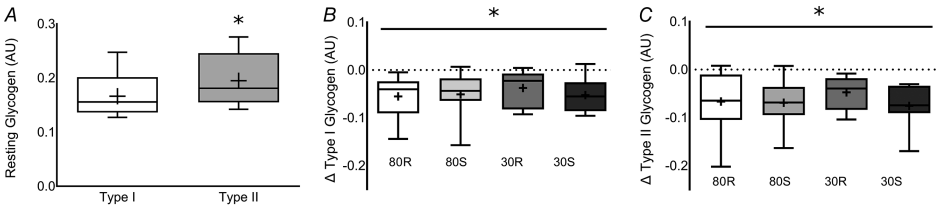

Figure 4. Muscle glycogen analyses
Histochemical analysis of skeletal muscle glycogen content at rest (*A*) and the change in glycogen content following each condition in type I (*B*) and type II (*C*) fibres. *Significantly different from type I fibres (*A*) or significantly different from rest (*B* and *C*) ($P < 0.01$). The data are presented as box and whisker plots with the median (line), mean (cross), inter-quartile range (box), and minimum and maximum values (tails).

of phosphorylated/total S6 ribosomal protein ($P = 0.04$), which was significantly higher post-exercise in 30R than 30S (Fig. 5*C*).

Correlational analysis

There were no significant correlations between initial EMG_{amp}, final EMG_{amp}, integrated EMG, change in EMG_{amp}, change in average EMG or change in MnPF with type I, type II or total (the sum of type I and type II) muscle fibre glycogen depletion (all $r < 0.24$; $P > 0.15$). However, there were weak to moderate correlations between type I and type II glycogen depletion and anabolic signalling (phosphorylation/total) and the reduction in peak torque (Table 2).

Discussion

We found that performing RE to task failure with varying loads and conventional (1:1:1) or longer (3:1:3) repetition durations (i.e. time under tension) resulted in no significant differences in fibre type-specific glycogen depletion, which is a direct measure of the 'use' (and preceding activation) of the fibres that we assessed (Gollnick *et al.* 1973; Gollnick *et al.* 1974*b*; Vollestad *et al.* 1984; Vollestad & Blom, 1985). By manipulating

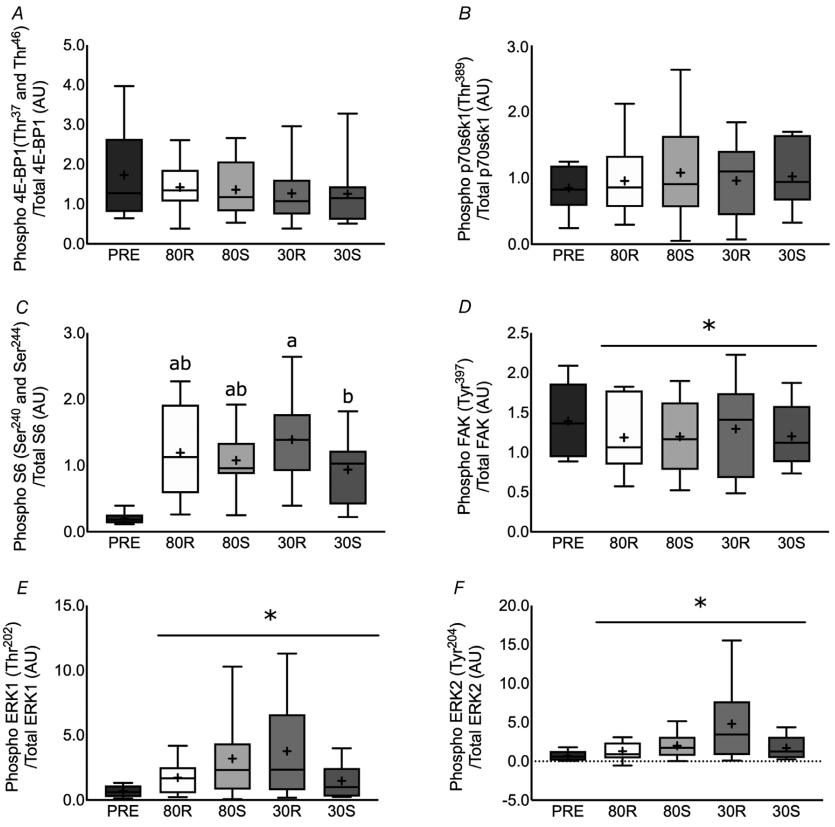

Figure 5. Acute anabolic signalling following each condition
Phosphorylation/total expressions for 4E-BP1 (*A*), p70s6k (*B*), S6 ribosomal protein (*C*), FAK (*D*), ERK1 (*E*) and ERK2 (*F*). *Indicates a main effect for time and the different lowercase letters above individual bars indicate a significant time by condition interaction between those conditions ($P < 0.05$). The data are presented as box and whisker plots with the median (line), mean (cross), inter-quartile range (box), and minimum and maximum values (tails).

Table 2. Correlations between anabolic signalling protein phosphorylation (relative to total protein), peak torque and glycogen depletion

	Δ Type I glycogen	Δ Type II glycogen	Δ Total glycogen
Δ p-mTOR	0.13	0.13	0.10
Δ p-4E-BP1	0.15	0.12	0.18
Δ p-p70 S6k	0.41*	0.37*	0.33*
Δ p-S6	0.30	0.37*	0.29
Δ p-FAK	−0.03	0.01	−0.02
Δ p-ERK1	0.37*	0.33*	0.32*
Δ p-ERK2	0.32	0.31	0.30
Δ peak torque	0.23	0.25	0.32*

Values are Pearson *r* values. *$P < 0.05$.

load and repetition duration, we were able to create substantial differences in a number of RE variables (i.e. number of repetitions, exercise volume, total TUL and impulse) (Table 1), allowing us to evaluate how such differences influenced surface EMG (Figs 2 and 3), force loss (Fig. 2*D*), muscle fibre activation (Fig. 4*B* and *C*) and anabolic signalling (Fig. 5). Our main finding was that, independent of load or repetition duration, performing RE to task failure resulted in substrate depletion (and therefore activation and recruitment of the innervating motor neurons) of both type I and type II fibres with no significant differences observed between conditions. In addition, we confirm that when RE is performed to task failure the maximal amplitude of surface EMG at the beginning or the end of a set is not related, as some have posited (Looney *et al.* 2016), to muscle fibre activation, reductions in peak torque, or signalling protein phosphorylation, which have been linked to protein synthesis and hypertrophy. Thus, as also previously concluded by ourselves (Vigotsky *et al.* 2016; Vigotsky *et al.* 2017) and others (Farina *et al.* 2004; Dideriksen *et al.* 2010; Dideriksen *et al.* 2011; Enoka & Duchateau, 2015), the current data suggest that surface EMG does not accurately assess type I or type II muscle fibre activation during RE.

Resistance training variables and muscle fibre activation

Previous research has demonstrated that isokinetic MVC (Bell & Jacobs, 1989), heavier load RE (Robergs *et al.* 1991; Koopman *et al.* 2006) and lighter load RE (Robergs *et al.* 1991) all result in glycogen depletion in both type I and type II fibres. Nonetheless, it has been hypothesized that type II muscle fibre activation is exclusive, or greater, when performing RE with heavier loads (Grgic & Schoenfeld, 2018). This proposal has been buoyed by measurements of greater surface EMG amplitude during resistance exercise (Jenkins *et al.* 2015; Looney *et al.* 2016; Haun *et al.* 2017) and isometric exercise with subsequent algorithmic decomposition of the EMG signal to track motor units (Muddle *et al.* 2018). We manipulated load and repetition duration to create three-fold differences in load, repetition duration, number of repetitions, exercise volume, total TUL and impulse (Table 1), as well as almost two-fold differences in EMG_{amp} at the beginning of each set, EMG_{amp} at the end of each set, and the integrated (or total) EMG between conditions (Fig. 2). However, performing RE to task failure resulted in similar magnitudes of glycogen depletion (i.e. the use and therefore activation) in type I and type II muscle fibres (Fig. 4) and similar levels of anabolic signalling protein phosphorylation (Fig. 5). Thus, we conclude that neither load repetition duration, nor the accompanying surface EMG_{amp}, align with muscle fibre type-specific activation when RE is performed to task failure.

Surface EMG and muscle fibre activation

Surface EMG records the electrical activity of numerous motor units, which can be decoded and modelled as an indirect measurement of individual neuron firing, individual neuron 'drop out' and individual neuron recycling (Enoka & Duchateau, 2015; Vigotsky *et al.* 2016; Muddle *et al.* 2018). However, the relationship between surface EMG_{amp} and motor unit recruitment is not easily determined during sustained/fatiguing contractions (Dideriksen *et al.* 2010; Dideriksen *et al.* 2011), it may be convoluted by non-random motor unit distribution in the VL (Knight & Kamen, 2005) and it is preferential to superficial motor neurons (Muceli *et al.* 2015). In the present study, we demonstrate that performing RE to task failure with lower loads does not result in 100 %MVE (Figs 2*B* and 3) but does result in the use/activation of type II muscle fibres that are part of larger, higher threshold motor units (Fig. 4*C*). Indeed, larger motor units produce larger action potentials and experience greater reductions in firing rates with sustained contractions (Potvin & Fuglevand, 2017); thus, it is not surprising that the increase in EMG signal is attenuated in higher loads (Fig. 3) and that lower-load contractions to task failure never reaches 100 %MVE (Fig. 2*B*). Evidently, particularly during sustained or repeated isotonic contractions, caution is warranted regarding the efficacy of EMG_{amp} to infer fibre type-specific motor unit recruitment (Farina *et al.* 2004; Dideriksen et al. 2010, 2011; Enoka & Duchateau, 2015; Vigotsky *et al.* 2016).

Anabolic signalling and muscle fibre activation

We have shown that type II muscle fibre hypertrophy occurs with low-load RE when loads are lifted to fatigue, which would require type II muscle fibre activation

(Mitchell *et al.* 2012; Morton et al. 2015, 2016). Here, we demonstrate that, independent of load or repetition duration, performing RE to task failure results in both type I and type II muscle fibre activation (Fig. 4) to an equivalent extent. We also observed equivalent increases in the phosphorylation of a number of canonical signalling proteins (Fig. 5) and we extend our findings to support the recommendation of submaximal loading in older or unhealthy populations (McLeod *et al.* 2019). Moreover, we found significant correlations between glycogen depletion *vs.* reduction in peak torque and increase in anabolic signalling protein phosphorylation and add that, in some cases, the proteins (e.g. p70 S6k) have established correlations with muscle protein synthesis (Burd *et al.* 2010) and changes in fibre cross-sectional area following RE training (Mitchell *et al.* 2013). Thus, we confirm our hypothesis that type II muscle fibre activation occurs when lighter loads are lifted to task failure and add that fibre-specific glycogen depletion is related to muscle fatigue and signalling protein activation in the VL, which highlights the subsequent type II muscle fibre hypertrophy reported previously (Mitchell *et al.* 2012; Morton *et al.* 2016).

Limitations

We have quantified muscle fibre activation via glycogen depletion because it is the primary substrate used during RE (Koopman *et al.* 2006) and it has been used extensively in studies to directly establish that fibres were activated and

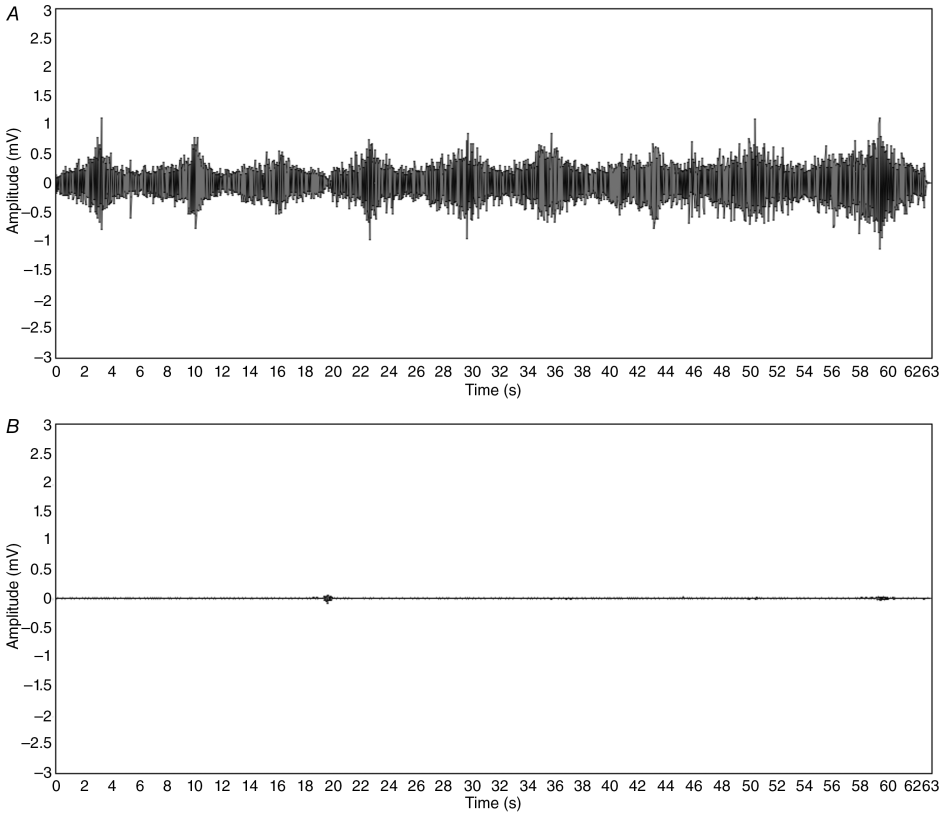

Figure 6. A representative raw EMG signal during the 80R condition
A, raw EMG signal in the VL of the exercising leg. *B*, raw EMG signal in the VL of the non-exercising leg.

used (Gollnick *et al.* 1973; Gollnick *et al.* 1974*b*; Vollestad et al. 1984, 1985; Robergs *et al.* 1991; Koopman *et al.* 2006; Prats *et al.* 2013). Indeed, substrate depletion in a muscle fibre is indicative that the fibre was used and therefore activated; however, we acknowledge that the method may lack sensitivity as an indication of muscle fibre depolarization and that glycogen is not the only substrate used during fatiguing contractions (Koopman *et al.* 2006). In addition, unilateral RE induces a small increase in strength in the contralateral limb (Munn *et al.* 2004); however, the best evidence of an origin for the cross-limb education effect is found in the central nervous system and not within the muscle itself (Carroll *et al.* 2006). Indeed, muscle activity is negligible in the non-contracting VL (Fig. 6), which reinforces evidence that post-exercise rates of protein turnover are exclusive to the muscle group that is contracting (Wilkinson *et al.* 2014; Holwerda *et al.* 2018). Thus, we see no reason to hypothesize that contracting one limb resulted in muscle fibre activation in the contralateral limb. Otherwise, we acknowledge that our intramuscular analyses is limited to ∼275 muscle fibres in the VL per biopsy, which may not be representative of all muscle fibres in the VL or of surrounding muscles (Burke & Tsairis, 1974). Finally, we elected to take muscle biopsies 1 h post-exercise to measure protein phosphorylation and to avoid waiting so long that significant glycogen resynthesis would occur (Robergs *et al.* 1991; Koopman *et al.* 2006; Camera *et al.* 2012; Cumming *et al.* 2014); however, we acknowledge that measuring protein phosphorylation one hour post-exercise provides only a snapshot in the time course of protein signalling and that minimal glycogenesis may have resulted in an underestimation of type II fibre glycogen depletion (Vollestad *et al.* 1989).

Conclusions

We show that performing RE to task failure, independent of load or repetition duration, resulted in equivalent type I and type II muscle fibre glycogen depletion, which is indicative of preceding fibre activation. Moreover, by manipulating load and repetition duration, we demonstrate that no specific RE variable (e.g. number of repetitions, exercise volume per session, total TUL per session, or impulse) affected fibre type-specific glycogen depletion in the VL when RE was performed to task failure. Indeed, despite similar magnitudes of glycogen depletion, there were substantial and significant differences in EMG amplitude, average EMG, mean power frequency and integrated (total) EMG in each condition. Thus, we also show that surface EMG amplitude, average EMG, mean power frequency and integrated (total) EMG are not indicative of fibre type-specific glycogen depletion in the VL. By contrast, and similar to glycogen depletion, RE-induced anabolic signalling was independent of load and repetition duration. Therefore, we conclude that muscle fibre activation is aligned with reductions in peak torque and anabolic protein signalling, and that neither is determined by load or repetition duration when RE is performed to task failure.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

RWM, CM, JRP and SMP designed the study. RWM, MWS, AFZ, AJ and SMP performed the data collection. RWM, MWS, AFZ, PJK and IYZM performed the data analysis. RWM and SMP drafted the manuscript. All authors critically revised the manuscript and have approved the final version of the manuscript submitted for publication. The authors 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.

Author contributions

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Translational perspective

Elwood Henneman first defined the 'size principle' as the systematic recruitment of motor units based on their morphology in a series of publications in and around 1965. Subsequently, exercise physiologists have corroborated the size principle by showing that increased fatigue when lifting submaximal loads necessitates an increase in the recruitment of larger, higher threshold motor units and their associated type II muscle fibres. Research in the realm of resistance exercise has found that, in contrast to the widely held belief, lifting relatively light loads to task failure (volitional fatigue) leads to hypertrophy of type II fibres. Thus, we hypothesized that, when resistance exercise was performed to task failure, the inevitable muscular fatigue would necessitate the recruitment of larger motor units and subsequent activation (use) of type II muscle fibres, which would be independent of load or repetition duration ('time under tension'). Indeed, we confirm these hypotheses and provide evidence that is in agreement with the size principle as described by Henneman. Specifically, we show that performing resistance exercise to task failure, independent of load or repetition duration, resulted in the activation of both type I and type II muscle fibres. Moreover, although surface EMG is an informative and accessible way to measure general muscular activity, we add to the existing literature that calls for caution against using EMG amplitude to infer the activation of individual motor units. Lastly, we demonstrate that muscle fibre activation and muscle anabolism are related, and that neither is affected by the load or repetition duration used during resistance exercise performed to task failure.

CHAPTER 6:

Variability in resistance training-induced hypertrophy and strength are independent of load and limb location in healthy young men

In preparation

Variability in resistance training-induced hypertrophy and strength are independent of load and limb location in healthy young men

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Running title: Exercise-induced muscle hypertrophy is endogenously mediated

Key words: load, proteomics, strength training, dynamic proteome profiling

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Key Points:

- There is considerable individual variability in resistance exercise training (RET)-induced muscle hypertrophy; however, RET-induced muscle hypertrophy is consistent within an individual (between the upper- and lower-body) and is independent of load when RET is performed to volitional fatigue.
- There is negligible shared variance between RET-induced increases in muscle size and strength. In addition, there are limited relationships between measures used to assess RET-induced increases hypertrophy and strength, respectively.
- We conclude that when effort is matched (i.e., working to muscular fatigue), RET-induced muscular hypertrophy is mediated by endogenous factors that explain the heterogeneity between individuals.

Abstract

Introduction: Regardless of load, performing resistance exercise training (RET) to volitional fatigue results in an increase in muscle size and strength; however, there is significant response variability. The purpose of this study was to determine if inter-individual differences in RET-induced muscle hypertrophy were dependent on load and limb location (upper versus lower body). **Methods:** Twenty healthy young men (22±3 y, 26±6 kg/m^2, means±SD) completed three resistance exercise sessions weekly for 10 wk. Each limb was randomly assigned to perform unilateral biceps curls or knee extensions with either higher-loads (8-12 repetitions at ~80% one repetition maximum [1RM]) or lower-loads (20-25 repetitions at ~40% 1RM) for three sets to volitional fatigue during each resistance exercise session. Muscle size (via dual x-ray absorptiometry, ultrasonography, and muscle biopsies from the vastus lateralis) and muscle strength (via unilateral 1RMs and isometric maximum voluntary contractions) were measured before and after 10 weeks of RET. **Results:** Following 10 weeks of RET there was an increase in every index of muscle size and strength with no difference between the higher- and lower-load conditions. In addition, the relative RET-induced increase in the upper- and lower-body for both changes in muscle size (R^2=0.49, P<0.01) and strength (R^2=0.35, P<0.01) were consistent despite considerable variability between participants (range [Δ%]; Arm FBFM: -3 to 13, Arm MT: -4 to 19, Leg MT: -4 to 24, Leg FBFM: -2 to 8, Arm US CSA: 1 to 24, Leg US CSA: -10 to 19, Type I CSA: -24 to 49, Type 2 CSA: -30 to 96, elbow flexion peak torque: -17 to 36, knee extension peak torque: -16 to 56, dumbbell biceps curl 1RM: 0 to 87, and knee extension 1RM: 12 to 171). Further, the

relative RET-induced increase in muscular hypertrophy showed no shared variance with the relative RET-induced increase in muscle strength (R^2=0.01, P=0.63) and we observed that redundant outcomes used to assess RET-induced increases in muscle size (DXA, CSA, MT, and fibre CSA) and muscle strength (1RM and MVC) were seldom correlated with each other. **Conclusion**: Performing RET to volitional fatigue resulted in an increase in muscle size and strength that was considerably different between individuals but was equivalent between higher- vs. lower-loads and the upper- vs. lower-body. We also observed that RET-induced increases in muscle size and strength shared minimal variance and that outcomes used to quantify RET-induced changes in muscle size and strength, respectively, were seldom correlated with each other. We conclude that an individual's propensity for hypertrophy and strength gains are primarily an endogenous process.

Introduction

Performing resistance exercise training (RET) is a robust way to augment and/or maintain muscle mass; however, similar to changes in aerobic capacity following endurance-type exercise (1), there is substantial inter-individual response variability in RET-induced adaptation (2). Indeed, it is unlikely that an individual is a complete (i.e., across all outcomes) 'non-responder' to RET (3), but it is also evident that there are individuals who, on average, experience significantly different RET-induced adaptations than others performing the same RET (4-8).

Hypertrophy is considered a hallmark response to RET, and a large volume of research has attempted to elucidate the exogenous factors that stimulate hypertrophy. Data show that increasing daily protein intake (9) and manipulating specific RET variables such as exercise volume-load (10,11), training frequency (12), training velocity (13), specific exercises (14), blood flow occlusion (15), and the load lifted per repetition (16)) have small (if any) effect on RET-induced increases in muscle size and strength. Instead, we propose that endogenous variables inherent to an individual are the greatest source of individual variability in RET-induced hypertrophy. For example, satellite cell number (17), intramuscular biomarkers (e.g., total RNA, myozenin 1 protein content, and androgen receptor content) (4, 5, 18), select microRNA expression (6), select mRNA expression (19), and individual genes (e.g., *ACE* and *ACTN3*) (7,8) have all been associated with the individual variability in RET-induced hypertrophy.

The purpose of this study was to determine the effect of external load (exogenous) and limb location (upper versus lower body: endogenous) on RET-induced changes in

muscle size and strength. To do so, we used a within-subject, unilateral design such that each participant's arms and legs were randomized to perform higher-load (HL) or lower-load (LL) RET for 10 weeks. We hypothesized that there would be considerable inter-individual heterogeneity in RET-induced muscle hypertrophy, which would not be determined by either load or limb location. In addition, we included a number of measurement methods to quantify changes in muscle size (arm FBFM, leg FBFM, vastus lateralis [VL] muscle thickness [MT], biceps brachii [BB] MT, VL CSA, BB CSA, type I VL fibre CSA, and type II VL fibre CSA) and muscle strength (knee extension 1RM, biceps curl 1RM, isometric knee extension peak torque, and isometric elbow flexion peak torque) to provide a comprehensive assessment of RET-induced adaptations. Although our measures of muscle size and strength are all muscle-related they use very different methods and measure fundamentally different outcomes (20, 21). Thus, we hypothesized that the absolute differences between different measurements of muscle size and muscle strength would not be correlated with each other, but that ranking each participant's score in each outcome would increase the consistency between measurements (22). Finally, we hypothesized that RET-induced changes in muscle size would share significant variance with RET-induced changes in muscle strength (23).

Methods

Study Participants. We recruited 20 healthy, recreationally active but not trained young men to complete this study. The study and consent form were approved by the Hamilton Integrated Research Ethics Board (HIREB 4774) and the study conformed to the most

recent Canadian Tri-Council policy statement on the use of human participants in research (http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/). Each participant was informed of the purpose, methodology, and potential risks before providing written consent to participate (Figure 1).

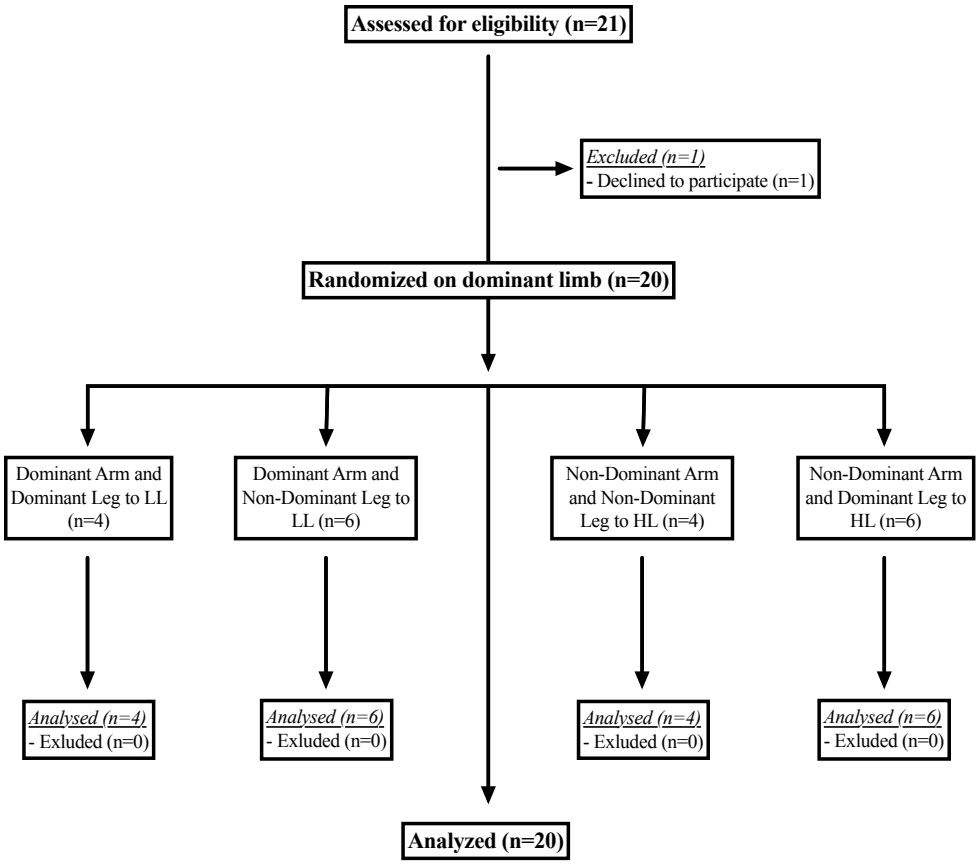

Figure 1. Consort Diagram

Experimental Design. Both muscle size and strength (variously measured) were recorded only twice: before the first RET session and >72 hours following the last RET session of the 10 wk RET intervention. Maximum voluntary contractions (MVC) were practiced

during a familiarization session on a Biodex dynamometer (System 3, Shirley, NY, USA) to record the physical set-up of the dynamometer for each participant and to expose each participant to the test; however, participants performed a second round of MVCs immediately before the first RET session, which, similar to the familiarization testing, consisted of three five-second isometric, unilateral MVCs on each limb (knee extension [60°] and elbow flexion [110°]; System 3, Shirley, NY, USA) with 60 s rest in between each attempt. In contrast, knee extension (Atlantis Inc., Laval, QC, CAN) and dumbbell preacher curl (York Barbell, York, PA, USA) 1RM were recorded during the familiarization session after a general warm-up (5 min on a cycle ergometer). Specifically, load was progressively increased (~4.5 kg [knee extension] or ~2.5 kg [biceps curl]) alongside a decreasing number of repetitions (~eight, five, three, and then one) with between 120 and 180 s of rest between each set/attempt until the participant could not perform a single successful repetition. Successful repetitions were defined as starting at 90° and finishing at 170° with their hips in contact with the seat (knee extension) and by starting at 180° and finishing at complete elbow flexion with their hips remaining in the seat, chest against the apparatus, and elbow in contact with the pad (dumbbell preacher curl).

Condition allocation and resistance exercise training. Each participant's dominant arm and leg were randomly assigned to one of two RET conditions while their non-dominant limbs were assigned to the opposite condition. The two conditions were either higher-

load, lower-repetition RET (HL: 8-12 repetitions at ~70-80% 1RM) or lower-load, higher-repetition RET (LL: 20-25 repetitions at ~30-40% 1RM).

Each participant completed three RET sessions each week (Monday, Wednesday, and Friday) for 10 wk. Each RET session consisted of three sets of unilateral resistance exercise, according to each limb's condition allocation, to volitional fatigue (i.e., the participant was unable to complete another concentric muscle action). Adherence to the RET regime was ensured with one-on-one supervision with trained study personnel. Each RET session started with knee extensions, but the starting condition alternated between RET sessions. Each set was separated by 90 s of rest and each repetition was performed to a cadence of 2:0:2 (seconds; eccentric: isometric: concentric) with proper form (full range of motion, staying seated, and keeping the chest and elbow in contact with the apparatus' pads [dumbbell preacher curls]).

Dietary Consideration. Dietary intake records were collected during familiarization, week 1, and week 10 and were analyzed using the NutriBase dietary analysis software (Nutribase11 Professional Edition, version 11.5, Cybersoft Inc., Phoenix, Arizona, USA). In addition, participants received 25 g of whey protein isolate (Leprino Foods, Denver, CO) twice per day (morning or post-exercise and pre-sleep) for the duration of the study in effort to ensure each participant was receiving adequate (>1.6 g/kg of body mass/day) daily protein intake (9).

Fat- and bone-free mass (FBFM). FBFM was assessed using dual energy x-ray absorptiometry (DXA) with a GE Lunar iDXA total body scanner (GE Medical Systems Lunar, Madison, WI, USA) following an overnight fast and >72 h following their last exercise bout both pre- and post-intervention. Data were analyzed in the medium scan mode (Lunar enCORE version 14.1, GE Medical Systems Lunar, Madison WI, USA) and each analysis region (i.e., head, torso, arms, and legs) was subdivided by the software before manual inspection by a blinded study investigator.

Muscle thickness and cross sectional area. Muscle thickness and CSA were assessed using B-mode ultrasonography and were performed by the same experienced technician. Each participant laid supine for 10 minutes before a BK3500 unit and 18L5 probe (BK Medical North America, Peabody, MA, USA) was used to measure muscle thickness and CSA. The settings were determined by pilot testing and remained constant at every measurement time and within the same participant. For the VL: 100 % size, 120 % sector, 32 Hz frequency rate, 9.0 MHz frequency B, 70 dB dynamic range, 59 mm focus, 1.5 MI, 9.0 cm depth, filter B3, and 57 % gain, BB: 85 % size, 100 % sector, 35 Hz frequency rate, 6.0 MHz frequency B, 65 dB dynamic range, 38 mm focus, 1.5 MI, 6.5 cm depth, 3 Filter B, and 50% gain. Video captures of the VL and BB were obtained in the axial plane at 70 % of the length between the apex of each participant's anterior superior iliac spine and lateral superior patellar boarder, and 60% of the length between the subject's acromioclavicular joint and the center of their antecubital fossa, respectively. For the VL, participants' feet were elevated 5 cm and aligned to point upwards with a custom foot suspension apparatus, which ensured consistent orientation, no compression of the leg

muscles against the bed, and the ability for our technician to obtain the posterior border of the VL. For BB video capture, participants laid supine with their legs hanging freely off the end, and their arms resting on the table at a 45° angle with their forearms supinated. To ensure axial orientation throughout each video capture, a goniometer and flexible ruler were used to draw a line at a 90° and a guiding, cross-sectional line down the muscle, respectively. An experienced technician used ample transmission gel (Aquasonic 100; Parker Laboratories, Fairfield, NJ, USA) to ensure the skin was not compressed at any point throughout video capture.

Each video file was converted to tiff frames via Filezigzag (www.filezigzag.com) before being stitched into a panoramic image via Autostitch (http://matthewalunbrown.com/autostitch/autostitch.html). To evaluate the validity of Autostitch, 20 unstitched sets of tiff frames (five baseline BB, five post-training BB, five baseline VL, five post-training VL) were manually stitched into a panoramic image using GIMP (www.gimp.org) in a manner similar to manual stitching methodologies reported elsewhere (24, 25). Regardless, the panoramic images were uploaded and calibrated in ImageJ (National Institute of Health, Bethesda, MD, USA) before being assessed for CSA (polygon tracing tool) and MT (a straight line at the thickest part [muscle belly] of each image). All images were stitched and analyzed by a single blinded reviewer. Each measurement in each limb was performed in duplicate (separately captured and stitched) and had good ICCs (two-way mixed, single-measures, consistency) for both the VL (CSA: 0.89; MT: 0.73) and BB (CSA: 0.92; MT: 0.83, all $P<0.05$).

Muscle fibre cross sectional area. Muscle fibre CSA was assessed using muscle biopsies that were obtained from the VL using a Bergström needle, that was custom modified for manual suction, under local anesthesia (1% xylocaine). Approximately 30 mg from each biopsy was cleared of connective tissue, mounted in optimal cutting temperature (OCT) media, frozen in liquid nitrogen-cooled isopentane, and stored in a -80ºC freezer until analysis. Cross sections were cut 5 μm thick using a Microm HM550 Cyrostat (Thermo Fisher Scientific, Waltham, MA) with particular care taken not to expose samples to any freeze-thaw cycles. Antibodies raised against dystrophin (MANDYS1 [3B7]), MHC I (BA-F8), MHC IIA/X (BF-35), and MHC IIX (6H1; Developmental Studies Hybridoma Bank, Iowa, USA) were combined with secondary isotype-specific antibodies (350 [A-21120], 488 [A-21131], 594 [A-21125], and 647 [A-21238]; Alexa Fluor, Invitrogen, Thermo Scientific) before being mounted with Prolong Diamond Antifade Reagant (Life Technologies, Toronto, ON, Canada) similar to previous publications (24, 26). Slides were kept in a dark drawer before being imaged the following day at wavelengths 488 nm (dystrophin), 350 nm (type IIA), 594 nm (type IIX), and 647 nm (type I) at 20x magnification with a Nikon Eclipse 90i fluorescent microscope (Nikon Instruments, Melville, NY, USA).

To determine fibre type and CSA, each dystrophin border was circled in Nike NIS Elements (Nikon Instruments, Melville, NY, USA) and exported as an individual region of interest (ROI). Exporting each fibre as a ROI provided us with a number of ROI characteristics including fibre area, circularity, and relative stain intensity in each channel (i.e., type I, IIA, and IIX MHC expression). The cut-off for inclusion in our CSA analysis

was a circularity of >0.85, which rendered 117±63 fibres per biopsy. In addition, we quantified the type of each fibre based on relative stain intensity (i.e., relative expression of each MHC isoform), which provided an objective way to determine hybrid fibre types and included an average of 244±98 fibres per biopsy. Where indicated, type II fibres include the weighted (according to distribution) average of type IIA, type IIA/X, and type IIX fibres. The image analysis for each participant was done by a single blinded study investigator.

Outcome Ranking. To assess the relative response of each limb in each participant we ranked (between 1 and 20) the increase in each outcome: FBFM (DXA; arms and legs; HL and LL), CSA (US; arms and legs HL and LL), MT (US; arms and legs HL and LL), type I fibre CSA (biopsy; legs only; HL and LL), and type II fibre CSA (biopsy; legs only; HL and LL), When comparing the upper- versus lower-body responses we summed the rank of each outcome in each condition in the upper- and lower-body, respectively. Similarly, when comparing hypertrophy versus strength outcomes we used the summed rank of each outcome in each limb for hypertrophy and strength, respectively.

Statistics. Statistical outliers were identified for the change in each outcome by multiplying 1.5 by the interquartile range and subtracting that number from the first quartile (low cutoff) and adding that number to the third quartile (high cutoff) (27). If an outlier was identified (<2 % of the data collected met the criteria of a statistical outlier), the data was replaced by the low cutoff or high cutoff value (28), which allowed us to

maintain statistical power and appropriately rank the participant (e.g., #1 or 20 for that outcome). One-way ANOVAs with either time or condition (HL vs. LL) as the independent variable were used to assess changes/differences in dietary outcomes, FBFM, appendicular FBFM, and exercise volume-load. Two-way repeated measures ANOVAs with time and condition (HL vs. LL) as the within-subject variables were run for changes in 1RM, peak torque, whole-muscle CSA, MT, leg FBFM, arm FBFM and fibre CSA. For outcomes that were evaluated in both the upper- and lower-body (FBFM, US CSA, US MT, 1RM, and peak torque), differences between the arms and legs were assessed via paired t-tests. In addition, linear regressions were used to detect the shared variance between each participant's upper- versus lower-body and between each participant's change in muscle size versus change in muscle strength. Finally, Pearson correlations between different individual measurements were used to assess the variability between different outcomes. All ANOVAs, t-tests, and correlations were performed in SPSS (version 20; Chicago, IL, USA) and all regressions were performed in Graphpad Prism (version 8; La Jolla, CA, USA). Significance was set at $P<0.05$. Where data are presented as box and whisker plots, the box is the interquartile range, the cross is the mean, the line is the median, and the tails are the minimum and maximum values. Where data are presented as waterfall plots, the relative change for each participant is marked with the same symbol in each outcome and the shaded area is the cumulative measurement error. Data in text are presented as means±SD.

Results

Participant and study characteristics. 20 young, recreationally-active men (22±3 y, 26±6 kg/m^2) completed this study with equal allocation of HL and LL conditions to dominant limbs. There was no change in protein (25±11 % total energy intake), carbohydrate (44±11 % total energy intake), or fat (34±10 % total energy intake) consumption throughout the study intervention. There was a significant difference between HL and LL total exercise volume-load in the arms (HL: 38335±849 versus LL: 58756±12351 kg, P<0.01) but not the legs (HL: 78583±22984 versus LL: 81969±26925 kg, P=0.20). There were no injuries reported throughout the intervention.

Strength. The RET intervention resulted in an increase in biceps curl 1RM (HL: 3.8±4.3, LL: 3.2±4.8 kg, P<0.01) and knee extension 1RM (HL: 24±11, LL: 22±9 kg, P<0.01) with a greater absolute (knee extension: 23±10, biceps curl: 3.5±4.5 Δkg, P<0.01) and relative (knee extension: 66±40, biceps curl: 22±27 Δ%, P<0.01) increase in the legs compared to the arms. However, there was no difference between HL and LL in either absolute or relative change in biceps curl or knee extension 1RM (all P>0.69). In addition, there was a significant increase in elbow flexion (HL: 11±10, LL: 12±15 Nm, P<0.01) and knee extension (HL: 38±53, LL: 42±41 Nm, P<0.01) peak torque with a greater absolute (knee extension: 40±47, elbow flexion: 12±13 ΔNm, P<0.01) but not relative (knee extension: 18±20, elbow flexion: 14±14 Δ%, P=0.36) increase in the legs compared to the arms. There was no difference in the absolute or relative increase

between HL and LL for either isometric elbow flexion or isometric knee extension peak torque (all P>0.48).

Muscular hypertrophy. The change in FBFM, VL CSA, VL MT, BB CSA, and BB MT are presented in Figure 1 and VL type I fibre CSA and type II fibre CSA are presented in Table 1. Broadly, there was an increase in whole-body FBFM (1.6±1.0 Δkg, P<0.01), appendicular FBFM (1.0±0.5 Δkg, P<0.01), leg FBFM (0.2±0.1 Δkg, P<0.01), and arm FBFM (0.3±0.3 Δkg, P<0.01) with a significant difference between the arms and legs (P=0.04) but not conditions (Figure 2, Panel A). In addition, there was a significant increase in VL CSA (1.1±2.2 Δcm^2, P<0.01), BB CSA (1.3±1.0 Δcm^2, P<0.01), VL MT (0.17±0.22 Δcm, P<0.01) and BB MT (0.17±0.22 Δcm, P<0.01) with no difference between the VL and BB or conditions (Figure 2, Panels B and C). Lastly, there was an increase in type IIA (607±1694 $\Delta \mu m^2$, P=0.03) and type II (pooled; 762±1729 $\Delta \mu m^2$, P=0.03) fibre CSA; however, there was no significant increase in type I (549±1381 $\Delta \mu m^2$, P=0.11), type IIA/X (987±1937 $\Delta \mu m^2$, P=0.08), or type IIX (676±2128 $\Delta \mu m^2$, P=0.38) fibre CSA (Table 1) and no differences between HL and LL in any fibre type (P>0.49).

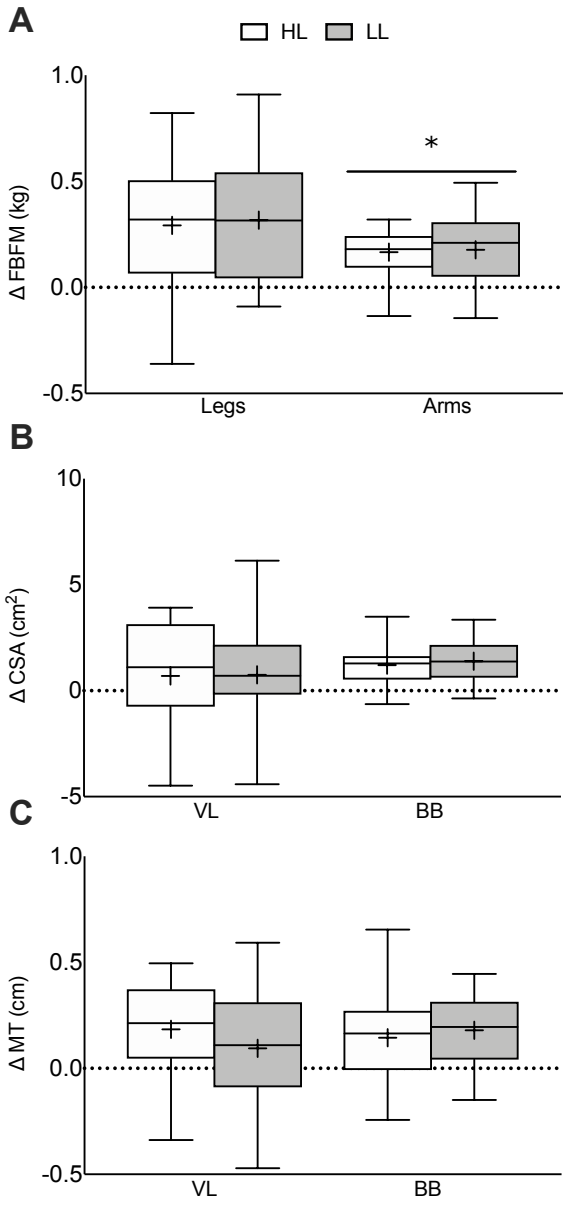

Figure 2. The absolute change in fat- and bone-free mass (FBFM; Panel A), US-measured cross sectional area (CSA; Panel B), and US-measured muscle thickness (MT; Panel C) between higher load (HL) and lower load (LL) in the lower- and upper-body. *Significant difference between the arms and the legs ($P<0.05$). VL: vastus lateralis and BB: biceps brachii.

Table 1. Changes in fibre CSA and distribution.

	Higher Load	Lower Load
Δ Fibre CSA (μm^2)		
Δ Type I CSA	495±1244	602±1537
Δ Type II CSA	811±1746*	712±1756*
Δ Type IIA CSA	704±1754*	510±1672*
Δ Type IIA/X CSA	1138±2422	875±1588
Δ Type IIX CSA	868±2203	483±2099
Δ Fibre Distribution (%)		
Δ Type I	-0.00±0.13	0.05±0.17
Δ Type II	0.47±2.04	0.12±0.67
Δ Type IIA	0.16±1.18	0.52±2.14
Δ Type IIA/X	3.83±5.98*	3.60±5.91*
Δ Type IIX	-4.17±5.47*	-4.21±4.77*

*Significant change pre- to post-intervention (P<0.05). CSA = cross sectional area.

Fibre Distribution. As illustrated in Table 1, there was no change in type I, IIA, IIX, or II fibres following RET; however, there was an increase in the number of hybrid type IIA/X fibres (4±6 Δ%, P<0.01) and a decrease in the number of type IIX fibres (-4±5 Δ%, P<0.01) with no differences between HL and LL (P>0.24).

Heterogeneity between outcomes and limbs. There was considerable individual variability between participants for each outcome assessing RET-induced change in muscle size (range [Δ%]; Arm FBFM: -3 to 13, Arm MT: -4 to 19, Leg MT: -4 to 24, Leg FBFM: -2 to 8, Arm US CSA: 1 to 24, Leg US CSA: -10 to 19, Type I CSA: -24 to 49, Type 2 CSA: -30 to 96; Figure 3) and muscle strength (range [Δ%]; elbow flexion peak torque: -17 to 36, knee extension peak torque: -16 to 56, dumbbell biceps curl 1RM: 0 to 87, and knee extension 1RM: 12 to 171). However, there was significant shared variance between the relative (rank) response between the upper- and lower-body in RET-induced change in muscle size (including FBFM, CSA, and MT; R^2=0.49, P<0.01) and strength (including 1RM and MVC; R^2=0.35, P<0.01; Figure 4, Panels A and B). For the interested reader, there is similar shared variance between the upper- and lower-body for RET-induced hypertrophy (R^2=0.39, P<0.01) and strength gains (i.e., R^2=0.18, P=0.07) when evaluated with cumulative percent change instead of a rank score. Interestingly, there was no shared variance between the rank (or cumulative percent change) in RET-induced changes in muscle size and strength (Figure 4, Panel C).

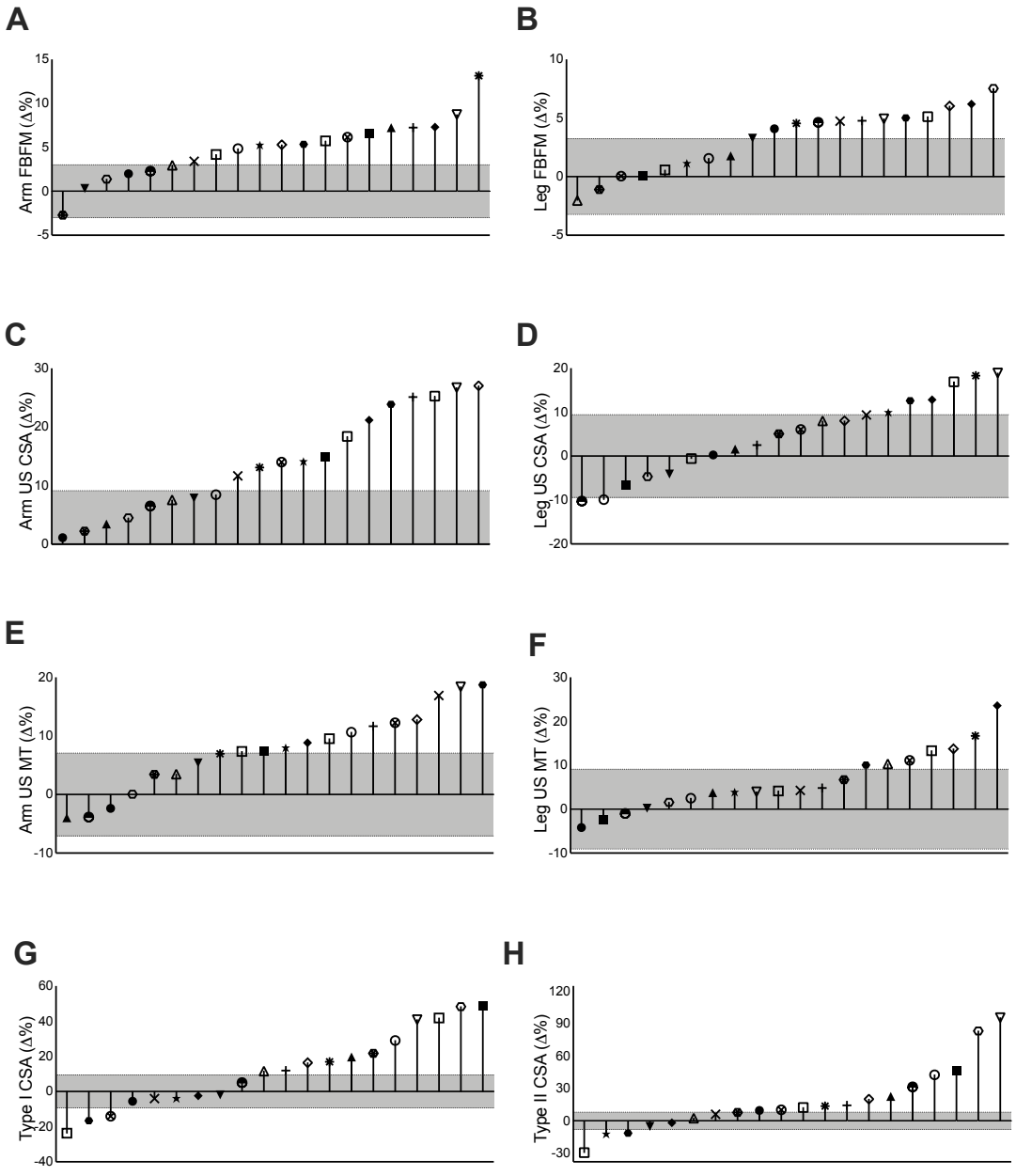

Figure 3. Waterfall plots with each participant marked with the same symbol across all outcomes assessing RET-induced changes in muscle size: Arm fat- and bone-free mass (FBFM; Panel A), Leg FBFM (Panel B), Arm US cross sectional area (CSA; Panel C), Leg US CSA (Panel D), Arm US muscle thickness (MT; Panel E), Leg US MT (Panel F),

Type I CSA (Panel G), and Type II CSA (Panel H). The shaded areas are the measurement error for each outcome.

Table 2. Correlations between the rank in lower-body hypertrophy outcomes

	Δ Leg FBFM	**Δ Leg CSA**	**Δ Leg MT**	**Δ Type 1 CSA**	**Δ Type 2 CSA**
Δ Leg FBFM	-	-0.04	-0.10	0.17	0.29
Δ Leg CSA	-0.04	-	0.74*	-0.44	-0.47*
Δ Leg MT	-0.10	0.74*	-	-0.43	-0.49*
Δ Type 1 CSA	0.17	-0.44	-0.43	-	0.79*
Δ Type 2 CSA	0.29	-0.47*	-0.49*	0.79*	-

*Significant correlation (P<0.05). FBFM: fat- and bone-free mass, CSA: cross sectional area, MT: muscle thickness.

Table 3. Correlations between the rank in upper-body hypertrophy outcomes

	Δ Arm FBFM	**Δ Arm CSA**	**Δ Arm MT**
Δ Arm FBFM	-	0.41	0.29
Δ Arm CSA	0.41	-	0.78*
Δ Arm MT	0.29	0.78*	-

*Significant correlation (P<0.05). FBFM: fat- and bone-free mass, CSA: cross sectional area, MT: muscle thickness.

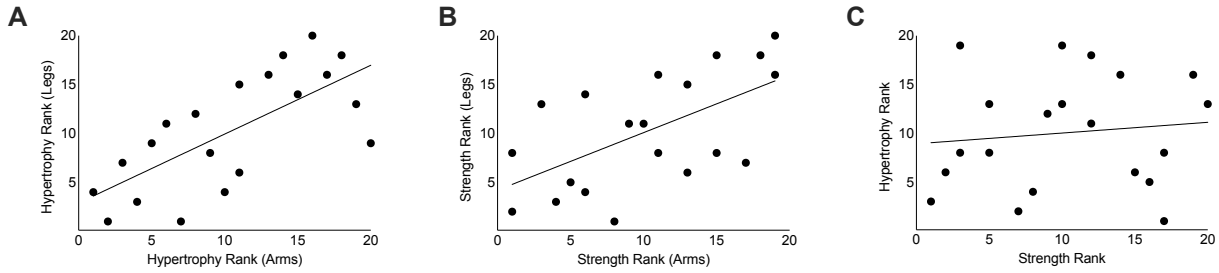

Figure 4. Correlation between each participants' cumulative leg rank versus their cumulative arm rank for RET-induced increases in muscle size (R^2=0.49, P<0.01, Panel A) and strength (R^2=0.35, P<0.01, Panel B). Correlation between RET-induced increases in muscle strength and muscle size (R^2=0.01, P=0.63, Panel C).

Correlations between resistance exercise training outcomes. Correlations between the ranks of each outcome assessing RET-induced hypertrophy in the upper- and lower-body are presented in Tables 2 and 3, respectively. Broadly, there were scarce correlations between different indices of muscle size measured with different methods; however, fibre CSA was inversely correlated with US-measured CSA and MT. In addition, there was a significant correlation between knee extension 1RM with knee extension peak torque (r=0.65, P<0.01) and no significant correlation between elbow flexion 1RM with elbow flexion peak torque (r=0.37, P=0.66; data not shown). For the interested reader, the correlations do not generally improve when run on the percent change in each outcome instead of the relative rank (data not shown).

Discussion

Similar to previous research (16) and contrary to RET guidelines (29, 30) we observed no difference between lifting higher- versus lower-loads on RET-induced changes in muscle size and muscle strength when the resistance exercise was performed to volitional fatigue (Figure 2 and Table 1). In addition, despite considerable variability between participants (Figure 3), there was significant shared variance between the RET-induced increases in muscle size and strength within an individual (i.e., between their arms and legs; Figure 4, Panels A and B, respectively). Interestingly, we observed negligible shared variance between RET-induced increases in muscle size and strength (Figure 4, Panel C) and limited correlations between common indices used to assess RET-induced increases in muscle size and strength (Tables 2 and 3). We conclude that when effort is matched, neither load nor limb mediate RET-induced hypertrophy, and reveal that RET-induced muscular hypertrophy is mediated in large part by endogenous differences.

Similar to a number of recent randomized controlled trials (15, 16, 26, 31-38) and meta-analyses (16), we observed that muscular hypertrophy is independent of load when the resistance exercises are performed to volitional fatigue (Figure 2). Further, we found similar RET-induced increases in 1RM tests between higher- versus lower-loads, which was unexpected (15, 16, 21, 35, 37) and may be a product of the documented cross-limb education effect on muscular strength (39). Nevertheless, the lack of difference between loading conditions in the unpracticed isometric peak torque tests is similar to previous RET studies (15, 16, 36, 37, 40, 41) and there is now substantial evidence that higher

loads while sufficient are not necessary for RET-induced increases in muscle size or strength when RET is performed to volitional fatigue.

We hypothesized that the percent change between our outcomes (e.g., FBFM and fibre CSA) would be unrelated, but the relative score (i.e., rank) of each participant in each outcome would show a better relationship (22). However, both with and without ranking our participants, we observed few correlations between common indices that are broadly used to measure muscle size (Tables 2 and 3). Furthermore, we show that indices of RET-induced increases in muscle size are unrelated to RET-induced increases in muscle strength (Figure 4, Panel C), which was contrary to our hypothesis (23) but supports current opinion (42). For the interested reader, the shared variance between the upper- and lower-body is not improved if each outcome is evaluated individually (e.g., US CSA in the legs and arms; all $R^2<0.11$, $P>0.17$). Furthermore, the only significant correlations between an individual index of muscle size with an individual index of muscle strength were inverse correlations between the RET-induced increase in type I CSA with the RET-induced increase in knee extension 1RM ($r=-0.60$, $P<0.01$) and arm MVC ($r=-0.46$, $P<0.05$; data not shown). We conclude that multiple methods of assessment are important to make general conclusions about RET-induced muscular hypertrophy and that RET-induced changes in muscle size and strength are unrelated at least within the first 10 wk of a RET protocol.

The principle aim of this study was to investigate the relative contribution of load and limb on RET-induced muscular hypertrophy, and we observed that neither load (Figure 2 and Table 1) nor limb location (Figure 4, Panel A) determine RET-induced

muscular hypertrophy. Our data broadly corroborate previous analyses that have revealed the limited (if any) contribution of exogenous factors to hypertrophy when considered relative to the change seen without the added exogenous stimulus, which would include: protein supplementation (9), load (16), exercise volume-load (10, 11), training frequency (12), time of training (43), velocity of contraction (13), type of exercise (14), days of recovery between training sessions (44), and occlusion of blood flow (15). We conclude that RET-induced muscular hypertrophy is primarily mediated by endogenous differences.

Limitations

The use of unilateral designs in exercise can be advantageous (45); however, a common critique of unilateral exercise designs is the contribution of a cross-limb education effect. Indeed, a recent meta-analysis concluded that unilateral strength training increases both isotonic and isometric strength of the contralateral non-exercising limb (46). Therefore, it is plausible that the difference in RET-induced increases in muscle strength between HL and LL limbs were diluted by a cross-education effect. In contrast, there is scant evidence that unilateral RET results in an increase in muscle size in the contralateral untrained limb (2); in fact, post-exercise rates of protein turnover are exclusive to the muscle group that is contracting (47, 48) and are unaffected by systemic endogenous hormone exposure (49).

Conclusion

We observed that there was considerable inter-individual variability in RET-induced increases in muscle size (range [Δ%]; Arm FBFM: -3 to 13, Arm MT: -4 to 19, Leg MT: -4 to 24, Leg FBFM: -2 to 8, Arm US CSA: 1 to 24, Leg US CSA: -10 to 19, Type I CSA: -24 to 49, and Type 2 CSA: -30 to 96) and muscle strength (range [Δ%]; elbow flexion peak torque: -17 to 36, knee extension peak torque: -16 to 56, dumbbell biceps curl 1RM: 0 to 87, and knee extension 1RM: 12 to 171). However, both RET-induced increases in muscle size (to a greater extent) and muscle strength (to a lesser extent) are independent of load and limb. In addition, we observed minimal correlations between different indices of muscle size and between different indices of muscle strength, and no correlation between RET-induced increases in muscle size and strength. Thus, we encourage future research to consider using multiple measures of muscle size and muscle strength for a more complete appraisal of RET-induced adaptations. We conclude that exogenous factors play a relatively small role in influencing RET-induced muscular hypertrophy. In particular, we show here that there are only small influences of exogenous variables such as load (but also protein supplementation (9), exercise volume-load (10, 11), training frequency (12), velocity of contraction (13), exercise selection (14), days of recovery between training sessions (44), and occlusion of blood flow (15)) on RET-induced muscular hypertrophy. Instead, we conclude that endogenous factors are primary determinants of RET-induced increases muscular hypertrophy.

Competing Interests

The authors have no competing interests financial or otherwise to declare.

Author Contributions

RWM and SMP designed the study, RWM, MDF, SRM, RSS, CM, and SMP performed the data collection, RWM, MDF, SRM, RSS, BF, JGB, and SMP performed the data analysis, RWM, JGB, and SMP drafted the manuscript, and all authors critically revised the manuscript and approved the final version.

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CHAPTER 7:

DISCUSSION

7.1 Increasing skeletal muscle mass and strength in humans

The main aim of this thesis was to explore the contribution of exogenous and endogenous variables on resistance exercise training (RET)-induced increases in muscle size and strength. The studies included in this thesis assessed the efficacy of protein supplementation (Study 1), external load (Study 2, 4, and 5), contraction cadence (Study 4), circulating hormone concentrations (Study 2 and 3), intramuscular hormone-related variables (Study 3), and limb location (Study 5) on acute and/or chronic indices of RET-induced adaptation. Collectively, these studies showed that protein supplementation, load, systemic hormones, and intramuscular hormones have relatively small (if any) effects on RET-induced adaptations over and above RET alone. The broad take-away message from these studies together with recent evidence is that endogenous factors, such as intramuscular androgen receptor (AR) content, appear responsible for a greater contribution to RET-induced hypertrophy than exogenous factors.

7.2 Dietary protein intake

Prior to Study 1 being published, there were conflicting narrative reviews (1-5) and meta-analyses (6-12) on the efficacy of protein supplementation on RET-induced adaptations. Therefore, the purpose of Study 1 was to provide a comprehensive assessment on the efficacy of protein supplementation during RET. Accordingly, the largest meta-analysis before Study 1 included 22 studies and 680 participants (7), which was less than half the number of studies and participants included in Study 1 (49 studies and 1863 participants). The principal finding from Study 1 is that protein supplementation slightly augmented

RET-induced adaptations: one repetition maximum (1RM; means [95% confidence interval]: 2.49 kg [0.64, 4.33]), fat-free mass (FFM; 0.30 kg [0.09, 0.52]), muscle fibre cross sectional area (CSA; 310 μm^2 [51, 570]), and mid-femur CSA (7.2 mm^2 [0.20, 14.30]). However, the effect of protein supplementation was relatively small compared to the benefit of RET alone (1RM: 27±22 kg; FFM: 1.1±1.2 kg; Appendix A, Supplementary Figure 4).

7.2.1. Considerations for protein provision during RET

A secondary aim of Study 1 was to explore the putative role of other variables that could mediate the efficacy of protein supplementation during RET using meta-regressions. We noted that protein dose (positively) (13), training status (negatively) (14), and age (negatively) (15) altered post-resistance exercise rates of muscle protein synthesis (MPS). Thus, we chose *a priori* to examine the impacts of protein dose, training status, and age as covariates in composite and univariate meta-regressions, which revealed that training status positively (0.75 kg [0.09, 1.40], P=0.03), age negatively (-0.01 kg [-0.02, -0.00], P=0.002), but not protein dose 0.02 (-0.01, 0.04) mediated the efficacy of protein supplementation on RET-induced increases in FFM. Indeed, resistance-trained participants have a smaller potential for muscle growth (16), so it is plausible that the increased efficacy of protein supplementation during RET in resistance-trained persons is reflective of an increased relative effect of the protein supplementation versus an effect of RET per se. In addition, older persons require a greater protein dose to achieve maximal stimulation of myofibrillar MPS (13) and may benefit from higher daily protein intakes to

stimulate MPS (17); however, in Study 1, the average protein bolus given to older persons was less than half of the protein bolus given to younger participants (~20 vs. ~40 g). Therefore, we hypothesize that the efficacy of protein supplementation during RET would have been augmented had the older persons been supplemented with a greater, or at least equal, protein dose as the studies carried out in younger individuals. We also noted that neither training status (5.77 [-2.96, 7.13]), age (0.01 [-0.09, 0.11]), nor protein dose (0.13 [-0.04, 0.31]), mediated the efficacy of protein supplementation on RET-induced increases in 1RM strength, which corroborates a lack of relatedness between changes in FFM and 1RM (Study 5 and (18)).

We recognized that there are a number of other factors that may mediate the efficacy of protein supplementation during RET beyond protein dose, age, and training status, so we ran additional univariate meta-regressions and included them here in Appendix A (Supplementary Table 3). Briefly, the change in protein intake, repetitions per set, sets per exercise, sessions per week, length of training intervention, total exercise volume, protein source, sex, type of supplementation (whole-food versus isolated protein source), whether the RET was whole-body or not, and whether the RET was supervised or not did not mediate the efficacy of protein supplementation on RET-induced increases in FFM. However, whole-body RET (positively) (4.41 [1.14, 7.68]) and supervised RET (negatively) (-3.80 [-7.56, -0.06]) effected the efficacy of protein supplementation on RET-induced increases in 1RM. In addition, we acknowledged that there is a dose-response relationship between protein supplementation and myofibrillar MPS (13), so we undertook an additional unadjusted bi-phasic linear segmented regression analyses to

determine if there was a dose-response relationship between daily protein intake and RET-induced changes in FFM. Indeed, we found that protein supplementation beyond total protein intakes of 1.62 g of protein/kg of body mass/d (95% CI: 1.03, 2.20) did not augment RET-induced increases in FFM, which included 42 study arms and 723 participants (both young and old with daily protein intakes ranging from 0.9 to 2.4 g /kg/d).

7.2.2. Practical implications for increased daily protein intake

The study durations included in Study 1 ranged from 6 to 52 weeks (13±8 wk), which resulted in an average increase in both 1RM (including bench press, leg press, shoulder press, and knee extension; 27±22 kg) and FFM (including air plethysmography, hydrostatic weighing, and dual-energy x-ray absorptiometry [DXA]; 1.1±1.2 kg). Evidently, Study 1 provided a comprehensive appraisal (1863 participants) of the modest effect of short-term RET interventions on RET-induced increases in 1RM and FFM, which can be been used as a benchmark for researchers and practitioners alike (19). Furthermore, we quantified the relative unadjusted effect of protein supplementation from the 49 studies and reported that protein supplementation provided a 2.49 kg (9%) and 0.30 kg (27%) additive effect for increases in 1RM and FFM, respectively (Appendix A, Supplementary Figure 3). Therefore, an important implication from Study 1, which is well-supported by acute rates of MPS (20), is that RET alone is a far more potent stimulus than protein supplementation. Lastly, the average daily protein intake of the 1863 participants was ~1.4 g protein/kg/d, which is 75% greater than the current

US/Canadian recommended dietary allowance (21); however, protein supplementation still augmented RET-induced changes in FFM. Therefore, we conclude that consuming protein at the RDA of 0.8 g of protein/kg of body mass/d is insufficient for those who have the goal of gaining greater strength and FFM with RET, and provide an evidence-based recommendation that daily protein intakes should be at least 1.6 g/kg/d. This recommendation has been recognized and implemented in both the International Society for Sports Nutrition (22) and International Olympic Committee (23) position stands.

7.3 External load and other RET-related variables

Data from our laboratory showed that load (i.e., the amount of mass lifted per repetition) does not determine acute post-resistance exercise rates of MPS (24) or RET-induced increases in muscle size (25) when the resistance exercise is performed to volitional fatigue (i.e., until the participant cannot perform another concentric repetition according to their own volition). However, the aforementioned studies were dismissed by some because the participants were training-naïve and the study was conducted using a within-subject, unilateral design (26). Therefore, the purpose of Study 2 was to compare RET-induced adaptations between participants performing RET with heavier loads (HL; ~75-90 % 1RM) or lighter loads (LL; ~30-50 %1RM) using a whole-body RET intervention in resistance-trained young men. The principal finding of Study 2, in accordance with our previous data, was that load did not determine RET-induced adaptations in resistance-trained men when resistance exercise was performed to volitional fatigue. In addition, we ran Study 4, independently, to test the hypothesis that acute muscle fibre activation would

be similar (despite dissimilar surface electromyography [EMG] amplitude) between resistance exercise with HL and LL performed to volitional fatigue. Indeed, we found that muscle fibre activation was independent of load and contraction cadence when the resistance exercise was performed to volitional fatigue. Finally, the purpose of Study 5 was to determine variability in hypertrophy within (upper versus lower body) and between individuals performing contractions to volitional fatigue with HL and LL. Data from Study 5 revealed that the relative hypertrophy within an individual (between arms and legs) showed relatively good agreement and was again independent of load (between RET with HL and LL).

7.3.1. RET-related variables and muscle hypertrophy

Following the publication of Study 2, a meta-analysis of 21 studies (27) and a number of studies since that meta-analysis (27-36) have shown that RET-induced increases in muscle size and strength are independent of load, particularly when both HL and LL are performed to volitional fatigue. Indeed, LL RET performed to volitional fatigue results in performance of a greater number of repetitions and therefore 'volume-load' (repetitions x load x sets), which may augment resistance exercise-induced increases in myofibrillar MPS (37). Indeed, volume-load differs when studies categorically manipulate the number of sets per session (38-41), the number of repetitions per set (31, 42, 43), the number of training sessions per week (44-46), the load lifted per repetition (28, 29, 34, 36, 47, 48), and the contraction cadence (49); however, an increase in volume-load does not necessarily result in increased RET-induced hypertrophy. Recently, a proof-of-concept

study showed that six weeks of thrice-weekly unilateral elbow flexion RET with relatively HL (70 %1RM) results in similar RET-induced increases in muscle thickness (MT) as performing the same number of repetitions with no load (but contracting as hard as possible throughout the range of motion) (35). Evidently, load and volume-load do not determine RET-induced increases in muscle size, at least in the short-term.

There are many other RET-related variables that can be manipulated in an attempt to induce specific changes in muscle size and strength, these include: training frequency (50), inter-set rest (51), the time of day the resistance exercise is performed (52), the cadence of contraction (49), eccentric versus concentric muscle actions (53), an individual's autonomy over RET variables (54), periodized versus non-periodized programs (55), the days of recovery between training sessions (56), single- vs. multi-joint resistance exercise (57), and whether blood flow is occluded or not (34, 58). However, a broad overview of the studies cited above shows that categorical manipulation of any single RET-related variable confers relatively small (if any) benefits on RET-induced increases in muscle mass or size. Though a detailed review of each RET-related variable is beyond the scope of this thesis discussion, our substantive hypothesis is that specific resistance exercise variables are secondary to the intensity of effort, no matter how specific variables are manipulated, during the RET (3).

7.3.2. Muscle fibre recruitment and RET-induced muscle fibre hypertrophy

Studies 2 and 5, and previous data from our laboratory (25), showed similar type II fibre hypertrophy between HL and LL RET interventions, which we hypothesized was

indicative of similar type II fibre activation. The size principle defines the characteristic recruitment of motor units based on their size (and depolarization threshold), which increases from small motor units to larger motor units with increased intensity of effort due to load lifted or progression to fatigue (reviewed elsewhere (59)). Indeed, data from a number of studies in both aerobic (60-65) and resistance (66-68) exercise showed substrate depletion in type II fibres, which is evidence of the size principle during exercise training. Nonetheless, some have assumed, based on higher EMG amplitudes during HL resistance exercise (69-71) or decomposition of the EMG signal during isometric contractions (72), that HL resistance exercise preferentially recruits [and leads to superior hypertrophy of (73)] type II muscle fibres. Therefore, the purpose of Study 4 was to test whether categorical manipulation of specific RET variables resulted in similar muscle fibre recruitment if resistance exercise was performed to volitional fatigue. There is evidence that both increased load (24) and contraction cadence (74) augment post-resistance exercise rates of myofibrillar MPS when the resistance exercise is not performed to volitional fatigue; thus, we chose to manipulate load and contraction cadence to challenge our hypothesis that performing resistance exercise to volitional fatigue results in similar muscle fibre activation.

The primary finding of Study 4 was that muscle fibre activation (as detected by glycogen depletion) is independent of load or contraction cadence when resistance exercise is performed to volitional fatigue. We also noted that EMG amplitude is a poor predictor of muscle fibre activation and challenge the assertion that EMG amplitude during resistance exercise with HL is indicative of greater motor unit recruitment (69-72).

Finally, we observed that acute anabolic protein signaling (1 h post-resistance exercise) was independent of load and contraction cadence.

7.3.3. Load and RET-induced increases in muscle strength

Data from our laboratory (25) and other laboratories (27, 32, 34, 35, 75) have shown that RET-induced increases in voluntary 1RM are superior when repeatedly performing RET with loads that are nearer-to-maximal loads (e.g., >85 %1RM). Nonetheless, the data in Study 5, our previous work (25), and that of others (27, 33-35, 48, 76) have shown that when strength is evaluated in an unpracticed test (e.g., isometric dynamometry) there is no difference between HL and LL RET on increases in muscle strength. Indeed, RET-induced increases in 1RM strength tests are seldom related to RET-induced increases in an unpracticed peak torque test (75) and reviewed here (77). As proof-of-concept, others have elucidated that simply practicing a 1RM test five times twice per week for 8 weeks results in similar RET-increases in 1RM strength as performing a typical high-volume RET regime (four sets of 8-12 repetitions) twice per week for eight weeks (76). Therefore, in Study 2 we tested the hypothesis that periodic practice of the 1RM tests (affording the LL condition 'practice' with lifting heavier loads) would result in similar post-RET increases in 1RM strength. Accordingly, we found similar increases in 1RM between HL and LL RET (with the exception of bench press), which we hypothesize is because we afforded both groups practice of the 1RM tests.

7.3.4. Practical implications for manipulating load during periods of RET

In contrast to the recommendations put forth by the American College of Sports Medicine (ACSM) (78) and National Strength and Conditioning Association (NSCA) (79), Study 2 and Study 5 demonstrate that, when RET is performed to volitional fatigue, RET-induced hypertrophy is independent of load. Indeed, we advocate that future RET guidelines include increased emphasis on effort during resistance exercise and decreased emphasis on the strength-endurance continuum (i.e., that RET with LL results in inferior RET-induced increases in muscle strength and size) (78, 79). In summary, together with previous publications from our laboratory (24, 25), Studies 2, 4, and 5 challenge current RET dogma and provide support for the thesis that muscle fibre activation and a subsequent program of events underpin RET-induced muscle hypertrophy.

7.4 Anabolic hormones and RET-induced adaptations

Following a bout of resistance exercise there is an increase in a number of circulating hormones including testosterone (T), growth hormone (GH), and insulin-like growth factor 1 (IGF-1) (80-87); however, data from our laboratory has revealed that the post-resistance exercise rise in those hormones does not correlate with the magnitude of resistance exercise-induced myofibrillar MPS (82, 85) or RET-induced hypertrophy (88, 89). Nonetheless, narrative reviews (90, 91) and RET guidelines (78) posit that circulating hormones are related to, and indeed are predictive of, RET-induced changes in muscle size. The support provided in these (90, 91) reviews comes from other narrative reviews (92), data from studies in which exogenous T results in increases in muscle size

(93-97), correlations from select small cohorts (98, 99), and, recently, statistical modeling on small data sets (26 participants) (100) that is usually reserved for very large data sets. The purpose of Studies 2 and 3 were to, in a large (the largest intervention datasets so far as we are aware) data set of 49 resistance-trained participants and 10 circulating biomarkers, test whether the resting concentration or post-resistance exercise rise in any hormone was related to any index of RET-induced adaptation. In addition, Study 3 included intramuscular analyses of free T, dihydrotestosterone, 5a reductase (an androgen-converting enzyme), and AR content on the top and bottom quintile of 'responders' to 12 weeks of RET (Appendix C, Supplementary Table 1). We found that there was no association between any of the circulating hormones we measured with RET-induced muscle hypertrophy and no difference in intramuscular free T, dihydrotestosterone, and 5a-reductase between the top and bottom quintile of responders to RET. However, we found significant correlations between AR content and the change in type I CSA, type II CSA, and fat- and bone-free mass (FBFM; measured by DXA), and that AR content was significantly higher before and after 12 weeks of RET in the top compared to the bottom quintile of responders.

7.4.1. Critical evaluation of support for the hormone hypothesis

There are several lines of evidence that show the flaws in evidence and logic that are ostensibly supportive of the hormone hypothesis. First, the post-resistance exercise rise in circulating hormones (e.g., T: 500 ng/dl) (82, 85) is four- to 10-fold lower than resting concentrations in individuals receiving exogenous T (e.g., 600 mg weekly injections of T

enanthate: 3000 ng/dl) (97). Further, the post-resistance exercise rise in circulating hormones is remarkably transient (~15-60 minutes; if detectable at all); thus, in a single week (of what is often a 10+-week cycle of exogenous T provision) there is over a one thousand-fold difference in systemic exposure to circulating hormones. Therefore, it is hard to reconcile how the post-exercise increase in circulating hormones is comparable to the physiological manifestations of exogenous T provision (discussed in more detail below and elsewhere (101)). Similarly, we view previous correlational analyses on ≤11 participants as largely inconclusive compared with much correlations performed with much larger data sets in 56 (89) and 49 (Study 2) participants. We also highlight and discuss in Study 3 that the use of partial least squares structural equation modeling (PLS-SEM) is inappropriate for small data sets (e.g., 26 participants (100)) primarily because PLS-SEM exaggerates the weights of spurious correlations (102). Indeed, between Study 2 and Study 3 we performed 120 correlations, 24 regressions on original data, and 24 regressions on principal components, which was intentional data dredging to show the scant and poor association between the post-resistance exercise rise in circulating hormones with RET-induced muscle hypertrophy.

7.4.2. Evidence in contrast to the hormone hypothesis

A persuasive argument against the hormone hypothesis is that the post-exercise rise in ostensibly anabolic hormones (e.g., T, GH, and IGF-1) is not limited to resistance exercise but to exercise in general. For example, aerobic exercise that is comparable in duration to a bout of RET produces a similar (if not oftentimes superior) increase in

circulating T (103-106), GH (104, 106-108), and IGF-1 (106, 107, 109). Indeed, a function of exercise-induced release of GH (and the simultaneous release of IGF-1 (110, 111)) into circulation is to promote the mobilization of lipid as a fuel (112). Similarly, the function of the exercise-induced release of T into circulation may be related to glucose uptake (113, 114). There is evidence showing that exogenous administration of GH results in increments of lean body mass and reductions in fat mass, but with no effect on strength (115). Thus, GH may enhance retention of water or promote the synthesis of non-force generating proteins, but there is no evidence that either exogenous GH and/or IGF-1 increase muscle mass for force-generating capacity in healthy humans [reviewed in detail elsewhere (115-117)].

Perhaps the most compelling argument that undermines the hormone hypothesis is a sex-based comparison of mechanisms and muscle mass gain in men and women. The major male androgenic hormone T is produced by Leydig cells of the testes; hence, men have 10-15-fold higher circulating T at rest and 45-fold higher circulating T following an acute bout of RET (85). However, men and women also have similar rates of MPS at rest (118-121), stimulation of rates of MPS following a bout of resistance exercise (85, 122), and relative (to their initial muscle mass) RET-induced muscle hypertrophy (123). Further, women experience similar RET-induced adaptations to a number of the aforementioned specific RET-related variables (33, 36, 39, 40, 42, 49, 124, 125). Thus, the similar acute and chronic response to resistance exercise between men and women is quite convincing evidence that circulating T does not play a strong role in determining resistance exercise-induced muscle anabolism.

7.4.3. Androgen receptors (AR) and RET-induced adaptations

Androgens (e.g., T) can passively diffuse through cell membranes to bind with ARs in the cytoplasm. When dimerized with the AR, the canonical action of an androgen-receptor complex is to translocate into the nucleus and bind to upstream elements of specific genes possibly interacting with various transcription factors involved in skeletal muscle growth and development (126). Indeed, in addition to an increase in the number of myonuclei (127), satellite cells (128), and muscle fibre number (129, 130), a characteristic consequence of exogenous T provision is an acute increase in AR mRNA (131) and AR content (after a period of weeks) (93, 132). Furthermore, there is evidence to suggest that AR content is a modulating factor in androgen-mediated regulation of skeletal muscle mass and size. For example, knocking out AR reduces muscle mass in male mice (133), a bout of resistance exercise can increase AR mRNA expression in both young (84, 85, 134) and older men (135), weeks of RET may increase intramuscular AR content in training-naïve men (136, 137), and the RET-induced increase in AR protein content has been correlated with RET-induced hypertrophy (137, 138). Accordingly, we observed in Study 3 that the top quintile of responders to RET had significantly more AR content (both pre- and post-RET) than the bottom quintile of responders. Therefore, our data broadly support a body of evidence that androgen-related muscle anabolism is affected by intramuscular AR content.

7.4.4. Practical implications for the role of endogenous hormones during RET

Studies 2 and 3, alongside numerous previous reports from our laboratory (82, 85, 88, 89), show that circulating hormones are not associated, or are in any way predictive, of RET-induced adaptations. Therefore, we advocate that exercise scientists, practitioners, and health professionals refrain from use of resting concentrations or the post-exercise rise in circulating hormones to infer the ability of an individual or particular exercise regime to experience/stimulate RET-induced adaptations in eugonadal healthy individuals. Moving forward, Study 3 offers a paradigm shift away from quantifying androgen availability (either in the blood or in the muscle) and towards considering other intramuscular indices (e.g., AR content) as an important step in RET-induced and androgen-mediated muscle hypertrophy.

7.5 Exogenous versus endogenous factors and RET-induced adaptations

To date, researchers have manipulated a number of exogenous variables (e.g., protein intake (124), exercise volume (39, 40), training frequency (50), training velocity (49), and external load (27)) but observed relatively small (if any) effects on RET-induced adaptations. Indeed, the data, when viewed in totality, in this thesis showed that protein supplementation (Study 1) and load (Study 2 and 5) have limited effects on RET-induced muscle hypertrophy. Meanwhile, there is emerging evidence that endogenous differences between individuals largely underpin the inter-individual variability in RET-induced muscle hypertrophy. There are a number of potential candidates for what within-person variables could be deterministic for RET-induced hypertrophy, however, including:

satellite cell number (139), intramuscular biomarkers (e.g., intramuscular total RNA and myozenin 1) (140, 141), mitochondrial volume (140), select microRNA expression (142), select mRNA expression (143), and individual gene variants (e.g., *ACE* and *ACTN3*) (144, 145)). Indeed, Study 3 showed that AR content may contribute to the degree of inter-individual variability in RET-induced muscle hypertrophy as well.

The notion that adaptations to aerobic exercise training are mediated endogenously is well-supported with studies between monozygotic twins (146-148) and with the predictability (based on the Heritage study) of aerobic exercise training-induced increases in cardiorespiratory fitness (149-152). Aerobic exercise training-induced increases in cardiorespiratory outcomes are estimated to be ~50% heritable, and the inter-individual variability in aerobic exercise training-induced increases in maximum oxygen uptake have given rise to comprehensive genomic and transcriptomic investigations (reviewed elsewhere (153)). In contrast, the relative contribution of endogenous factors on RET-induced muscle hypertrophy remains uncertain. Therefore, the primary purpose of Study 5 was to establish the relative effect of exogenous (load) and endogenous (limb location) factors on RET-induced muscle hypertrophy. We found that RET-induced hypertrophy is consistent within an individual (between the upper- and lower-body; R^2=0.49) and independent of load despite considerable inter-individual variability in indices of RET-induced muscle hypertrophy (e.g., range [Δ%]; leg FBFM: -2 to 8, VL MT: -4 to 24, VL CSA: -10 to 19, and Type II fibre CSA: -30 to 96). Therefore, we conclude that endogenous (within-person) factors are principal mediators of RET-induced

muscle hypertrophy and advocate for further investigation of the endogenous determinants of RET-induced increases in muscle mass and size.

7.6 Methodological considerations in RET research

As discussed above, RET-induced increases in strength are largely dependent on the habitual practice of the strength test [Study 2, Study 5, and (76)] and are seldom related (e.g., 1RM versus isometric peak torque) (75). Furthermore, a recent review highlighted that there is considerable discrepancy between different indices of RET-induced increases in muscle size as well (154). Therefore, in Study 5 we used ultrasonography (US), DXA, histochemistry, 1RM testing, and isometric dynamometry to assess RET-induced adaptations and observed there were only weak to moderate correlations between the relative RET-induced increase in 1RM and peak torque, and no positive correlations between different modalities used to measure changes in muscle size (e.g., between DXA and US). Notably, the RET-induced change in type I and type II fibre CSA were not related to the RET-induced change in leg FBFM (type I: r=0.17, P=0.47; type II: r=0.29 and P=0.22) and were inversely correlated with the change in US-measured VL CSA (type I: -0.44, P=0.06; type II: r=-0.47, P=0.04) and VL MT (type I: r=-0.43, P=0.06; type II: r=--0.49, P=0.03). Therefore, in support of previous research, we conclude that 1RM and peak torque measurements are weakly (at best) related and that RET-induced increases in fibre CSA are not related to other indices of RET-induced muscle hypertrophy.

The percent change in each variable commonly used to assess RET-induced muscle hypertrophy varied markedly (Appendix C, Supplementary Figure 1) and each variable had its own measurement error (Study 5, Figure 4). Importantly, many exercise scientists interpret RET-induced increases in DXA-derived lean body mass as RET-induced increases in muscle mass; however, DXA uses x-rays to assess bone density and only through a series of subtractive attention does it estimate fat mass versus FBFM. Indeed, FBFM is subject to changes in fluid balance, intramuscular fat, and non-skeletal muscle organs (e.g., heart, liver, intestines, and other organs). In fact, muscle mass only makes up ~50 % FBFM (155). Similarly, despite strong correlations between US-measured CSA and magnetic resonance imaging-quantified CSA (156-158), US MT and CSA are affected by muscle edema (34), measurement location (i.e., muscle) (159), and the technician technique (for example, taking care not to depress the muscle tissue during the measurement: Appendix D, Supplementary Figures 1 and 2). Finally, changes in fibre CSA are dependent on the orientation of the fibres during microscopy, on the location of the biopsy, and are particularly subject to sampling error given the relatively small area of the muscle assessed (~150 cells, approximately 50 % of which are type I and type II fibres, respectively). Notably, the RET-induced histologically-determined changes in muscle fibre CSA are the most extreme, which may reflect the relatively high susceptibility of this method to sampling error (Appendix C, Supplementary Figure 1). In conclusion, along with others (75, 77, 154, 160), we advocate that future research include multiple methods of assessment of muscle hypertrophy for a more comprehensive

appraisal of RET-induced increases in muscle strength (i.e., practiced versus not practiced) and muscle size (i.e., FBFM, US, and fibre CSA).

7.7 Future directions

Study 1 identified only two studies in older persons that included a RET intervention and daily protein intake near what we consider optimal in elderly individuals (~1.2-1.6 g/kg/d) (5). Indeed, given the protective effects of increased skeletal muscle mass and function against sarcopenia (161), loss of independence (162), obesity/insulin resistance (163), cardiovascular disease (164), and mortality (164-167), and acknowledging that there is no increased risk of kidney damage (168) cancer (169), cardiovascular disease, or mortality (170) with increased protein intake, there is a clear need for more (and larger) randomized controlled trials in elderly persons performing RET with higher daily protein intakes. In addition, though there is substantial evidence that challenges the hormone hypothesis, our finding that AR content may be the rate-limiting step in androgen-mediated anabolism warrants future mechanistic research and continued study of AR in different situations (e.g., RET, immobilization, aging, and critical illness). Finally, there is emerging evidence that endogenous factors (e.g., Study 3, Study 5, and (139-145)), as opposed to exogenous factors (Study 1, Study 2, Study 4, Study 5, and (27, 34, 39, 40, 49, 50, 57)) underpin the inter-individual variability in RET-induced muscle hypertrophy. Therefore, larger cohort studies and follow-up studies may help reveal the repeatability of RET-induced responsiveness, and should be paired with larger ‘omic’ analyses (similar to aerobic exercise training studies (153)) to provide a more comprehensive assessment of

the endogenous mediators of RET-induced hypertrophy. In addition, we acknowledge that endogenous and exogenous factors that underpin RET-induced adaptations may also include psychology (e.g., effort and motivation) and sociology (e.g., peer motivation), respectively, and should be included in future studies. Finally, it is prudent to acknowledge that the efficacy of an exogenous factor to augment RET-induced adaptation, although inferior to endogenous factors, may provide a clinically-significant benefit, so we encourage researchers and practitioners to continue seeking exogenous variables that affect RET-induced increases in muscle mass and strength.

7.8 Conclusion

The collective conclusion of this thesis is, when RET is performed with significant effort, that the categorical manipulation of specific exogenous factors such as protein supplementation (Study 1), load (Study 2, 4 and 5), and contraction cadence (Study 4) provide small (if any) benefit on the acute and chronic response to RET. In addition, we add to a growing body of evidence that show androgen receptor content, but not circulating hormones, is associated with RET-induced increases in muscle size (Study 2 and 3). Further, we observed that inter-individual response variability is underpinned primarily by endogenous factors (Study 5). In summary, endogenous factors appear to be the principal determinants of adaptation to RET, but performing RET with a high degree of effort and with sufficient daily protein intake (~1.6 g/kg/d) are effective strategies to improve RET-induced increases in muscle size and strength.

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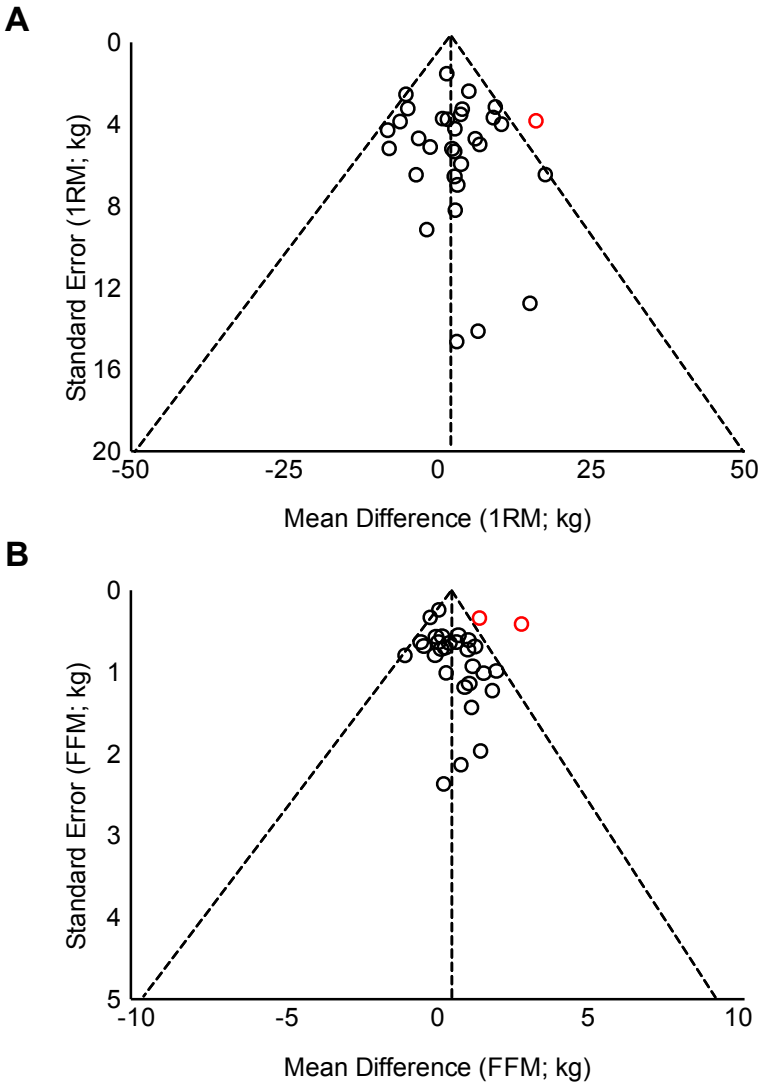
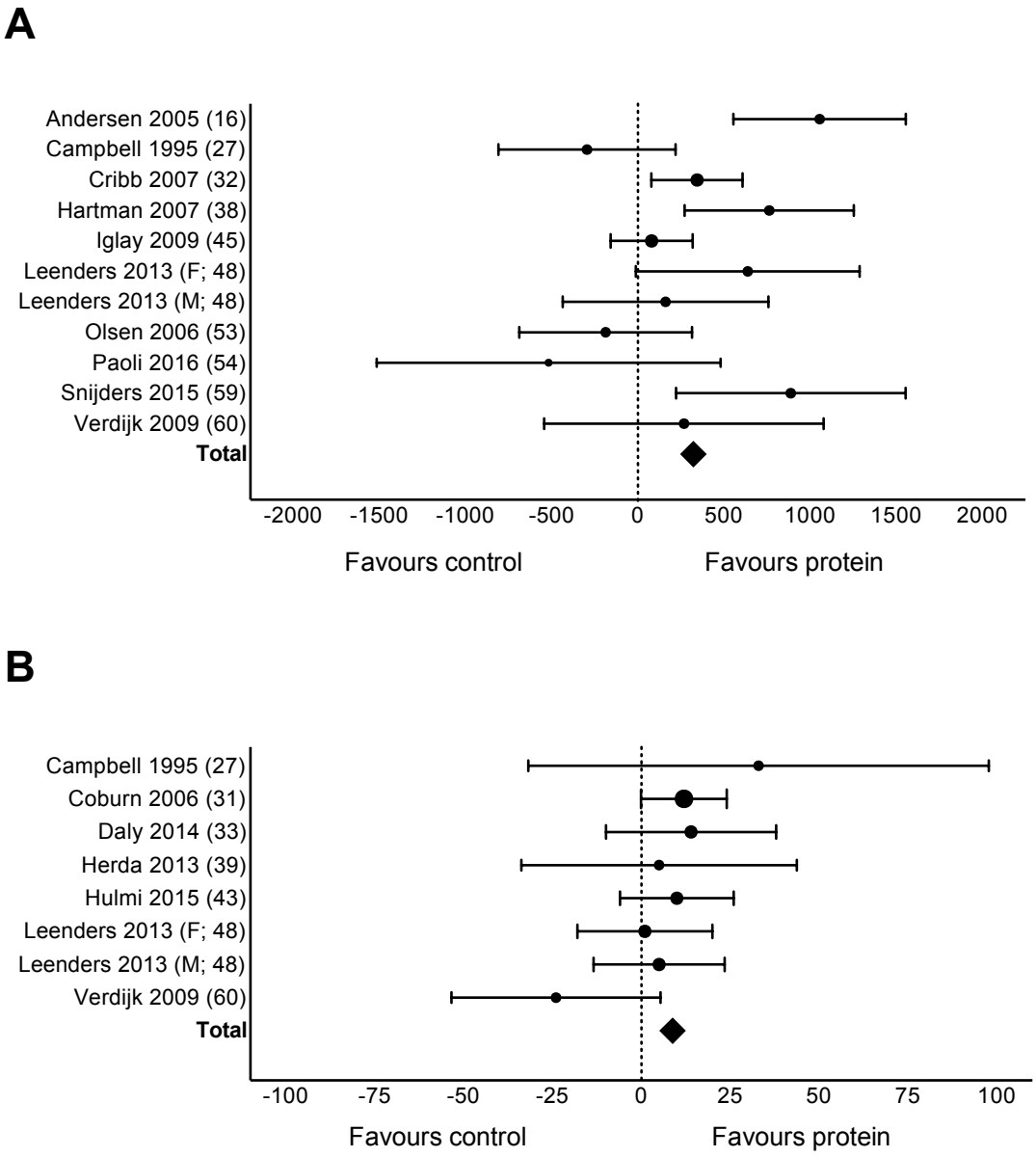
APPENDIX A:

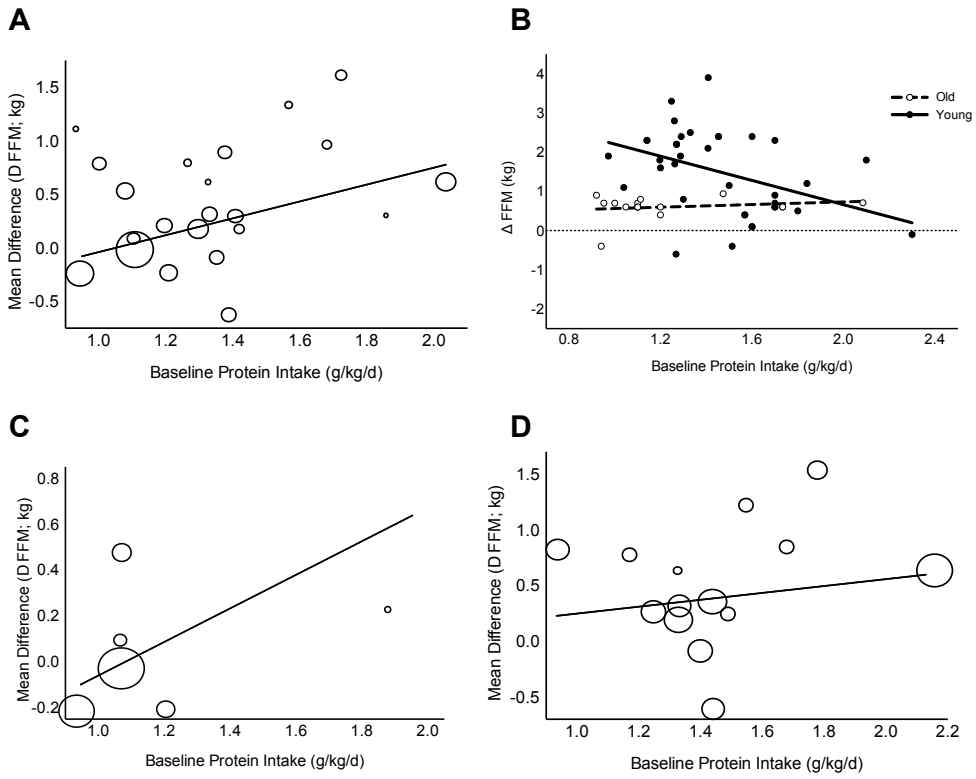
Supplementary Data from Study 1



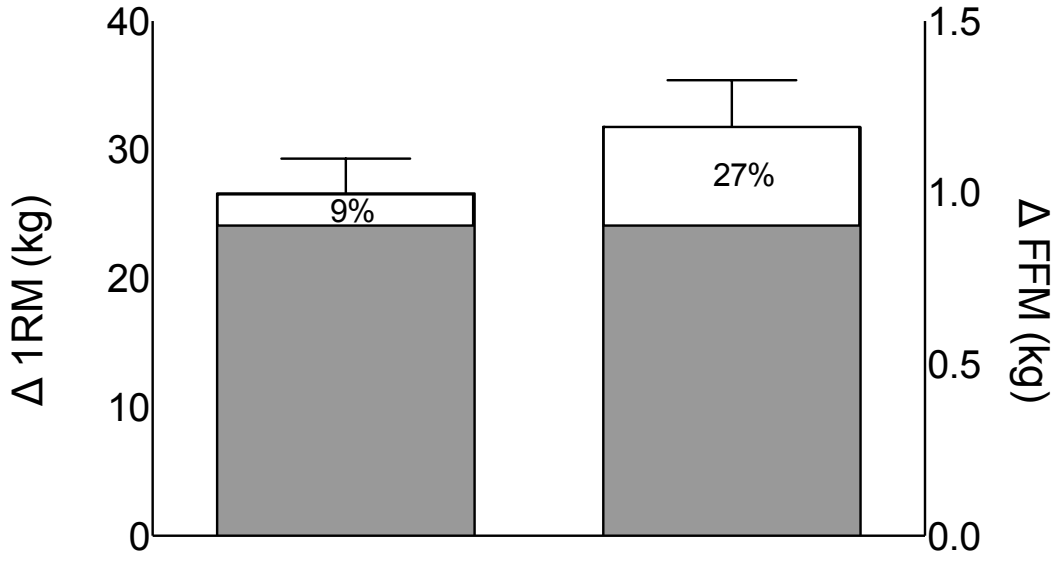
Supplementary Figure 1. Funnel plots of the main outcomes (one repetition-maximum [1RM] and fat-free mass [FFM]) used to determine publication/reporting bias. The inverse of the standard error is plotted on the y axis with the mean difference between groups plotted on the x axis. Funnel plots were generated using fixed-effect meta-analyses in RevMan (Review Manager [RevMan]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) to generate a triangular 95% confidence region. Each circle represents an individual study, with the highlighted circles outside of the of the confidence region identified as having some degree of publication bias. Panel A: Funnel plot for changes in 1RM with one study (32) identified and removed. Panel B: Funnel plot for changes in FFM with two studies (24, 63) identified and removed.



Supplementary Figure 2. Forest plots of the results from random-effects meta-analyses on fibre cross sectional area (CSA; μm^2; Panel A) and mid-femur CSA (mm^2; Panel B). Data is shown as mean difference with 95% CIs. For each study, the circle represents the mean difference of the intervention effect with the horizontal line intersecting it as the lower and upper limits of the 95% CI. The size of each circle represents the relative weight that study carried in the meta-analysis. The rhombi represent the weighted total mean difference in fibre CSA (Panel A; 310 μm^2 [51, 570], P=0.02) and mid-femur CSA (Panel B; 7.2 mm^2 [0.20, 14.30], P=0.04).



Supplementary Figure 3. Panel A, C and D contain random-effects univariate meta-regressions between baseline protein intake (g/kg/d) and the mean difference in ΔFFM (kg) between the protein and control groups. Each circle represents a study and the size of the circle reflects the influence of that study on the model (inversely proportionate to the standard error of that study). The regression prediction is represented by solid lines. Panel A: all participants (0.64 kg [0.02, 1.26], P=0.045), Panel D: old participants (0.46 kg [-4.07, 5.00], P=0.79) and Panel C: young participants (0.33 kg [-0.55, 1.22], P=0.43). Panel B contains two linear regressions between baseline protein intakes (g/kg body mass/d) and the changes in fat-free mass (ΔFFM; measured by dual x-ray absorptiometry) in old (open circles and dotted line) and young (solid circles and line) participants, respectively. Each circle represents a single group from a study. Linear regressions explained significantly more variance than biphasic regressions in both young (slope=-1.54 g/kg/d, R^2=0.17, df=34) and old (slope=0.16 g/kg/d, R^2=0.04, df=14) participants with a difference between age groups (P=0.042).



Supplementary Figure 4. Relative contribution of protein supplementation on RET-induced changes in one repetition maximum strength (1RM) and fat-free mass (FFM). The average change in 1RM was (mean±SE, [95%CI]; 27±3 kg [22, 32]) with the mean difference between the protein and control groups being only (2.49 kg [0.64, 4.33], P=0.01), or 9% of the total improvements (28 studies, 1078 participants). The average change in FFM was (1.1±0.1 kg [0.94, 1.45]) with the mean difference between the protein-supplemented and control groups being only (0.30 kg [0.09, 0.52], P=0.007), or 27% of the total improvements (28 studies, 1107 participants).

APPENDIX 1. Search strategy for CINAHL, EMBASE, Medline and SportDiscus.

Database: OVID Medline Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present> (982 citations)

Search performed: January 2, 2017. Total citations: 3056

1 exp Dietary Proteins/
2 (protein* adj3 (diet* or supplement* or intake* or consum*)).ti,ab,kf.
3 (protein* adj3 ingest*).ti,ab,kf.
4 or/1-3
5 Resistance Training/
6 Weight Lifting/
7 Isometric Contraction/
8 ((weight* or isometric or strength or resistance) adj3 (train* or lift* or exercise*)).ti,ab,kf.
9 exp muscle strength/
10 ((muscle* or muscular) adj3 strength*).ti,ab,kf.
11 or/5-10
12 4 and 11
13 animals/ not (humans/ and animals/)
14 12 not 13
15 limit 14 to english language
16 remove duplicates from 15

Database: Embase <1974 to 2016 December 30> (1161 citations)

Search performed: January 2, 2017

1 protein intake/
2 (protein* adj3 (diet* or supplement* or intake* or consum* or ingest*)).ti,ab,kw.
3 1 or 2
4 resistance training/
5 weight lifting/
6 ((weight or isometric or strength or resistance) adj3 (train* or lift* or exercise*)).ti,ab,kw.
7 muscle strength/
8 ((muscle* or muscular) adj3 strength*).ti,ab,kw.
9 isometric contraction*.ti,ab,kw.
10 or/4-9
11 3 and 10
12 exp animal/
13 human/
14 11 not (12 not (12 and 13))
15 remove duplicates from 14

16 limit 15 to english language

Database: CINAHL (406 citations)

Search performed: January 2, 2017

S15 S12 NOT S13 Limiters - English Language
S14 S12 NOT S13
S13 (MH "Animals+") NOT ((MH "Human") AND (MH "Animals+"))
S12 S4 and S11
S11 S5 OR S6 OR S7 OR S8 OR S9 OR S10
S10 (muscle* or muscular) N3 strength*
S9 (MH "Muscle Strength+")
S8 (weight* or isometric* or strength* or resistance) N3 (train* or lift* or exercise*)

S7 (MH "Weight Lifting")
S6 (MH "Muscle Strengthening+")
S5 (MH "Resistance Training")
S4 S1 OR S2 OR S3
S3 protein N3 (intake* or injest* or supplement* or diet* or consum)
S2 (MH "Dietary Proteins+")
S1 (MH "Diet, High Protein")

Database: SPORTDiscus (507 citations)

Search performed: January 2, 2017

S10 S4 AND S9 Limiters - Language: English
S9 S5 OR S6 OR S7 OR S8
S8 (muscle* or muscular) N3 (strength*)
S7 DE "MUSCLE strength" OR DE "GRIP strength" OR DE "KRAUS-Weber test"
S6 (weight* or isometric* or strength* or resistance) N3 (train* or lift* or exercise*)
S5 DE "RESISTANCE training (Physical training & conditioning)" OR DE "ISOMETRIC exercise" OR DE "WEIGHT training"
S4 S1 OR S2 OR S3
S3 protein* N3 (injest* or diet* or supplement* or intake* or consum*)
S2 DE "HIGH-protein diet" OR DE "FOOD -- Protein content"
S1 DE "LOW protein diet

Appendix 2. Risk of bias assessment.

	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete Outcome Data	Selective reporting	Other bias
Andersen 2005 (16)	L	L	L	L	L	L
Antonio 2014 (18)	L	H	H	L	L	H
Antonio 2015 (17)	H	H	H	L	L	L
Arazi 2011 (19)	L	L	L	L	L	L
Arnarson 2013 (20)	L	L	L	L	L	L
Babault 2014 (21)	L	L	L	L	L	L
Babault 2015 (22)	L	L	L	L	L	L
Bemben 2010 (23)	L	L	L	L	L	L
Brown 2004 (24)	L	L	L	L	L	H
Bunout 2004 (25)	H	U	H	L	U	L
Burke 2001 (26)	H	L	L	H	L	H
Campbell 1995 (27)	H	L	L	L	L	L
Candow 2006 (28)	H	L	L	L	L	L
Candow 2006a (29)	L	L	L	L	L	L
Carter 2005 (30)	L	L	L	L	L	L
Coburn 2006 (31)	L	L	L	L	L	L
Cribb 2007 (32)	L	L	L	L	L	L
Daly 2014 (33)	L	H	H	L	L	L
Deibert 2011 (34)	L	H	H	L	L	L
Eliot 2008 (35)	L	L	L	L	L	L
Erksine 2012 (36)	L	L	H	L	L	L
Farup 2014 (37)	L	L	L	L	L	L
Hartman 2007 (38)	L	L	H	L	L	L
Herda 2013 (39)	L	L	L	L	L	L
Hoffman 2007 (40)	L	L	L	L	L	L
Hoffman 2009 (41)	L	H	H	L	L	L
Hulmi 2009 (42)	L	L	L	L	L	L
Hulmi 2009a (44)	L	L	L	L	L	L
Hulmi 2015 (43)	L	L	L	L	L	L
Iglay 2009 (45)	L	H	H	L	L	L
Josse 2010 (46)	L	L	H	L	L	L
Kerksick 2006 (47)	L	L	L	L	L	L
Leenders 2012 (48)	L	L	L	L	L	L
Mielke 2009 (49)	L	L	L	L	L	L
Mitchell 2015 (50)	U	L	H	L	L	H
Negro 2014 (51)	L	H	H	L	L	L
Oesen 2015 (52)	L	H	H	L	L	L
Olsen 2006 (53)	L	L	L	L	L	L
Paoli 2015 (55)	L	H	H	L	L	L
Paoli 2015 (54)	L	H	H	L	L	L
Rankin 2004 (56)	L	U	L	L	L	H
Reidy 2016 (57)	L	L	L	L	L	L
Rozenek 2002 (58)	L	L	L	L	L	L
Snijders 2015 (59)	L	L	L	L	L	L
Verdijk 2009 (60)	L	L	L	L	L	L
Volek 2013 (61)	L	L	L	L	L	L
Weisgarber 2012 (62)	L	L	L	L	L	L
White 2009 (63)	L	H	H	L	L	H
Willoughby 2007 (64)	L	L	L	L	L	L

PRISMA 2009 Checklist

APPENDIX 3

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3 and 4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5 and 6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5 and 6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7 and 8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7 and 8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8 and 9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10, 11 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10, 11 and Supplementary Tables 1 and 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11, 12 and Appendix 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	12-14 and Figures 2-4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	12 and 13
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11 and 12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12-14, Table 1, Figure 4 and Figure 5
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	14-20
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	19 and 20
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	20 and 21
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	21

Supplementary Table 1. Participant characteristics, resistance exercise training details and individual study outcomes.

	Subject Details			Resistance Exercise Training Details			Performance		Body Composition Outcomes				
Author and Year	**Age**	**Sex**	**Training Status**	**Length (wk)**	**Frequency (d/wk)**	**Sets x Reps or %RM**	**1RM**	**MVC**	**Mass**	**FFM**	**FM**	**Fibre CSA**	**Mid-Femur CSA**
Andersen 2005 (16)	23	M	UT	14	3	3-4 x 4-15		→				↑	
Antonio 2014 (18)	24	M (29) F (11)	T	8	?	?			→	→	→		
Antonio 2015 (17)	24	M (37) F (11)	T	8	5	3 x 5-15	→		↓	→	↓		
Arazi 2011 (19)	22	M	T	8	3	3 x 8	↑		↑				
Arnason 2013 (20)	74	M (67) F (94)	UT	12	3	3 x 6-8		→		→			
Babault 2015 (whey; 22)	22	M	UT	12	3	2-5 x 5-15	→	→					
Babault 2015 (pea; 22)	22	M	UT	12	3	2-5 x 5-15	→	→					
Babault 2014 (milk; 21)	22	M	UT	10	3	3-5 x 6-20	→		→				
Babault 2014 (casein; 21)	22	M	UT	10	3	3-5 x 6-20	→		→				
Bemben 2010 (23)	57	M	UT	14	3	3 x 8	→						
Brown 2004 (whey; 24)	21	M	T	9	?	3 x 4-6				↑			
Brown 2004 (soy; 24)	21	M	T	9	?	3 x 4-6				↑			
Bunout 2004 (25)	74	M (14) F (33)	UT	52	2	3-10 x 10-15		→		→	→		
Burke 2001 (26)	?	M	T	6	4	4-5 x 6-12	→	↑	↑	↑	→		
Campbell 1995 (27)	65	M (8) F (4)	UT	6	3	3 x 80%			→	→	→	→	→
Candow 2006 (whey; 28)	24	M (9) F (18)	UT	6	4	4-5 x 6-12	↑			↑	→		
Candow 2006 (soy; 28)	23	M (9) F (18)	UT	6	4	4-5 x 6-12	↑			↑	→		
Candow 2006a (pre-ex; 29)	63	M	UT	12	3	3 x 10	→			→			
Candow 2006a (post-ex; 29)	67	M	UT	12	3	3 x 10	→			→			
Carter 2005 (30)	57	M	UT	16	3	3 x 8		→		→			→
Coburn 2006 (31)	22	M	UT	8	3	3-5 x 80%	↑		→	→	→		→
Cribb 2007 (32)	24	M	T	11	?	?	↑		→	→	→	↑	
Daly 2014 (33)	73	F	UT	16	2	3 x 8-12	↑			↑	→		→
Deibert 2011 (34)	56	M	UT	12	2	? x 10-25		↑	→	↑	→		
Eliot 2008 (35)	?	M	?	14	3	3 x 8			→	→	→		
Erksine 2012 (36)	23	M	UT	12	3	2-3 x 8-10	→	→					
Farup 2014 (37)	24	M	UT	12	3	6-12 x 6-15		→					
Hartman 2007 (milk; 38)	?	M	UT	12	5	2-4 x 4-12	→		→	↑	↓	↑	
Hartman 2007 (soy; 38)	?	M	UT	12	5	2-4 x 4-12	→		→	→	→	↑	
Herda 2013 (39)	21	M	UT	8	3	1-5 x 80%	→		→	→			→
Hoffman 2007 (40)	21	M	T	12	4	2-5 x 3-10	↑		→	→			
Hoffman 2009 (morning + night; 41)	20	M	T	10	4	2-4 x 4-10	→		→	→			
Hoffman 2009 (pre- + post-ex; 41)	20	M	T	10	4	2-4 x 4-10	→		→	→			
Hulmi 2009 (42)	26	M	UT	21	2	2-5 x 5-20	→	→	→				↑
Hulmi 2009a (44)	25	M	UT	21	2	2-5 x 5-20			→			→	
Hulmi 2015 (PRO; 43)	34	M	UT	12	2 or 3	2-5 x 4-12	→	→		→	↓		→
Hulmi 2015 (PRO+CHO; 43)	34	M	UT	12	2 or 3	2-5 x 4-12	→	→		→	↓		→
Iglay 2009 (45)	61	M (17) F (19)	UT	12	3	2 x 8				→	→	→	

Supplementary Table 1 Continued.

	Subject Details			Resistance Exercise Training Details			Performance		Body Composition Outcomes				
Author and Year	**Age**	**Sex**	**Training Status**	**Length (wk)**	**Frequency (d/wk)**	**Sets x Reps or %RM**	**1RM**	**MVC**	**Mass**	**FFM**	**FM**	**Fibre CSA**	**Mid-Femur CSA**
Josse 2010 (46)	23	F	UT	12	5	2-4 x 4-12	→		→	↑	↓		
Kerksick 2006 (whey + casein; 47)	31	M	T	10	4	3 x 6-10	→		→	↑	→		
Kerksick 2006 (whey + EAA; 47)	31	M	T	10	4	3 x 6-10	→		→	→	→		
Leenders 2013 (men; 48)	70	M	UT	24	3	2-4 x 8-15	→		→	→		→	→
Leenders 2013 (women; 48)	72	F	UT	24	3	2-4 x 8-15	→		→	→		→	→
Mielke 2009 (49)	23	M	UT	8	3	1 or 2 x 6-8	→		→	→	→		
Mitchell 2015 (young; 50)	22	M	UT	12	3	3-4 x 75-85%	→	→				→	
Mitchell 2015 (old; 50)	74	M	UT	12	3	3-4 x 75-85%	→	→				→	
Negro 2014 (51)	24	M (19) F (7)	UT	9	3	4 x 8	→			↑ (BIA)	↓ (BIA)		
Oesen 2015 (52)	82	M (9) F (47)	UT	24	2	1-2 x 15		→					
Olsen 2006 (53)	24	M	?	16	3	3-5 x 6-12		→				→	
Paoli 2015 (55)	25	M	UT	8	2 or 3	2-4 x 6-11		→		→	→		
Paoli 2016 (54)	25	M	UT	8	2 or 3	2-4 x 6-11	→			→	→	→	
Rankin 2004 (56)	21	M	UT	10	3	3-5 x 3-12	→		→	→	→		
Reidy 2016 (whey; 57)	25	M	UT	12	3	3-4 x 8-10	→	→	→	→			→
Reidy 2016 (soy; 57)	25	M	UT	12	3	3-4 x 8-10	→	→	→	→			→
Rozenek 2002 (58)	23	M	UT	8	4	4 x 8	→		→	→	→		
Snijders 2015 (59)	22	M	UT	12	3	2-4 x 8-15	↑		→	→		↑	↑
Verdijk 2009 (60)	72	M	UT	12	3	4 x 8-15	→		→	→		→	→
Volek 2013 (whey; 61)	23	M (37) F (26)	UT	36	2 or 3	3-5 x 3-15	→		→	↑	→		
Volek 2013 (soy; 61)	24	M (37) F (26)	UT	36	2 or 3	3-5 x 3-15	→		→	→	→		
Weisgarber 2012 (62)	24	M (9) F (8)	UT	8	4	3 x 6-10	→		→	→	→		
White 2009 (yogurt; 63)	21	F	UT	8	3	?	→		→	→	→		
White 2009 (whey; 63)	19	F	UT	8	3	?	→		→	→	→		
Willoughby 2007 (64)	19	M	UT	10	4	3 x 6-8	↑		↑	↑	→		

Note: PRO = protein, CHO = carbohydrate, M = male, F = female, T = trained, UT = untrained, 1RM = one-repetition-maximum, MVC = maximum voluntary contraction, FFM = fat-free mass, FM = fat mass, CSA = cross sectional area, → no difference between groups, ↑ protein group increased more than the control group, and ↓ protein group decreased more than the control group.

Supplementary Table 2. Experimental and control condition details

	Supplement/Dietary Details				Placebo/Control Details	
Author and Year	**Composition**	**Amount**	**Timing**	**N**	**Amount**	**N**
Andersen 2005 (16)	Protein blend (whey, casein, egg white protein and glutamine)	25 g	Training days = pre- and post-exercise; non-training days = morning	11	25 g CHO	11
Antonio 2014 (18)	Instructed to consume high protein diet	4.4 g/kg/day PRO diet	N/A	20	1.8 g/kg/day PRO diet	10
Antonio 2015 (17)	Instructed to consume high protein diet	3.4 g/kg/day PRO diet	N/A	31	No instruction - 2.3 g/kg/day PRO	17
Arazi 2011 (19)	Whey protein provided to increase dietary protein intake	1.8 g/kg/day was supplemented to increase PRO in diet	Training days = morning, post-exercise and night; non-training days = morning and night	20	CHO (unknown g)	20
Arnason 2013 (20)	Whey protein beverage	20 g (+20 g CHO and 1 g fat)	Training days = post-exercise	75	40 g CHO, 1 g fat	66
Babault 2015 (whey; 22)	Whey protein	25 g	Training days = morning and post-exercise; non-training days = morning and afternoon	54	37.125 g CHO (74.25 g/d)	54
Babault 2015 (pea; 22)	Pea protein	25 g	Training days = morning and post-exercise; non-training days = morning and afternoon	53	37.125 g CHO (74.25 g/d)	54
Babault 2014 (milk; 21)	Milk protein beverage	10 g (+20 g CHO)	Training days = morning, pre- and post-exercise; non-training days = morning and night	22	30 g CHO	24
Babault 2014 (casein; 21)	Casein protein beverage	10 g (+20 g CHO)	Training days = morning, pre- and post-exercise; non-training days = morning and night	22	30 g CHO	24
Bemben 2010 (23)	Whey protein + Gatorade	35 g (+480 mL Gatorade)	Training days = post-exercise	11	480 mL Gatorade	10
Brown 2004 (whey; 24)	Whey protein bar	11 g	Three times daily	9	N/A	9
Brown 2004 (soy; 24)	Soy protein bar	11 g	Three times daily	9	N/A	9
Bunout 2004 (25)	Provided soup or porridge	6.5 g	Two times daily	31	N/A	28
Burke 2001 (26)	Whey protein	additional 1.2 g/kg/day	Four times daily	12	1.2 g/kg/day CHO	11
Campbell 1995 (27)	Milk beverage to increase dietary protein intake	additional 1 g/kg/day	?	6	Milk with reduced additional PRO - 0.2 g/kg/day PRO	6
Candow 2006 (whey; 28)	Whey protein	additional 1.2 g/kg/day	Training days = pre-exercise, post-exercise and pre-sleep; non-training days = three times spread throughout the day	9	1.2 g/kg/day CHO	9
Candow 2006 (soy; 28)	Soy protein	additional 1.2 g/kg/day	Training days = pre-exercise, post-exercise and pre-sleep; non-training days = three times spread throughout the day	9	1.2 g/kg/day CHO	9
Candow 2006a (pre-ex; 29)	Whey protein + Myoplex + Cocoa	additional 0.3 g/kg PRO	Training days= pre-exercise with a placebo post-exercise	9	0.63 g/kg CHO	10
Candow 2006a (post-ex; 29)	Whey protein + Myoplex + Cocoa	additional 0.3 g/kg PRO	Training days= post-exercise with a placebo pre-exercise	10	0.63 g/kg CHO	10
Carter 2005 (30)	Whey protein + Gatorade	35 g (+480 mL Gatorade)	Training days = post-exercise	11	480 mL Gatorade	10
Coburn 2006 (31)	Whey and leucine beverage	20 g (+6.2 g leucine)	Training days = morning, pre- and post-exercise; non-training days = morning	11	26.2 g CHO	12
Cribb 2007 (32)	Whey protein	1.29 g/kg/day	Morning, post-exercise and pre-sleep	5	1.325 g/kg/day CHO	7
Daly 2014 (33)	Provided red meat	45 g/day	Food was supplied and consumed it in two different meals	48	25-35 g CHO	43
Deibert 2011 (34)	Soy protein	13.35 g (+7.65 g CHO)	Post-exercise or evening	15	N/A	15
Eliot 2008 (35)	Whey protein	35 g (+480 mL Gatorade)	Training days = post-exercise	11	480 mL Gatorade	10
Erksine 2012 (36)	Whey protein	20 g (+6.7 g lactose)	Training days = pre- and post-exercise	17	6.8 g lactose	16
Farup 2014 (37)	Whey protein beverage	19.5 g (+19.5 g CHO)	Training days = half pre- and half post-exercise	11	39 g CHO	11
Hartman 2007 (milk; 38)	Milk protein beverage	17.5 g (+25.7 g CHO and 0.4 g fat)	Training days = immediately and 1h post-exercise	18	9% CHO	19
Hartman 2007 (soy; 38)	Soy protein beverage	17.5 g (+25.7 g CHO and 0.4 g fat)	Training days = immediately and 1h post-exercise	19	9% CHO	19
Herda 2013 (39)	Whey protein	20 g	Training days = pre- and post-exercise; non-training days = once with no specific time	22	27 g CHO	21
Hoffman 2007 (40)	Protein blend (glutamine, whey, egg and milk protein)	42 g (+18 g CHO and 3 g fat)	Training days = morning and post-exercise; non-training days = morning	11	63 g CHO, 2 g PRO and 2 g fat	10
Hoffman 2009 (morning + night; 41)	Protein blend (collagen, whey and casein)	42 g (+2 g CHO)	Morning and evening daily	13	N/A	7
Hoffman 2009 (pre- + post-ex; 41)	Protein blend (collagen, whey and casein)	42 g (+2 g CHO)	Training days = pre- and post-exercise; non-training days = taken at similar two similar times	13	N/A	7
Hulmi 2009 (42)	Whey protein	15 g	Training days = pre- and post-exercise	11	Nonenergetic placebo drink	10
Hulmi 2009a (44)	Whey protein	15 g	Training days = pre- and post-exercise	9	Nonenergetic placebo drink	9
Hulmi 2015 (PRO; 43)	Whey protein	30 g (+5 g lactose)	Training days = post-exercise	22	34.5 g CHO	21
Hulmi 2015 (PRO+CHO; 43)	Whey protein beverage	30 g (+34.5 g CHO)	Training days = post-exercise	25	34.5 g CHO	21
Iglay 2009 (45)	Instructed to consume high protein diet	1.6 g/kg/day PRO diet	N/A	18	0.8 g/kg/day PRO	18

Supplementary Table 2 Continued.

	Supplement/Dietary Details				Placebo/Control Details	
Author and Year	**Composition**	**Amount**	**Timing**	**N**	**Amount**	**N**
Josse 2010 (46)	Fat-free milk	18 g (+24 g CHO)	Training days = immediately and 1h post-exercise	10	CHO (unknown g)	10
Kerksick 2006 (whey + casein; 47)	Whey and casein beverage	40 g (+8 g casein)	Training days = post-workout; non-training days = morning	10	48 g CHO	11
Kerksick 2006 (whey + EAA; 47)	Whey and AA beverage	40 g (+3 g BCAA and 5 g glutamine)	Training days = post-workout; non-training days = morning	15	48 g CHO	11
Leenders 2013 (men; 48)	Milk	15 g (+0.5 g fat, 7.13 g lactose and 0.42 g calcium)	Morning daily	15	7.13 g lactose, 0.42g calcium	14
Leenders 2013 (women; 48)	Milk	15 g (+0.5 g fat, 7.13 g lactose and 0.42 g calcium)	Morning daily	12	7.13 g lactose, 0.42g calcium	12
Mielke 2009 (49)	Whey and leucine beverage	20 g (+6.2 g leucine)	Training days = pre- and post-exercise; non-training days = morning and night	13	20 g CHO	13
Mitchell 2015 (young; 50)	500ml chocolate milk	14 g (+54 g CHO and 5 g fat)	Training days = post-exercise; non-training days = taken after breakfast	?	0.4 g PRO, 5 g fat and 66 g CHO	?
Mitchell 2015 (old; 50)	500ml chocolate milk	14 g (+54 g CHO and 5 g fat)	Training days = post-exercise; non-training days = taken after breakfast	?	0.4 g PRO, 5 g fat and 66 g CHO	?
Negro 2014 (51)	Daily 135 g serving of lean beef	20 g (+1.7 g fat)	Training days = post-exercise	12	N/A	14
Oesen 2015 (52)	Protein beverage	20.7 g (+9.3 g CHO, 3 g fat, 800 IU Vit D, 2.9 mg Vit B6 and 3 ug Vit B12)	Training days = morning and post-exercise; non-training days = morning	25	N/A	31
Olsen 2006 (53)	Milk	20 g (+80 g CHO)	Training days = half pre-exercise and half post-exercise	8	80 g CHO	8
Paoli 2015 (55)	Provided whey protein to increase dietary protein intake	1.8 g/kg/day PRO diet	Training days = pre- and post-exercise	9	0.85 g/kg/day CHO	9
Paoli 2016 (54)	Provided whey protein to increase dietary protein intake	1.8 g/kg/day PRO diet	Training days = pre- and post-exercise	9	0.85 g/kg/day CHO	9
Rankin 2004 (56)	Low fat chocolate milk	0.21 g/kg (+0.92 g/kg CHO and 0.06 g/kg fat)	Training days = post-exercise	10	1.25 g/kg CHO	9
Reidy 2016 (whey; 57)	Whey protein	22 g	Training days = post-exercise; non-training days = between meals	23	22 g CHO	23
Reidy 2016 (soy; 57)	Soy protein	22 g	Training days = post-exercise; non-training days = between meals	22	22 g CHO	23
Rozenek 2002 (58)	Protein beverage	106 g (+356 g CHO and 18 g fat)	Daily with half consumed between the morning and afternoon and half consumed pre-sleep	26	450 g CHO, 24 g PRO, 14 g fat	25
Snijders 2015 (59)	Casein protein	27.5 g (+15 g CHO)	Daily pre-sleep	20	Nonenergetic placebo drink	19
Verdijk 2009 (60)	Casein protein	10 g	Training days = pre- and post-exercise	13	citric acid and CHO (unknown g)	13
Volek 2013 (whey; 61)	Whey protein	21.6 g (+22.5 g CHO and 1.9 g fat)	Training days = post-exercise; non-training days = morning	19	45.2 g CHO	22
Volek 2013 (soy; 61)	Soy protein	20 g (+24.5 g CHO and 1.3 g fat)	Training days = post-exercise; non-training days = morning	22	45.2 g CHO	22
Weisgarber 2012 (62)	Whey protein	0.3 g/kg body mass	Training days = half pre-exercise and the rest throughout the training session	9	0.2 g/kg cornstarch and 0.1 g/kg sucrose	8
White 2009 (yogurt; 63)	Yogurt	5 g (+19 g CHO, 200 mg calcium and 80 IU Vit D)	Three yogurts per day	12	25 g CHO	11
White 2009 (whey; 63)	Whey protein	5 g (+20 g CHO)	Training days = post-exercise	12	25 g CHO	11
Willoughby 2007 (64)	Protein blend (whey, milk, casein and AAs)	40 g	Training days = pre- and post-exercise; non-training days = morning	10	40 g CHO	9

Note: EAA = essential amino acids, Vit = vitamin, CHO = carbohydrate, and PRO = protein,

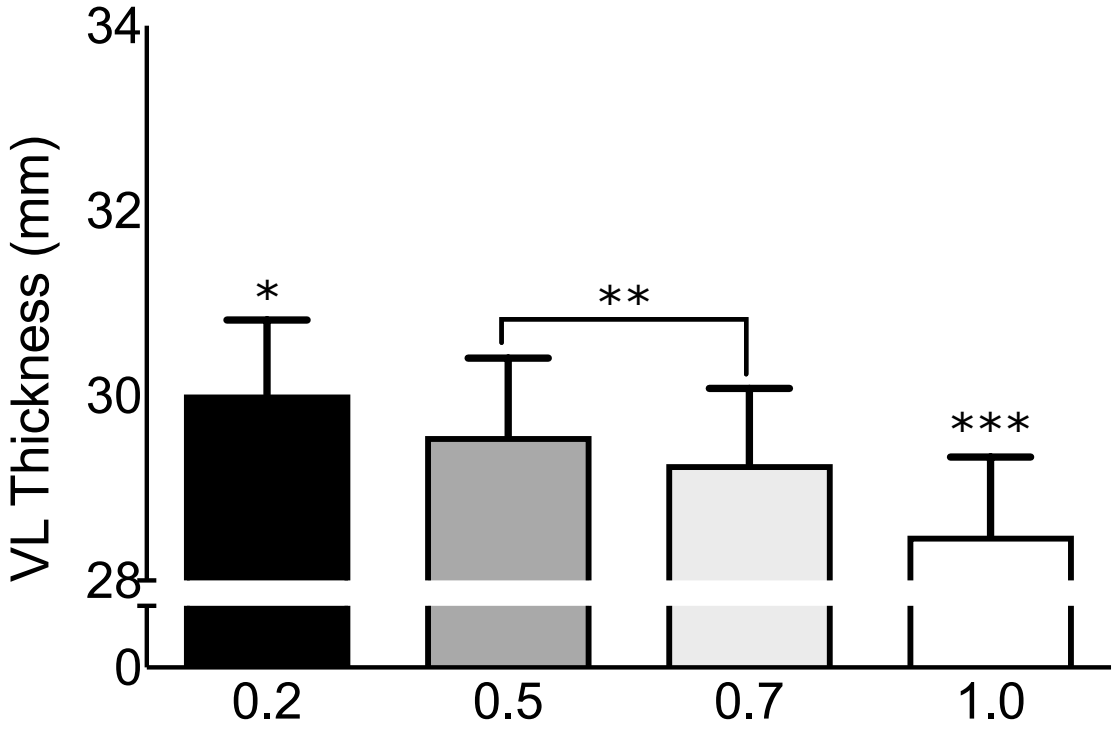
Supplementary Table 3. Additional univariate meta-regressions.

		1RM					Fat Free Mass			
Univariate	N	Coeff. (95% CI)	Adj. R^2	I^2	p-value	N	Coeff. (95% CI)	Adj. R^2	I^2	p-value
Mean Diff - Total PRO intake (g/kg/d)	16	2.54 (-8.01, 13. 07)	-2%	55%	0.61	18	-0.01 (-1.14, 1.13)	-20%	17%	0.99
Mean Diff - △PRO intake (g/d)	13	-0.03 (-0.13, 0.08)	0%	0%	0.60	14	-0.01 (-0.02, 0.01)	0%	0%	0.70
Repetitions per set	28	-0.14 (-1.25, 0.98)	-9%	34%	0.80	26	0.02 (-0.07, 0.10)	-6%	0%	0.74
Sets per exercise	28	1.97 (-0.62, 4.57)	31%	16%	0.13	25	-0.01 (-0.51, 0.48)	-18%	0%	0.96
Exercises per session	25	0.33 (-0.21, 0.87)	15%	29%	0.23	23	0.02 (-0.06, 0.09)	-29%	2%	0.17
Frequency (sessions per week)	27	2.54 (-0.95, 6.03)	22%	28%	0.15	26	0.12 (-0.17, 0.41)	7%	0%	0.39
Length (number of weeks)	29	-0.21 (-0.51, 0.08)	9%	47%	0.15	27	-0.02 (-0.05, 0.01)	10%	2%	0.24
Total Volume	24	-0.00 (-0.00, 0.00)	-14%	45%	0.69	23	-0.00 (-0.00, 0.00)	-15%	3%	-0.90
Source (whey or soy)	17	-1.95 (-10.98, 7.08)	-12%	59%	0.65	14	-0.12 (-0.95, 0.71)	-36%	31%	0.85
Sex (male or female)	24	-0.27 (-7.61, 7.06)	-6%	36%	0.94	21	-0.04 (-0.72, 0.63)	-23%	15%	0.90
Type (diet or exercise-supplement)	28	-2.12(-5.75, 1.52)	20%	21%	0.24	27	0.05 (-0.42, 0.52)	-21%	10%	0.83
Whole-body (yes or no)	27	4.41 (1.14, 7.68)	76%	2%	0.01	25	-0.22 (-1.15, 0.71)	-2%	1%	0.63
Supervision (yes or no)	28	-3.80 (-7.56, -0.06)	58%	5%	0.05	27	0.48 (-0.28, 1.24)	21%	0%	0.21

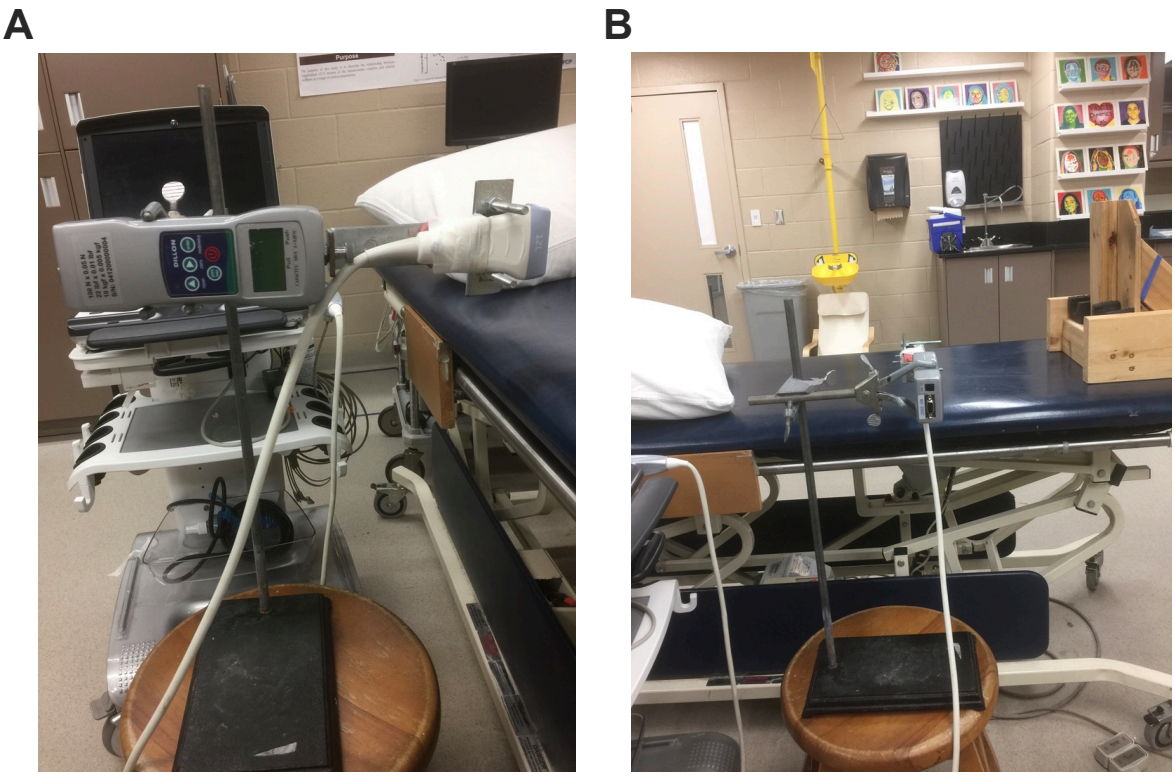
Note: Diff = difference, PRO = protein, and 1RM = one-repetition-maximum

APPENDIX B:

Supplementary Data from Study 2



Supplementary Figure 1. Vastus lateralis thickness (mm) is affected by the pressure applied to the skin (measured here in Newtons with a force transducer attached to the ultrasound probe).



Supplementary Figure 2. Panel A: Lateral view of the mount used to assess pressure during ultrasonography. Panel B: Frontal view of the mount used to assess pressure during ultrasonography including the apparatus used to elevate the participant's leg from the bed.

APPENDIX C:

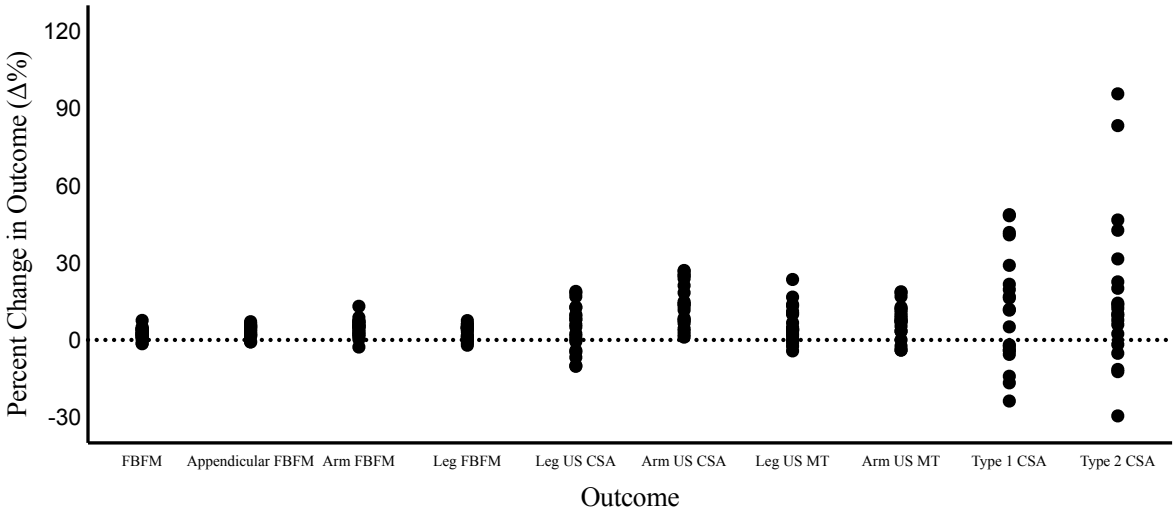
Supplementary Data from Study 3

Supplementary Table 1. The change in each hypertrophy outcome from Study 3. Each outcome was individually ranked, the ranks were summed, and each participant was assigned a 'composite rank'. The top- and bottom-quintile of respondants are highlighted in green and red, respectively. NB: there was a similar number of top- and bottom-responders in the high- and low-load conditions.

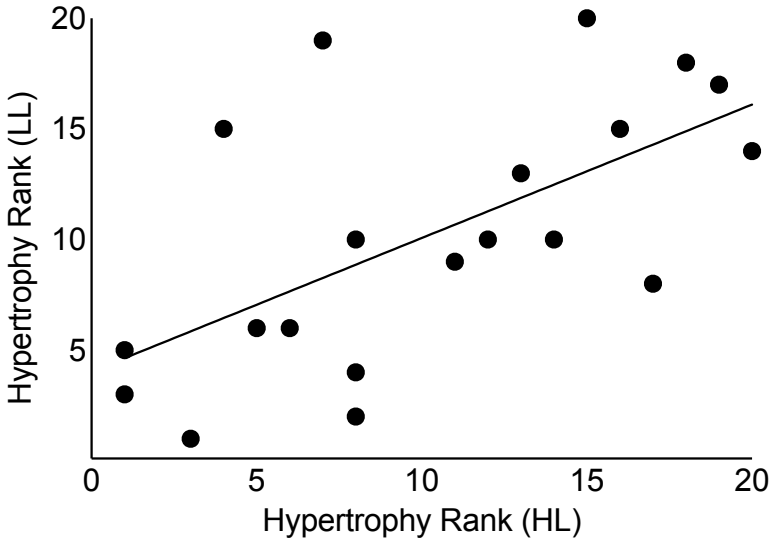
	Subject ID	Δ Type 1 CSA	Rank	Δ Type 2 CSA	Rank	Δ LBM	Rank	Sum of Ranks	Composite Rank
Low Load	S4	866.793125	31	69.83071747	13	1.183	22	66	28
	S11	-163.4996757	16	37.3507619	11	0.928	19	46	38
	S15	377.8283824	21	-89.6853539	10	0.61	17	48	37
	S10	1920.145881	39	2317.398274	40	2.157	39	118	7
	S13	1236.035667	35	1666.389946	36	-0.037	5	76	24
	S14	2152.526432	45	2494.03465	43	-0.184	3	91	18
	S18	825.7148571	29	1692.942074	37	1.69	32	98	12
	S19	159.7879231	18	3186.442984	45	0.59	15	78	23
	S21	-799.7725	4	-1051.622508	4	1.79	34	42	40
	S24	1975.761853	40	1716.841866	38	1.63	31	109	8
	S27	-328.7764596	12	114.969624	14	-0.13	4	30	43
	S28	-269.5173764	14	1599.016393	34	0	6	54	33
	S29	-415.3484787	11	152.7324638	16	1.52	28	55	32
	S30	858.7020357	30	1458.775452	32	0	6	68	27
	S31	1063.0074	32	1176.032218	27	0	6	65	29
	S34	-446.5853472	8	-883.802935	6	1.58	29	43	39
	S35	2391.699667	47	1914.769726	39	2.45	43	129	4
	S45	460.9673182	23	219.6058333	20	0	6	49	36
	S48	-446.9934527	7	-847.4123943	7	2.086	38	52	34
	S50	600.7998125	25	772.9073988	22	1.287	25	72	26
	S51	1982.595	41	1093.950201	26	1.47	27	94	15
	S52	-1074.920765	1	-1197.912371	1	0	6	8	49
	S55	712.7899659	27	938.0002466	24	2.073	37	88	20
	S57	1247.644252	36	2477.613165	42	1.435	26	104	9
High Load	S1	-963.6734211	2	-443.2360884	8	1.019	20	30	43
	S2	2024.560891	44	3559.618152	49	2.358	42	135	2
	S5	-439.601875	9	212.7964118	18	-0.812	1	28	46
	S7	431.947051	22	1441.551362	31	2.735	44	97	13
	S8	1224.910862	34	668.8894771	21	1.136	21	76	24
	S5	-439.601875	9	212.7964118	18	-0.812	1	28	46
	S17	-302.6036171	13	-1141.97919	2	1.195	24	39	41
	S20	677.7941558	26	965.7151744	25	0.18	13	64	30
	S22	1309.700667	37	2435.956585	41	0	6	84	21
	S23	-816.3123626	3	-938.4225	5	1.19	23	31	42
	S32	1317.584583	38	60.15590774	12	5.43	49	99	11
	S33	2713.674037	49	3463.446905	48	2.8	46	143	1
	S37	2154.015389	46	1649.627074	35	3.67	48	129	4
	S38	1088.461131	33	1207.540409	28	1.72	33	94	15
	S39	748.8850104	28	138.0879247	15	0.59	15	58	31
	S40	-624.2017577	6	205.4971926	17	0	6	29	45
	S41	1991.274337	42	2546.166581	44	0.652	18	104	9
	S43	33.13197379	17	-1135.271856	3	1.599	30	50	35
	S49	2023.698522	43	3405.741829	47	2.312	41	131	3
	S46	212.967708	20	1243.313422	29	2.212	40	89	19
	S47	-235.3179008	15	1394.894806	30	3.355	47	92	17
	S54	167.3450278	19	1534.377372	33	2.737	45	97	13
	S56	-742.7195084	5	-133.6119588	9	0.58	14	28	46
	S58	547.1725	24	917.3191273	23	1.965	36	83	22
	S59	2618.399286	48	3368.790322	46	1.808	35	129	4

APPENDIX D:

Supplementary Data from Study 5



Supplementary Figure 1. Considerable difference in response variability within different indices of RET-induced changes in muscle size. Each black circle represents a participant.



Supplementary Figure 2. Significant shared variance between the relative (rank) increase in HL and LL limbs within a participant with all outcomes considered (FBFM, US CSA, US MT, and fibre CSA; R^2=0.38, P<0.01).

Supplementary Table 2. An example of how each outcome was ranked in Study 5. Each limb was ranked separately based on percent change. From there, the limbs could be summed to compare arms versus legs, the conditions could be summed to compare LL versus HL, and the total rank could be used to assess overall rank in that outcome.

	Fat- and Bone-Free Mass																	
	LL Arm		HL Arm		LL Leg		HL Leg		Arm	Arm	Leg	Leg	LL	LL	HL	HL	FBFM	Total
Subject ID	△%	Rank	△%	Rank	△%	Rank	△%	Rank	Sum	Rank	Sum	Rank	Sum	Rank	Sum	Rank	Sum	Rank
1	0.00	17	3.98	12	3.92	8	4.24	9	29	**17**	17	**9**	**25**	**15**	**21**	**11**	**46**	**12**
2	6.59	7	3.11	14	0.83	15	2.29	13	21	**11**	28	**14**	**22**	**11**	**27**	**14**	**49**	**15**
3	2.78	14	1.81	18	2.09	13	7.19	1	32	**18**	14	**6**	**27**	**16**	**19**	**8**	**46**	**12**
4	6.61	6	6.61	4	0.15	16	0.00	17	10	**2**	33	**17**	**22**	**11**	**21**	**11**	**43**	**10**
5	5.46	9	5.99	6	4.11	7	6.12	5	15	**5**	12	**4**	**16**	**5**	**11**	**4**	**27**	**3**
6	7.86	5	6.60	5	1.04	14	2.50	12	10	**2**	26	**13**	**19**	**8**	**17**	**6**	**36**	**9**
7	5.31	10	3.07	15	2.79	12	-1.66	19	25	**14**	31	**16**	**22**	**11**	**34**	**18**	**56**	**17**
8	-0.66	18	1.31	19	5.90	5	0.65	15	37	**19**	20	**12**	**23**	**14**	**34**	**18**	**57**	**18**
9	1.16	16	4.76	10	-0.40	17	-3.69	20	26	**15**	37	**19**	**33**	**19**	**30**	**17**	**63**	**19**
10	13.95	2	3.51	13	3.77	9	6.09	6	15	**5**	15	**8**	**11**	**3**	**19**	**8**	**30**	**5**
11	4.56	13	10.04	1	6.15	4	6.22	4	14	**4**	8	**1**	**17**	**6**	**5**	**1**	**22**	**1**
12	8.27	4	2.33	17	5.28	6	6.75	2	21	**11**	8	**1**	**10**	**2**	**19**	**8**	**29**	**4**
13	4.95	11	5.73	9	3.63	10	6.40	3	20	**9**	13	**5**	**21**	**9**	**12**	**5**	**33**	**7**
14	-4.26	20	7.02	3	9.09	1	5.94	7	23	**13**	8	**1**	**21**	**9**	**10**	**2**	**31**	**6**
15	11.65	3	2.82	16	6.99	3	2.54	11	19	**8**	14	**6**	**6**	**1**	**27**	**14**	**33**	**7**
16	18.80	1	7.47	2	3.40	11	5.68	8	3	**1**	19	**11**	**12**	**4**	**10**	**2**	**22**	**1**
17	4.61	12	5.88	8	-0.58	18	2.82	10	20	**9**	28	**14**	**30**	**18**	**18**	**7**	**48**	**14**
18	6.38	8	5.92	7	-0.92	20	1.00	14	15	**5**	34	**18**	**28**	**17**	**21**	**11**	**49**	**15**
19	-2.76	19	-2.68	20	-0.77	19	-1.44	18	39	**20**	37	**19**	**38**	**20**	**38**	**20**	**76**	**20**
20	2.24	15	4.58	11	8.87	2	0.58	16	26	**15**	18	**10**	**17**	**6**	**27**	**14**	**44**	**11**

Supplementary Table 3. When each outcome was ranked similar to Supplementary Table 2, the total arm, leg, LL, HL, and hypertrophy score could be calculated as shown here.

	Overall Hypertrophy Score									
Subject ID	Total Arm Sum	Total Arm Rank	Total Leg Sum	Total Leg Rank	Total LL Sum	Total LL Rank	Total HL Sum	Total HL Rank	Hypertrophy Sum	Hypertrophy Rank
1	55	**20**	41	**16**	72	**20**	49	**14**	36	**19**
2	30	**13**	45	**19**	44	**13**	46	**13**	32	**16**
3	53	**18**	44	**18**	60	**19**	53	**17**	36	**19**
4	24	**9**	52	**20**	26	**4**	50	**15**	29	**13**
5	18	**6**	33	**11**	31	**6**	35	**6**	17	**8**
6	39	**14**	38	**15**	50	**14**	42	**10**	29	**13**
7	26	**11**	25	**6**	32	**7**	59	**19**	17	**8**
8	47	**16**	43	**17**	54	**15**	64	**20**	33	**18**
9	46	**15**	33	**11**	55	**16**	50	**15**	26	**12**
10	24	**9**	24	**5**	34	**8**	17	**2**	14	**6**
11	16	**4**	5	**1**	19	**1**	25	**3**	5	**2**
12	15	**3**	18	**4**	19	**1**	33	**5**	7	**3**
13	14	**1**	13	**2**	34	**8**	26	**4**	3	**1**
14	47	**16**	36	**13**	38	**11**	40	**9**	29	**13**
15	14	**1**	26	**7**	28	**5**	35	**6**	8	**4**
16	22	**7**	14	**3**	25	**3**	16	**1**	10	**5**
17	23	**8**	29	**9**	58	**17**	37	**8**	17	**8**
18	16	**4**	30	**10**	39	**12**	42	**10**	14	**6**
19	53	**18**	37	**14**	59	**18**	57	**18**	32	**16**
20	28	**12**	27	**8**	34	**8**	42	**10**	20	**11**