FACTORS INFLUENCING SKELETAL MUSCLE REMODELLING WITH LOADING

FACTORS INFLUENCING SKELETAL MUSCLE REMODELLING WITH LOADING

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LAY ABSTRACT

Skeletal muscle is critical for good health. Skeletal muscle can adapt to the environment that it is placed in. For example, strength training increases skeletal muscle mass, whereas reducing physical activity (due to injury, bed rest, sedentary, or sedentary lifestyle) decreases skeletal muscle mass. There are several factors that influence skeletal muscle size. We demonstrated that manipulating resistance training variables, such as load (i.e., how heavy), volume (i.e., how many sets), and weekly frequency (i.e., how many times per week) influence skeletal muscle adaptations. We also discovered that RNAs that do not code for protein likely play a role in facilitating resistance training increases in skeletal muscle mass. Lastly, we demonstrated that just a few sessions of resistance exercise increases muscle protein synthesis, whereas 2 weeks of unloading – via a knee brace – decreases muscle protein synthesis.

ABSTRACT

Skeletal muscle is a plastic tissue that can adapt to several stimuli by changing its metabolic and contractile properties. Increased skeletal muscle mass can be brought on by loading through resistance exercise training, whereas decreased skeletal muscle mass can be brought on by reducing skeletal muscle contractile activity. Rates of muscle protein synthesis can be influenced by several factors broadly categorized as external or internal system variables. External system variables are environmental perturbations indispensable for activating internal system variables. Internal system variables are local, skeletal muscle-specific, biological processes that mechanistically underpin skeletal muscle adaptations. The overarching objective of the experiments conducted as part of this thesis was to discover the influence of external variables (resistance training program variables) and internal variables (long noncoding RNA) on skeletal muscle adaptations and characterize changes in skeletal muscle protein synthesis under various scenarios. In studies 1 and 2, we used systematic review and network meta-analytical methodology and discovered that resistance exercise training load, volume, and weekly frequency were important determinants of skeletal muscle adaptations. As an internal variable, the long-noncoding transcriptome is poorly characterized in skeletal muscle biology, and for study 3, we used five independent exercise studies to identify a novel long-noncoding RNA signature associated with resistance exercise-induced changes in lean mass. Lastly, in study 4, we characterized integrated rates of bulk muscle protein synthesis under distinct loading states in young, healthy men. We found that 14 days of single-leg immobilization was sufficient to induce rapid declines in muscle protein synthesis, whereas 4 sessions of resistance exercise increased muscle protein synthesis. Taken together, the findings of this thesis contribute substantially to our understanding the role of external and internal variables on skeletal muscle remodeling.

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LIST OF ABBREVIATIONS

1RM	1-Repetition Maximum
4EBP1	eIF4E Binding Protein 1
ACSM	American College of Sports Medicine
AMSTAR	A Measurement Tool to Assess Systematic Reviews
APE	Atom Percent Excess
ATF4	Activating Transcription Factor 4
BIA	Bioelectrical Impedance
CAPN1	Calpain 1
CAPN-AS1	lncRNA Antisense to Calpain 1
CDF	Chip Definition File
cDNA	Copy DNA
CrI	Credible Interval
CSA	Cross-Sectional Area
CTRL	Non-Exercise Control
CYTOR	Cytoskeleton Regulator RNA
D_2O	Deuterium Oxide
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DE	Differential Expression
DIC	Deviance Information Criterion
DNA	Deoxy-Ribonucleic Acid
DPP	Dynamic Proteomic Profiling
DXA	Dual Energy X-ray Absorptiometry
EAA	Essential Amino Acids
eIF2α	Eukaryotic Initiation Factor 2 Alpha
ENST	ENSEMBL Transcript
ERK1/2	Extracellular-Related Signaling Kinase 1/2
fCSA	Fibre-Cross-Sectional Area
FDR	False Discovery Rate
FSR	Fractional Synthetic Rate
GO	Gene Ontology
HIIT	High-Intensity Interval Training
HTA2.0	Human Transcriptome Array 2.0
LEU	Leucine
LLM	Leg Lean Mass
LM	Lean Mass
lncRNA	Long-Non Coding RNA
MAPK	Mitogen-Activated Protein Kinase
MCMC	Markov chain Monte Carlo
MEGENA	Multi-Scale Embedded Gene Co-Expression Network Analysis
MJ	Multi Joint
MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
MRI	Magnetic Resonance Imaging

mRNA	Messenger Ribonucleic Acid
mTORC1	Mammalian Target of Rapamycin Pathway Complex 1
MVC	Maximum Voluntary Contraction
MyoFSR	Myofibrillar Fractional Synthetic Rate
NCSA	National Strength and Conditioning Association
NMA	Network Meta Analysis
NMR	Network Meta Regression
p70S6K1	Protein 70 kDa S6 Kinase 1
PICOS	Population, Intervention, Comparator, Outcomes, and Study Design
PRAS40	Proline-Rich AKT Substrate 40 kDa
PRISMA-NMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension statement for network meta-analyses
QoE	Quality of Evidence
Raptor	Regulatory-Associated Protein of mTOR
RE	Resistance Exercise
RE	Resistance Exercise
RET	Resistance Exercise Training
RETx	Resistance Training Prescription
RM	Repetition Maximum
RNA-seq	RNA Sequencing
rpS6	Ribosomal Protein S6
rRNA	Ribosomal Ribonucleic Acid
RTx	Resistance Training Prescription
SD	Standard Deviation
SD_{change}	Standard Deviation Change
SES	Standardized Effectiveness Statement
SJ	Single Joint
SMD	Standardized Mean Difference
SUCRA	Surface Under the Cumulative Ranking Curve
TEAD1	TEA domain transcription factor 1
TIF-1α	Transcriptional Intermediary Factor-1α
TRIM28	Tripartite Motif-Containing 28
tRNA	Transfer Ribonucleic Acid
UME	Unrelated Mean Effects
US	Ultrasonography
VL_{CSA}	*vastus lateralis* cross-sectional area
WHO	World Health Organization

PREFACE:

DECLARATION OF ACADEMIC ACHEIVEMENT

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, four original research papers prepared in journal article format, and a general discussion. The candidate is joint first author on chapters 2 and 3, and lead author on chapters 4 and 5. At the time of thesis preparation, chapter 2 was accepted at a peer-reviewed journal and chapter 3 is under review at peer-reviewed journal.

CONTRIBUTIONS TO PAPERS WITH MULTIPLE AUTHORSHIPS

CHAPTER 1 – INTRODUCTION

Mcleod JC, Stokes T, Phillips SM. Resistance Exercise Training as a Primary Countermeasure to Age-Related Chronic Disease. Front Physiol. 2019;10:645.

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McKendry J, Currier BS, Lim C, **Mcleod JC**, Thomas ACQ, Phillips SM. Nutritional Supplements to Support Resistance Exercise in Countering the Sarcopenia of Aging. Nutrients. 2020;12(7).

McKendry, J., Stokes, T., **Mcleod, J.C.**, Phillips, S.M. (2021) Resistance Exercise, Aging, Disuse, and Muscle Protein Metabolism. Compr. Physiol. Jun 30;11(3):2249-2278.

Lim C, Nunes EA, Currier BS, **Mcleod JC**, Thomas ACQ, Phillips SM. An Evidence-Based Narrative Review of Mechanisms of Resistance Exercise-Induced Human Skeletal Muscle Hypertrophy. Med Sci Sports Exerc. 2022;54(9):1546-59.

Contributions: J.C.M wrote the sections of the reviews that are included in this section of the thesis.

CHAPTER 2: STUDY 1

The influence of resistance exercise training prescription variables on skeletal muscle mass, strength, and physical function in healthy adults: An umbrella review. Accepted at the *Journal of Sport and Health Sciences.*

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JCM and BSC designed and executed the systematic search, with the assistance of CVL screened articles, extracted data, evaluated articles using standardized effectiveness statements and AMSTAR and along with SMP conceived the review and drafted the manuscript. All authors critically revised the manuscript. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

CHAPTER 3: STUDY 2

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JCM, BSC and SMP conceived the review. JCM, BSC, and LB designed and executed the systematic search. JCM, BSC, ACD, SMP, JAJK, LL, GC, LC, KJL, and AV screened articles and extracted data. JCM, BSC, AY, and AW completed within-study risk of bias assessments. JCM and BSC conducted the statistical analysis with assistance from JB and NJW. JCM, BSC,

and SMP drafted the manuscript. All authors critically revised the manuscript. All authors approved the submission of this manuscript.

CHAPTER 4: STUDY 3

A long non-coding-RNA signature is related to human skeletal muscle load-induced remodelling.

Contribution:

The project analysis strategy was established by J.A.T., J.C.M., and S.M.P. The clinical studies were designed by S.M.P., J.A.T., B.P., and P.J.A. T.S., C.M., S.O., R.W.M., C.L., J.C.M., The transcriptome methods and primary statistical analysis was carried out by J.CM., and J.A.T. The manuscript was drafted by J.C.M., and S.M.P.

CHAPTER 5: STUDY 4

Changes in Protein Synthesis During Short-term Unloading and Loading in Human Skeletal Muscle.

Contribution:

J.C.M., S.M.P., P.J.A., K.S., and D.J.W., designed the study. J.C.M., C.L., J.M., S.M.P., V.Z., and A.D., performed data collection. J.C.M., V.Z., C.L., D.J.W., and K.S. performed data analysis. J.C.M., and S.M.P., wrote the manuscript.

CHAPTER 1: INTRODUCTION

1.1 Skeletal muscle and its importance for human health

Skeletal muscle is the largest organ in the human body, accounting for 40% of total body mass (1). Further, skeletal muscle plays a paramount role in various mechanical and metabolic functions. From a mechanical standpoint, skeletal muscle converts chemical and tensile signals into contractile force. The contractile force produced by skeletal muscle is then articulated onto the skeletal system, enabling locomotion, respiration, posture maintenance, and carrying out physical activities of daily living. From a metabolic perspective, skeletal muscle is an important reservoir for insulin-stimulated-glucose-uptake (2) and amino acids to buffer against catabolic perturbations (3). Skeletal muscle is also the largest contributor to resting energy expenditure (4). Throughout the first 3-4 decades of life, skeletal muscle size and strength is largely maintained. However, lower than predicted norms of skeletal muscle size and strength in late(r) life are prognostic for non-communicable disease risk (e.g., cardiovascular disease, type II diabetes, and cancer), all-cause mortality, physical disability, and reduced quality of life (5). Therefore, concerted efforts to maintain skeletal muscle mass throughout life and during catabolic conditions (such as those experienced during periods of disuse atrophy) are important for human health.

Skeletal muscle structure can be viewed as the hierarchical organization of contractile machinery. At the macroscopic level, skeletal muscle is attached to the skeletal system via tendons. The outermost layer of connective tissue that surrounds skeletal muscle is referred to as the epimysium (6). Beneath the epimysium resides bundles of myofibres (i.e., fascicles) that are covered by another layer of connective tissue called the perimysium (6). At the microscopic level, myofibres are multinucleated cells that are sheathed by the endomysium (6). The myofibres are surrounded by many interstitial cells, such as fibroblasts, immune cells,

endothelial cells, pericytes, and fibro-adipogenic progenitor cells (6). Importantly, satellite cells are a population of muscle-resident stem cells residing between the endomysium and the sarcolemma (i.e., the myofiber plasma membrane) that are essential for adding new myonuclei to postnatal skeletal muscle tissue (7).

The intracellular matrix of myofibres contains a gelatinous sarcoplasm that contains the primary ultrastructural elements of the myofibre. Although the intracellular matrix of myofibres has a mixed pool of muscle proteins, estimates suggest that ~ 30 – 40% of intracellular proteins are mitochondrial, t-tubule, and sarcoplasmic reticulum-related proteins, while ~ 60 – 70% of the total mixed muscle protein are used to create an array of rod-like structures called the myofibrils (8): myofibrils house sarcomeres, the primary contractile unit of skeletal muscle.

1.2 Skeletal muscle plasticity

Skeletal muscle is a plastic organ that can adapt to numerous stimuli by altering its mass and contractile and metabolic properties. Indeed, all the structural elements that make up skeletal muscle (e.g., connective tissue, myofibres, interstitial cells, satellite cells, intracellular proteins) are dynamically regulated by environmental stimuli, such as loading (e.g., resistance exercise training [RET]) and unloading (e.g., disuse atrophy). Further, external to skeletal muscle are adaptive structural events that will directly impact skeletal muscle function. Consequently, increased loading or unloading directly impacts the skeletal muscle phenotype, resulting in skeletal muscle hypertrophy, or skeletal muscle atrophy, respectively.

1.2.1 Resistance exercise training – loading (hypertrophy)

As alluded to above, the increase in skeletal muscle size – commonly referred to as hypertrophy – is a favourable outcome to combat chronic disease risk and maintain physical independence

(5). RET, defined as the contraction of skeletal muscle against external resistance, is undoubtedly the most potent non-pharmacological intervention for promoting increased skeletal muscle size (9). Unsurprisingly, various physical activity guidelines advocate for the regular performance of RET.

At the molecular level, skeletal muscle hypertrophy can be defined as the addition of sarcomeres in parallel, which can be attributed to the accumulation of structural and contractile proteins (10). However, the accumulation of structural and contractile proteins following a resistance training program are rarely measured. Rather, whole-body macroscopic assessments (e.g., dual energy x-ray absorptiometry [DXA], bioelectrical impedance [BIA], etc.) report a crude estimate of body size and composition including bone, fat, and lean mass (i.e., fat-and-bone-free). More direct interrogation of muscle cross-sectional area (CSA) and volume, and fibre-cross-sectional-area (fCSA) can be performed using magnetic resonance imaging (MRI), ultrasonography (US), and immunohistochemistry of muscle tissue, respectively. Therefore, commonly employed methods of skeletal muscle mass may serve as surrogates for structural and contractile protein accumulation.

Although the longitudinal growth of skeletal muscle is apparent during development (11) and to an extent during load-induced hypertrophy (12), excellent work demonstrates that in adult skeletal muscle, RET-induced muscle accretion is predominately driven by the radial growth (i.e., increase in cross-sectional area) of skeletal muscle (13, 14). Further, it has been speculated that the greatest increases in skeletal muscle mass occurs during the early phases of RET (3 – 12 weeks) (15, 16). However, Damas and colleagues (17) demonstrated marked z-band streaming (i.e., a direct marker of muscle damage) following 3 weeks of RET, with no evidence of increased fCSA. Interestingly, increases in fCSA were only observed following 10 weeks of

RET, with no evidence of z-band streaming (17). Therefore, it is reasonable to suggest that only after prolonged periods (≥ 6 weeks) of RET, and after attenuation of muscle damage, is when measurable muscle growth (i.e., the addition of contractile machinery) be detected in human skeletal muscle.

1.2.2 Disuse - unloading (disuse atrophy)

Skeletal muscle atrophy is a hallmark consequence of many chronic illnesses (e.g., sepsis, burn injuries, organ failure). Under scenarios of chronic illnesses, delineating the atrophy-inducing factors intrinsic to skeletal muscle are difficult to discern from systemic factors (e.g., increased serum cytokines and cortisol) (18, 19). In contrast, skeletal muscle atrophy can arise from a variety of uncomplicated models of disuse, including single-leg immobilization (20), microgravity (21), step reduction (i.e., voluntary increase in sedentary behaviour) (22), and bed rest (23), in otherwise healthy individuals. Periods of uncomplicated disuse presents a unique opportunity to delineate the atrophy-inducing factors intrinsic to skeletal muscle. For this thesis, I focus exclusively on muscle atrophy arising from uncomplicated models of disuse.

Compared with RET-induced skeletal muscle hypertrophy, which is a relatively gradual process, disuse-induced skeletal muscle atrophy is rapid. Stokes and colleagues (20) demonstrated that the magnitude of muscle gained during 10 weeks of unilateral RET was equivalent to the atrophic losses following just 2 weeks of single-leg immobilization. Two independent meta-analyses (24, 25) consistently demonstrated that the greatest losses of muscle mass arising from uncomplicated disuse occur within the first 14 days. Indeed, Hardy and colleagues (24) demonstrated that during the first 14 days of skeletal muscle disuse, atrophic losses in quadriceps volume average ~ 0.46% per day, thereafter, skeletal muscle disuse extending beyond 2 weeks result in atrophic losses of quadriceps volume that slow to ~ 0.3 %

per day. Taken together, skeletal muscle maintenance is clearly dependent upon the presence of contractile activity, and removal of contractile activity induces rapid muscle atrophy that eventually slows once muscle mass reaches nadir.

1.2.3 Exercise and disuse heterogeneity

When skeletal muscle is exposed to an environmental perturbation (such as RET), there is remarkable individual heterogeneity among the outcomes observed in physiological adaptation. Indeed, the HERITAGE Family Study demonstrated that over 20% of individuals did not improve their insulin sensitivity following 20 weeks of supervised aerobic exercise training (26). Following supervised RET, gains in skeletal muscle size are also variable between individuals (27, 28). In five hundred eighty-five subjects undergoing 12 weeks of progressive RET, the relative percent change in biceps brachii CSA ranged from -2 to 59% (29). Skeletal muscle unloading also leads to highly heterogenous atrophic losses in muscle size (30, 31). The implications of heterogeneous muscle remodeling are substantial, underpinning musculoskeletal frailty and insulin sensitivity (32) and, potentially, a blunted hypertrophic response to RET in older adults (33). Therefore, it is critical to identify the factors that contribute to inter-individual skeletal muscle remodelling in humans.

1.3 The impact of acute resistance training program variables on resistance training-induced muscle plasticity

Several factors regulate skeletal muscle mass and size, which can be broadly categorized into internal or external system variables. Internal system variables can be defined as local, skeletal muscle-specific, biological processes that mechanistically underpin skeletal muscle adaptations, such as the mammalian target of rapamycin pathway complex 1 (mTORC1) activation, ribosome

biogenesis, mechano-transduction, and proteomic and transcriptomic responses (34). On the other hand, external system variables are environmental perturbations that are indispensable for activating internal system variables. External variables include diet-related factors (e.g., dietary protein), consumption of anabolic supplements (e.g., creatine), and administration of anabolic hormones (e.g., testosterone) (34); RET is the most potent external stimulus for skeletal muscle mass accrual (35), and much attention (36) has been given to understanding how manipulating RET training program variables can be leveraged to optimize RET-induced adaptions. RET program variables that can be manipulated, include: volume (number of sets) (36), load (37), training frequency (36), time under tension (38, 39), set configuration (40), muscle action (i.e., eccentric vs concentric vs traditional; (41), set-end point (42), rest between sets (43), and periodization (44).

1.3.1 Volume (number of sets)

RET volume is the total work performed in each resistance exercise (RE) session. RET volume can be expressed in several ways; however, the number of sets performed is the most used definition for RET volume (36). Data from acute studies demonstrate volume-dependent increases in ribosome biogenesis (45), anabolic signalling (45, 46), and MPS (46). Longitudinal studies further support that RET volume is a primary variable dictating skeletal muscle adaptations. In a meta-analysis comprising 15 studies, performing 10 weekly sets was associated with a9.8% increase in muscle mass, whereas performing less than 5 weekly sets was associated with a 5.4% increase in muscle mass (47). Similarly, a meta-analysis of 14 studies and 440 participants found that multiple sets/exercise was associated with significantly greater strength gains compared with a single set/exercise (48).

The dose-response relationship between RET volume and skeletal muscle adaptations has been suggested to follow an inverted-U shape (36). In a meta-regression, Krieger and colleagues showed that performing 2-3 sets/exercise or 4-6 sets/exercise was superior to performing 1 set/exercise, but there was no difference in hypertrophy adaptations when comparing 2-3 sets/exercise to 4-6 sets/exercise (48). The results from Krieger and colleagues suggest that higher volume RET confers an increasingly additive hypertrophic advantage but plateaus, after which there are diminishing returns (less gain per volume increase) for hypertrophy and possibly detrimental outcomes (36).

1.3.2 Load

RET load is the amount of resistance undertaken during a RE set. RET load is commonly expressed as a percentage of maximum strength achieved on a strength test (e.g., maximum voluntary contraction [MVC] or 1-repetition maximum [1RM]). It is well established that high-load RET (≥ 80% 1RM or ≤ 8 repetition maximum [RM]) appears to be more effective than low-load RET for muscular strength gains. For example, in a recent network meta-analysis (NMA), Lopez and colleagues (49) found that, compared with lower-load RET (<60% of 1RM, or >15 RM), higher-load RET (≥80% 1RM, or ≤ 8RM) and moderate-load RET (60% - 79% 1RM, or 9–15 RM) promoted superior muscular strength gains. The superiority of higher-load RET for muscular strength gains can be attributed to the principle of specificity (i.e., participants in higher-load groups regularly train using loads that are closer to the test of maximal [1RM] strength) (50).

A common misconception is the long-proposed presence of a “hypertrophy loading zone”, such that maximal increases in muscular size are optimized when training in a narrow loading, ranging between 6 to 12 repetitions (51). However, accumulating evidence suggests that

similar RET hypertrophic adaptations may occur across a broad spectrum of loading schemes. A NMA (49) demonstrated no significant difference in RET-induced muscle hypertrophy between high-load RET and low-load RET; moderate-load resistance training and low-load RET; high-load RET and moderate-load RET. The lack of importance of load for hypertrophy is supported by other analyses (52-55), but performing RET to momentary muscular failure (fatigue) has been posited as a key component for RET-induced hypertrophy with lower loads (55).

1.3.3 Weekly frequency

Training frequency is usually defined as the number of RET sessions performed within a week. A meta-analysis of 22 studies demonstrated that higher RET frequencies (e.g., 4 days/week) resulted in larger strength gains than lower RET frequencies (e.g., 1 day/week) (56). However, the effect of weekly training frequency on muscular adaptations is difficult to discern because training frequency is embedded in weekly RET volume. It has been suggested that weekly training frequency does not independently influence skeletal muscle adaptations, but increasing training frequency can be manipulated to increase within-session volume, leading to muscle mass and strength accrual (36). Indeed, Schoenfeld and colleagues (57) found that when RET volume was matched, there was no difference between higher-, and lower-RET frequency. Further, similar to resistance training volume, at some point, weekly training frequency becomes redundant, and increases in strength and hypertrophy would plateau.

1.4 The molecular regulation of muscle protein synthesis

Skeletal muscle proteins are constantly being synthesized and broken down. The constant turnover of skeletal muscle proteins aids in effective repair and renewal of damaged proteins, ensuring a proper functioning proteome. Further, the dynamic turnover of skeletal muscle proteins underpins the plasticity of the tissue to environmental perturbations. Mathematically,

muscle protein turnover is the difference between muscle protein synthesis (MPS) and muscle protein breakdown (MPB) (58). In the post-absorptive state (i.e., fasted), rates of MPB exceed that of MPS, and skeletal muscle is in a state of negative protein balance (58). During negative protein balance, skeletal muscle is in a catabolic state and proteins are broken down to supply amino acids for gluconeogenesis and for energy production (58). During the presence of anabolic stimuli – namely dietary protein ingestion paired with RE – rates of MPS exceed rates of MPB, and skeletal muscle is in a state of positive protein balance (59), enabling skeletal muscle protein, and eventually mass, accrual to proceed.

Generally, protein synthesis can be described as translating genetic information contained within messenger ribonucleic acid (mRNA) templates into polypeptide chains. There are three primary steps in MPS: (1) initiation; (2) elongation; and (3) termination. Translation initiation begins with eukaryotic initiation factors being recruited to the start codon of the mRNA, which aids in ribosomal subunit assembly to form a translationally competent ribosome (9). A translationally competent ribosome (80S) contains two subunits (one large (60S) and one small (40S)), formed by the intricate association of over 80 ribosomal-associated proteins and 4 ribosomal ribonucleic acids (rRNAs) (9). Thereafter, elongation can begin. During elongation, the 60S ribosome subunit recognizes each subsequent mRNA codon and recruits the corresponding aminoacyl-transfer ribonucleic acid (tRNA), whereas the 40S subunit catalyzes peptide bond formation between subsequent amino acids (9). Following peptide bond formation, eukaryotic elongation factors facilitate ribosome movement along the mRNA template (9). Once the ribosome reaches one of three stop codons, release factors are recruited to the ribosome to initiate termination, promoting the release of the ribosome from the mRNA transcript and disassociation into its catalytic subunits (9).

To meet the cellular demands of the cell, and control rates of MPS, there are a variety of intracellular signalling cascades that are receptive to environmental stimuli, such as nutrients, growth factors, and mechanical loading (34). A major focal point for the detection and convergence of environmental stimuli is mTORC1. mTORC1 is a serine/threonine kinase that is a rapamycin-sensitive regulator of mammalian cell size (60). mTORC1 contains subunits regulatory-associated protein of mTOR (Raptor) and proline-rich AKT substrate 40 kDa (PRAS40) (60). Activation of mTORC1-dependent regulation of MPS serves to activate downstream kinases such as the ribosomal protein 70 kDa S6 kinase 1 (p70S6K1), eIF4E Binding Protein 1(4EBP1), and ribosomal protein S6 (rpS6), which serves to promote translation initiation and elongation (60). In addition, mTORC1 activates transcriptional intermediary factor-1α (TIF-1α), which promotes transcription and synthesis of the ribosomal machinery necessary for translation (60).

It is important to recognize that control of MPS and cellular growth may also be mediated via rapamycin insensitive complexes. The aforementioned notion is supported by work in pre-clinical models demonstrating that rapamycin administration did not completely ablate RE-induced increases in MPS (61) or RET-induced increases in skeletal muscle hypertrophy (62). Most of the mTORC1-independent mechanisms governing protein translation are thought to occur via the mitogen-activated protein kinase/extracellular signal-regulated kinases 1/2 (MAPK/ERK1/2) pathway (34, 35). Recently, tripartite motif-containing 28 (TRIM28) has been identified as a rapamycin-insensitive regulator of mechanical load-induced hypertrophy (63). Therefore, at the molecular level, mTORC1 dependent, and independent mechanisms mediate increases in MPS.

1.5 The application of stable isotope tracers to monitor muscle protein synthesis

Stable isotopes are elements that are chemically (i.e., same number of protons and electrons) and metabolically similar to another naturally occurring element on the periodic table but differ in mass due to a different number of neutrons within the atomic nucleus (64). Abundant stable isotopes (^{1}H, ^{12}C, etc.) make up the majority of our biological environment; however, rare, low abundant stable isotopes (e.g., ^{2}H, ^{13}C, etc.) make up a small portion of our natural environment. Unlike radio isotopes, stables isotopes do not emit ionizing radiation, making their mass constant and safe for human consumption (64). Pairing the oral ingestion or intravenous infusion of stable isotope tracers with skeletal muscle biopsies and high-sensitivity mass spectrometers provides an invaluable opportunity to interrogate MPS under a variety of scenarios, such as muscle loading and unloading.

Early work utilizing stable isotope tracers to monitor MPS relied on substrate-specific sterile infusions of stable isotope tracers (e.g., ^{13}C-phenylalanine), skeletal muscle biopsies, and repeated blood sampling. Although the infusion of stable isotope-labelled compounds provided unprecedented insight into the acute regulation of MPS with loading (46), unloading (65), and nutritional/hormonal administration (66), intravenous infusions of stable isotopes are restricted to a laboratory setting and unable to provide insight into the regulation of MPS regulation under "free-living" conditions (i.e., days-to-weeks). Recent advances in mass spectrometry techniques have led to the reintroduction of deuterium oxide (D_2O) (67-69), which enables the assessment of metabolic flux in response to various stimuli, such as skeletal muscle loading, unloading, and feeding, under longer-term, free-living conditions. Further, D_2O is not substrate-specific and can be used to simultaneously measure the metabolic flux across a numerous of substrates, including protein, lipid, RNA, and deoxy-ribonucleic acid (DNA) (67).

Regardless of the tracer used (i.e., sterile infusion of substrate-specific tracer or D_2O ingestion), the underlying methodological premise is the same. Stable isotope infusion or ingestion increases the plasma enrichment and is then taken up by skeletal muscle to be incorporated into protein (64). Serial muscle biopsies allow the determination of the stable isotope tracer into skeletal muscle over time, and the change in isotopic enrichment of skeletal muscle proteins over time reflects the rates of MPS (64).

1.5.1 Muscle protein synthesis during resistance training

In the fasted state, MPB (~ 0.11 %/hr) exceeds that of MPS (~ 0.06 %/hr), and skeletal muscle is in a state of net negative protein balance (~ -0.06%/hr) (70). RE is a potent stimulator of MPS. Phillips and colleagues (70) demonstrated that, when compared to the rested state, skeletal muscle fractional synthetic rate (FSR) was elevated above basal levels 3 – 48 hours following a single bout of RE in humans. When RE is performed in the fasted state, rates of MPB are also elevated – albeit to a lesser extent than rates of MPS (70). Despite a strong anabolic response following a bout of RE in the post-absorptive state, skeletal muscle remains in a state of negative protein balance (70). Ingestion of an adequate dose of protein, containing essential amino acids (EAA) – particularly leucine (LEU) – results in a transient hyperaminoacidemia and stimulation of MPS (59). Further, dietary protein consumption suppresses MPB resulting in a positive protein balance (71). Despite the positive effect of EAA ingestion on rates of MPS and MPB, sufficient EAA ingestion for 3 and 6 months was not sufficient to increase skeletal muscle mass (72, 73). Skeletal muscle mass accrual may only occur when acute, periodic bouts of RE are paired in close temporal proximity to sufficient dietary protein ingestion (66, 74).

Evidence suggests that the MPS response can be modified by several RET program variables. Burd and colleagues (46) randomly assigned healthy young men to undergo unilateral knee extension using a low-volume protocol (i.e., 1 set), or a high-volume protocol (i.e., 3 sets). The authors demonstrated that higher volume RE augmented the MPS response to a greater extent, than with the low volume RE protocol (46). Further, Hammarstron and colleagues (45) demonstrated marked increases in anabolic signalling markers with higher volume RE training. These findings may align with high(er) volume chronic RET protocols leading to more muscle mass accrual.

It is commonly recommended that high-load RET is required for maximal stimulation of MPS (75). Indeed, some studies demonstrate a greater MPS response with high(er) loads versus low(er) loads (76). In contrast, other studies suggest no effect of load on MPS responses (37, 77). Discrepancies across the aforementioned studies may be attributed to between-group differences in the degree of effort. For example, Burd and colleagues (37) demonstrated that performing RET at 30% 1RM to volitional fatigue resulted in a similar increase in myofibrillar MPS as performing 90% 1RM to volitional fatigue. Studies displaying a benefit of heavy load RE on MPS responses are volume-matched, and lighter load groups undoubtedly complete their sets well short of failure. With the current state of evidence, there appears to be a negligible impact of load on rates of MPS, given that RE is performed with a high degree of effort.

Chronic RET modifies the post-absorptive MPS response. Compared with the untrained state, post-absorptive MPS is greater in the trained state (78, 79), and this has recently been put forward as a primary contributor to RET-induced skeletal muscle hypertrophy (80). However, identical to what is seen in untrained individuals (70), trained individuals have a net negative protein balance due to the concomitant rise in MPB (79). In addition, the MPS response to a bout

of RE is altered following RET. Tang and colleagues demonstrated that 8 weeks of RET changed the temporal pattern of MPS in response to an acute bout of RE (66). Specifically, trained individuals experience a greater initial peak in MPS following RET; however, the duration of elevated MPS was reduced compared to untrained individuals (9, 58). Further, protein turnover during the early stages of a RET program is directed towards repairing of damaged muscle proteins (17). In contrast, the homeostatic perturbation is dampened in trained individuals, leading to a more refined MPS directed to muscle protein accretion (17). Therefore, chronic RET alters the post-absorptive and RE-induced MPS response.

At the molecular level, the RE-induced stimulation of MPS may occur owing to two mechanisms. Increased translational efficiency, with more mRNA being translated for a fixed pool of ribosomes, or increased translational capacity, with a greater pool of ribosomes available to translate mRNA into protein. It should be noted that measuring RNA content represents a crude readout of translational capacity, as rRNA makes up 85% of total RNA (81). Chesley and colleagues were the first to demonstrate that following a bout of RE, the acute rise in MPS was attributed to increased RNA activity (i.e., translation efficiency), as a rise in MPS occurred without increases in RNA (82). Further, following a bout of forced lengthening and shortening contractions in mice, the abundance of mRNAs being translated increased, and this was concordant with elevated levels of p70S6K1 (83). Work in pre-clinical models of synergist ablation demonstrated large increases in rRNA content (84). However, models of synergist ablation induce non-physiological rates of hypertrophy, such that muscle mass has been reported to increase up to 40% in a matter of days (84), which is clearly not aligned with RET-mediated increases in hypertrophy in humans.

Several groups have demonstrated that chronic RET results in increased total RNA (45, 85-88), rRNA content (45), regulators of rRNA synthesis (8, 85, 88), and pre-rRNA species (89). In contrast, decreases (90) or no change (87) in ribosomal content have been reported. In a series of papers, Brook and colleagues (67, 85) demonstrated that rates of RNA synthesis were increased above basal rates over the 0- to 6-wk period with RET; however, this showed discordance with long-term measures of MPS and muscular adaptations. We have previously hypothesized (58) that early on in a RET program, translational capacity may be increased as a general response to a need for greater rates of global MPS, but once protein synthetic responses and transcriptional programs become more refined, further increasing ribosomal capacity is not required and RNA concentration would either stabilize or possibly decline. It should be noted that stabilization of ribosomal concentration does not necessarily reflect a decline in the ribosomal pool, per se, but instead demonstrates a dilution of ribosome content by larger, hypertrophied myofibres (58) (Figure 1).

1.5.2 Muscle protein synthesis during disuse atrophy

Mechanistically, the loss of skeletal muscle mass can be underpinned by imbalances in MPS and MPB (9). Work from our group (20, 91-93) and others (94-97) have attributed the loss of skeletal muscle mass with muscle disuse, for the most part, to a reduction in rates of MPS. Gibson et al. (94) were the first to report a 25% reduction in fasted rates of MPS following 5 weeks of unilateral lower-limb immobilization in healthy young men with tibial fractures. The findings from Gibson et al. (94) have been corroborated with immobilization lasting as little as one week in healthy young men (95, 96).

Reductions in rates of MPS of the *Vastus Lateralis* during muscle disuse are not limited to the fasted state and extend to fed periods. Indeed, fed rates of MPS are decreased by 25% to

50% in response to 5 days (96), or 14 days (65) of immobilization. Thus, skeletal muscle disuse depresses both fed- and fasted-rates of MPS, which strongly suggests reductions in daily-integrated rates of MPS. Consistent with skeletal muscle disuse-induced anabolic resistance, integrated rates of MPS (i.e., fed and fasting) are rapidly decreased during periods of reduced skeletal muscle activity. As little as 2 weeks of step reduction (<1000 steps/day) was sufficient to reduce integrated rates of MPS by 13%-26% from baseline in healthy older adults, which is not recovered following 2 weeks of return to habitual activity (93, 98). We demonstrated that 2 weeks of single-leg immobilization was sufficient to depress integrated rates of MPS by a similar but opposite magnitude as 10 weeks of RET in healthy young men (20).

Despite an overwhelming amount of evidence suggesting that diminished rates of MPS are a primary driver of disuse-induced skeletal muscle atrophy, the molecular mechanisms governing this decrement remains unclear. Evidence is conflicting regarding the role of translational capacity in disuse-induced deficits in MPS. Following a period of unloading in humans and pre-clinical models, studies have demonstrated either decreased (99), or unchanged (94, 100) markers of ribosomal biogenesis. The conflicting evidence may be attributed to various markers used for interrogating ribosomal biogenesis (e.g., total RNA content, pre-rRNA species, ribosomal biogenesis-related transcription factors, ribosomal-associated proteins, etc) and biopsy sampling time points used, implicating a defect in translational efficiency with unloading.

The diminished MPS response during skeletal muscle disuse may be paralleled by changes in the activation of mTORC1 pathway. In the hour following protein ingestion, there is blunted activation of mTORC1, and all its downstream targets (4EBP1, p70S6K1, rpS6), after 10 days or 5 days of bed rest (101), or single-leg immobilization (97), respectively. In contrast, Wall et al. (97) reported no changes in phosphorylation levels of mTORC1 following 5 days of

immobilization. While the differences in biopsy sampling time points may explain the aforementioned discordant findings across studies, a lack of mTORC1 pathway activation could be indicative of mTORC1 independent mechanisms driving decrements in MPS with unloading. One example is the intermuscular accumulation of lipid species. The anabolic effect of amino acid administration on MPS is blunted with lipid infusion, with no obvious decrement in mTORC1 signaling (102). Further, Black and colleagues found increased intermyofibrillar lipid accumulation following 2 weeks of unloading in healthy young women (103).

In C2C12 cells, palmitate-induced ceramide accumulation was associated with a decreased MPS response, yet eukaryotic initiation factor 2 alpha (eIF2α) was phosphorylated (104).

Although eIF2α phosphorylation downregulates the activity of most cellular proteins, it increases the synthesis activating transcription factor 4 (ATF4) (105). ATF4, among other molecules, up regulates Sestrin2, an intracellular sensor of LEU (106). It has been hypothesized that the increase in Sestrin2 would increase the "LEU threshold" required to stimulate MPS, however, this has yet to be confirmed in clinical models of unloading. Nonetheless, this hypothesis parallels nicely with work demonstrating that unloading blunts the anabolic response to dietary protein administration (96, 97). Collectively, the data point toward a disuse-induced defect in the anabolic intracellular signaling pathway underpinning the attenuation in rates of MPS during periods of physical inactivity (Figure 1).

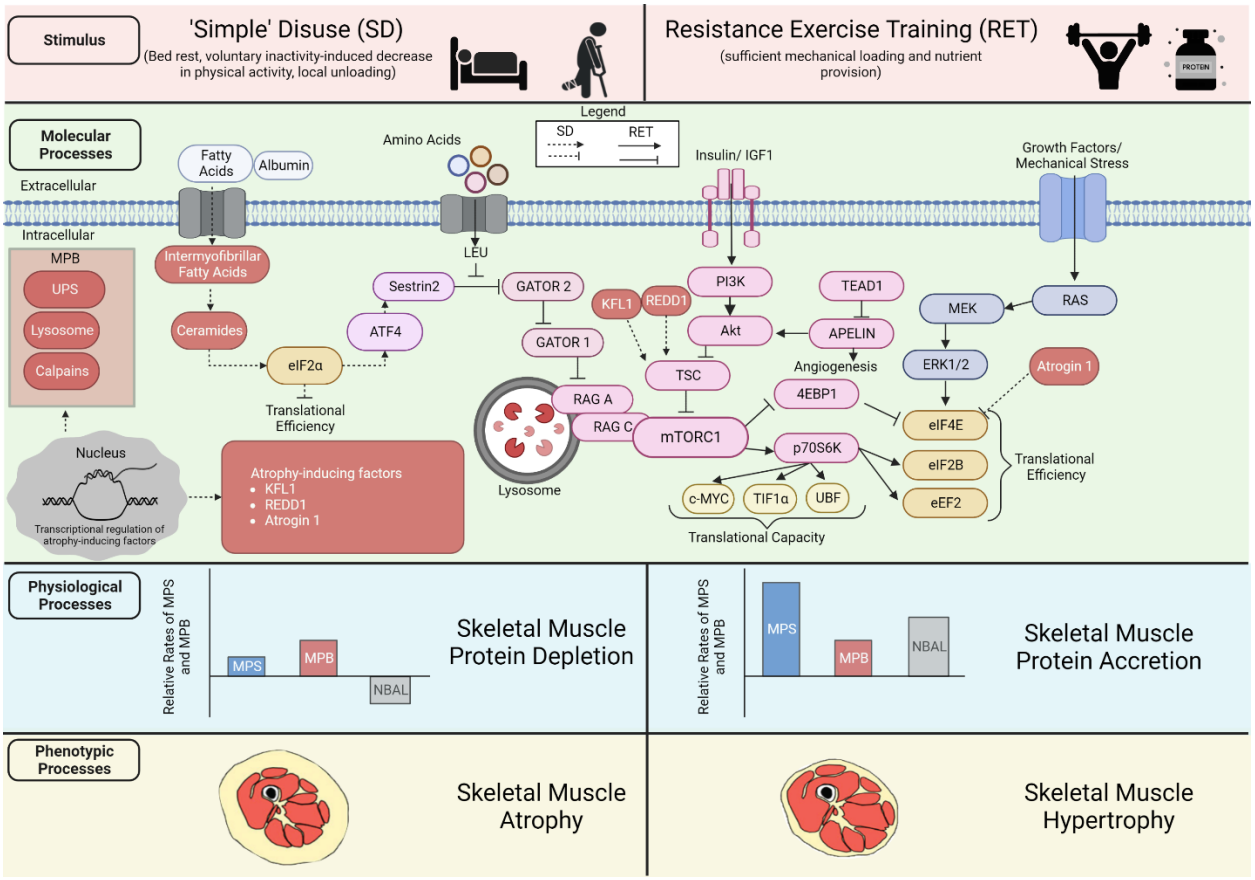

Figure 1. A simplified overview contrasting molecular, physiological, and phenotypic processes occurring with simple disuse atrophy and with resistance training induced skeletal muscle hypertrophy.

1.6 Exercise heterogeneity and insight from transcriptomic studies.

In addition to changes in protein synthesis and protein abundance, skeletal muscle plasticity is a complex process involving satellite cell-derived myonuclear accretion, angiogenesis, and extracellular matrix modelling. Indeed, proteins are the biological actors in cells, responsible for almost all cellular activities, and it is unsurprising that measuring protein dynamics may provide biological insight during physiological perturbations, such as disease. However, our ability to measure proteomic expression is not without its limitations. For example, the top 10 most abundant myofibrillar proteins in human skeletal muscle comprise approximately 45% of the spectral abundance (107), which may result in low abundance regulatory molecules – such as transcription factors – being unreliably quantified (108).

Our ability to quantify the proteome has lagged behind our ability to quantify the transcriptome, which can be defined as the set of all RNA transcripts, coding, and non-coding. For example, one can reliably quantify ~40,000 RNA species with microarray technology (32). A broad misconception is that capturing RNA expression has a small-to-moderate correlation with protein abundance data (109). However, when modelled properly, the variation in RNA could explain up to 73% of protein abundance changes (110). Further, poor measurement reproducibility of the proteome accounts for a substantial fraction of unexplained variance between RNA expression and protein abundance (108). Measuring the transcriptome extends beyond simply serving as a surrogate for protein expression. Instead, changes in RNA expression reflect an integration of environmental (20), genetic (111), and epigenetic (112) perturbations. Therefore, capturing RNA expression in lieu of protein expression may serve as a useful strategy to uncover the molecular regulators of skeletal muscle adaptation.

Pillon et al. (113) used published transcriptomic profiling data sets (n = 66) with more than 1000 individuals and demonstrated 2000 genes affected by RET. Downstream Gene Ontology (GO) analysis characterized that RE training mainly upregulated mRNA genes involved in extracellular matrix remodeling (113). Despite the large sample size, Pillon and colleagues (113) studied only differential expression (DE) of genes and failed to incorporate relevant physiological changes, such as skeletal muscle hypertrophy. Thus, based on this analysis (113) whether the 2000 genes affected by RET affect hypertrophic responses are unknown. Raue and colleagues (114) identified over 600 genes that correlated with muscle growth after 12 weeks of RET, but many genes were generic adaptations of exercise, and not specific to skeletal muscle mass, *per se;* these genes include growth factor genes, cell cycle-related genes, cytokine signaling genes, and several genes involved in ubiquitin-proteasome signaling and substrate metabolism. Critically, it has been known for quite some time that averaging the transcriptional responses across individuals displaying wide phenotypic variability is a flawed procedure (115).

Examining group mean transcriptional responses across individuals displaying varying degrees of hypertrophy hides important variance. It may be more beneficial to leverage the response-heterogeneity to identify the change in molecular responses that scale proportionally with physiological adaptations. For example, we (20) recently discovered a set of 141 genes correlated with the muscle growth response to chronic muscle loading in humans (n = 100), and these activated genes form functional networks that were observed to be associated with extracellular matrix remodeling, angiogenesis, and mitochondrial function. Further analysis by Phillips and colleagues (116) led to the surprising finding that higher responders to RET exhibit a gene signature consistent with reduced activation of mTORC1 signaling. Collectively, utilizing

transcriptome technology and leveraging individual heterogeneity in response to resistance training may help determine molecular regulators for RET-induced skeletal muscle hypertrophy.

1.6.1 Appreciating the mammalian long non-coding transcriptome.

Protein coding genes account for a small proportion of the genome (2%), and the number of protein coding genes does not scale with biological complexity (117). When early global transcriptomic analyses determined that most of the mammalian genome is transcribed into RNAs that have little-to-no protein coding potential (118), it was widely speculated that majority of the mammalian transcriptome was redundant, non-functional, and labelled as "transcriptional noise" (119). By contrast, Liu and colleagues demonstrated that there is a strong and statistically significant correlation between the proportion of the genome that is non-coding and organismal complexity (117), suggesting that non-coding RNAs may serve as regulatory molecules in a wide array of cellular processes.

The non-coding transcriptome consists of RNAs that do not code for proteins, such as rRNAs, tRNAs, small nucleolar RNAs, spliceosomal RNAs, small interfering RNAs, and small PIWI-interacting RNAs (120). Long non-coding RNA (lncRNA) are a specific class of non-coding transcripts that are more than 200 nucleotides in length, which conveniently cuts off most infrastructural RNAs (such as 5S rRNAs, tRNAs, snRNAs and snoRNAs, as well as miRNAs, siRNAs and piRNAs) (121). There is an extensive list of distinct sub-groups of lncRNAs, with different structural and functional characteristics, making annotation of lncRNAs relatively difficult (120). For example, lncRNA can be classified based on their: "mRNA-like" properties; or location at which the lncRNA functions relative to its transcription site (i.e., trans or cis); or with respect to its protein coding genes (i.e., intronic, intergenic, antisense). A wide variety of mechanistic and gene expression studies have demonstrated that lncRNA are involved in several

cellular functions, including transcriptional and post-transcriptional regulation, X chromosome inactivation, developmental processes, cell differentiation, and disease states, such as cancer, and metabolic disease (32, 122).

Due to their dynamic expression and key regulatory roles in gene expression, lncRNA may be important regulators of skeletal muscle processes. For example, cytoskeleton regulator RNA (CYTOR) has been identified as an exercise induced lncRNA that mitigates age-associated skeletal muscle atrophy of type II fibres by preventing the binding of TEA domain transcription factor 1 (TEAD1) transcription factors to its target genes, which promotes the differentiation of myoblasts (123). Further, the master transcription factor myoblast determination protein, which is central to skeletal muscle differentiation *in vivo*, is regulated by lncRNA (124). Combined, the data support exploration of lncRNA candidates that are associated with RET-induced increases in skeletal muscle hypertrophy.

1.6.2 Methodological considerations for studying the long non-coding transcriptome

The most commonly used methods to interrogate the transcriptome are short-read RNA sequencing (RNA-seq) and modern high-density arrays. High density arrays contain oligonucleotide probes immobilized to a glass surface that bind complementary DNA – an exact copy of RNA as strand-specific DNA – in a non-competitive manner (125). Thereafter, fluorescent emissions are generated in proportion to the concentration of copy DNA (cDNA) (125). Modern high-density arrays rely on ~7million probes to cover the transcriptome, with multiple copies of 25-mer probes and a minimum of three probes combined into probe-sets (126). Chip definition files are then used to determine the exact composition of each probe-set (127).

On the other hand, short-read RNA-seq relies on fragmenting and amplification. Short-read RNA-seq begins by fragmenting RNA, which is followed by making cDNA using non-

linear polymerase chain reaction amplification (128). The cDNA fragments are then hybridized to a flow cell, where DNA clusters are formed using bridge amplification (128). Each cluster of amplified nucleotides are then read to generate sequencing information (128). The counts generated with short-read RNA seq reflect the expression of the cDNA library within a sequencing run (127). Short-read RNA seq has become the method of choice for bulk transcriptomics studies, replacing microarray technology (127).

However, RNA-seq is not without some drawbacks. For example, short-read RNA-seq does not profile RNA, rather, it profiles a DNA library. Additionally, rRNA makes up >80% (81) of total RNA in a sample, and to overcome this RNA-seq methods often call for rRNA depletion and/or poly-A enrichment prior to polymerase chain reaction (129). Interestingly, most lncRNA lack poly-A tails and will therefore not be efficiently incorporated into the cDNA library if a poly-A enrichment strategy is used. In contrast to short-read RNA-seq, modern high-density arrays do not require RNA processing selection prior to linear amplification (127). Additionally, short-read RNA-seq is susceptible to a variety of biases, including "jackpotting", incomplete coverage of the transcriptome in the cDNA library, and preferential amplification of certain higher abundance RNA molecules (127).

A lack of appreciation of biases in short-read RNA-seq can compromise downstream analyses such as GO analysis (130, 131), and DE analysis (127). Further, using short-read RNA-seq has led the scientific community to describe *all* lncRNA as low abundance transcripts (132). However, using modern high-density arrays, Stokes and colleagues demonstrated a broad range in the expression of lncRNA detected by short-read RNA-seq (127). Further, the authors illustrate that short-read RNA-seq detects lncRNA exhibiting less variable expression across samples (127). Despite the popularity of short-read RNA-seq (133), short-read RNA-seq

provides highly variable coverage of the non-coding transcriptome, whereas modern-high density arrays provide superior > 75% coverage of non-coding RNA (>75%) (127), including lncRNA (32). Therefore, modern high-density arrays are more robust than short-read RNA-seq when profiling lncRNA.

1.7 Thesis objectives and hypotheses

The overarching objective of the experiments conducted as part of this thesis was to study the influence of external variables (RET program variables) and internal variables (lncRNA) on skeletal muscle adaptations, and characterize changes in MPS during differential loading. To fulfill this objective, we first conducted a systematic umbrella review to summarize the current state of the evidence on the influence of various RET program variables on skeletal muscle adaptation (Chapter 2; Study 1). Next, we conducted a Bayesian NMA to statistically determine the influence of several combinations of RET program variables (i.e., resistance training prescription RETx) on RET induced skeletal muscle adaptations (Chapter 3; Study 2). Using five independent exercise studies, we leveraged the variability in RET induced changes in lean mass (LM) to generate a lncRNA signature (Chapter 5; Study 4). Finally, we aimed to characterize integrated rates of bulk MPS in skeletal muscle under distinct loading states in young healthy individuals (Chapter 4; Study 3). The following four studies make up this Ph.D. thesis:

Chapter 2 – Study 1: The influence of resistance exercise training prescription variables on skeletal muscle mass, strength, and physical function in healthy adults: an umbrella review.

RET program variables have been a longstanding focus of exercise science; however, the contribution of many manipulatable RET program variables to muscular adaptations remains to be established. To date, a synthesis of systematic reviews integrating multiple RET program variables is lacking. Umbrella reviews extend upon systematic reviews by identifying,

synthesizing, and evaluating evidence from multiple systematic reviews and meta-analyses on a common topic. Therefore, the aim of study 1 was to summate evidence from existing systematic reviews and meta-analyses investigating RET program variables and muscular adaptations. Thus, the purpose of the current umbrella review was two-fold: (1) to determine the influence of resistance training on skeletal muscle mass, strength, and physical function, compared with a non-exercise control group, and (2) to determine the impact individual RET program variables may have on RET-induced increases in muscle mass, muscle strength, and physical function. We looked for evidence suggesting that RET was superior to a non-exercise control group for increasing muscle mass, strength, and physical function. We hypothesized that there will be some or sufficient evidence in favour of certain RET program variables in augmenting skeletal muscle adaptations; in contrast there will be insufficient evidence to determine an effect, or insufficient evidence in favour of, other RET program variables for augmenting skeletal muscle adaptations.

Chapter 3 – Study 2: Resistance Training Prescription for Muscle Strength and Hypertrophy in Healthy Adults: A Systematic Review and Bayesian Network Meta-Analysis.

Various meta-analyses have provided seminal evidence on the *univariate* impact of load (52, 54, 134, 135), sets (47, 136-138), or frequency (57, 139-142) to improve muscle strength, mass, and physical function. However, these univariate analyses limit RET guideline development because acute RET programs variables are neither mutually exclusive nor prescribed independently; rather, several variables are collectively inherent to any Resistance exercise training prescription (RETx). Comparisons between multivariate RETx are needed to advance, or support, current RET guidelines. Pairwise meta-analyses are methodologically

constrained to only comparing two RET prescriptions (143). NMA expands upon pairwise meta-analysis by permitting the simultaneous comparison of multiple treatments (144). Therefore, the aim of this study this systematic review and NMA was to determine how different RETx affect muscle strength, hypertrophy, and physical function in healthy adults. We compared distinct combinations of RETx variables – load, sets, and frequency – and non-exercising control groups. For each outcome, NMA was used to compare thirteen distinct conditions with data from randomized trials.

Chapter 4 – Study 3: A long non-coding-RNA signature is related to human skeletal muscle load-induced remodelling.

lncRNA contain little-to-no coding potential, are more than 200 nucleotides in length, and are poorly characterized in skeletal muscle biology. The purpose of study 3 was to interrogate changes in the lncRNA transcriptome following supervised exercise training. We used the human transcriptome 2.0 array (HTA2.0) chip to profile the lncRNA transcriptome of 144 individuals, from 5 independent studies, before and after supervised exercise training. LM was quantified using DXA, and we used the measurement error of the DXA to identify individuals with exercise-induced changes in LM. We then leveraged interindividual heterogeneity in exercise induced increases in LM to identify DE lncRNA. We used data-driven networks to elucidate potential biological features of lncRNA, performed an extensive literature search, and correlated select lncRNA candidates with cell-type specific gene markers. We also used four in vivo trials from human skeletal muscle unloading in an attempt to validate lncRNA genes.

Chapter 5 – Study 4: Changes in Protein Synthesis During Short-term Unloading and Loading in Human Skeletal Muscle.

The aim of this study was to characterize the changes in integrated rates of MPS in skeletal muscle under a variety of scenarios in young, healthy adults. We adopted a paired model, such that, within an individual, one limb was exposed to unloading to induce atrophy (14 days), whereas the contralateral, non-immobilized limb was first used as a short-term *bona fide* control (6 days), followed by exposure to short-term loading (8 days). We aimed to determine if short-term (6 days) unilateral skeletal muscle unloading was sufficient to alter MPS of the immobilized and contralateral, non-immobilized limbs. We hypothesized that 6 days of unloading would alter MPS; in contrast, MPS would remain largely unchanged. The second objective of this study was to contrast the MPS of muscle subjected to single-leg immobilization with the contralateral limb subjected to a short-term hypertrophic stimulus (i.e., short-term resistance exercise). We hypothesized that there would be differential regulation of MPS by both loading and unloading.

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CHAPTER 2:

The influence of resistance exercise training prescription variables on skeletal muscle mass, strength, and physical function in healthy adults: An umbrella review

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ABSTRACT

Objective: The aim of this umbrella review was to determine the impact of RET and individual RET prescription variables on muscle mass, strength, and physical function in healthy adults.

Methods: Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we systematically searched and screened eligible systematic reviews reporting the effects of differing RET prescription variables on muscle mass (or its proxies), strength, and/or physical function in healthy adults aged >18 years.

Results: We identified 44 systematic reviews that met our inclusion criteria. The methodological quality of these reviews was assessed using A Measurement Tool to Assess Systematic Reviews (AMSTAR); standardized effectiveness statements were generated. We found that RET was consistently a potent stimulus for increasing skeletal muscle mass (4/4 reviews provide some or sufficient evidence), strength (4/6 reviews provided some or sufficient evidence), and physical function (1/1 review provided some evidence). RET load (6/8 reviews provided some or sufficient evidence), weekly frequency (2/4 reviews provided some or sufficient evidence), volume (3/7 reviews provided some or sufficient evidence), and exercise order (1/1 review provided some evidence) impacted RET-induced increases in muscular strength. We discovered that 2/3 reviews provided some or sufficient evidence that RET volume and contraction velocity influenced skeletal muscle mass while 4/7 reviews provided insufficient evidence in favor of RET load impacting skeletal muscle mass. There was insufficient evidence to conclude that time of day, periodization, inter-set rest, set configuration, set end point, contraction velocity/time under tension, or exercise order (only pertaining to hypertrophy) influenced skeletal muscle adaptations. A paucity of data limited insights into the impact of RET prescription variables on physical function.

Conclusion: Overall, RET increased muscle mass, strength, and physical function compared to no exercise. RET intensity (load) and weekly frequency impacted RET-induced increases in muscular strength but not muscle hypertrophy. RET volume (number of sets) influenced muscular strength and hypertrophy.

INTRODUCTION

Skeletal muscle is integral to many locomotive and metabolic processes critical for good health. Performing regular resistance training (RET)—muscle contraction against external resistance—improves muscular health[1]; in particular, RET increases skeletal muscle mass (i.e., hypertrophy), strength, and physical function (gait speed, timed up-and-go, chair sit-to-stand, *etc*.). RT prescription (RETx) involves multiple programming variables, such as load, sets, frequency, rest intervals, muscle action type, and velocity.[2] Understanding how RETx variables impact muscular adaptations to RET is critical for effective exercise programming.

RETx has been a longstanding focus of exercise science; however, the contribution of many manipulatable RETx variables to muscular adaptations remains to be established. Systematic reviews have aimed to determine how individual RETx variables influence the development of strength and hypertrophy. These reports contributed to the development of advice by the World Health Organization (WHO) for healthy adults to engage in moderate-to-vigorous RT at least twice weekly.[3] Furthermore, the American College of Sports Medicine (ACSM) and National Strength and Conditioning Association (NCSA) have offered prescriptive position statements that advise adults to consider few (load and frequency[3,4]) or several RETx variables (load, frequency, sets, muscle action type/velocity, and rest intervals[5,6]). Systematic reviews provide high-quality evidence by collating and evaluating data with replicable search strategies and synthesis methods[7]; however, a synthesis of systematic reviews integrating multiple RETx variables is lacking.

Umbrella reviews extend upon systematic reviews by identifying, synthesizing, and evaluating evidence from multiple systematic reviews and meta-analyses on a common topic. We sought to summarize the evidence from existing systematic reviews and meta-analyses

investigating RET program variables and muscular adaptations to aid resistance training programming and guideline development for healthy adults. Thus, the purpose of the current umbrella review was two-fold: (a) to determine the influence of RET on skeletal muscle mass, strength, and physical function, compared with a non-exercise control group, and (b) to determine the impact individual RET program variables may have on RET-induced increases in muscle mass, muscle strength, and physical function.

METHODS

Protocol and registration

This review was prospectively registered on the International Platform of Registered Systematic Review and Meta-analysis Protocols (INPLASY202220028; https://inplasy.com/) and conducted under the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[8]

Information sources

A systematic search of Ovid MEDLINE, SPORTDiscus, and Web of Science was conducted from inception to December 9, 2021. No publication status, language, nor study design limits were used when conducting each search, and references from relevant systematic reviews were screened manually. The complete search strategy for Ovid MEDLINE is provided in the supplementary material (Supplementary Table 1).

Eligibility criteria

Eligibility was assessed by the predetermined Population, Intervention, Comparator, Outcomes, and Study Design (PICOS) criteria detailed in Table 1. Eligible reviews were published in English and investigated how muscle mass, strength, and/or physical function were impacted by

RET, compared to a non-exercising control, and/or the manipulation of individual RETx variables in healthy adults.

1

Table 1. Population, intervention, comparator, outcomes, and study design (PICOS) criteria

	Criteria	Description
Population	Does the review involve healthy adults?	*Included* - Adults aged ≥18 years who are generally healthy (no disease condition indicated other than sarcopenia) and community-dwelling adults *Excluded* - Athletes/military persons - Persons with or at risk for comorbidities (e.g., cardiovascular disease, type II diabetes, type I diabetes, cancer, peripheral artery disease, osteoarthritis, *etc.*) - Persons who are injured (e.g., musculoskeletal-related fracture and/or repair) - Explicitly mentions obese and/or overweight participants - Individuals who are hospitalized (inpatient/outpatient/rehabilitation, *etc.*) - Individuals living in long-term care homes - Animal studies (e.g., murine models)
Intervention	Does the review involve resistance training prescription?	*Included* - Resistance training programs (defined as the contraction of skeletal muscle against an external load/resistance, which can be provided by body mass, free-weights, guided motion machines, and elastic bands)
Comparator	Does the review include a different resistance training prescription or non-exercise control?	*Included* - A different resistance training prescription is when at least one programming variable is differentially prescribed for the duration of the intervention - A non-exercise control group continues a habitual lifestyle with no additional interventions
Outcomes	Does the review report on muscle-related outcomes?	*Included* - Muscle hypertrophy - Strength - Physical function (e.g., timed up and go test, chair rise, sit to stand test, gait speed test(s), walking test(s), short-performance physical battery test, Berg balance test, *etc.*)
Study design	Is the review secondary research?	*Included* - Only systematic reviews, meta-analyses, meta-regressions, network meta-analyses, and/or umbrella reviews are included *Excluded* - Non-systematic reviews (e.g., narrative review) or primary research (e.g., RCT, case report, case-control, cohort study)

2

Study selection and data extraction

Per PICOS criteria, 2 reviewers (SMP and BSC) independently screened records at the title/abstract and full text stages, and any discrepancies were resolved by consensus with a third reviewer (JCM). Two reviewers (BSC and CL) independently extracted information regarding the methods, results, and quality of all included articles, and any discrepancies were resolved by consensus with a third reviewer (JCM). Article screening and data extraction was completed using the Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia; available at www.covidence.org).

Methodological quality assessment and evidence synthesis

The methodological quality of all included reviews was determined in duplicate using A Measurement Tool to Assess Systematic Reviews (AMSTAR),[9] as previously described,[10,11] to yield a score ranging from 1 to 11. Three authors (BSC, CL, and JCM) systematically synthesized the evidence from each review to produce a standardized effectiveness statement (SES; sufficient evidence, some evidence, insufficient evidence, insufficient evidence to determine; see Supplementary Table 2) for each outcome.[10,11] Two authors (BSC and JCM) then considered each SES to generate a bottom-line statement for the impact of each RET prescription variable on muscle mass, strength, and function. The quality of evidence (QoE) derived from each article was determined by a method based on the Grading of Recommendations Assessment, Development and Evaluation[12] approach for primary evidence (1 = very low; 2 = low; 3 = moderate; or 4 = high). This method incorporates the review design (meta-analysis: yes/no) and methodological quality (AMSTAR score) of included reviews[10,11] (Supplementary Table 3). RET and RETx variables were judged on the strength of evidence and number of participants with increased muscle mass, strength, and/or function.[13]

RESULTS

Included reviews

The literature search identified 837 records, and 362 were removed as duplicates. There were 407 records removed on title/abstract screening, 2 records could not be retrieved, and 23 records were excluded on full-text screening. Fourty-four[14–57] reviews (5 systematic reviews, 2 meta-regressions, 35 meta-analyses, 1 network meta-analysis, and 1 umbrella review) met the eligibility criteria and were included (Figure 1). The AMSTAR scores and QoE for the included systematic reviews range from 2 to 10 and 1 to 4, respectively (Supplementary Fig. 1, Table 2). The average RT duration within the included reviews ranged from 6 weeks to 24 weeks. For details of the 44 systematic reviews included in the umbrella review, see Supplementary Table 4.

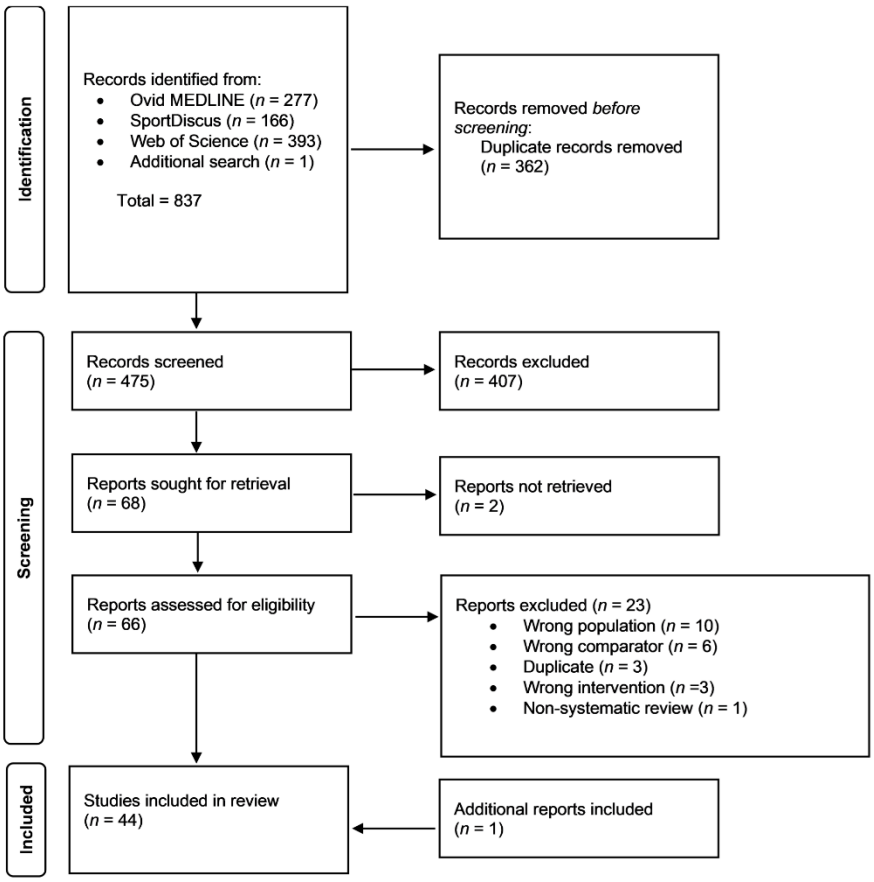

Figure 1. PRISMA flowchart of reviews identified, screened, removed, and included in the review. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Table 2. Summary of included studies.

Study	AMSTAR	Standardized effectiveness statements			QoE (/4)
		Hypertrophy	Strength	Physical function	
RT vs CTRL					
Beckwee, et al (2019)[15]	5	Sufficient evidence in favor of RT variable	Sufficient evidence in favor of RT variable	Some evidence in favor of RT variable	2
Borde, et al (2015)[16]	7	Some evidence in favor of RT variable	Insufficient evidence to determine	ND	3
Csapo, et al (2016)[18]	6	Some evidence in favor of RT variable	Some evidence in favor of RT variable	ND	3
Guizelini, et al (2018)[30]	4	ND	Sufficient evidence in favor of RT variable	ND	3
Hagstrom, et al (2020)[31]	8	Sufficient evidence in favor of RT variable	Sufficient evidence in favor of RT variable	ND	4
Silva, et al (2014)[54]	5	ND	Insufficient evidence to determine	ND	3
Load					
Csapo, et al (2016)[18]	6	Some evidence in favor of RT variable	Some evidence in favor of RT variable	ND	3
Grgic, et al (2020)[22]	4	Insufficient evidence to determine	ND	ND	3
Lacio, et al (2021)[36]	6	Insufficient evidence in favor of RT variable	Some evidence in favor of RT variable	ND	2
Lopez, et al (2021)[37]	8	Insufficient evidence in favor of RT variable	Sufficient evidence in favor of RT variable	ND	4
Refalo, et al (2021)[44]	7	Insufficient evidence in favor of RT variable	Sufficient evidence in favor of RT variable	ND	3
Schoenfeld, et al (2017)[48]	6	Insufficient evidence in favor of RT variable	Sufficient evidence in favor of RT variable	ND	3
Schoenfeld, et al (2016)[53]	3	Insufficient evidence to determine	Insufficient evidence to determine	ND	2
Steib, et al (2010)[55]	7	ND	Some evidence in favor of RT variable	Insufficient evidence to determine	3
Borde, et al (2015)[16]	7	Insufficient evidence to determine	Insufficient evidence to determine	ND	3
Set end point (momentary muscular failure)					
Davies, et al (2016)[19]	8	ND	Insufficient evidence in favor of RT variable	ND	4
Grgic, et al (2021)[28]	6	Insufficient evidence in favor of RT variable	Insufficient evidence in favor of RT variable	ND	3
Contraction velocity / time under tension / power training					
Orssatto, et al (2019)[40]	8	ND	ND	Insufficient evidence to determine	4
Schoenfeld, et al (2015)[51]	4	Insufficient evidence in favor of RT variable	ND	ND	3
Steib, et al (2010)[55]	7	ND	Insufficient evidence to determine	Insufficient evidence to determine	3
Borde, et al (2015)[16]	7	Insufficient evidence to determine	Insufficient evidence to determine	ND	3
Volume (number of sets)					
Krieger, et al (2010)[35]	5	Some evidence in favor of RT variable	ND	ND	3
Ralston, et al (2017)[41]	5	ND	Some evidence in favor of RT variable	ND	3
Schoenfeld, et al (2017)[50]	4	Sufficient evidence in favor of RT variable	ND	ND	3
Wolfe, et al (2004)[57]	5	ND	Some evidence in favor of RT variable	ND	3
Steib, et al (2010)[55]	7	ND	Insufficient evidence to determine	ND	3
Androulakis-Korakakis, et al (2020)[14]	5	ND	Insufficient evidence to determine	ND	2
Borde, et al (2015)[16]	7	Insufficient evidence to determine	Insufficient evidence to determine	ND	3
Ralston, et al (2019)[43]	7	ND	Insufficient evidence to determine	ND	3
Krieger, et al (2009)[34]	6	ND	Some evidence in favor of RT variable	ND	3
Set configuration					
Davies, et al (2021)[20]	8	Insufficient evidence in favor of RT variable	Insufficient evidence in favor of RT variable	ND	4
Jukic, et al (2021)[32]	9	Insufficient evidence to determine	Insufficient evidence in favor of RT variable	ND	4

Inter-set rest					
Grgic, et al (2017)[24]	6	Insufficient evidence to determine	ND	ND	2
Grgic, et al (2018)[29]	7	ND	Insufficient evidence in favor of RT variable	ND	2
Borde, et al (2015)[16]	7	Insufficient evidence to determine	Insufficient evidence to determine	ND	3
Periodization					
Grgic, et al (2018)[25]	5	Insufficient evidence in favor of RT variable	ND	ND	2
Grgic, et al (2017)[26]	8	Insufficient evidence in favor of RT variable	ND	ND	4
Rhea, et al (2004)[45]	2	ND	Some evidence in favor of RT variable	ND	2
Williams, et al (2017)[56]	7	ND	Insufficient evidence to determine	ND	3
Weekly frequency					
Grgic, et al (2018)[27]	7	ND	Sufficient evidence in favor of RT variable	ND	3
Kneffel, et al (2021)[33]	6	Insufficient evidence in favor of RT variable	Some evidence in favor of RT variable	ND	2
Ralston, et al (2018)[42]	9	ND	Insufficient evidence in favor of RT variable	ND	4
Schoenfeld, et al (2019)[47]	5	Insufficient evidence in favor of RT variable	ND	ND	3
Schoenfeld, et al (2016)[49]	4	Some evidence in favor of RT variable	ND	ND	3
Steib, et al (2010)[55]	7	ND	Insufficient evidence to determine	ND	3
Borde, et al (2015)[16]	7	Insufficient evidence to determine	Insufficient evidence to determine	ND	3
Muscle action type					
Buskard, et al (2018)[17]	3	ND	Insufficient evidence to determine	ND	2
Douglas, et al (2017)[21]	2	Some evidence in favor of RT variable	Some evidence in favor of RT variable	ND	1
Molinari, et al (2019)[38]	7	ND	Insufficient evidence to determine	ND	3
Roig, et al (2009)[46]	7	Insufficient evidence to determine	Insufficient evidence in favor of RT variable	ND	3
Schoenfeld, et al (2017)[52]	5	Some evidence in favor of RT variable	ND	ND	3
Time of day					
Grgic, et al (2019)[23]	8	Insufficient evidence in favor of RT variable	Insufficient evidence in favor of RT variable	ND	4
Exercise order					
Nunes, et al (2021)[39]	10	Insufficient evidence in favor of RT variable	Some evidence in favor of RT variable	ND	4

Notes: See supplementary materials for definitions.Abbreviations: AMSTAR = A Measurement Tool to Assess Systematic Reviews; ND = not determined; QoE = quality of evidence (/4; see Online Supplementary Table 3 for details); RT= resistance training.

Table 3. Effects of resistance training and resistance training prescription variables on muscle hypertrophy, strength, and physical function.

	Hypertrophy	Strength	Physical function
RT *vs.* non-exercising control	**Overall: ✓** 2 reviews (*n* = 2651) ↑↑ 2 reviews (*n* = 304) ↑	**Overall: ✓** 3 reviews (*n* = 3929) ↑↑ 1 review (*n* = NR) ↑ 2 reviews (*n* = 991) ?	**Overall: ✓** 1 review (*n* = 404) ↑
Load	**Overall: X** 1 review (*n* = 213) ↑ 4 reviews (*n* = 1895) ↔ 3 reviews (*n* = 470) ?	**Overall: ✓** 3 reviews (*n* = 2431) ↑↑ 3 reviews (*n* = 1111) ↑ 2 reviews (*n* = 251) ?	**Overall: ?** 1 review (*n* = 168) ?
Set end point (momentary muscular failure)	**Overall: X** 1 review (*n* = 219) ↔	**Overall: X** 2 reviews (*n* = 593) ↔	
Contraction velocity / time under tension	**Overall: X** 1 review (*n* = 204) ↔ 1 review (*n* = 159) ?	**Overall: ?** 2 reviews (*n* = 140) ?	**Overall: ?** 2 reviews (*n* = 692) ?
Volume (number of sets)	**Overall: ✓** 1 review (*n* = 418) ↑↑ 1 review (*n* = 235) ↑ 1 review (*n* = 159) ?	**Overall: ✓** 3 reviews (*n* = 1284) ↑ 4 reviews (*n* = 1074) ?	
Set configuration	**Overall: X** 1 review (*n* = 195) ↔ 1 review (*n* = NR) ?	**Overall: X** 2 reviews (*n* = 764) ↔	
Inter-set rest	**Overall: ?** 2 reviews (*n* = 115) ?	**Overall: X** 1 review (*n* = 491) ↔ 1 review (*n* = NA) ?	
Periodization	**Overall: X** 2 reviews (*n* = 417) ↔	**Overall: ?** 1 review (*n* = NR) ↑ 1 review (*n* = 612) ?	
Weekly frequency	**Overall: X** 1 review (*n* = 200) ↑ 2 reviews (*n* = 711) ↔ 1 review (n = 159) ?	**Overall: ✓** 1 review (*n* = 912) ↑↑ 1 review (*n* = 314) ↑ 1 review (*n* = 271) ↔ 2 reviews (*n* = 504) ?	
Muscle action type	**Overall: ✓** 2 reviews (*n* = 795) ↑ 1 review (*n* = 73) ?	**Overall: X** 1 review (*n* = NR) ↑ 2 reviews (*n* = 738) ↔ 2 reviews (*n* = 313) ?	
Time of day	**Overall: X** 1 review (*n* = 112) ↔	**Overall: X** 1 review (*n* = 221) ↔	
Exercise order	**Overall: X** 1 review (*n* = 177) ↔	**Overall: ✓** 1 review (*n* = 207) ↑	

Notes: Resistance training and resistance training prescription variables important (✓), not important (**X**), or unable to determine (**?**) for each outcome. The decision whether resistance training or a resistance training prescription variable was important or not was based on the total number of participants affected positively across the relevant trials (i.e., one trial (*n* = 200) showed an increase (↑); two trials (*n* = 711) showed no effects (↔); overall decision **X**). ↑↑ sufficient effect; ↑ some effect; ↔ no effect; ? cannot determine effect. Abbreviations: NR = not reported.

Muscle hypertrophy

RET vs. non-exercising control

Four reviews[15,16,18,31] ranging from low quality of evidence (QoE: level 2) to high quality of evidence (QoE: Level 4) provided some[16,18] or sufficient[15,31] evidence that RET increases skeletal muscle mass compared to non-exercising controls (Table 3). In a high-quality meta-analysis (QoE: Level 4) comprising 15 original studies, Hagstrom and colleagues[31] found that RET resulted in a significant increase in skeletal muscle mass (standardized mean difference (SMD) = 0.52; 95% confidence interval (95%CI): 0.2–0.78; p = 0.002) compared with non-exercising controls in young women. Csapo and colleagues[18] conducted a meta-analysis including 5 original studies and found that, compared to non-exercising controls, gains in muscle size were small following higher-intensity RET (SMD = 0.199; 95%CI: 0.046–0.343; p = 0.011), and lower-intensity RET (SMD = 0.108; 95%CI: –0.050 to 0.261; p = 0.179) in healthy older adults.

Load

One low-quality review (QoE: Level 2),[36] 2 moderate-quality reviews (QoE: level 3),[44,48] and 1 high-quality review (QoE: Level 4)[37] provided evidence that RET load does not impact RET-induced skeletal muscle hypertrophy (Table 3). In a high-quality NMA (QoE: Level 4; 24 studies and n = 747 participants), Lopez and colleagues[37] compared 3 load prescriptions (high load, ≥80% of 1-repetition maximum (1RM) or ≤8 repetition maximum (RM); moderate load, 60%–79% of 1RM or 9–15 RM; low load, <60% of 1RM or >15 RM) and found no significant difference in muscle hypertrophy between high-load RET and low-load RET (SMD = 0.12; 95%CI: –0.06 to 0.29; p = 0.241); moderate-load RET and low-load RET (SMD = 0.20; 95%CI:

–0.04 to 0.44; p = 0.113); high-load RET and moderate-load RET (SMD = –0.09; 95%CI: –0.33 to 0.16; p = 0.469). A moderate-quality meta-analysis (QoE: Level 3) conducted by Schoenfeld et al.[48] reported similar hypertrophic adaptations between high-load RET (>60% 1RM or <15 RM) and low-load RET (≤60% 1RM or ≥15 RM; SMD = 0.03; 95%CI: –0.08 to 0.14; p = 0.56). Lacio et al.[36] highlighted that 14/16 randomized studies included in their review found no differences across low-load RET (<67% 1RM or >12RM), moderate-load RET (67%–85% 1RM or 6–12 RM), and high-load RET (>85% 1RM or <6 RM) performed to volitional fatigue in muscle cross-sectional area or muscle thickness (QoE: Level 2) in young adults.

In contrast, a meta-analysis of moderate-quality (QoE: Level 3; 7 studies and n = 213 participants)[18] provided some evidence suggesting that high-load RET (≥80% 1RM) provoked larger gains in muscle size than low-load RET (≤60% 1RM), although the difference in hypertrophy was trivial (SMD = 0.136; 95%CI: 0.009 –0.259; p = 0.036). Three reviews[16,22,53] provided insufficient evidence to form a conclusion on the effects of RET load on skeletal muscle hypertrophy due to a limited number of studies[16,53] and imprecise effect estimates.[22]

Set end point (momentary muscular failure)

One high-quality meta-analysis (QoE: Level 4; comprising 7 studies (n = 219 participants)[28] concluded that performing RET to volitional fatigue had no impact on skeletal muscle hypertrophy (SMD = 0.22; 95%CI: –0.11 to 0.55; p = 0.152) (Table 3).

Contraction velocity/time under tension

A moderate-quality meta-analysis (QoE: Level 3)[51] found no significant differences (p = 0.94) when training with repetition durations ranging from 0.5 s to 8 s (Table 3). One review[16]

contained insufficient evidence to determine the effect of time under tension on muscle hypertrophy in older adults.

Volume (number of sets)

A moderate-quality meta-analysis (QoE: Level 3; 15 studies) by Schoenfeld and colleagues[50] found that RET with a higher number of weekly sets promoted greater skeletal muscle mass gains than RET with a lower number of weekly sets (SMD = 0.241 ± 0.10; 95%CI: 0.026–0.457; p = 0.03). Using meta-regression, the authors found there was a significant effect of the number of weekly sets on changes in skeletal muscle mass such that performing RET with 10+ sets per muscle group per week (SMD = 0.520 ± 0.13; 95%CI: 0.226–0.813; equivalent percent gain: 9.8%) elicited larger increases in skeletal muscle hypertrophy than performing RET with <5 sets per muscle group per week (SMD = 0.307 ± 0.07; 95%CI: 0.152–0.462; equivalent percent gain = 5.4%). Krieger and colleagues[35] found that performing RET with multiple sets per exercise were associated with significantly larger increases in skeletal muscle than performing RET with a single set per exercise (SMD = 0.11 ± 04; 95%CI: 0.02, 0.19; p = 0.016), with no difference in performing 4 to 6 sets per exercise *vs.* 2 to 3 sets per exercise (SMD = 0.10 ± 0.10; 95%CI: 20.09, 0.30; p = 0.29). One meta-regression[16] provided insufficient evidence to form a conclusion on the effect of RET volume on skeletal muscle hypertrophy in older adults (QoE: Level 3).

Set configuration

Davies and colleagues[20] found similar improvements in skeletal muscle hypertrophy between cluster-set RET and traditional-set RET (SMD = –0.05 ± 0.14; 95%CI: –0.32 to 0.23; p = 0.73). Another meta-analysis[32] investigating cluster sets contained insufficient information to determine

the impact of set configuration on skeletal muscle hypertrophy due to high heterogeneity (I^2: 52%–87%) and a small number of studies synthesized for analysis (<3 studies).

Inter-set rest

Two reviews, 1 of moderate quality (QoE: Level 3)[16] and 1 of low quality (QoE: Level 2),[24] contained insufficient evidence to determine the impact of inter-set rest on muscle hypertrophy due to the limited number of studies synthesized for analysis (<6 studies) (Table 3).

Periodization

A high-quality meta-analysis (QoE: Level 4) by Grgic and colleagues[26] found no significant differences between linear periodization and undulating periodization on measures of hypertrophy (SMD = 0.02; 95%CI: –0.25 to 0.21; p = 0.848). Similarly, a low-quality systematic review (QoE: Level 2)[25] concluded that periodized and non-periodized RET programs may yield similar hypertrophic adaptations (Table 3).

Weekly frequency

Two moderate-quality reviews (QoE: Level 3)[33,47] provided evidence that weekly RET frequency does not impact skeletal muscle hypertrophy (Table 3). In a meta-analysis comprising 13 primary studies, Schoenfeld and colleagues[47] found that when RET volume was matched, there was no difference between higher- and lower-RET frequency (SMD = 0.07 ± 0.06; 95%CI: –0.08 to 0.21; p = 0.32). Similarly, in healthy older adults, Kneffel et al.[33] found there to be no significant difference of RET frequency for muscle hypertrophy (p = 0.51), with an estimate of 0.02 (95%CI: –0.04 to 0.07) for each day increase in frequency (QoE: Level 2). In contrast, Schoenfeld et al.[49] conducted a moderate-quality meta-analysis (QoE: Level 3) and found that higher-frequency RET was associated with a greater effect size than lower-frequency RET (SMD

= 0.19 ± 0.03; 95%CI: 0.11 –0.2; p = 0.003) (QoE: Level 3). One meta-regression[16] provided insufficient evidence to form a conclusion on the effect of RET frequency on skeletal muscle hypertrophy in older adults.

Muscle action type

Two reviews provided some evidence[21,52] that muscle action type might influence skeletal muscle hypertrophy (Table 3). In a moderate-quality meta-analysis (QoE: Level 3; 15 studies), Schoenfeld and colleagues[52] reported that, compared with concentric RET, eccentric RET modestly increased skeletal muscle hypertrophy (SMD = 0.25; 95%CI: –0.03 to 0.52; p = 0.076). One very low-quality systematic review (QoE: Level 1) concluded that "Eccentric training appears to elicit greater increases in muscle CSA (cross-sectional area) than concentric or traditional RET.... Selective increases in fast-twitch fibre size have been reported and there is evidence to suggest that a shift towards a fast phenotype can occur as a result of chronic eccentric training."[21] One moderate-quality meta-analysis (3 studies and n = 73 participants)[46] provided insufficient evidence to determine the effect of muscle action type on skeletal muscle hypertrophy.

Time of day

One high-quality meta-analysis (QoE: Level 4; 6 primary studies (n = 112 participants))[23] found no significant difference between morning-RET and evening-RET (SMD = 0.20, 95%CI: –0.40 to 0.40; p = 0.958).

Exercise order

One high-quality meta-analysis (QoE: Level 4; 7 primary studies; n = 177 participants)[39] found no significant influence of exercise order on skeletal muscle hypertrophy (SMD = -0.02; 95%CI: −0.45 to 0.41; p = 0.937; I^2 = 0%).

Strength

RET vs. non-exercising control

One low-quality review (QoE: Level 2),[15] 2 moderate-quality reviews (QoE: Level 3),[18,30] and 1 high-quality review (QoE: Level 4)[31] provided sufficient evidence[15,30,31] or some evidence[18] that RET increases skeletal muscle strength compared to non-exercising controls (Table 3).

In a high-quality meta-analysis (QoE: level 4), Hagstrom and colleagues[31] found that compared to non-exercising controls, RET resulted in significant increases in upper body strength (SMD = 1.70; 95%CI: 1.28–2.13; p < 0.001) and lower body strength (SMD = 1.40; 95%CI: 1.03–1.76; p < 0.001) in young women. Similarly, 2 other moderate-quality meta-analyses (QoE: Level 3)[18,30] demonstrated similar benefits in muscular strength with RET, compared to non-exercising control groups, in healthy older adults. A low-quality umbrella review (QoE: Level 2; 7 reviews and n = 2869 participants) concluded there is a high quality ~~of~~ evidence in support of RET for increasing muscle strength in older adults.[15] Csapo and colleagues[18] provided some evidence that high-load RET (SMD = 0.778: 95%CI: 0.447–0.921; p < 0.001) or low-load RET (SMD = 0.663; 95%CI: 0.396–0.826: p < 0.001) provoked greater increases in muscle strength, compared with non-exercising controls, in healthy older adults. Two moderate-quality reviews (QoE: Level 3)[16,54] provided insufficient evidence due to high heterogeneity (I^2 >80%).

Load

One high-quality review (QoE: Level 4),[37] 4 moderate-quality reviews,[18,44,53,55] and 1 low-quality review (QoE: Level 2)[36] provided some[18,36,55] or sufficient[37,44,53] evidence that RET load impacts RET-induced muscular strength gains (Table 3). In a high-quality network meta-analysis (QoE: Level 4), Lopez and colleagues[37] found that compared with low-load RET (<60% of 1RM or >15 RM), high-load RET (≥80% 1RM or ≤ 8 RM; SMD = 0.60; 95%CI: 0.38–0.82) and moderate-load RET (60%–79% 1RM or 9–15 RM; SMD = 0.34; 95%CI: 0.05–0.62) resulted in larger muscular strength improvements ($p < 0.001$ and < 0.003, respectively). Refalo and colleagues[44] found that higher-load RET is superior to lower-load RET for increasing 1RM (QoE: level 3; 36 studies, 1187 participants; SMD = 0.34; 95%CI: 0.15–0.52; $p = 0.0003$; favors high-load) and isometric maximal voluntary contraction (MVC; 14 studies, 302 participants; SMD = 0.41; 95%CI: 0.07–0.76; $p = 0.02$) but not isokinetic MVC (10 studies, 264 participants; SMD = 0.19; 95%CI: –0.10 to 0.49; $p = 0.20$). In a systematic review, Lacio and colleagues[36] demonstrated that most studies (11/18 studies for 1RM strength; 6/9 studies for isometric MVC) concluded that moderate- and high-load RET were superior to low-load RET for increasing 1RM strength and isometric MVC (QoE: Level 2) in young adults. Meta-analyses by Steib and colleagues[55] and Csapo and colleagues[18] provided some evidence that RET with heavier loads may be required to maximize RET-induced skeletal muscle strength gains in older adults. Two reviews[16,53] provided inconclusive evidence regarding the impact of RET load on muscle strength due to a small number of studies being synthesized for analysis[53] or high heterogeneity (I^2 >80%).[16]

Set end point (momentary muscular failure)

In a meta-analysis of 15 reviews, Grgic and colleagues[28] showed no significant difference between failure RET or non-failure RET on skeletal muscle strength (SMD = –0.09; 95%CI: –0.22 to 0.05; $p = 0.198$). Similarly, a high-quality meta-analysis (QoE: Level 4; 8 studies)[19]

found similar increases in muscle strength between failure RET (effect size = 0.33; 95%CI: 0.06–0.61) and non-failure RET (effect size = 0.34; 95%CI: 0.06–0.62) (Table 3).

Contraction velocity/time under tension

Two moderate-quality reviews (QoE: Level 3)[16,55] provided insufficient evidence to determine an effect in older adults due to highly heterogenous effects (I^2 >80%; Table 3).

Volume (number of sets)

A moderate-quality meta-analysis (QoE: Level 3; 14 studies and n = 440 participants)[34] found that performing multiple sets/exercise was associated with significantly greater strength gains compared with performing a single set/exercise (SMD = 0.26 ± 0.05; 95%CI: 0.150.37: p = 0.0001). The same review[34] found that performing 2 to 3 sets/exercise was associated with a significantly greater effect size than performing 1 set/exercise (SMD = 0.25 ± 0.06; 95%CI: 0.14–0.37; p = 0.0001), but performing 4 to 6 sets/exercise was not significantly better to performing 1 set/exercise (SMD = 0.35 ± 0.25; 95%CI: –0.05 to 0.74; p = 0.17) or 2 to 3 sets/exercise (0.09 ± 0.20; CI: –0.31 to 0.50; p = 0.64). In a moderate-quality meta-analysis (QoE: Level 3; 16 studies and n = 621 participants), Wolfe and colleagues[57] found that multiple sets were superior to single sets for trained individuals (p < 0.001) and RET programs with an extended duration (p < 0.05).

Four additional reviews, ranging from low quality (QoE: Level 2)[14] to moderate quality (QoE: Level 3)[16,43,55] provided insufficient evidence to determine an effect due to systematic reviews containing high heterogeneity (I^2 >80%),[16,43] a low number of studies synthesized for review,[55] and 1 review[14] reporting inconclusive results with no effect present in 3/6 studies included (Table 3).

Set configuration

In a high-quality meta-analysis (QoE: Level 4), Davies and colleagues[20] showed no difference in muscular strength gains between cluster-set RET and traditional-set RET (SMD = –0.05 ± 0.08; 95%CI: –0.21 to 0.11; p = 0.56). Similarly, Jukic and colleagues[32] found that neither cluster (SMD = –0.07; 95%CI: –0.21 to 0.07; p = 0.300; I^2 = 0%) nor rest redistribution (SMD = –0.04; 95%CI: –0.20 to 0.12; p = 0.641; I^2 = 51%) set structures were more effective than traditional set structures in promoting muscular strength adaptations.

Inter-set rest

One low-quality systematic review (QoE: Level 2; 23 studies; n = 491 participants)[29] concluded that rest interval duration does not impact skeletal muscle strength. Another review[16] provided insufficient evidence to determine an effect in older adults due to considerably large heterogeneity (I^2 >80%; Table 3).

Periodization

In a low-quality meta-analysis (QoE: Level 2), Rhea and colleagues[45] found that periodized RET programs improved muscle strength over non-periodized RET programs. A moderate-quality review (QoE: Level 3)[56] provided insufficient evidence to determine an effect due to highly heterogenous effects (Q = 213.56; p < 0.001; I^2 = 62.5%; Table 3).

Weekly frequency

In a moderate-quality meta-analysis (QoE: Level 3; 22 studies; n = 912 participants), Grgic et al.[27] found a significant (p = 0.003) effect of weekly training frequency on muscular strength gains. Specifically, the authors concluded that higher RET frequencies (e.g., 4 days/week)

resulted in larger strength gains than lower RET frequencies (e.g., 1 day/week). Similarly, a low-quality meta-regression (QoE: Level 2; 9 studies and n = 314 participants)[33] found a significant impact of weekly training frequency, such that for every daily increase in training frequency there was an 0.14 increase in effect size for muscular strength (Table 3).

In contrast, a high-quality meta-analysis (QoE: Level 4; 12 studies; *n* = 299 participants) done by Ralston and colleagues[42] concluded there was no significant impact of weekly frequency on muscular strength, regardless of whether RET volume was equated or not. Two moderate-quality reviews (QoE: Level 3) provided insufficient evidence to determine the impact of RET frequency on muscular strength gains in older adults.[16,55]

Muscle action type

In a very low-quality systematic review (QoE: Level 1), Douglas and colleagues concluded that "Eccentric training may improve overall strength to a greater extent than concentric and traditional modalities, although there is a mode-specificity (i.e., muscle action type and velocity) of improvements."[21] In contrast, a moderate-quality meta-analysis (QoE: Level 3)[46] found no differences between eccentric- or concentric-RET for improvement in peak torque (weighted mean difference: 3.71 N•m; 95%CI: –0.27 to 7.70; *p* = 0.07; *n* = 333) or 1RM (weighted mean difference: 1.07 kg; 95%CI: –0.22 to 2.37; *p* = 0.10; *n* = 72). Two moderate-quality reviews (QoE: level 3)[17,38] provided insufficient evidence to determine an effect due to the low number of studies synthesized for the analysis (<5 studies) (Table 3).

Time of day

One high-quality meta-analysis (QoE: Level 4; 11 primary studies; *n* = 221 participants)[23] found no significant differences between morning-RET and evening-RET, regardless of whether

strength was assessed in the morning (SMD = –0.08; 95%CI: –0.40 to 0.25; I^2 = 1%; p = 0.643) or the evening (SMD = 0.19, 95%CI: –0.11 to 0.50; I^2 = 0%; p = 0.220) (Table 3).

Exercise order

One high-quality meta-analysis (QoE: Level 4; 8 primary studies; n = 207 participants)[39] found no significant influence of exercise order on dynamic strength (SMD = −0.02; 95%CI: −0.45 to 0.41; p = 0.937; I^2 = 0%) when all performed strength tests were considered (i.e., multi-joint (MJ) and single-joint (SJ)). However, exercise order did influence MJ dynamic strength (SMD = 0.32; 95%CI: 0.02–0.62; p = 0.034; I^2 = 0%; favors performing MJ exercises first) and SJ dynamic strength (SMD = −0.58; 95%CI:−1.11 to −0.05; p = 0.032; I^2 = 0%; favors performing SJ exercises first).

Physical function

Evidence was available for the influence of RET compared to no exercise and for the impact of RETx variables "load" and "contraction velocity/time under tension." No evidence was available for the influence of other RETx variables on physical function.

RET vs. non-exercising control

A low-quality systematic review (QoE: Level 2; 3 studies and n = 404 participants)[15] concluded that there is high-quality evidence to support the role of RET in improving physical function in older adults compared to non-exercising controls (Table 3).

Load

One moderate-quality meta-analysis (QoE: Level 3)[55] contained insufficient evidence to determine an effect due to the low number of studies synthesized for analysis (<2 studies).

Contraction velocity/time under tension

One moderate-quality review (QoE: Level 3)[55] and 1 high-quality review (QoE: Level 4)[40] contained insufficient evidence to determine an effect in older adults due to the limited number of studies synthesized for analysis (<3 studies)[55] or due to high heterogeneity and small-study publication bias.[40]

DISCUSSION

This umbrella review incorporated evidence from 44 systematic reviews and meta-analyses to determine the impact of RET and individual RETx variables on skeletal muscle mass, strength, and physical function in healthy adults. RET was consistently found to be a potent stimulus for increasing skeletal muscle mass, strength, and physical function compared to non-exercising controls. RET load, weekly frequency, volume (number of sets), and muscle action type were the most studied RETx variables. Load, weekly frequency, and exercise order impacted RET-induced increases in muscular strength but not muscle hypertrophy. RET volume (number of sets) influenced muscular strength and hypertrophy. Muscle action type also impacted skeletal muscle hypertrophy (eccentric favored). In contrast, several other RETx variables—including inter-set rest, periodization, set end point, contraction velocity/time under tension, and set configuration—did not appear to affect muscle hypertrophy and strength gains. In many cases, a paucity of data limited our ability to shed insight on the impact of several RETx variables on physical function.

RET load was the most investigated RETx variable (8/44 systematic reviews) across all outcomes. Traditionally, training with higher loads has been a key strategy to optimize neuromuscular adaptations. Six out of 8 systematic reviews contained some[18,36,55] or sufficient[37,44,48] evidence supporting the notion that higher-load RET is pertinent for maximizing muscular strength gains. The superiority of higher-load RET for muscular strength gains can be attributed to the principle of specificity (i.e., participants in higher-load groups regularly train using loads that are closer to the test of maximal (1RM) strength)[48] and neural adaptations that come with exercising at higher relative loads.[58] In contrast to muscular strength gains, muscle hypertrophy occurred independent of RET load (Table 3). Only 1 review examined the impact of

RET load on physical function, and the low number of studies synthesized (<3 studies) prevented critical appraisal. However, the authors reported no differences between higher- and lower-load RET for improving stair climbing, timed up-and-go, chair rise, and walking speed in healthy older adults.[55] Further work is needed to clarify the impact—or lack thereof—of RET load on physical function. While the reviews included in our umbrella review provided important insight into the effects of RET load on skeletal muscle adaptations, RET load is primarily classified in binary terms (e.g., heavy load *vs.* light load), yet during an RET program, individuals may employ a spectrum of RET loading zones. It has been hypothesized that the amalgamation of a variety of RET loading zones may have synergistic effects on skeletal muscle adaptions[59]; however, future studies are needed to draw stronger inferences.

RET volume is the total amount of work performed in a resistance exercise session (or sometimes summed per week), and it is often defined by the number of sets performed. Our results herein suggest that RET volume impacts skeletal muscular strength and hypertrophic gains (Table 3). It has been suggested that the dose-response relationship between RET volume and skeletal muscle hypertrophy follows an inverted-U shape.[59] Krieger and colleagues[34] showed that performing 2 to 3 sets/exercise and 4 to 6 sets/exercise was superior to performing 1 set/exercise, but there was no difference in hypertrophy adaptations when comparing 2 to 3 sets/exercise and 4 to 6 sets/exercise. The results from Krieger and colleagues suggest that higher volume RET confers an increasingly additive hypertrophic advantage but then plateaus, after which there are diminishing returns (less gain per volume increase) for hypertrophy and possibly detrimental outcomes.[59] A recent umbrella review by Bernárdez-Vázquez et al.[60] also observed a dose-response relationship between RET volume and hypertrophy and suggested that at least 10 sets per muscle group is optimal to increase muscle mass. Without considering blood flow

restriction (excluded herein), our review strengthens this observation by including 32 (as opposed to 12[60]) systematic reviews on RETx variables and hypertrophy. Bernárdez-Vázquez et al.[60] included 1 review not captured by our search strategy that showed exercise order might influence strength (favoring exercises performed at the beginning of a training session) but not hypertrophy.[60] Overall, our finding that RET volume is critical for hypertrophy supports and expands upon the findings from Bernárdez-Vázquez et al.[60]

RET frequency was found to have a negligible impact on muscle hypertrophy but a potential influence on muscle strength. The discrepancy between muscle mass and strength outcomes might be attributed to including systematic reviews regardless of whether volume-equated studies were included. For instance, a meta-analysis of 22 studies[27] found a dose-response relationship between RET frequency and muscular strength gains; however, a subgroup analysis of volume-equated studies suggested that RET frequency negligibly impacted muscular strength gains. Furthermore, Schoenfeld et al. [49] demonstrated that when RET volume was *not* equated across protocols, higher training frequencies (e.g., 3 days per week) were consistently superior to lower training frequencies (e.g., 1 day per week). The same group conducted another meta-analysis[47] on only volume-equated studies and found no effect of RET frequency on muscular hypertrophy. The effect of weekly training frequency is difficult to discern because training frequency is related to RET volume. Therefore, we propose that weekly training frequency does not independently influence skeletal muscle adaptations, but that increasing training frequency can be manipulated to permit higher total weekly volume (with equal, or even reduced, within-session volume) and subsequent muscle mass and strength accrual. We also propose that, as with RET volume, at some point frequency becomes redundant and increases in strength and hypertrophy plateau. However, future studies are needed to determine whether splitting weekly

RET volume across additional weekly training sessions can maximize skeletal muscle adaptations.

Muscle action type (eccentric RET *vs.* concentric RET) was an impactful RET variable for muscle hypertrophy (Table 3). However, a limitation of the current umbrella review is that we did not consider limiting our inclusion to reviews that matched muscle action type for total work or maximum load, an oversight that could lead to divergent effects. Greater forces can be generated with eccentric contractions than concentric contractions, so utilizing eccentric loads (greater than concentric 1RM) could yield different workloads and subsequent adaptations[21]; on the other hand, adaptations are similar when the 2 muscle action types are matched for total work or maximum load.[61] Performing isolated, supra-maximal, eccentric contractions is pragmatically complex and may require special equipment (e.g., isokinetic dynamometers, iso-inertial devices) or external assistance (e.g., a spotter).[52,62] However, flywheel training has emerged as a RET modality that is particularly effective for implementing high eccentric loads, which are difficult to achieve with traditional RET equipment.[63] Nonetheless, combining eccentric and concentric contractions (i.e., conventional RET) is more practical for practitioners.

There was either no impact or insufficient evidence to determine the impact of contraction-velocity/time-under tension on muscle hypertrophy, strength, and physical function. Diverse ranges of repetition durations are practical for promoting skeletal muscle adaptations, though very slow repetition durations (~10s) appear to be detrimental. Considering that preserving muscle power appears to be important for maintaining physical function and the activities of daily living,[6,64] older adults may benefit from high concentric-velocity RET (i.e., power training). High-quality studies must be conducted to provide more evidence clarifying the role of contraction velocity on skeletal muscle adaptations.

Non-periodized RET programs with adequate volume and progressive overload are sufficient to elicit muscular adaptations.[59] However, per session (or weekly) volume does not have to remain consistent throughout a training program, and periodizing volume has been hypothesized as a viable strategy for maximizing the dose-response relationship between volume and muscular adaptations. A~~n~~ RET program may be periodized using one or more conventional methods, such as linear periodization, daily undulating periodization, or block periodization. Overall, periodized and non-periodized RET programs elicit similar increases in hypertrophy. Furthermore, linear and undulating periodization approaches will yield similar skeletal muscle adaptations.[26] Early work from Rhea and colleagues[45] suggested that periodized RET is superior to non-periodized RET for strength development. The mechanisms behind augmented strength gains with periodized RET remain unclear, but periodization may aid with augmenting recovery and preventing overtraining.[6,59] It has been suggested that superior strength gains with periodized RET are not due to the systematic variation of training, but could be attributed to the principle of specificity; participants may be training with heavier loads in the last mesocycle (i.e., near post-testing).[65] In the current study, none of the included reviews controlled for the principle of specificity, and future studies should attempt to control for this phenomenon to properly determine whether periodized RET is an important variable to consider for maximizing strength gains.

Set configuration methods (traditional set distribution, rest redistribution, cluster sets) and rest intervals represent advanced techniques to disperse training volume within a resistance exercise session. However, we found less evidence supporting these variables than volume for optimizing skeletal muscle adaptations. Therefore, periodized or non-periodized training

approaches, including various set configurations and rest intervals, appear to induce similar adaptations, provided adequate volume is employed.

Performing resistance exercises to momentary muscular failure has been posited as important for increasing muscular strength and mass. In contrast to this hypothesis, the current umbrella review suggests that RET-induced increases in muscle mass can be achieved *without* going to a set end point of momentary muscular failure. Training to muscular failure does not appear to have detrimental effects on training-induced adaptations *per se*, but studies on the chronic (i.e., >3 months) impact of momentary failure training are lacking. Training to momentary muscular failure may also elicit discomfort, pose safety risks, and lead to neuromuscular fatigue, particularly for older adults.[6] Indeed, training to muscular fatigue is not required for older adults to observe training-induced neuromuscular adaptations.[66,67] Training to momentary muscular failure may become increasingly important for trained individuals,[28,59] but the findings here should be translated to athletic populations with caution and diligence to avoid excess fatigue and overtraining.

Human exercise performance[68] and strength[69] appear to peak in the evening (~18:00 h), and preclinical studies suggest the timing of exercise over the day can influence the beneficial effects of training.[70] However, the results presented herein demonstrate that morning or evening produces similar increases in muscle strength and mass (Table 3). Only 2 systematic reviews were identified on this topic; so, additional research is needed to determine whether there are differences between morning and evening training. Time of day for training does not appear to impact muscular adaptations and is best selected by personal preference.

We found that exercise order impacted RET-induced increases in muscular strength. Specifically, Nunes and colleagues[39] found that increases in dynamic strength were greater in

exercises performed at the beginning of a resistance exercise session, which relates back to the principle of specificity. When exercises are performed at the beginning of a resistance exercise session, individuals are less fatigued and able to utilize higher relative loads and effort. To augment RET-induced increases in skeletal muscle hypertrophy, it has been hypothesized that individuals should prioritize performing MJ as opposed to SJ exercises at the beginning of a resistance exercise session as this allows for the accumulation of greater training volume.[71] In contrast, to augment site-specific skeletal muscle hypertrophy (e.g., triceps) others have suggested that it is better to perform SJ exercises (e.g., triceps extension) prior to MJ exercises (e.g., bench press).[72] The results of the current umbrella review suggest that RET-induced increases in skeletal muscle hypertrophy are similar regardless of exercise order. Therefore, exercise order impacted RET-induced increases in muscular strength but not muscle hypertrophy. Individuals wanting to improve their maximal strength for a given exercise should perform that exercise at the beginning of the resistance exercise session.[39]

The strengths of this umbrella review include the comprehensive search strategy utilized and the large number of systematic reviews included. Several limitations require acknowledgement and consideration when interpreting the results of this umbrella review. Potentially relevant reviews published since the last search (December 9, 2021 to current)[60,73–77] were not captured by our search strategy, which prevented us from determining the impact of additional RET-variables, such as exercise selection,[73,76] exercise variation,[74] and range of motion.[75,77] Future work should be aimed at characterizing the influence of the aforementioned RETx variables on hypertrophy, strength, and physical function. We identified limited evidence from which we could draw conclusions on the impact of different RET variables on physical function, which may be attributed to including systematic reviews with only healthy older adults

(e.g., not frail). Additionally, well-trained elite athletes/military persons were excluded because these populations commonly perform RET in addition to their sport-specific training, which would make it difficult to discern the influence of RETx variables from alternative modalities of exercise training. The current study also excluded individuals with chronic disease. To provide additional insight, future work is required to determine the impact of different RETx variables on these populations. The average range for RET duration was 6 to 24 weeks, and any inter-pretations of the results for longer durations should be viewed with caution. About 1/3 of the systematic reviews in the current study contained either high levels of heterogeneity or included a limited number of studies; thus, we were unable to draw satisfactory conclusions from these reviews. We also acknowledge that additional RET program variables not investigated in the current review (e.g., blood flow restriction) may influence outcomes. Nonetheless, based on observations from the current review, we propose that the impact of these other variables is likely limited in terms of effects on strength and hypertrophy. Directions for future research are apparent when we note the limited data for physical function and specific RETx variables (e.g., time of day, set configurations, and inter-set rest intervals). Additionally, not presenting a list of included and excluded studies (40 reviews), not including *a priori* design (38 reviews), and the use of publication status as an inclusion criterion (if the search included grey literature; 33 reviews) were the most common factors detracting from the quality of included reviews. Researchers may wish to consider these points before embarking on future systematic reviews.

CONCLUSION

This umbrella review found that RET promotes increased muscle hypertrophy, strength, and physical function in healthy adults compared to no exercise. RET volume appears to be important for both muscular strength and hypertrophic gains. RET load and weekly frequency

appeared to be important for muscle strength. Muscle action type seems to be important for hypertrophy but not strength. Inter-set rest, periodization, set end point, contraction velocity/time under tension, and set configuration were not important to RET-induced adaptations. Less is known regarding which RET variables are important for optimizing improvements in physical function. We conclude that RET largely increases muscle hypertrophy, strength, and physical function compared to no exercise, and that very few RETx variables impact muscular adaptations.

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CHAPTER 3: STUDY 2

Resistance Training Prescription for Muscle Strength and Hypertrophy in Healthy Adults: A Systematic Review and Bayesian Network Meta-Analysis.

Under review at *the British Journal of Sports Medicine*

ABSTRACT

Objective: To determine how distinct combinations of resistance training prescription (RETx) variables (load, sets, and frequency) affect muscle strength and hypertrophy.

Data Sources: MEDLINE, Embase, Emcare, SPORTDiscus, CINAHL, and Web of Science were searched until February 2022.

Eligibility Criteria: Randomized trials that included healthy adults, compared at least two predefined conditions (non-exercise control [CTRL] and 12 RETx, differentiated by load, sets, and/or weekly frequency), and reported muscle strength and/or hypertrophy were included. **Analyses:** Systematic review and Bayesian network meta-analysis methodology was used to compare resistance training prescriptions and CTRL. Surface under the cumulative ranking curve values were used to rank conditions. Confidence was assessed with threshold analysis.

Results: The strength network included 178 studies (n = 5,097; women = 45%). The hypertrophy network included 119 studies (n = 3,364; women = 47%). All resistance training prescriptions were superior to CTRL for muscle strength and hypertrophy. Higher-load (>80% of single repetition maximum) prescriptions maximized strength gains, and all prescriptions comparably promoted muscle hypertrophy. While the calculated effects of many prescriptions were similar, higher-load, multiset, thrice-weekly training (standardized mean difference [95% credible interval]; 1.60 [1.38, 1.82] vs CTRL) was the highest-ranked RETx for strength, and higher-load, multiset, twice-weekly training (0.66 [0.47, 0.85] vs CTRL) was the highest-ranked RETx for hypertrophy. Threshold analysis demonstrated these results were extremely robust.

Conclusion: All RETx promoted strength and hypertrophy compared to no exercise. The highest-ranked prescriptions for strength involved higher loads, whereas the highest-ranked prescriptions for hypertrophy included multiple sets.

Registration: PROSPERO CRD42021259663 and CRD42021258902.

INTRODUCTION

Skeletal muscle is critical for numerous functional and metabolic processes essential to good health. Resistance training (RET), muscle contraction against external weight, potently increases muscle strength and mass (hypertrophy), improves physical performance, provides a myriad of metabolic-health benefits, and combats chronic disease risk [1-4]. Although endogenous biological and physiological factors are pertinent to maximizing RET-induced skeletal muscle adaptations [5, 6], RET programming variables can affect RET adaptations [7-13]. Therefore, a resistance training prescription (RETx) should be determined appropriately. Each RETx is comprised of a distinct combination of RET variables, and the most-studied RETx variables include the load lifted per repetition, sets per exercise (generally involving a single RET maneuver or muscle group), and weekly frequency (the number of RET sessions completed per week).

Guideline developers rely on systematic reviews and meta-analyses for determining recommendations, as these study designs are, in most cases, the most robust forms of evidence [14]. Indeed, various meta-analyses have provided seminal evidence on the *univariate* impact of load [15-18], sets [19-22], or frequency [23-27] to improve muscle strength, mass, and physical function. However, these univariate analyses limit RETx guideline development because individual RET variables are neither mutually exclusive nor prescribed independently; rather, several variables are collectively inherent to any resistance training prescription. Comparisons between multivariate RET prescriptions are needed to advance optimal RETx guidelines.

Pairwise meta-analyses are methodologically constrained to only comparing two RET prescriptions [28]. Several RET prescriptions are conceivable, and multiple pairwise meta-analyses are unlikely to yield congruent insights. Network meta-analysis (NMA) expands upon

pairwise meta-analysis by permitting the simultaneous comparison of multiple treatments [29]. NMA leverages direct and indirect evidence to produce enhanced effect estimates between all treatments, even when some comparisons have never been tested in randomized trials [30]. Additionally, NMA permits the rank-ordering of all included treatments and the incorporation of data from multi-arm trials [28]. Within exercise science, NMA has been used to compare different types of exercise [31-34]; within RET, NMA has only been used to compare different load doses [35]. Importantly, NMA can compare several multivariate RET prescriptions.

The purpose of this systematic review and NMA was to determine how different resistance training prescriptions affect muscle strength, hypertrophy, and physical function in healthy adults. Specifically, we sought to compare distinct combinations of resistance training prescription variables – load, sets, and frequency – and non-exercising control groups. For each outcome, we used network meta-analysis to integrate data from randomized trials.

METHODS

Protocol and Registration

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension statement for network meta-analyses (PRISMA-NMA) [36] and Cochrane Handbook for Systematic Reviews of Interventions [37]. The PRISMA-NMA checklist is provided in online supplementary appendix 1. This review combines network meta-analyses registered in the International Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/prospero/; CRD42021259663 and CRD42021258902).

Eligibility Criteria

The eligibility criteria are detailed in Table 1. Only trials that included healthy adults ≥18 years old, were randomized, compared at least two of 13 unique conditions (Table 2), and measured muscle strength, size, and/or physical function were included. Physical function was subdivided into three domains: mobility, the ability to physically move; balance, the ability to maintain a body position during a task; and gait speed, the time taken to locomote over a given distance. Trials that included athletes, persons with comorbidities, or military persons; spanned <6 weeks; involved unsupervised resistance training (e.g., home-based exercise); were reported in a non-English language; or were non-randomized were excluded.

Condition Coding Framework

Arms of included studies were classified as one of twelve resistance training prescriptions or non-exercise control (CTRL). Each RETx was classified based on the load, set, and frequency prescription (Table 2). Resistance training prescriptions were denoted with a three-character acronym – XY# – where X is load (H, ≥80% 1-repetition maximum [1RM]; L, <80% 1RM); Y is sets (M, multi-set; S, single-set); and # is the weekly frequency (3, ≥3 d/wk; 2, 2 d/wk; 1, 1 d/wk), respectively. For example, HM2 denotes higher-load, multi-set, twice-weekly resistance training within this framework. CTRL was comprised of subjects who received no intervention.

Table 1. Study inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Population - Humans ≥18 years old - Generally healthy (no disease condition indicated other than sarcopenia) - Community-dwelling adults *Intervention* - Upper-body, lower-body, and/or whole-body resistance training - RETx aligns with one predefined node; specifically, exercises performed: - with high [H; ≥80% 1RM or ≥8 RM] or low [L; <80% 1RM or > 8 RM] load, AND - for a single [S] or multiple [M] sets, AND - once- [1], twice- [2], or at least thrice-weekly [3] - Intervention duration ≥6 weeks *Comparison* - RETx variable (load, sets, or frequency) differentially prescribed between training groups - Eligible RETx compared to CTRL *Outcome* - Eligible outcome(s) assessed pre- and post-intervention. Muscle Strength: • 1RM test • Isometric maximum voluntary contraction • Isokinetic maximum voluntary contraction Muscle size: Fat-free mass, fat- and bone-free mass, lean mass, whole-muscle cross-sectional area or volume or thickness, or muscle fibre cross-sectional area. Eligible measurement instruments: • Ultrasonography • Magnetic resonance imaging • Computed tomography • Bioelectrical impedance • Dual-energy X-ray absorptiometry • Hydrostatic weighing • Air displacement plethysmography • Microscopy Physical Function: Assessed physical function in older adults (mean age ≥55 years old) in the domain(s): • Mobility: (defined as a person's ability to move physically, e.g., Timed Up and Go Test, Chair Rise Sit to Stand) • Balance: (defined as the ability to maintain a controlled body position during a given task, e.g., Berg Balance Test, Sit and Reach Test) • Gait Speed: (defined as the time it takes to cover a given distance, e.g., 6 Minute Walk Test, or 25 Foot Walk Test) *Study Design* - Randomized trial - Reported in English	*Population* - Non-human species - <18 years old - Persons with or at risk for comorbidities (e.g., cardiovascular disease, type II diabetes, type I diabetes, cancer, peripheral artery disease, osteoarthritis) - Persons that are injured (e.g., musculoskeletal-related fracture and/or repair) - Athletes or military personnel - Explicitly mentions obese and/or overweight participants - Individuals that are hospitalized (inpatient/outpatient/rehabilitation) - Individuals living in long-term care homes *Intervention* - Resistance training involved added intervention (e.g., blood flow restriction) - RETx does not align with one node (e.g., load 60-90% 1RM) - Explicitly mentions unsupervised resistance training - Resistance training familiarization/lead-in >4 weeks - CTRL received treatment beyond habitual lifestyle (e.g., nutritional advice, lifestyle consultation) *Comparison* - Eligible RETx not compared to another eligible RETx nor CTRL *Outcome* - No measure of muscle strength, size, mobility, gait speed, or balance *Study Design* - Non-randomized trials - Systematic reviews (i.e., systematic reviews; meta-analyses review; meta-regressions; umbrella reviews; network meta-analyses) - Narrative reviews - Observational studies (e.g., retrospective, prospective, or longitudinal)

Abbreviations: 1RM, one-repetition maximum; CTRL, non-exercise control RM, repetition maximum; RETx, resistance training prescription.

Table 2. Description of predefined conditions.

Condition Acronym – Condition Description
CTRL – Non-exercise control
LS1 – Lower load, single set/exercise, 1 day/week resistance training
LS2 – Lower load, single set/exercise, 2 days/week resistance training
LS3 – Lower load, single set/exercise, ≥ 3 days/week resistance training
LM1 – Lower load, multiple sets/exercise, 1 day/week resistance training
LM2 – Lower load, multiple sets/exercise, 2 days/week resistance training
LM3 – Lower load, multiple sets/exercise, ≥ 3 days/week resistance training
HS1 – Higher load, single set/exercise, 1 day/week resistance training
HS2 – Higher load, single set/exercise, 2 days/week resistance training
HS3 – Higher load, single set/exercise, ≥ 3 days/week resistance training
HM1 – Higher load, multiple sets/exercise, 1 day/week resistance training
HM2 – Higher load, multiple 2 sets/exercise, 2 days/week resistance training
HM3 – Higher load, multiple sets/exercise, ≥ 3 days/week resistance training

Search Strategy

MEDLINE, Embase, Emcare, SPORTDiscus, CINAHL, and Web of Science were systematically searched until 7 February 2022. Multiple experts developed the search strategy, which included subject headings and keywords specific to the research question and each database. No language nor study design limits were used in the search strategy. The complete search strategy is provided in online supplementary appendix 2. Relevant systematic reviews (online supplementary appendix 3) were manually selected, and the references were scrutinized for eligibility.

Study Selection and Data Extraction

All records underwent title/abstract screening by two independent reviewers, with discrepancies resolved by a third reviewer. The full text of potentially eligible reports was then assessed for inclusion by two independent reviewers, with discrepancies resolved by a third reviewer. Reports deemed eligible for inclusion then underwent data extraction.

Data from included studies were extracted independently by pairs of reviewers, with any discrepancies resolved by consensus with a third reviewer (BSC or JCM). Extracted data included study and participant characteristics, RETx details, and measurements of muscle strength and/or size (online supplementary appendix 4). Measures of mobility, balance, and/or gait speed were extracted when the mean participant age was ≥55 years old. Authors of studies with missing data were contacted twice with a request for the missing data. The systematic review software Covidence (Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org) was used for record screening and data extraction.

Mean change from baseline and standard deviation (SD) change (SD_{change}) from baseline were the outcomes of interest and extracted when reported. When unreported, SD was calculated with standard errors, confidence intervals, p-values, or t-statistics [37], and SD_{change} was imputed from pre- and post-SD values with a correlation coefficient of 0.5 [38]. Resistance training loads reported as repetition maximum (RM) were converted to a percentage of one-repetition maximum (%1RM) with the equation: %1RM = 100-(RM(2.5)) [39]. The highest-ranked measurement was extracted, per predetermined hierarchy (online supplementary appendix 5), when multiple measurements were reported for a single outcome (e.g., magnetic resonance imaging and ultrasonography for muscle size). The longest period that all conditions were unchanged from baseline was analyzed when the outcome(s) of interest were measured at multiple time points [37]. Cohorts randomized separately but reported together (e.g., young and old [40]) were analyzed independently. Within-group outcomes reported by participant sex were grouped by condition [37, 41].

Risk of Bias

Reviewers independently evaluated the within-study risk of bias using the Cochrane Risk of Bias V.2.0. tool [42]. Signalling questions and criteria were followed to inform the risk of bias appraisals for the intention-to-treat effect. Articles were assessed in duplicate at the strength and hypertrophy outcome level for bias: 1) arising from the randomization process, 2) due to deviations from intended interventions, 3) due to missing outcome data, 4) in the measurement of the outcome, and 5) in the selection of reported result. Every domain was determined to be of high, moderate (some concerns), or low risk of bias, and studies were subsequently given an overall classification of high, moderate, or low risk of bias. Any disagreement was resolved by consensus (BSC and JCM).

Statistical Analysis

Standardized mean differences (SMD), adjusted for small-sample size bias [43], were calculated as the summary statistic because each outcome was measured with various tools [37]. The direction of effect was standardized to analyze mobility, gait speed, and balance to ensure consistency of desirable outcomes [44]. When multiple studies compared two conditions, random-effects pairwise meta-analyses were conducted to identify comparison-level heterogeneity, publication bias, outliers and influential cases [41, 45]. To account for within-trial correlations in multi-arm trials (≥3 conditions), the standard error in the base/reference arm was calculated as the square root of the covariance between calculated effects [46], assuming a correlation of 0.5 between effect sizes [47].

Network meta-analysis integrated all direct evidence, with one network constructed for each outcome. NMA models were fitted within a Bayesian framework using Markov chain Monte Carlo (MCMC) methods [48]. Four chains were run with non-informative priors. There were 50,000 iterations per chain; the first 20,000 were discarded as burn-in iterations. Values were collected with a thinning interval of 10. Convergence was evaluated by visual inspection of trace plots [49] and the potential scale reduction factor. Both fixed- and random-effects models were fit, and the more parsimonious model was used for analysis [50]. Model fit was assessed with the deviance information criterion (DIC) and posterior mean residual deviance [50, 51]. Heterogeneity was assessed by examining the between-study SD (τ) and 95% credible intervals (95% CrI). Global inconsistency was assessed by comparing model fit, DIC and variance parameters between the NMA model and an unrelated mean effects (UME) model [52]. Local inconsistency was assessed with the node-splitting method [53], and inconsistency was considered to be detected when the Bayesian P value < 0.05. Forest plots and league tables were

generated to display relative effects. Surface under the cumulative ranking curve (SUCRA) values were used to rank-order each condition from top-to-bottom; additionally, the probability of each condition ranking in the top three was calculated as a percentage of the area under the curve. NMA results were presented as posterior SMD and 95% CrI, interpreted as a range in which a parameter lies with a 95% probability [54].

Confidence in Recommendations

The robustness of recommendations was assessed with threshold analysis [48, 55]. Several factors, including bias and sampling error, can influence NMA results. Threshold analysis determines how much the included evidence could change – for any reason – before treatment recommendations differ and identifies the subsequent treatment recommendation [56]. Identifying the robustness of results with threshold analysis permits guideline developers to have appropriate confidence levels in the reported recommendations.

Sensitivity Analysis and Network Meta-Regression

Sensitivity analyses were conducted to explore the impact of outliers, influential cases, and sources of network inconsistency on model fit, relative effects, and treatment rankings. The first sensitivity analysis excluded studies identified during pairwise meta-analyses and node-splitting, and the second sensitivity analysis excluded node(s) comprised of only one study. Network meta-regression (NMR), assuming independent treatment interactions [57], was performed to determine if additional factors improved model fit and altered treatment effects. NMR covariates included age, training status, the proportion of females, duration, volitional fatigue, relative weekly volume load, outcome measurement tool, outcome measurement region, and publication year. Missing data on covariates were managed through multivariate imputation

by chained equations (n imputations = 20) [58]. NMR is detailed in online supplementary appendix 12.

All analyses were performed in R version 4.0.4 using the packages: *'esc'* [59], to calculate SMD; *'dmetar'* [41], to conduct pairwise meta-analyses and assess comparison-level heterogeneity; *'multinma'* [48], to conduct NMA, NMR and consistency testing; *'nmathresh'* [55], to perform thresholding; and *'mice'* [60], to perform multiple imputation. Figures were created with *multinma* [48], *metafor* [61], *ggplot2* [62], and GraphPad Prism (version 9.1.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com). All code was made publicly available (see Data Sharing Statement).

Equity, Diversity, and Inclusion (EDI) Statement

Our author group comprises various disciplines, career stages, and genders. Data collection, analysis, and reporting methods were not altered based on regional, educational, or socioeconomic differences of the community in which the included studies were conducted. The only consistently reported EDI-relevant variable on which we have analyzed the data is biological sex.

RESULTS

Included Studies

The systematic search yielded 16,880 records after duplicates were removed. Following title/abstract screening, 1,051 full texts were assessed for inclusion. A total of 192 articles were included in this review (Figure 1). Characteristics of included studies are detailed in the online supplementary appendix 6.

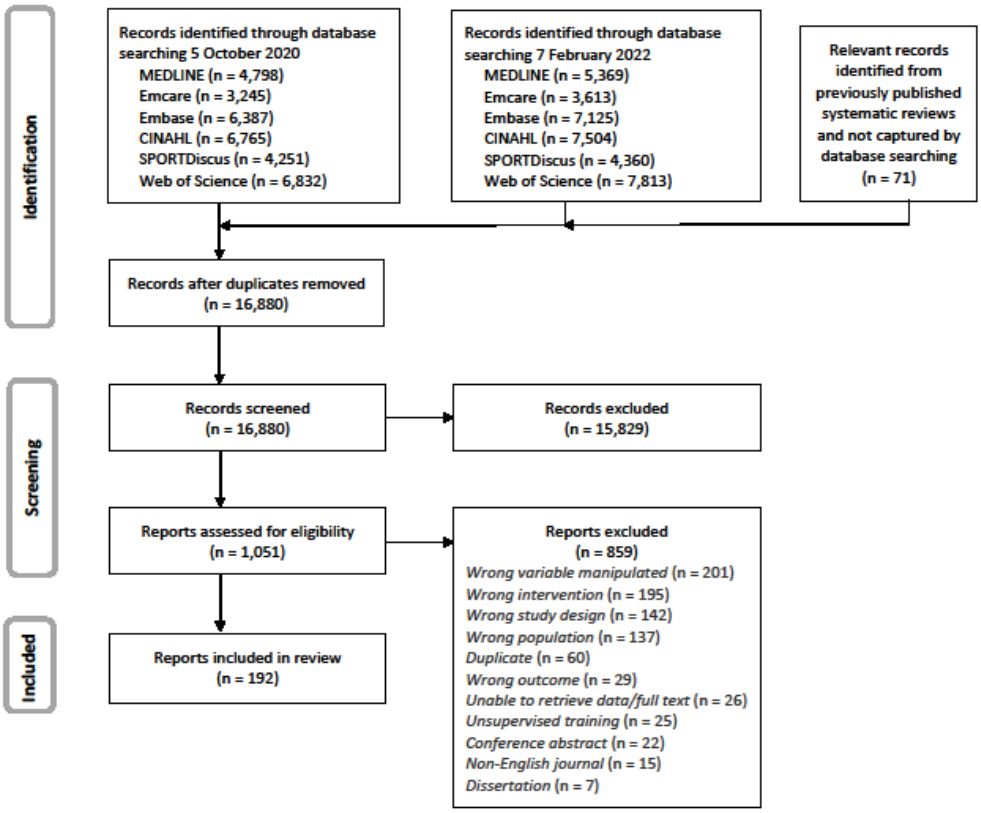


Figure 1. PRISMA flow diagram of study selection.

Network Geometry

Network geometry for strength is displayed in Figure 2A. The strength NMA (178 studies, n = 5,097) included 13 conditions and 32 direct comparisons. The three largest nodes were CTRL (n = 1,321), LM3 (n = 1,133), and LM2 (n = 710), and the three smallest nodes were HM1 (n = 54), LS1 (n = 34), and HS1 (n = 13). The most common comparisons were LM3 vs CTRL (51 studies), HM3 vs LM3 (32 studies), HM3 vs CTRL (30 studies), and LM2 vs CTRL (30 studies).

Network geometry for hypertrophy is displayed in Figure 2B. The hypertrophy NMA (119 studies, n = 3,364) included 11 conditions – no studies included HS1 or LS1 – and 24 direct comparisons. The three largest nodes were CTRL (n = 847), LM3 (n = 810), and LM2 (n = 548), and the three smallest nodes were HS3 (n = 60), HS2 (n = 21), and HM1 (n = 11). The most common comparisons were LM3 vs CTRL (35 studies), HM3 vs LM3 (22 studies), LM2 vs CTRL (18 studies), and HM3 vs CTRL (17 studies).

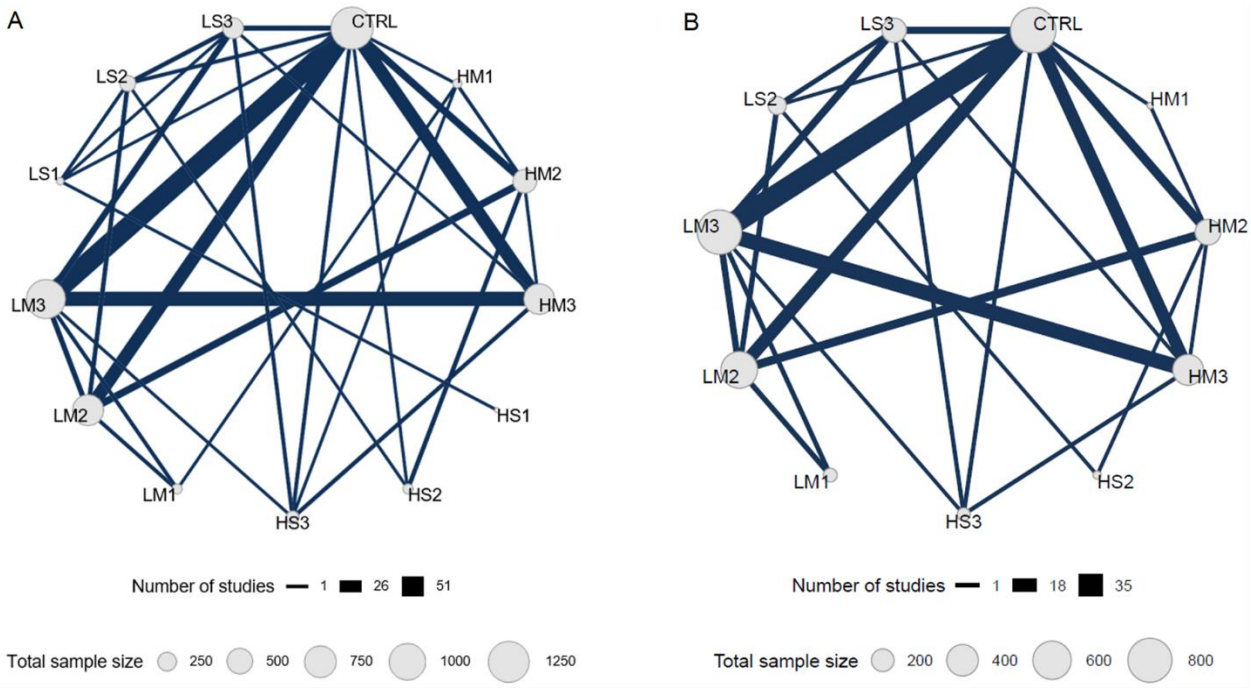


Figure 2. Network geometry for all available studies evaluating strength (A) and hypertrophy (B). Each node represents a unique condition, and the size of each node is proportional to the sample size per condition. Each edge represents direct evidence, and the width of each edge is proportional to the number of studies comparing connected nodes. Resistance training prescriptions are denoted with a three-character acronym – XY# – where X is load (H, ≥80% 1-repetition maximum [1RM]; L, <80% 1RM); Y is sets (M, multi-set; S, single-set); and # is the weekly frequency (3, ≥3 d/wk; 2, 2 d/wk; 1, 1 d/wk), respectively. For example, "HM2" denotes higher-load, multi-set, twice-weekly training. CTRL, non-exercising control group.

Risk of Bias

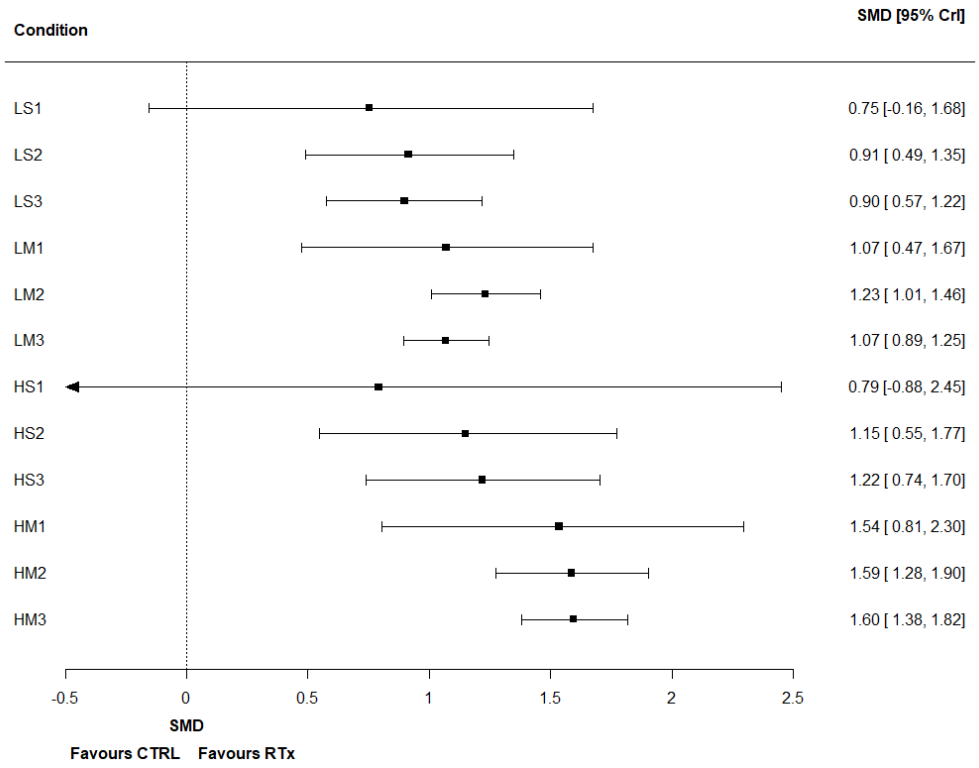
Within-study risk of bias was moderate-high for both strength and hypertrophy outcomes. In the strength network, 22%, 67%, and 1% of studies had a high, moderate, or low risk of bias, respectively. In the hypertrophy network, 18%, 82%, and 0% of studies had a high, moderate, or low risk of bias, respectively. Study-level risk of bias assessments for both strength and hypertrophy is detailed in online supplementary appendix 7.

Resistance Training Prescriptions versus CTRL

The relative effect of each RETx compared to CTRL on muscle strength is displayed in Figure 3A. The posterior SMD for all prescriptions ranged from 0.75 to 1.60, with the largest relative effect from HM3 (1.60 [1.38, 1.82]). Compared to CTRL, the relative effect of LS1 (0.75 [-0.16, 1.68]) and HS1 (0.79 [-0.88, 2.45]) were the only comparisons where the 95% CrI crossed zero.

The relative effect of each RETx compared to CTRL on muscle hypertrophy is displayed in Figure 3B. The posterior SMD for all RETx ranged from 0.10 to 0.66, with the largest relative effect from HM2 (0.66 [0.47, 0.85]). Compared to CTRL, the relative effect of HS2 (0.10 [-0.57, 0.80]), HS3 (0.34 [-0.02, 0.71]), and HM1 (0.40 [-0.35, 1.17]) were the only comparisons where

the 95% CrI crossed zero.


Condition
SMD [95% CrI]
LS1
LS2
LS3
LM1
LM2
LM3
HS1
HS2
HS3
HM1
HM2
HM3
0.75 [-0.16, 1.68]
0.91 [0.49, 1.35]
0.90 [0.57, 1.22]
1.07 [0.47, 1.67]
1.23 [1.01, 1.46]
1.07 [0.89, 1.25]
0.79 [-0.88, 2.45]
1.15 [0.55, 1.77]
1.22 [0.74, 1.70]
1.54 [0.81, 2.30]
1.59 [1.28, 1.90]
1.60 [1.38, 1.82]
-0.5
0
0.5
1
1.5
2
2.5
SMD
Favours CTRL
Favours RTx

A)

B)

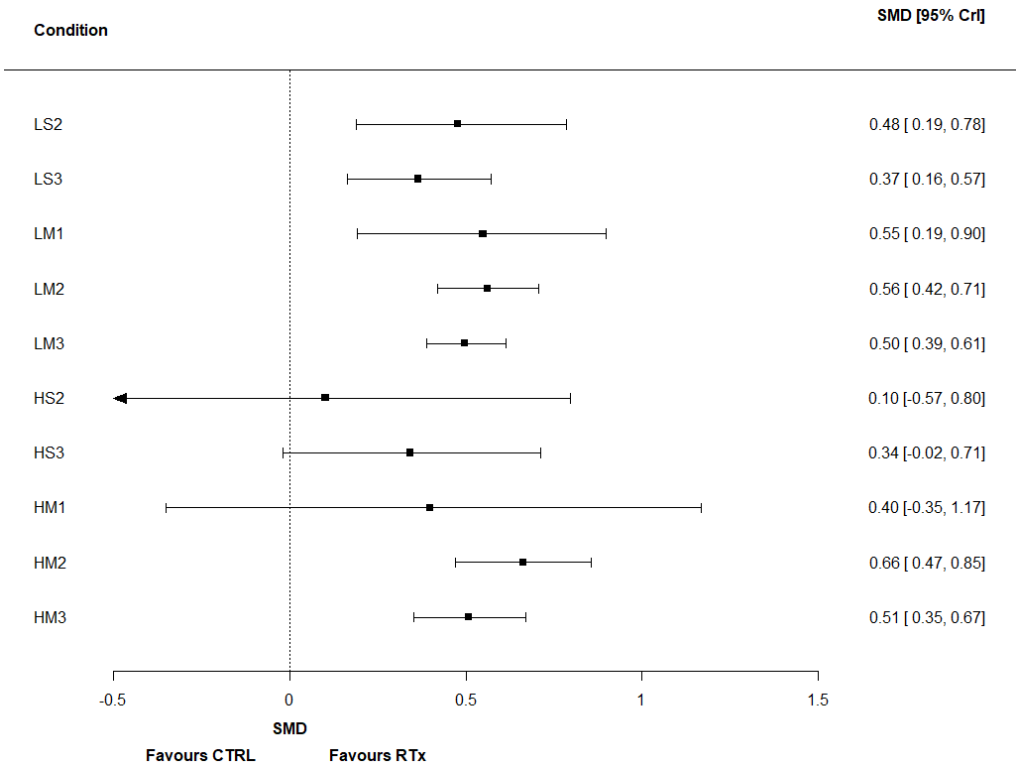

Condition
SMD [95% CrI]
LS2
LS3
LM1
LM2
LM3
HS2
HS3
HM1
HM2
HM3
0.48 [0.19, 0.78]
0.37 [0.16, 0.57]
0.55 [0.19, 0.90]
0.56 [0.42, 0.71]
0.50 [0.39, 0.61]
0.10 [-0.57, 0.80]
0.34 [-0.02, 0.71]
0.40 [-0.35, 1.17]
0.66 [0.47, 0.85]
0.51 [0.35, 0.67]
-0.5
0
0.5
1
1.5
SMD
Favours CTRL
Favours RTx

Figure 3. Forest plots displaying network estimates for relative effects of resistance training prescriptions versus non-exercising control for strength (A) and hypertrophy (B). Each resistance training prescription (RTx) is denoted with a three-character acronym – XY# – where X is load (H, ≥80% 1-repetition maximum [1RM]; L, <80% 1RM); Y is sets (M, multi-set; S, single-set); and # is the weekly frequency (3, ≥3 d/wk; 2, 2 d/wk; 1, 1 d/wk), respectively. For example, "HM2" denotes higher-load, multi-set, twice-weekly training. CTRL, non-exercising control; SMD, standardized mean difference; 95% CrI, 95% credible interval.

Comparing Resistance Training Prescriptions

The relative effects from all 133 network comparisons for muscle strength and hypertrophy are displayed in Table 3. For comparisons between resistance training prescriptions (i.e., not CTRL), the 95% CrI excluded zero for 13.6% (9/66) and 2.2% (1/45) of comparisons in the strength and hypertrophy NMA, respectively. For muscle strength, there was a 95% probability that HM2 yields a larger relative effect than LS1, LS2, LS3, LM2, and LM3 and that HM3 yields a larger relative effect than LS2, LS3, LM2, and LM3. There was a 95% probability for muscle hypertrophy that HM2 yields a larger relative effect than LS3.

Table 3. League table of all relative effects.

		STRENGTH												
		CTRL	HM1	HM2	HM3	HS1	HS2	HS3	LM1	LM2	LM3	LS1	LS2	LS3
HYPERTROPHY	CTRL		**1.54 (0.81, 2.30)**	**1.59 (1.28, 1.90)**	**1.60 (1.38, 1.82)**	0.79 (-0.88, 2.45)	**1.15 (0.55, 1.77)**	**1.22 (0.74, 1.70)**	**1.07 (0.47, 1.67)**	**1.23 (1.01, 1.46)**	**1.07 (0.89, 1.25)**	0.75 (-0.16, 1.68)	**0.91 (0.49, 1.35)**	**0.90 (0.57, 1.22)**
	HM1	0.40 (-0.35, 1.17)		0.05 (-0.71, 0.79)	0.06 (-0.71, 0.82)	-0.74 (-2.54, 1.08)	-0.39 (-1.32, 0.55)	-0.32 (-1.13, 0.50)	-0.47 (-1.37, 0.42)	-0.31 (-1.07, 0.44)	-0.47 (-1.23, 0.28)	-0.78 (-1.97, 0.38)	-0.62 (-1.48, 0.21)	-0.64 (-1.45, 0.15)
	HM2	**0.66 (0.47, 0.85)**	0.26 (-0.50, 1.02)		0.01 (-0.35, 0.37)	-0.79 (-2.46, 0.91)	-0.44 (-1.00, 0.14)	-0.37 (-0.91, 0.20)	-0.52 (-1.16, 0.12)	**-0.36 (-0.66, -0.04)**	**-0.52 (-0.87, -0.17)**	**-0.83 (-1.77, 0.11)**	**-0.67 (-1.16, -0.18)**	**-0.69 (-1.12, -0.24)**
	HM3	**0.51 (0.35, 0.67)**	0.11 (-0.67, 0.88)	-0.15 (-0.39, 0.09)		-0.80 (-2.48, 0.86)	-0.45 (-1.09, 0.21)	-0.38 (-0.86, 0.11)	-0.53 (-1.14, 0.09)	**-0.37 (-0.66, -0.07)**	**-0.53 (-0.74, -0.31)**	-0.84 (-1.77, 0.08)	**-0.68 (-1.14, -0.22)**	**-0.70 (-1.06, -0.34)**
	HS1	N.D.	N.D.	N.D.	N.D.		0.36 (-1.42, 2.09)	0.43 (-1.27, 2.16)	0.28 (-1.47, 2.05)	0.44 (-1.25, 2.12)	0.28 (-1.38, 1.95)	-0.04 (-1.45, 1.34)	0.12 (-1.53, 1.77)	0.11 (-1.54, 1.76)
	HS2	0.10 (-0.57, 0.80)	-0.30 (-1.28, 0.66)	-0.56 (-1.23, 0.14)	-0.41 (-1.08, 0.29)	N.D.		0.07 (-0.69, 0.84)	-0.08 (-0.91, 0.75)	0.08 (-0.54, 0.68)	-0.08 (-0.72, 0.55)	-0.40 (-1.46, 0.65)	-0.24 (-0.94, 0.44)	-0.25 (-0.94, 0.42)
	HS3	0.34 (-0.02, 0.71)	-0.06 (-0.90, 0.75)	-0.32 (-0.74, 0.09)	-0.17 (-0.54, 0.22)	N.D.	0.24 (-0.51, 0.99)		-0.15 (-0.88, 0.60)	0.01 (-0.51, 0.53)	-0.15 (-0.64, 0.33)	-0.47 (-1.49, 0.54)	-0.31 (-0.93, 0.31)	-0.32 (-0.84, 0.19)
	LM1	**0.55 (0.19, 0.90)**	0.15 (-0.68, 0.94)	-0.11 (-0.49, 0.25)	0.04 (-0.34, 0.41)	N.D.	0.45 (-0.33, 1.18)	0.21 (-0.31, 0.69)		0.16 (-0.43, 0.75)	-0.00 (-0.58, 0.59)	-0.32 (-1.40, 0.77)	-0.16 (-0.88, 0.56)	-0.17 (-0.83, 0.48)
	LM2	**0.56 (0.42, 0.71)**	0.16 (-0.62, 0.92)	-0.10 (-0.29, 0.11)	0.05 (-0.16, 0.26)	N.D.	0.46 (-0.23, 1.12)	0.22 (-0.18, 0.59)	0.01 (-0.31, 0.35)		-0.16 (-0.42, 0.10)	-0.48 (-1.39, 0.44)	-0.32 (-0.75, 0.12)	-0.33 (-0.70, 0.04)
	LM3	**0.50 (0.39, 0.61)**	0.10 (-0.66, 0.85)	-0.16 (-0.38, 0.05)	-0.01 (-0.16, 0.14)	N.D.	0.40 (-0.30, 1.07)	0.16 (-0.22, 0.52)	-0.05 (-0.39, 0.29)	-0.06 (-0.22, 0.10)		-0.32 (-1.24, 0.60)	-0.15 (-0.59, 0.29)	-0.17 (-0.49, 0.15)
	LS1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		0.16 (-0.73, 1.01)	0.15 (-0.77, 1.06)
	LS2	**0.48 (0.19, 0.78)**	0.08 (-0.71, 0.88)	-0.19 (-0.51, 0.16)	-0.03 (-0.35, 0.29)	N.D.	0.37 (-0.28, 1.01)	0.13 (-0.33, 0.60)	-0.07 (-0.51, 0.37)	-0.08 (-0.37, 0.22)	-0.02 (-0.31, 0.28)	N.D.		-0.02 (-0.46, 0.42)
	LS3	**0.37 (0.16, 0.57)**	-0.03 (-0.82, 0.76)	**-0.30 (-0.57, -0.02)**	-0.14 (-0.39, 0.10)	N.D.	0.26 (-0.39, 0.95)	0.02 (-0.37, 0.39)	-0.18 (-0.57, 0.22)	-0.20 (-0.44, 0.05)	-0.13 (-0.34, 0.08)	N.D.	-0.11 (-0.40, 0.17)	

Network estimates for all relative effects of resistance training prescriptions are displayed for strength (column header versus row header; values > 0 favour the column condition) and hypertrophy (row header versus column header; values > 0 favour the row condition). Data are displayed as posterior standardized mean difference (95% credible interval). Bolded numbers indicate a 95% probability one intervention yields a larger relative effect. Resistance training prescriptions are denoted with a three-character acronym – XY# – where X is load (H, ≥80% 1-repetition maximum [1RM]; L, <80% 1RM); Y is sets (M, multi-set; S, single-set); and # is weekly frequency (3, ≥3 d/wk; 2, 2 d/wk; 1, 1 d/wk), respectively. For example, "HM2" denotes higher-load, multi-set, twice-weekly training. Abbreviations: CTRL, non-exercise control; N.D., no data.

Ranking Conditions

Figure 4 displays the probability that each condition would rank in the top three best interventions for muscle strength and hypertrophy, such that scores closer to 100% indicate a greater chance of ranking in the top three. HM3 (85.5%), HM2 (83.5%), and HM1 (60.5%) were most likely to rank in the top three for muscle strength. HM2 (86.9%), LM1 (48.7%), and LM2 (48.3%) were most likely to rank in the top three for muscle hypertrophy. CTRL was the only condition with a 0% chance for strength and hypertrophy. Posterior rankings and distribution curves for all conditions are reported in the online supplementary appendix 8.

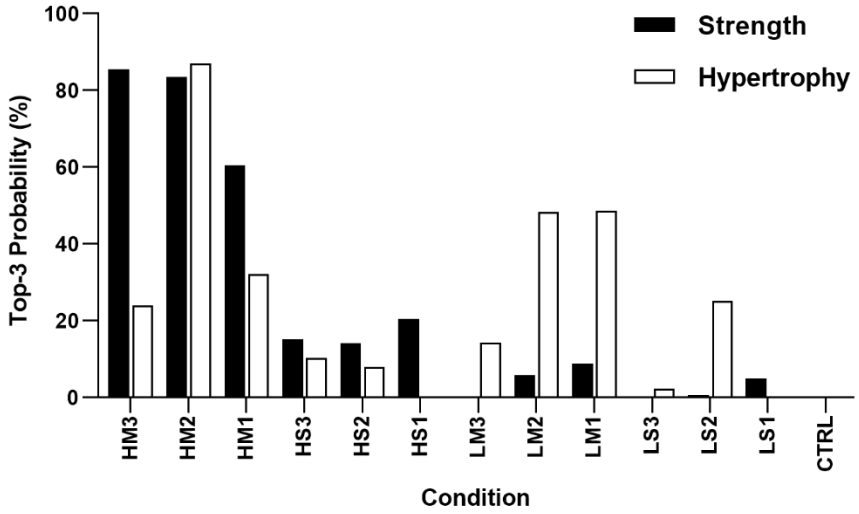


Figure 4. Probability for each condition ranking in the top three most effective for strength (A) and hypertrophy (B). Scores closer to 100% indicate a greater chance of being ranked in the top three. Resistance training prescriptions are denoted with a three-character acronym – XY# – where X is load (H, ≥80% 1-repetition maximum [1RM]; L, <80% 1RM); Y is sets (M, multi-set; S, single-set); and # is the weekly frequency (3, ≥3 d/wk; 2, 2 d/wk; 1, 1 d/wk), respectively. For example, "HM2" denotes higher-load, multi-set, twice-weekly training. CTRL, non-exercising control group.

Network Inconsistency

Model fit outputs and node-splitting plots are reported in the online supplementary appendix 9. In the strength network, the UME model (DIC = 402.3) was not meaningfully different than the random-effects NMA model (DIC = 400.8). Node-splitting was performed on 29 comparisons; the only significant difference was LM1 vs HM1 ($P < 0.01$). In the hypertrophy network, the UME model (DIC = 143.1) was meaningfully different than the random-effects NMA model (DIC = 137.8). Node-splitting was performed on 22 comparisons; the only significant difference was LS2 vs CTRL ($P < 0.01$).

Threshold Analysis

Threshold analysis results for strength and hypertrophy are shown in online supplementary appendix 10. HM3 was the top-ranked condition for strength; however, 65 comparisons indicated some sensitivity to the level of uncertainty and potential biases in the evidence. The revised top-ranked strength condition was HM2 in 92% (60/65) or HM1 in 8% (5/65) of comparisons. HM2 was the top-ranked condition for hypertrophy, and this finding was robust. Two comparisons indicated some sensitivity to the level of uncertainty and potential biases in the evidence, and HM1 was the revised top-ranked condition in both cases.

Sensitivity Analyses

Sensitivity analysis results are displayed in the online supplementary appendix 11. For both the strength and hypertrophy NMAs, the second sensitivity analysis (discussed herein) most improved model fit. The strength network included 155 studies (n = 4,397) and 11 conditions (LS1 and HS1 excluded). The relative effects for all RETx versus CTRL were tempered, such that posterior SMDs ranged from 0.77 to 1.49, with the largest relative effect from HM2 (1.49

[1.29, 1.70]) and smallest from LS3 (0.77 [0.56, 0.98]). The 95% CrI for each RETx versus CTRL excluded zero. There was a 95% probability that HM2 yields larger relative effects than LS2, LS3, LM1, LM2, LM3, and HS3; that HM3 was superior to LS2, LS3, LM1, LM2, and LM3; and that LM2 was superior to LS3. HM2 (99.9%) and HM3 (95.7%) remained most likely to rank in the top three for muscle strength.

The hypertrophy network included 115 studies (n = 3,240) and 9 conditions (HS2 and HM1 excluded). The relative effect for each RETx versus CTRL was mostly unchanged, with the largest relative effect from HM2 (0.59 [0.39, 0.78]) and the smallest from HS3 (0.30 [-0.05, 0.66]). Between prescriptions, there was a 95% probability that LM2 was superior to LS3. HM2 (82.8%) and LM2 (80.4%) remained most likely to rank in the top three for muscle hypertrophy.

Network Meta-Regression

Network meta-regression results are displayed in the online supplementary appendix 12. Model fit was not meaningfully different than the unadjusted model for all covariates, except relative weekly volume load, which worsened model fit. Age, training status, proportion of females, duration, volitional fatigue, relative weekly volume load, outcome measurement tool, outcome measurement region, and publication year did not yield any obvious modifying effect on the relative effect for each RETx versus CTRL, and data-sparse nodes reduced estimate precision.

Physical Function

Physical function results are reported in the online supplementary appendix 13. Few studies assessed mobility (25 studies, n = 859, age (mean) = 68 years), gait speed (15 studies, n = 488, 68 years), and balance/flexibility (11 studies, n = 323, 68 years). Compared to CTRL, there

was a 95% probability that LM2, LM3 and HM3 improved mobility and gait speed, while HM3 was the only condition that improved balance/flexibility. No differences were found between RT prescriptions for any physical function outcome.

DISCUSSION

Twelve distinct RET prescriptions and non-exercising control groups were compared using network meta-analysis to determine their effect on gains in muscle strength, hypertrophy, and improvements in physical function in healthy adults. Compared to no exercise, most load, sets, and frequency combinations increased muscle strength and hypertrophy, indicating that several RETx resulted in beneficial skeletal muscle adaptations. RET with higher loads characterized the top-ranked strength prescriptions, and RET with multiple sets characterized the top-ranked hypertrophy prescriptions. A diverse range of RET prescriptions improved physical function, but evidence scarcity limited insights. Guideline developers and practitioners may consider these results when forming recommendations and prescribing resistance training for healthy adults.

Network meta-analysis has previously been used to compare different types of exercise [31-34] and doses of RET load [35]. In the NMA by Lopez et al. [35], 23 (n = 582) and 24 (n = 604) studies were included in the strength and hypertrophy networks, respectively. The present strength (178 studies, n = 5,097) and hypertrophy (119 studies, n = 3,364) networks were much larger, and this is likely attributable to Lopez et al. [35] excluding studies not including RET to momentary muscular failure and our more comprehensive search strategy (2,629 [35] versus 16,880 records identified). This NMA, to our knowledge, represents the largest synthesis of RET data from randomized trials.

All loads, sets, and frequency combinations increased muscle strength and size compared to CTRL. There was a 95% probability that RET with at least two sets *or* two sessions per week increased strength (Figure 3A), and training with at least two sets and two sessions per week resulted in hypertrophy (Figure 3B). Considering only the lower credible interval limit, each

RETx induced at least a moderate (SMD > 0.47) and small (SMD > 0.16) increase in muscle strength and mass, respectively. Such certainty is not possible for all prescriptions, though, because the 95% credible interval crossed zero for two RETx for strength (HS1 and LS1) and three RETx for hypertrophy (HM1, HS2, and HS3), meaning these prescriptions might increase, not change, or decrease muscle strength and size. However, we posit that this is unlikely to represent an ineffectiveness of those particular RETx and that imprecise network estimates confound these findings. These strength (HS1 and LS1) and hypertrophy (HM1, HS2, and HS3) nodes included <60 participants and contributed little direct evidence (Figure 2). Within each study testing these prescriptions, strength increased significantly compared to CTRL/baseline in all cases, and hypertrophy increased from baseline in most cases. Those prescribing resistance training can be confident that all resistance training prescriptions increased strength and hypertrophy compared to no exercise.

Network comparisons suggest that most RET prescriptions were comparable for strength and hypertrophy. The 95% credible intervals contained zero for a striking 91% (101/111) of all between-RETx comparisons (Table 3). Nine of the 10 comparisons that did not contain zero were between HM2 or HM3 and a lower-load RETx for strength, suggesting higher-load, multiset programs caused the largest strength gains. This result remained after sensitivity analyses (online supplementary appendix 11) and aligned with previous meta-analyses that found higher-load RET yields the largest strength gains [17, 18, 35]. A critical point for practitioners is that lower-load RET prescriptions increase strength compared to no exercise. All RET prescriptions may comparably promote muscle hypertrophy, and the influence of load was less apparent. The lack of importance of load for hypertrophy is supported by other analyses [16, 17, 35, 63], but performing RET to momentary muscular failure (fatigue) has been posited as a key

component for RET-induced hypertrophy with lower loads [63]. Network meta-regression for exercise 'failure' (fatigue) did not improve model fit nor substantially alter network estimates, suggesting that lifting to fatigue does not suitably explain the observed hypertrophic response. Our finding in this domain agrees with previous work [64], suggesting that untrained individuals still achieve large gains in skeletal muscle mass without performing RET to failure. Performing RET to momentary muscular failure may, however, be increasingly important for trained individuals [13]. For both strength and hypertrophy, though, there was a large credible interval surrounding the non-significant effect estimate for many comparisons between resistance training prescriptions, so a wide range of different effects are possible for these comparisons. The available evidence does not permit definitive, statistically valid conclusions about the equivalency of each RETx, despite most comparisons between resistance training prescriptions not being statistically significantly different from each other.

Prescriptions for RET with higher loads were more likely to rank in the top three for strength than all lower-load prescriptions, and RET prescriptions with multiple sets per exercise were most likely to rank in the top three for hypertrophy (Figure 4). Rankings are sensitive to uncertainties within the network [28], but posterior ranking credible intervals supported higher-load, multi-set programs being the highest-ranked for strength and multiple sets or multiple sessions being the highest-ranked for hypertrophy. Notably, sets and frequency are major components of resistance training volume, a key factor for hypertrophy [21, 65-67]. The probability of each condition ranking in the top three was calculated because the top-ranked RETx does not necessarily reflect the best intervention for all individuals [68]. Personal preferences, including disliking higher loads or time constraints, including an inability to train more than once weekly, can be observed while still benefiting from RET. In our view, especially

given the low participation rates in RET, practitioners should not avoid prescribing, nor should individuals be discouraged from completing non-top-ranked RETx. While all prescriptions increased muscle strength and mass, the top-ranked prescriptions involved higher loads for strength and higher volume for hypertrophy. We do not know how these RETx affect relevant health outcomes. Some data suggest that health benefits exist with low time commitment (30-60min/wk) to RET and greater time commitment with reduced health benefits [4, 69].

Ours is the first review to assess confidence in RETx recommendations with threshold analysis. Several factors can influence NMA results [56], and the robustness of treatment recommendations should be considered when interpreting results. Previous methods to evaluate the confidence of meta-analytical findings do not consider how potentially influencing factors can change treatment recommendations [56, 70, 71] or are not yet developed for Bayesian NMA [72]. Threshold analysis determines how much the available evidence could change before recommendations differ and identifies a new top-ranked treatment [55, 56]. Sixty-five direct comparisons were identified that could potentially impact the recommendation of HM3 as the top-ranked strength treatment; however, the revised treatment recommendation was HM2 in 60 of these cases and HM1 in the other five cases (online supplementary appendix 10), suggesting that performing RET with higher loads and multiple sets/exercise are robust recommendation for optimizing RET-induced strength gains. The top-ranked RETx for hypertrophy – HM2 – was sensitive to the uncertainty of only two comparisons, and HM1 was the revised recommendation because both comparisons were from the same multi-arm study [73]. Furthermore, 127 of the 161 direct comparisons would need to change by more than four standard deviations to alter HM2 as the top recommendation for hypertrophy. The optimized recommendations of higher load, multiple-set programs for strength and HM2 for hypertrophy were extremely robust.

Current guidelines collectively advise healthy adults to complete RET at least twice weekly [10-12, 74]. The results herein support these recommendations and should not deter practitioners from promoting existing guidelines to improve strength and hypertrophy, nor do these results contradict the effectiveness of guidelines incorporating additional RETx variables, such as rest intervals and contraction type and velocity [10, 12]. However, our results support resistance training at less than recommended often-cited levels for enhancing strength and hypertrophy. Most individuals do not meet current guidelines, and RETx complexities may impede the adoption of resistance training. Minimal-dose approaches have been proposed to reduce barriers to resistance training [75], and our results strongly support the World Health Organization's claim, "Doing some activity is better than none" [74]. While others attempt to optimize resistance training prescription [76], we propose that, for most adults, regularly engaging in any RETx is more important than training to optimize strength and hypertrophy outcomes. Our analysis found multiple RETx comparable for healthy adults to increase muscle strength and mass. Thus, adults should engage in resistance training, even if they cannot meet existing recommendations.

LIMITATIONS

Risk of bias was frequently introduced by protocol deviations, randomization procedures, and selection of the reported result for both outcomes (online supplementary appendix 7). All three domains were regularly rated "*Some concerns*" because participants were aware of the intervention, appropriate analyses to estimate the effect of assignment were not performed, and randomization, concealment and pre-specified analysis procedures were rarely reported. Double-blinding RET is unfeasible, but the remaining issues are prevalent and reoccurring in RET

research [77]. Researchers should preregister analysis plans and report randomization procedures to reduce bias.

Several limitations require acknowledgement and consideration when interpreting the findings of this review. Well-trained elite athletes/military persons and individuals with chronic disease were excluded, so the results should be translated to these populations with caution and additional insights [13, 78-80]. Mobility, gait speed and balance/flexibility findings should also be interpreted with caution due to the limited evidence available, which could be attributed to including only healthy older (>55yr) adults (e.g., not frail). The coding framework for RET prescriptions prevented the inclusion of periodized RET programs overlapping conditions (e.g., loads ranging from 60-90% 1RM) from being captured in the network. Initially, our objective was to further divide the load and set prescriptions; however, this yielded sparse, disconnected networks, violating a critical assumption of NMA [50]. The continuous RETx variables investigated herein (load, sets, frequency) were classified categorically, so future work could utilize dose-response/model-based NMA methods to explore these RETx variables as continuous predictors [81, 82]. Several acute RET variables were not factored into the included RET prescriptions (e.g., inter-set rest, time under tension, repetition velocity, volitional fatigue, tempo); where possible, NMR was used to explore if these factors improved model fit and altered effects. Results from NMR are correlative, however, and should be interpreted cautiously [83]. Nonetheless, many variables (inter-set rest, tempo, time under tension) were reported too infrequently for inclusion as covariates. Calculating the relative weekly volume load (i.e., load • repetitions/set • number of sets • number of exercises • weekly frequency), which should impact results [21], also required approximations that hindered model fit. The principle of specificity [17] (i.e., the similarity between training and testing movement) and approximations of muscle

mass ([84], e.g., lean mass) could infringe on transitivity assumptions [37] when integrating results from multiple studies and NMR with the covariates measurement tool and region were imperfect solutions. Including one measurement per outcome for each study may limit the totality of evidence captured by this review, so future methodological work could explore the integration of multiple correlated effect sizes in NMA, as in recent pairwise meta-analyses [64, 85]. Increasingly, within-subject models are used due to their increased statistical power [86]. To our knowledge, however, no methods are available to account for the additional correlation when including within- and between-subject comparisons in NMA. With consideration for these limitations, guideline developers and practitioners can obtain meaningful insights from this analysis.

CONCLUSION

This network meta-analysis represents the largest synthesis of resistance training prescription data from randomized trials. Most RETx increased muscle strength and mass compared to no exercise. Top-ranked prescriptions for muscle strength were characterized by lifting heavier loads, and multiple sets characterized top-ranked prescriptions for muscle hypertrophy. Guideline developers and practitioners should encourage the adoption of resistance training since all RETx can increase muscle strength and mass in healthy adults. The effects on health outcomes of various RETx remain largely unknown.

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CHAPTER 4: STUDY 3

A long non-coding-RNA signature is related to human skeletal muscle load-induced remodelling

ABSTRACT

Background: The molecular responses responsible for muscle remodelling resulting in load-induced hypertrophy are poorly understood. Long non-coding RNAs (lncRNA) are a class of non-coding transcripts >200 nucleotides in length with no protein-coding potential. Recent work demonstrates that lncRNA may be important regulators of various cellular processes; however, the lncRNA transcriptome is poorly characterized in skeletal muscle biology.

Purpose: We aimed to investigate changes in the lncRNA transcriptome following supervised resistance exercise training and identify lncRNA associated with exercise-induced lean mass (LM) change.

Methods: We used the human transcriptome 2.0 array (HTA2.0) chip to profile the lncRNA transcriptome of 144 individuals, from 5 independent studies, before and after supervised exercise training. LM was quantified using DXA, and 88 individuals exhibited a > 2.5% change in LM (greater than the measurement error of the DXA). We also identified 50 individuals with a < 2.0% change in LM. Genes encoding lncRNA associated with LM growth were determined through differential expression analyses. Gene-gene co-expression networks and gene ontology were used to identify networks and biological processes.

Results: The average change in LM across our five independent exercise studies was 3.9 ± 4.7% (range: -4.9% to 24.7%). We identified a core set of 91 lncRNA genes associated with LM growth. The majority of these lncRNA genes were novel and uncharacterized. Several of our identified lncRNA genes were nested within gene networks related to biological processes central to phenotypic changes in load-induced remodelling of skeletal muscle, including angiogenesis, extracellular remodelling, and mitochondrial translation. *LINC00390* and *ENST00000655610* are two genes that were positively correlated with type II muscle fibre and T-

cell gene markers, respectively, suggesting these novel lncRNA genes may be cell-specific.

Conclusion: Ours is the first clinical transcriptomics study to interrogate the lncRNA transcriptome and leverage heterogeneous exercise-induced changes in LM to identify lncRNA genes uniquely associated with LM accretion.

INTRODUCTION

Skeletal muscle is the largest organ in the human body, accounting for ~40% of total body mass (1) and is paramount in various mechanical and metabolic functions. Skeletal muscle is a highly plastic tissue that can adapt to numerous stimuli by altering its mass, contractile and metabolic properties. Loading of muscle through resistance training (RET) results in skeletal muscle hypertrophy, whereas unloading (i.e., bed rest, limb immobilization) of skeletal muscle results in atrophy. Nonetheless, when skeletal muscle is exposed to loading/unloading stimuli, there is remarkable individual heterogeneity among the phenotypic response. For example, following supervised RT, gains in skeletal muscle size are highly variable between individuals (2, 3). In 585 participants undergoing 12 weeks of progressive RT, the relative percent change in biceps brachii muscle cross-sectional area (CSA) ranged from -2 to 59% (4). The key molecular responses responsible for heterogenous muscle remodelling need to be better understood, as these identified regulators may influence insulin sensitivity and age-related musculoskeletal frailty.

Rather than averaging molecular responses across individuals displaying wide phenotypic variability (a procedure that has been flawed for quite some time (5)), leveraging individual heterogeneity in response to RT may help determine molecular regulators that scale proportionally with physiological adaptations. For example, using data from 100 individuals we (6) recently discovered a set of 141 genes correlated with the muscle growth response to chronic muscle loading in humans, and these activated genes formed functional networks that were observed to be associated with extracellular matrix remodelling, angiogenesis, and mitochondrial function.

Most of the mammalian genome is transcribed into RNAs with little-to-no protein-coding potential (7), and there is a strong and statistically significant correlation between the proportion of the genome that is non-coding and organismal complexity (8). Long non-coding RNAs (lncRNA) are a specific class of non-coding transcripts that are >200 nucleotides in length that have no protein-coding potential. Several mechanistic and gene expression studies have demonstrated that lncRNA are involved in cellular functions, including transcriptional and post-transcriptional regulation, X chromosome inactivation, developmental processes, cell differentiation, and disease states, such as cancer and metabolic disease (9, 10). Due to their dynamic expression and key regulatory roles in gene expression, lncRNAs may be important regulators of skeletal muscle processes.

Most of the lncRNA transcriptome is under-characterized, particularly in skeletal muscle, with only a handful of studies published to date (11, 12). Therefore, the purpose of the present study was to interrogate changes in the lncRNA transcriptome following supervised exercise training. We used microarray technology to profile RNA from 144 participants before and after exercise training from 5 independent studies. We then leveraged interindividual heterogeneity in exercise induced increases in lean mass (LM) to identify differentially expressed lncRNAs. We used data-driven networks to elucidate potential biological features of lncRNAs and used four in vivo trials from human skeletal muscle unloading in an attempt to validate lncRNA genes.

MATERIALS AND METHODS

Clinical Samples and the Human Transcriptome Array 2.0. For an overview of the analysis process, please refer to Figure 1. The present study relied on 382 human skeletal muscle biopsy samples. We used 288 skeletal muscle biopsy samples from five individual exercise trials (four published studies (6, 13-15); one unpublished study). We also used 94 skeletal muscle biopsy samples from healthy individuals undergoing skeletal muscle atrophy (three published studies (6, 16, 17); one unpublished study). Array data for the following studies (6, 13-15, 17) are deposited at GEO (GSE154846); Array data for (16) is available at GSE148152.

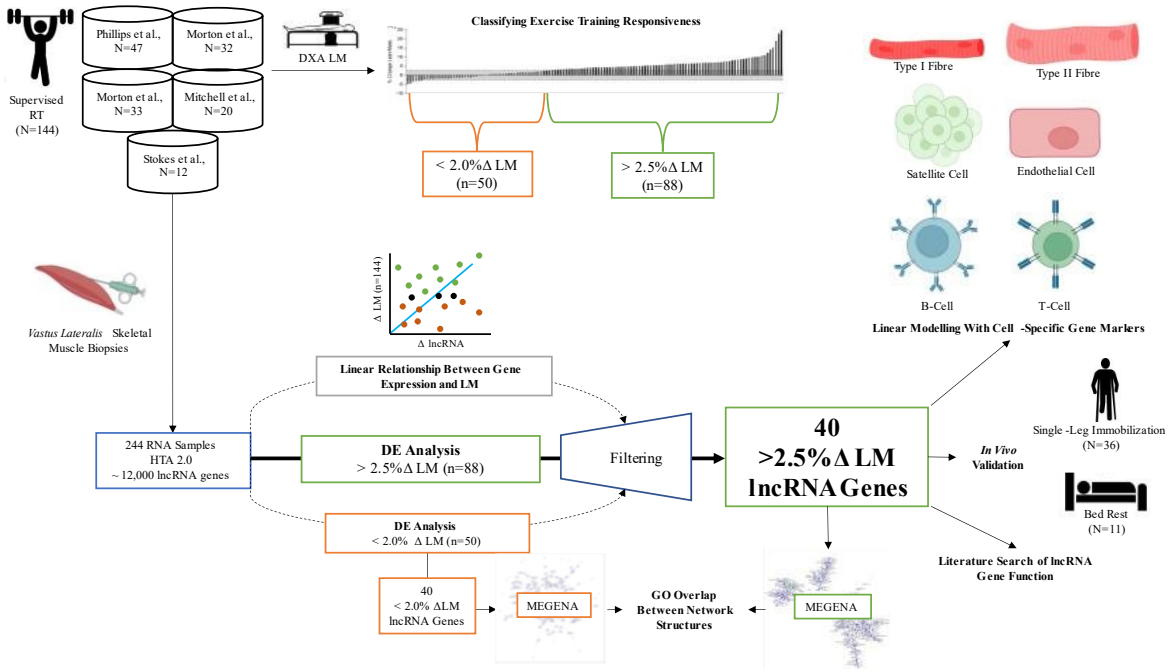


Figure 1. Overview of the analysis process. We used 144 participants from 5 independent RT clinical studies. Lean mass (LM) was quantified using dual-energy x-ray absorptiometry (DXA), and 88 individuals exhibited a > 2.5% ΔLM (greater than the measurement error of the DXA). We also identified 50 individuals with a < 2.0% ΔLM. *Vastus lateralis* skeletal muscle biopsies were obtained pre- and post-RT, RNA was extracted, and ~12,000 lncRNA genes were profiled on the Human transcriptome 2.0 array (HTA2.0). We used differential expression analyses and linear modelling to identify 40 lncRNA genes associated with the > 2.5% ΔLM. PubMed searches were conducted on each of the 40 lncRNA genes to identify any relevant gene function. We also correlated the expression of our lncRNA genes with 6 different muscle fibres and mononuclear cell types. We used gene co-expression analysis to determine Gene ontology overlap between network structures across the > 2.5% ΔLM group and the < 2.0% ΔLM group. Lastly, we attempted to validate our lncRNA genes using RNA samples from 47 individuals undergoing reduced contractile activity via single-leg immobilization or bed rest.

Description of Independent Exercise Training Studies.

Morton et al. Healthy recreational men completed this study (age: 22 ± 3 years; body mass index: 26 ± 7 kg/m^2). Each participant's dominant leg was randomly assigned to a high-load, low-repetition (8-12 repetitions at ~70-80% 1RM), RET protocol or a low-load, high-repetition (20-25 repetitions at ~30-40% 1RM) RET protocol. The contralateral leg was assigned to the opposite condition. Participants underwent RET three days a week (Monday, Wednesday, and Friday) for 10 weeks. Each RET session consisted of 3 sets of unilateral knee extensions, according to each leg's condition allocation, to volitional fatigue. The starting leg alternated between RET sessions. RET-induced skeletal muscle hypertrophy did not differ between allocation conditions and was not considered in the present muscle samples. Participants received 25 g of whey protein isolate twice daily (morning or post-exercise and pre-sleep) for the duration of the study. Unilateral leg LM for each condition was quantified using dual-energy X-ray absorptiometry (DXA) pre-RET and post-RET. At baseline, a *vastus lateralis* skeletal muscle biopsy was obtained from one leg, selected at random. Thereafter, *vastus lateralis* skeletal muscle biopsies were obtained from both legs following 10 weeks of RET.

Morton et al (14); for a detailed description of the protocol, please refer to (14). Participants were 23 ± 2 years old, with a BMI of 26.9 ± 2 kg/m2 and had 4-5 y of RET experience. Participants performed full-body RET 4 days/week (Mon, Tues, Thurs, Fri) for 12 weeks that targeted all the major muscle groups. Each session included 5 exercises performed for 3 sets to volitional failure. Participants were randomly assigned to complete either low-load, high-repetition (20-25 repetitions per set at ~ 30% - 50% 1-RM) RET or high-load, low-repetition RET (8-12 repetitions per set at ~ 75%-90% 1-RM). Indices of muscle hypertrophy did not significantly differ between the groups post-RET, so group allocation was not considered in the present use of

muscle tissue samples. Participants consumed 30 g of protein after each exercise bout. *Vastus lateralis* skeletal muscle biopsies were obtained at baseline and 72 hours following the final RE session. Leg LM was assessed using DXA at baseline and 72 hours following the final resistance exercise session.

Phillips et al (15); for a detailed description of the protocol, please refer to (15). Participants performed high-intensity interval training 3 days per week (Monday, Wednesday, and Friday) for 6 weeks. Each session consisted of a 2 min warmup at 50 W followed by 5 sets of high-intensity cycling at 125% $VO_{2\ peak}$ for 1 minute. Each set was separated by 90 s of rest. Biopsy tissue was available at pre- and post-training for 47 participants, including 16 males and 31 females with an average age of 39 years (range 20-51 years) and a BMI of 31 kg/m^2 (range 27 - 43 kg/m^2). Unilateral leg LM was assessed using DXA at baseline and following the final high-intensity interval training session.

Mitchell et al (13); for a detailed description of the protocol used, please refer to Mitchell and colleagues (13). Young, recreationally active, men completed the study. Participants were 24 ± 1 years old, with a BMI of 26.4 kg/m^2. Participants performed whole-body RET 4 days per week for 16 weeks. Resistance exercise sessions consisted of two upper-body and two lower-body training sessions per week. The program progressed from 3 sets of 12 repetitions to 4 sets of 6 repetitions of each exercise. The last set of each exercise was performed to volitional failure. Participants consumed 30 g of protein immediately after each exercise session. *Vastus lateralis* skeletal muscle biopsies were obtained before and following 16 weeks of RET (~48 – 72 hours following the final resistance exercise session). Whole body LM was assessed using DXA before RET and ~48 – 72 hours following the last resistance exercise session.

Stokes et al (6); for a detailed protocol description, please refer to (6). Young, healthy men completed this study (age: 21 ± 3 years; body mass index: 24 ± 3 kg/m^2). Participants underwent unilateral leg extension and leg press resistance exercise sessions three days a week (Monday, Wednesday, and Friday) for 10 weeks. Specifically, each session consisted of 3 sets of 8-12 repetitions of leg extension and 3 sets of 8-12 repetitions on a leg press. The last set of RET was performed to volitional failure. Following each exercise bout, participants ingested 25 g of whey protein isolate. *Vastus lateralis* skeletal muscle biopsies were obtained from the resistance-trained leg, and leg LM of the RET leg was assessed using DXA at baseline and ~72 h following the final resistance exercise session.

RNA Extraction and Transcriptome Profiling. For (6, 14) and Morton et al., samples, approximately 20 mg of muscle was used to extract total RNA. Muscle samples and 1000 uL of TRIzol were added to Lysing Matrix D tubes containing ceramic microbeads (MP Biomedicals, Solon, OH, USA) and homogenized using a FastPrep tissue homogenizer (MP Biomedicals, Solon, OH, USA). 200 uL of chloroform was added, and the tubes were hand-shaken vigorously for 15 s and incubated at room temperature for 5 min. Samples were then centrifuged at 12 000 g for 10 min at 4°C, and the upper aqueous phase containing RNA was transferred to an RNase-free tube. RNA was purified using E.Z.N.A Total RNA Isolation kit (Omega Bio-Tek, Norcross, GA, USA). RNA was processed for transcriptome profiling using the GeneChip WT Plus Reagent Kit according to the manufacturer's instructions. First and second-strand cDNA synthesis were performed using 100 ng of RNA and a spike-in Poly-A control, followed by reverse transcription into cRNA. cRNA was purified using magnetic beads and quantified using spectrophotometry (Nanodrop UV-Vis, Thermo Fisher Scientific). 15 mg of cRNA was then amplified and hydrolyzed using RNase H (leaving single-stranded cDNA) and purified with

magnetic beads. cDNA (5.5 mg) was then fragmented and labelled. A hybridization master mix was prepared and added to the fragmented and labelled cDNA. 200 uL of the mixture was applied to the HTA 2.0 cartridge and hybridized at 45°C for 16 h rotating at 60 rpm. The cartridge was washed and stained using the FS450_001 fluidics protocol on the GeneChip Fluidics Station 450 (Thermo Fisher) and scanned using a GeneChip Scanner 3000 7G (Thermo Fisher).

For (13, 15), RNA was extracted from whole muscle samples as described above. Thereafter, samples dissolved in RNase-free water were processed to single-stranded sense fragmented DNA using the GeneChip WT PLUS Reagent Kit, which relies on a reverse transcription priming strategy that primes the poly-A and non-poly-A RNA. HTA 2.0 chips were processed according to the manufacturer's protocol. Fragmented (5 mg) end-labelled sense strand target cDNA was hybridized to each array and scanned using a Gene Chip Scanner 30007G (Affymetrix Core, MPI A/S, Denmark).

All RNA samples used for the current analysis were profiled on the Human Transcriptome Array 2.0 (HTA 2.0). The HTA 2.0 gene chip contains 6.9 million, 25-mer 'probes,' which are computationally combined into groups (probe-sets) representing individual RNA transcripts (or part of a transcript) (18). Standard quality control processes were performed for each study before being used for downstream analyses. Probes with extreme GC content or probes mapped to more than one part of the genome were removed. All gene-chip samples were normalized using an iterative rank-order methodology (19). The exact composition of each probe set is defined using a 'map,' referred to as the chip definition file (CDF). For the present analyses, we relied on a GENCODE CDF to summarize the transcript-level data, and each probe-set was based on ENSEMBL Transcript (ENST) definitions, and R Bioconductor packages were

used to update, assemble, and summarize the expression data. In total, we measured 40,675 lncRNA ENSTs, which corresponded to 11,980 lncRNA genes.

Classifying Exercise Training Responsiveness. To identify lncRNA genes associated with exercise-induced LM change, we used the changes in DXA LM across individuals from our 5 independent exercise trials. For all individuals, we first calculated the percentage change in LM at the post-exercise training time point from the baseline value. Previous studies (2, 20) that leverage heterogeneous physiological adaptations to study skeletal muscle biology typically use only higher and lower quartile responders for downstream analyses. However, under such circumstances, sample sizes are extremely limited (e.g., 8 higher and 8 lower responders identified (20)), leading to underpowered "omic" analyses (21). Therefore, to determine a threshold for responsiveness while maximizing the sample size used for downstream analyses, we relied on the reported measurement error of 2.0 – 2.4% for DXA-derived changes in LM (22, 23) to classify individuals into one of three groups. Individuals that exhibited > 2.5% LM change were allocated to the group: " > 2.5% delta lean mass (dLM)", whereas individuals that showed < 2.0% LM change were assigned to the group called "< 2.0% dLM". These two groups were used for differential expression (DE) analysis. Individuals that fell within the range of reported precision error of the DXA (2.0 – 2.4%) (22, 23) were not used for DE analysis but were included in our linear modelling analysis.

Differential Expression (DE) Analysis. To generate an initial list of lncRNA genes associated with exercise-induced LM change, we conducted a DE analysis on the > 2.5% dLM group (n= 88; 176 paired RNA samples). We use samR (two class paired, 10k permutations) for DE analyses because permutation-based false discovery proportion estimation methods have better

false discovery rates and greater sensitivity than traditional p-value correction methods (21). To identify differentially expressed lncRNAs, we used a q-value cutoff of 5% and a $\log_2$ fold change ≥ |1.2|. We also conducted a DE analysis (using the same procedures as above) on the < 2.0% dLM group (n = 50; 100 paired RNA samples). We applied the following heuristics to assess differentially expressed lncRNA genes uniquely regulated in the > 2.5% dLM group. First, the lncRNA displayed a $\log_2$ fold change in the opposite direction of the < 2.0% dLM group. Or, the lncRNA gene showed a $\log_2$ fold change ratio ≥ |1.2| absolute $\log_2$ fold change ratio, compared with the < 2.0% dLM group. Similar heuristics were applied to identify uniquely regulated lncRNA genes in the <2.0% dLM group. Therefore, the final output from our DE analyses and heuristic-based filtering were two lists of uniquely regulated lncRNA genes: one lncRNA gene list from the > 2.5% dLM group and another lncRNA gene list from the < 2.0% dLM group.

Assessing the Linear Relationship Between Gene Expression and Lean Mass. For each study, we evaluated the linear relationship between the ΔlncRNA expression and ΔdLM using a type III analysis of variance in R. We use the following linear model in R: delta lncRNA expression ~ deltaLM. For each ENST, we calculated a Pearson correlation coefficient (r) and a corresponding p-value per study. To produce a reliable (24) and significant sub-set of LM lncRNA genes, we carried out a meta-analysis across the three largest cohorts (Phillips et al., (15) n=47; Morton et al. n=32; Morton et al.,(14) n=33), combining p-values using the Stouffer method, in the R package, poolr (25). lncRNA genes significantly associated with LM contained an absolute 3-study median r ≥ 0.1 and a significant Stouffer P value (≤ 0.05). The two smaller cohorts (Stokes et al., (6)n=12; Mitchell et al., (13)n=20) were not included in our meta-analysis

because sample sizes of at least n=30 are required to produce stable correlations (24); however, all five studies were used to identify lncRNA ENSTs with consistent directionality.

Gene Co-Expression Network Analysis: MEGENA. Regrettably, lncRNAs are not annotated in biological feature detection methods (such as gene ontology [GO]) and cannot be mined for biological interpretation. To overcome this limitation, we used a data-driven network analysis of all 42,400 genes annotated by our GENCODE CDF. We selected 40 > 2.5% dLM lncRNA genes and 40 < 2.0% dLM lncRNA genes and used multi-scale embedded gene co-expression network analysis (MEGENA; (26)) to identify network structures that these lncRNA genes belonged to (FDR < 1% for spearman correlation; $p < 0.01$ for module significance and $p < 0.01$ for network connectivity and 10,000 permutations for calculating FDR and connectivity p values). Network data plots were produced using Fruchterman-Reingold force-directed plotting within MEGENA (26). Biological interpretation of significant gene network structures was carried out using GO. The gene list for each significant network module was submitted to the Database for Annotation, Visualization, and Integrated Discovery (DAVID; https://david.ncifcrf.gov/) to identify enriched biological processes. A custom background file of 14,780 protein-coding genes was used. GO biological processes with a false discovery rate (FDR) of $\leq 5x10^{\wedge-3}$ were considered enriched. We then used REVIGO (http://revigo.irb.hr/) to reduce redundant GO terms (27), which was used to compare and contrast biological features between the > 2.5% dLM group and the < 2.0% dLM group.

Identification of lncRNA Gene Function. We performed a systematic literature review to identify whether a direct biochemical interaction or mechanistic insight had been previously established in our 40 lncRNA genes uniquely regulated in the > 2.5% dLM group. Specifically, each lncRNA gene symbol was searched on PubMed (April 2023) with and without the term; 'skeletal muscle,' followed by an extensive screening of whether meaningful biology had been reported. Further, for lncRNA genes classified as antisense, we executed the same search strategy as above, albeit on the corresponding protein-coding gene. For long-noncoding genes identified as "intergenic" or "novel," we first checked Ensembl (https://useast.ensembl.org/index.html; Ensembl Release 109 <Feb 2023>) to determine if the description or gene name had been updated.

Linear Modelling Between lncRNA Genes and Cell-Specific Gene Markers. We used linear modelling to determine if lncRNA genes uniquely regulated in the > 2.5% dLM group were associated with cell-specific gene markers at baseline. The cell-specific gene markers (Supplementary Data S1) we used for linear modelling were: *MYH1* (type II fibres; (28-30)), *MYH2* (type II fibres; (28-30)), *ATP2A1* (type II fibres; (28)), *TNNT1* (type I fibres; (29, 31)), *TPM3* (type I fibres; (29, 31)), *MYH7* (type I fibres; (28, 29)), *PDLIM1* (type I fibres; (28, 32)), *PAX7* (satellite cells; (33)), *MYF5* (satellite cells; (33)), *MYF6* (satellite cells; (33)), *MYOG* (satellite cells; (33)), *EDN1* (endothelial cells), *ENG* (endothelial cells; (34)), *ECSCR* (endothelial cells; (28)), *PECAM1* (endothelial cells; (35)), *CD4* (T-cells; (36-39)), *CD8B* (T-cells; (36-39)), *CD8A* (T-cells; (36-39)), *CD79α* (B-cells; (28, 36)), and *MS4A1* (B-cells; (28, 36)). Selected lncRNA genes were regressed against individual cell-specific markers in R, using the following formula: lncRNA expression ~ cell-specific marker expression. For each

regression model, r and p values were generated. For each lncRNA, p values were adjusted according to the number of cell-specific gene markers interrogated using Benjamini Hochberg (40). Absolute r values ≥ 0.3 and an FDR of $\leq 5.0x10^{-3}$ were considered meaningful.

Independent Validation of lncRNA Genes Using Skeletal Muscle Disuse Studies. We used four independent studies (n=47; 94 paired RNA samples) to establish if lncRNA genes uniquely regulated in the > 2.5% dLM group were altered during periods of skeletal muscle disuse atrophy – arising from either single-leg immobilization or prolonged bed rest – *in vivo*. Specifically, in three studies (Stokes et al., (6) [n=12; 24 paired samples]; Mcleod et al., (unpublished) [n=6; 12 paired samples]; Lim et al., (17) [n=18; 36 paired samples]), young, healthy men underwent two-weeks of unilateral unloading using a knee brace. Skeletal muscle biopsies were obtained from the *vastus lateralis* at baseline and following single-leg immobilization. Lim and colleagues (17) provided their participants with a nutritional supplement but found that it did not mitigate unloading-induced atrophy, compared with a placebo, so we did not consider group allocation for the present study. Further, we used a study (16) that subjected healthy young men (n=11; 22 paired samples) to 84 days of bed rest. Vastus lateralis skeletal muscle biopsies were obtained before and after 84 days of bed rest (16). These additional studies were all profiled on the same gene chip technology (HTA 2.0) and subjected to the same pre-processing, quality control, and normalization procedures as our independent exercise trials. We measured gene expression before and after skeletal muscle disuse, and to measure statistical significance, we conducted paired t-tests and adjusted p values using the Benjamini Hochberg procedure (40).

RESULTS

Heterogenous Changes in Lean Mass Across Five Independent Exercise Studies. Five independent exercise studies with 144 participants were used in our primary analyses (Table 1). The average change in LM across our five independent exercise studies was 3.9 ± 4.7% (range: -4.9% to 24.7%; Figure 2a). There was also considerable variation in changes in lean mass within each study (Figure 2b-f). 88 individuals fell into the > 2.5% dLM group, and 50 individuals were classified as falling into the < 2.0% dLM group (Figure 2a).

Table 1. Demographics of the independent exercise studies used for establishing lncRNA genes associated with LM change. For all clinical studies, LM change was assessed using DXA. The total sample consisted of 144 participants and 288 paired muscle samples processed on the HTA 2.0 gene-chip platform. Please refer to the methods for a detailed description of each independent exercise study.

	Morton et al. (unpublished)	Morton et al. (14)	Phillips et al.(15)	Mitchell et al. (13)	Stokes et al.(6)
Sample Size, n	32	33	47	20	12
Age, years	22 ± 3 (19 – 28)	23 ± 3 (20 – 29)	38 ± 9 (21 – 51)	24 ± 3 (20 – 30)	21 ± 3 (18 – 29)
Gender, M/F	32/0	33/0	18/29	20/0	12/0
Body mass index, kg/m^2	25 ± 6 (18 – 38)	26 ± 9 (41)	32 ± 4 (26 – 43)	24 ± 4 (15 – 32)	24 ± 3 (20 – 31)
> 2.5% dLM group, n	20	20	24	14	10
< 2.0% dLM group, n	10	13	20	5	2
2.0 – 2.4% dLM [a], n	2	0	3	1	0

Age, and body mass index are displayed as mean ± SD (min - max). Sample size, Gender, > 2.5% dLM group, < 2.0% dLM group, and 2.0 – 2.4% dLM group are displayed as counts. NR, not reported; dLM, delta lean mass.

[a] These individuals fall within the range of the reported precision error of the DXA, and were not used for DE analyses, but were used for linear modelling analyses.

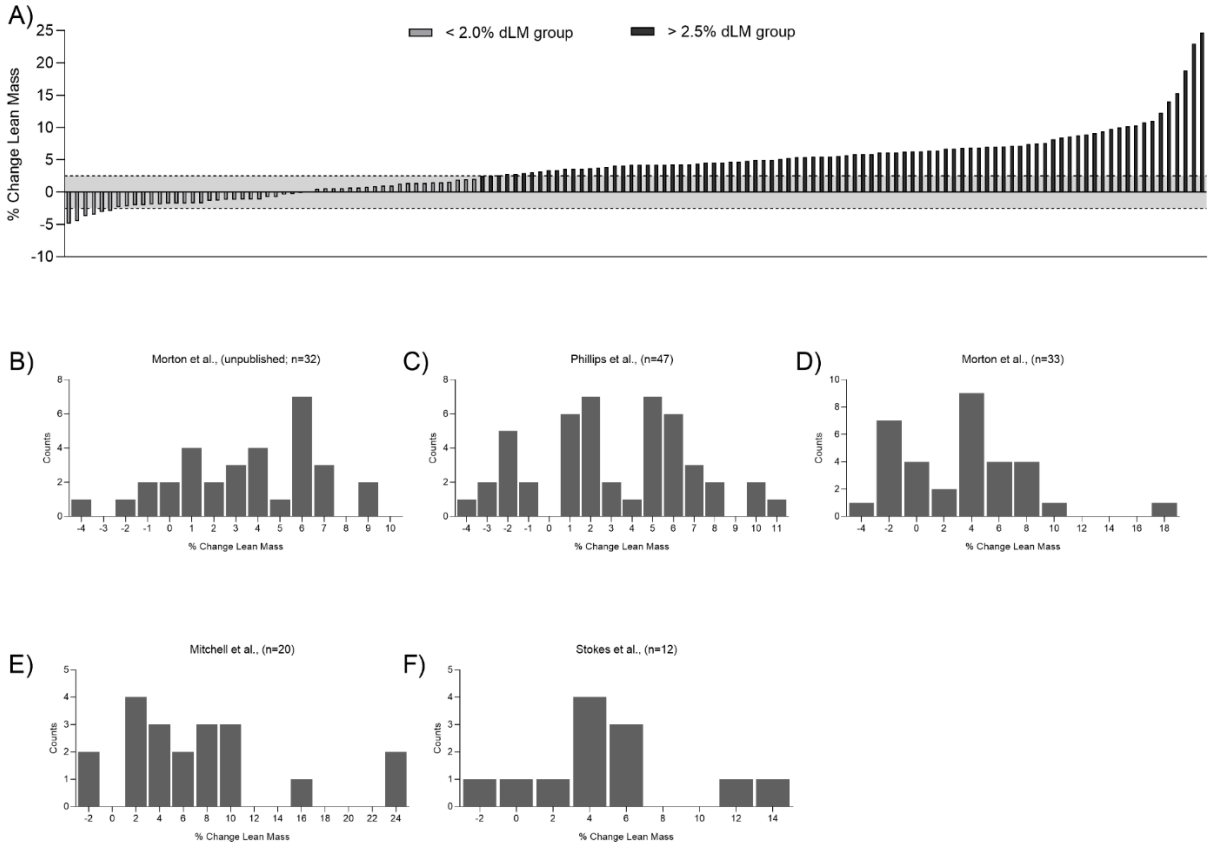


Figure 2. Exercise-induced changes in LM. A) Waterfall plot depicting changes in lean mass for 144 participants (n=88 > 2.5% dLM group, n=50 <2.0% dLM group. The shaded line depicts the measurement error of the DXA. B-F) study-level histograms depicting counts for changes in lean mass.

Establishing a LncRNA Gene Signature Associated With > 2.5% dLM. We performed DE analysis on the >2.5% dLM group (n=88) to identify lncRNA genes associated with exercise-induced LM change. Using stringent cutoffs for DE analysis (Q-value ≤ 5% and $\log_2$ fold change ≥ 1.2 or ≤ -1.2), 340 lncRNA genes were differentially expressed. Notably, 76 lncRNA genes such as *H19* (>2.5% dLM - $\log_2$ fold change: 1.41, Q-value: 0%; < 2.0% dLM: $\log_2$ fold change: 1.27, Q-value: 0%) and *LANCL1-AS1* (>2.5% dLM - $\log_2$ fold change: -1.88 Q-value: 0%; < 2.0% dLM - $\log_2$ fold change: -1.84, Q-value: 0%) were differentially expressed in both the >2.5% dLM group and the < 2.0% dLM group (and displayed a $\log_2$ fold change in the same direction) (Figure 3). Following heuristic-based filtering, we identified 91 lncRNA genes that were uniquely regulated in the >2.5% dLM group and 28 of these genes were also significantly associated with changes in lean mass (Stouffer p value ≤ 0.05, absolute r ≥ 0.1; Supplementary Data S2). From the list of 91 lncRNA genes, 40 >2.5% dLM lncRNA genes were selected – based on the magnitude of their difference from the < 2.0% dLM group – and used for downstream analyses (Table 2).

Table 2. 40 lncRNA genes uniquely regulated in the > 2.5% dLM group.

Gene ID	Log_2 Fold Change		Q-value (%)		dLM vs [Gene ID]		
	> 2.5% dLM	< 2.0% dLM	> 2.5% dLM	< 2.0% dLM	3 study Median R	3 study Stouffer	Published Biochemistry and/or Physiology
MEG3	1.4	1.1	0	40	0.21	0.05	• Maternally Expressed 3 • Regulates myoblast differentiation *in vitro.*(42) • MEG3 knockdown impairs injury-induced skeletal muscle regeneration; knockdown in mice leads to mesenchymal stromal differentiation in skeletal muscle (42). • MEG3 modulates the repression of anti-myogenic factors, TGFβ, RhoA, to promote myoblast plasticity and differentiation (42).
CYTOR	1.5	1.1	0	52	0.08	0.51	• Cytoskeleton Regulator RNA. • Expression increases following a bout of exercise (12). • Overexpression promotes type II fibre maturation (12). • Reduces chromatin accessibility at binding motifs for TEAD1 (12).
LINC00390	-1.2	-1.0	0	66	-0.30	0.02	• Long intergenic non-protein coding RNA 390. • There is no literature available on the lncRNA.
DOCK8-AS2	1.2	-1.0	0	70	0.30	0.05	• Antisense to DOCK8. • There is no literature available on the lncRNA. • Guanine nucleotide exchange factors regulate Rho GTPases, such as CDC 42 and Rac1 (43).
ENST00000662781	-1.2	1.1	1	23	-0.42	0.00	• Novel lncRNA. • There is no literature available on the lncRNA.
SERPINB9P1	1.3	1.0	0	64	0.29	0.01	• Serpin Family Member 9 Pseudogene. • There is no literature available on the lncRNA
LINC00332	1.2	-1.2	0	19	0.28	0.03	• Long intergenic non-protein coding RNA 332. • There is no literature available on the lncRNA.
DNMT3L-AS1	-1.3	1.1	1	64	-0.25	0.02	• Antisense to DNMT3L. • DNMT3L is part of the DNMT3 family but has no DNA methyltransferase activity (44) • Mouse embryonic stem cells deficient in DNMT3L reveal hypomethylated sites at many targets of DNMT3A (44).

							• DNMT3L forms a complex with DNMT3A2 and prevents it from being degraded (44).
LINC02802	1.4	-1.1	0	56	0.21	0.26	• Long-intergenic non-protein coding RNA 2802. • LINC02802 was upregulated following VEGF stimulation; knockdown significantly reduces sprouting activity (45). • LINC2802 prevents the anti-angiogenic effect of miR-486-5p by competitive binding post-transcriptional regulation of MAML3 (45).
ENST00000609241	1.2	-1.2	2	23	0.16	0.06	• Antisense to PGM5. • There is no literature available on the lncRNA. • PGM5 lacks enzymatic activity but is known as a dystrophin-binding protein. • PGM5 levels increase with chronic stimulation of the Rat TA, and decrease with denervation (46). • Knockdown of PGM5 in myotubes led to failure in myofibril assembly, alignment and membrane attachment and a massive reduction in myofibril number (47).
LINC01961	-1.2	1.1	1	27	-0.43	0.00	• Long intergenic non-protein coding RNA 332. • There is no literature available on the lncRNA
ENST00000412666	-1.2	1.1	4	36	-0.29	0.05	• Novel lncRNA. • There is no literature available on the lncRNA
TTN-AS1	-1.3	-1.1	0	40	-0.11	0.34	• Antisense to Titin • There is no literature available on the lncRNA. • Large structural protein in skeletal muscle. Serves as an adhesion molecule for contractile machinery.
ARHGEF26-AS1	1.2	-1.1	2	56	0.13	0.69	• Antisense to ARHGEF26 • ARHGEF26 is pro-angiogenic; essential to VEGF-induced in human endothelial cells (48). • Specifically, this protein is responsible for VEGF-induced cell-surface VEGFR-2 internalization by micropinocytosis (48). • ARHGEF26 is a guanine nucleotide exchange factor that activates Rho GTPases by exchanging GDP with GTP. Activated by VEGF in endothelial cells (48).

CARMN	1.2	-1.1	0	19	0.36	0.00	• Cardiac Mesoderm Enhancer-associated non-coding RNA. • In cervical cancer cells, CARMN overexpression inhibits miR-92a-3p expression via binding to miR-92a-3p, reducing the binding of miR-92a-3p to BTG2 to upregulate BTG2 transcription, which downregulated protein levels of Wnt3a and b-catenin to block the Wnt/b-catenin signalling pathway, consequently mitigating cervical cancer cell proliferation, migration, and invasion, and promoting apoptosis (49).
ENST00000515184	-1.6	-1.2	0	36	-0.28	0.02	• Novel lncRNA • There is no literature available on the lncRNA
CAPN1-AS2	1.3	1.1	0	48	0.24	0.11	• Antisense to Calpain-1 • Calpains are calcium-dependent cysteine proteases primarily responsible for the degradation of cytoskeletal and z-disk-associated proteins. (50) • Required for initial liberation of myofibrils. (50)
ENST00000537616	1.2	-1.0	0	68	0.15	0.02	• Novel lncRNA
ENST00000581351	-1.2	1.0	0	70	-0.24	0.05	• Novel lncRNA
LINC01905	-1.2	1.1	1	64	-0.30	0.03	• Long intergenic non-protein coding RNA 1905. • There is no literature available on this lncRNA.
LINC01255	1.3	1.0	1	72	0.21	0.04	• Long intergenic non-protein coding RNA 1255. • There is no literature available on this lncRNA.
ENST00000590024	-1.4	-1.1	0	44	-0.15	0.33	• Novel lncRNA, Antisense to Titin • There is no literature available on the lncRNA. Large structural protein in skeletal muscle. Serves as an adhesion molecule for contractile machinery.
LINC02018	1.2	-1.1	1	56	0.12	0.74	• Long intergenic non-protein coding RNA 2018. • There is no literature available on this lncRNA.
ENST00000610240	1.3	-1.0	0	62	0.34	0.04	• Novel lncRNA. • There is no literature available on this lncRNA.
ENST00000655610	1.2	1.0	1	72	0.15	0.44	• Novel lncRNA. • There is no literature available on this lncRNA.
ENST00000623720	1.2	-1.1	1	48	0.25	0.04	• Novel lncRNA. • There is no literature available on

							this lncRNA.
ENST00000568189	1.4	1.1	0	59	0.32	0.01	• Novel lncRNA. • There is no literature available on this lncRNA.
ENST00000648684	1.2	-1.0	0	64	0.18	0.06	• Novel lncRNA, antisense to TTC34. • There is no literature available on this lncRNA. • TTC34 is a structural motif of 34 amino acid tandem repeats, which form scaffolds to mediate protein-protein interactions and aid in the assembly of multiprotein complexes (51).
IDI2-AS1	1.2	-1.0	0	66	0.21	0.06	• Antisense to IDI2 • There is no information on the lncRNA. • Catalyzes the conversion of isopentenyl diphosphate to dimethylallyl diphosphate, a precursor for the synthesis of cholesterol (52).
ENST00000576873	-1.2	1.0	2	72	-0.06	0.62	• Novel lncRNA, antisense to GOLG8N. • No information available on the lncRNA. • GOLG8N is involved with golgi apparatus organization.
ENST00000444356	1.2	-1.1	1	48	0.25	0.04	• Novel lncRNA. • No literature available on this lncRNA.
MIR99AHG	-1.2	1.0	2	72	-0.17	0.03	• mir-99a-let-7c cluster host gene. • There is no literature available on this lncRNA.
KLHL30-AS1	1.2	-1.0	0	59	0.08	0.50	• KLHL30 antisense RNA 1 • There is no literature available on this lncRNA. • Proteins of KLHL30 contain a BACK domain (no known function); BTB/POZ domain (facilitates protein binding dimerization), and kelch domains (extracellular functions and binding to other proteins) (53).
CPB2-AS1	1.2	-1.1	2	36	0.06	0.17	• CPB2 antisense RNA 1 • There is no literature available on this lncRNA. • CPB2 hydrolyzes C-terminal peptide bonds.
ENST00000656127	-1.7	-1.2	0	10	-0.24	0.05	• Novel lncRNA. • There is no literature available on this lncRNA.
LINC02193	-1.2	-1.0	1	70	-0.25	0.14	• Long intergenic non-protein coding RNA 2193. • There is no literature available on this lncRNA.

DIRC3-AS1	1.2	-1.0	1	64	0.13	0.13	• DIRC3 antisense RNA 1. • No literature available on this lncRNA. • DIRC3 is also a lncRNA. • In differentiated thyroid cancer cells, DIRC3 may be related to IGF1 signaling by upregulating IGF1BP5, a suppressor of cancer (54).
ENST00000667754	-1.2	1.1	4	44	-0.30	0.16	• Novel lncRNA. • There is no literature available on this lncRNA.
ENST00000667385	1.2	-1.0	2	62	0.24	0.16	• Novel lncRNA. • There is no literature available on this lncRNA.
FAM27C	-1.2	1.0	4	66	-0.34	0.11	• family with sequence similarity 27 member C. • There is no literature available on this lncRNA.

Abbreviations: TGFβ, Transforming growth factor beta; DOCK8, director of cytokinesis 8; PGM5, phospho-glucomutase like 5;TA, tibalis anterior; DNMT, DNA Methyltransferase; VEGF, Vascular endothelial growth factor; MAML-3, Mastermind-like-3; BTG-2, B-cell translocation gene 2; TTC34, tetratricopeptide repeat domain 34; IDI2, isopentenyl-diphosphate delta isomerase 2; ARHGEF26, Rho Guanine Nucleotide Exchange Factor 26; GOLGA8N, Golgin A8 Family Member N; KLHL30, kelch like family member 30; CBP2, carboxypeptidase B2; DIRC3, disrupted in renal carcinoma 3; IGF1, insulin like growth factor 1; IGF1BP5, insulin like growth factor binding protein 5.

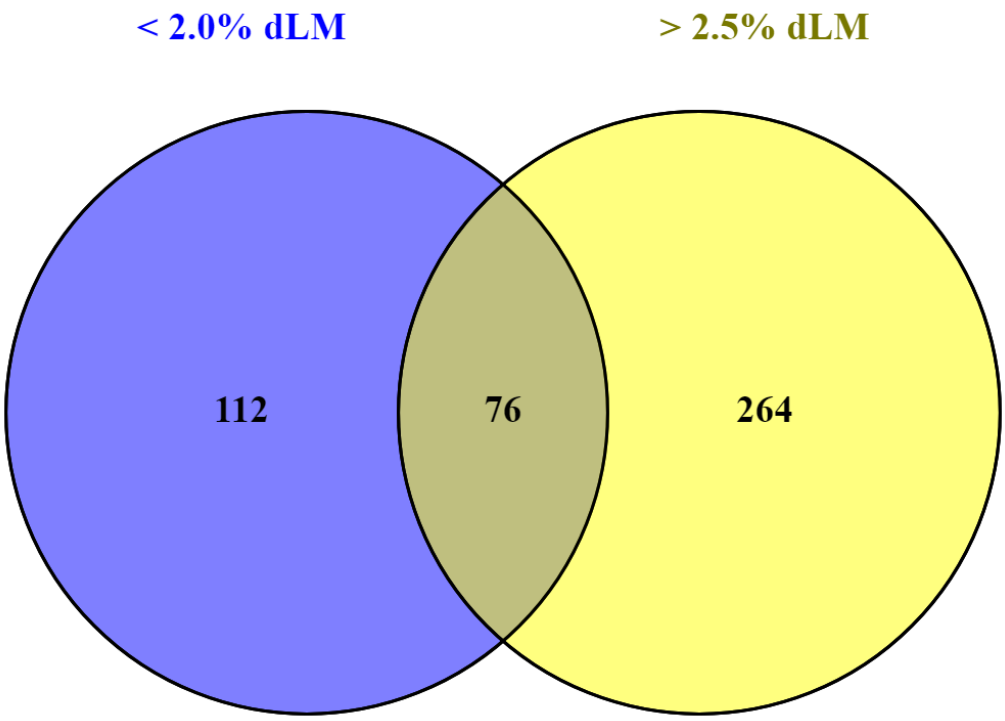


Figure 3. The number of lncRNA genes differentially expressed (DE) in the > 2.5% dLM and < 2.0% dLM groups and their overlap.

Data-Driven Network Analyses Yields Biological Insight Into > 2.5% dLM lncRNA Genes. *LINC00390* belonged to a module of 380 genes that consisted of genes centered around skeletal muscle contraction. The significant ontology categories that this network included, but were not limited to: muscle contraction (GO:0006936: FDR = 8.05 x 10^{-09}; fold enrichment (FE) = 11.5), muscle filament sliding (GO:0030049: FDR = 5.73 x 10^{-08}; FE = 32.4), sarcomere organization (GO:0045214: FDR = 7.73 x 10^{-03}; FE = 10.6), and transition between fast and slow fibre (GO:0014883: FDR = 8.19 x 10^{-08}; FE = 57.6) (Figure 4).

ENST00000655610 was nested within a large planar-filtered network of 1093 genes associated with positive regulation of T-cell proliferation (GO:0042102: FDR = 3.85 x 10^{-04}; FE = 6.10), adaptive immune response (GO:0002250: FDR = 2.96 x 10^{-05}; FE = 3.19), inflammatory response (GO:0002250: FDR = 2.96 x 10^{-05}; FE = 3.19), and neutrophil chemotaxis (GO:0002250: FDR = 2.96 x 10^{-05}; FE = 3.19). Another module of 130 genes contained the lncRNA gene, *IDI2-AS1*, and ontology analyses suggested that this module is related to the regulation of the mitochondrial transcriptome (e.g., ATP synthesis coupled proton transport, hydrogen ion transmembrane transport, mitochondrial translation).

Next, we aimed to elucidate whether there was any ontological overlap between our 40 > 2.5% dLM lncRNA genes and 40 <2.0% dLM lncRNA genes. In particular, two lncRNA genes *LINC02364* (<2.0% dLM lncRNA gene), and KLHL30-AS1 (> 2.5% dLM lncRNA gene) were nested within a module of 186 genes that yielded significant biological processes related to protein folding (GO:0006457: FDR = 2.77 x 10^{-03}; FE = 11.48), and protein stabilization (GO:0050821: FDR = 2.77 x 10^{-03}; FE = 10.36), suggesting that these biological processes may be related to general exercise adaptations, and are not reflective of exercise-induced LM change, *per se*. Crucially, we identified differences in biological processes between our 40 > 2.5% dLM

lncRNA genes and 40 < 2.0% dLM genes (Figure 5). Specifically, the network structures that > 2.5% dLM lncRNA genes belonged to were associated with several biological processes that were not enriched in the network structures that < 2.0% dlM lncRNA genes belonged to, including extracellular matrix organization (GO:0030198; FDR = 8.16×10^{-11}, FE = 15.50), angiogenesis (GO:0001525; FDR = 6.51×10^{-07}, FE = 8.63), mitochondrial translation (GO:0032543; FDR = 2.38×10^{-03}, FE = 12.23), adaptive immune response (GO:0002250; FDR = 2.96×10^{-05}, FE = 3.19), cellular response to amino acid stimulus (GO:0071230; FDR = 3.54×10^{-04}, FE = 17.70), and transition between fast and slow fibre (GO:0014883: FDR = 8.19×10^{-08}; FE = 57.6) (Supplementary Data S4).

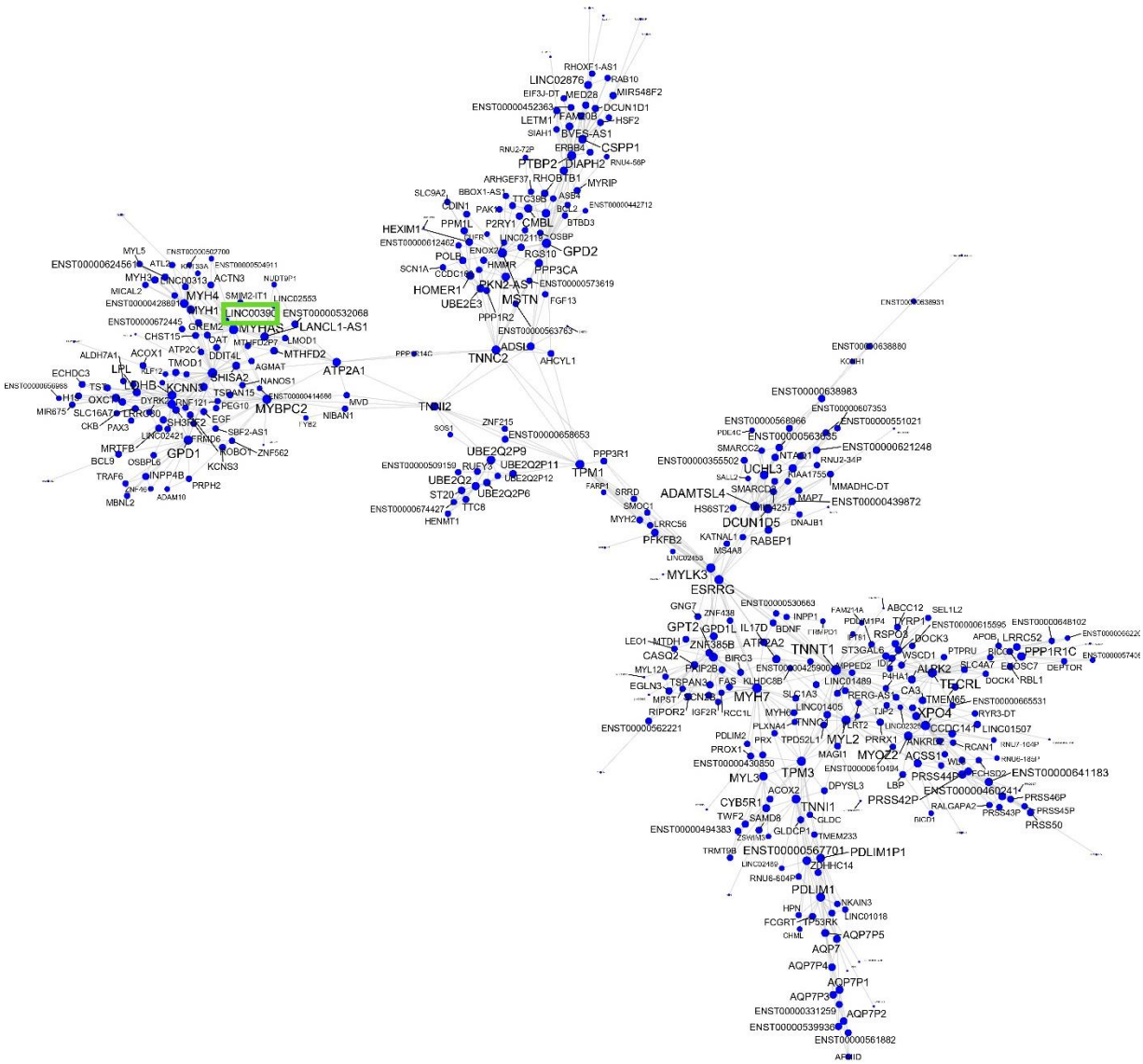


Figure 4. *LINC00390* belongs to a gene network structure associated with skeletal muscle contraction. An example network of gene interactions that belong to *LINC00390* (highlighted in green), a 2.5% dLM lncRNA-related gene. MEGENA (26) was used to identify discrete planar filtered networks using 407 pre-training RNA samples as input (FDR<1% and Spearman gene correlation; $P < 0.01$ for module significance and $P < 0.01$ for network connectivity). This network was associated with significant ontologies centred around skeletal muscle contraction (e.g., the transition between fast and slow fibre, sarcomere organization, regulation of skeletal muscle contraction, etc.). Node size is proportional to node degree.

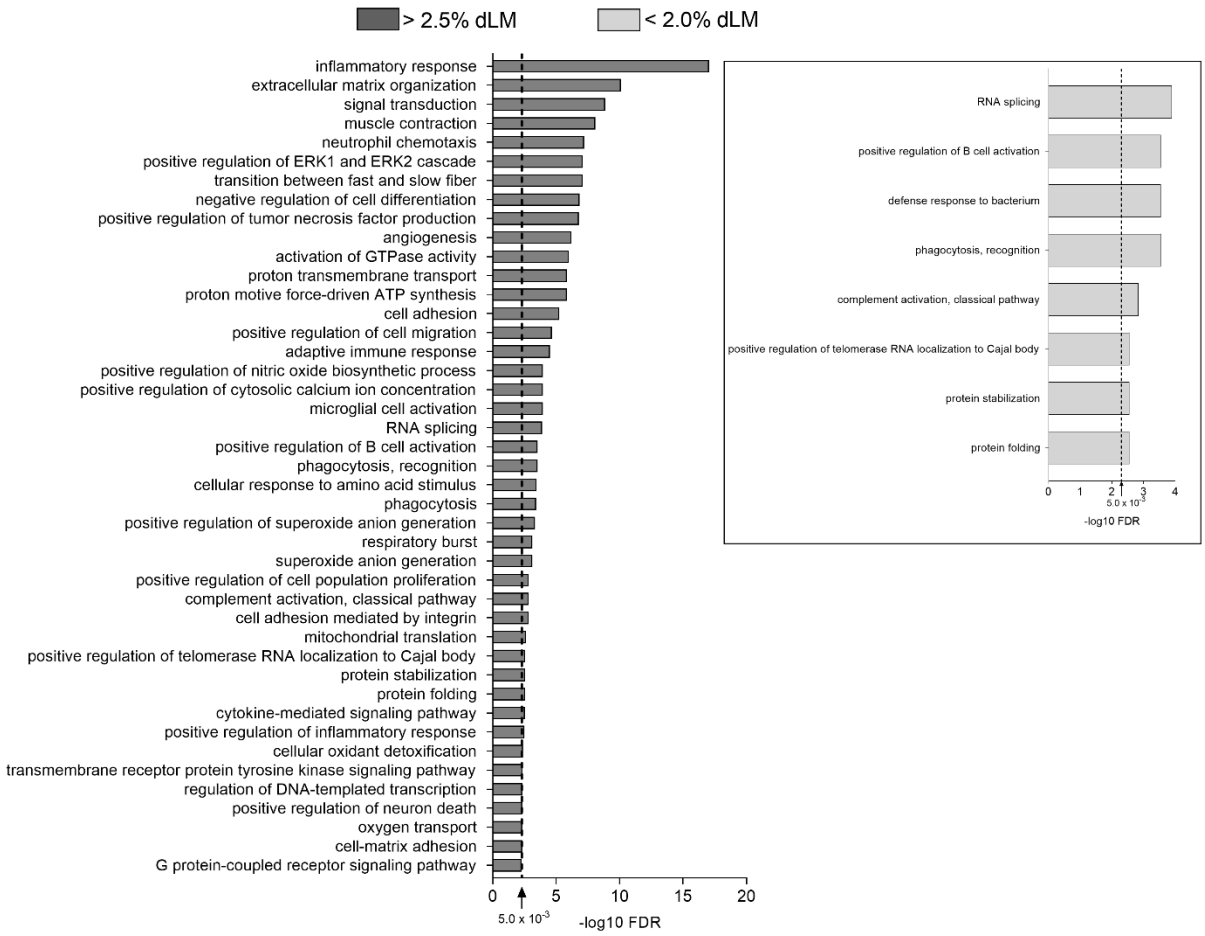


Figure 5. > 2.5% dLM lncRNA genes belong to network structures associated with unique biological processes not found in network structures that < 2.0% dLM lncRNA genes belong to. Significant (FDR ≤ 5.0 x 10^{-3}) REVIGO reduced ontology terms for network structures that ≥ 2.5% dLM lncRNA genes (inset: < 2.0% dLM lncRNA genes). The dotted vertical line indicates the -$\log_{10}$ FDR equivalent for 5.0 x 10^{-3}. Only network structures (and their ontologies) associated with 40 ≥ 2.5% dLM lncRNA genes and 40 < 2.0% dLM lncRNA genes were interrogated.

Low-Expressed lncRNA Genes Are Associated with Cell-Specific Gene Markers.

We observed a wide range in the average baseline expression (derived from 144 participants) of 40 > 2.5% dLM genes (Figure 6), which suggests that lower expressed lncRNA genes may be expressed in specific skeletal muscle fibre types or mononuclear cell type populations. Therefore, we used a linear modelling strategy to determine the association between several low-expressed lncRNA genes (Figure 6; highlighted in orange) and gene markers representative of distinct fibre or cell types. Notably, we found that *LINC00390,* a novel intergenic lncRNA gene, was positively correlated with type II fibre gene markers, *ATP2A1* ($r = 0.51$; FDR= 3.60×10^{-18}), *MYH1* ($r = 0.34$; FDR= 1.38×10^{-7}), and *MYH2* ($r = 0.34$; FDR= 6.4×10^{-03}), and negatively correlated with a type I fibre gene marker (*TNNT1*), and endothelial cell gene marker (*ENG;* Figure 7). *TTN-AS1*, a lncRNA gene antisense to the structural protein titin, was positively correlated with *ATP2A1* ($r = 0.37$; FDR= 1.24×10^{-08}) and *MYF6* ($r = 0.32$; FDR= 8.44×10^{-07}), a type II fibre and satellite cell gene marker, respectively (Supplementary Data S6). A novel lncRNA gene, *ENST00000655610,* was strongly associated with a T-cell gene marker, *CD4* ($r = 0.37$; FDR= 2.52×10^{-08}). The exercise-induced lncRNA gene, CYTOR, was not associated with any fibre type or cell population gene markers (Supplementary Data S6).

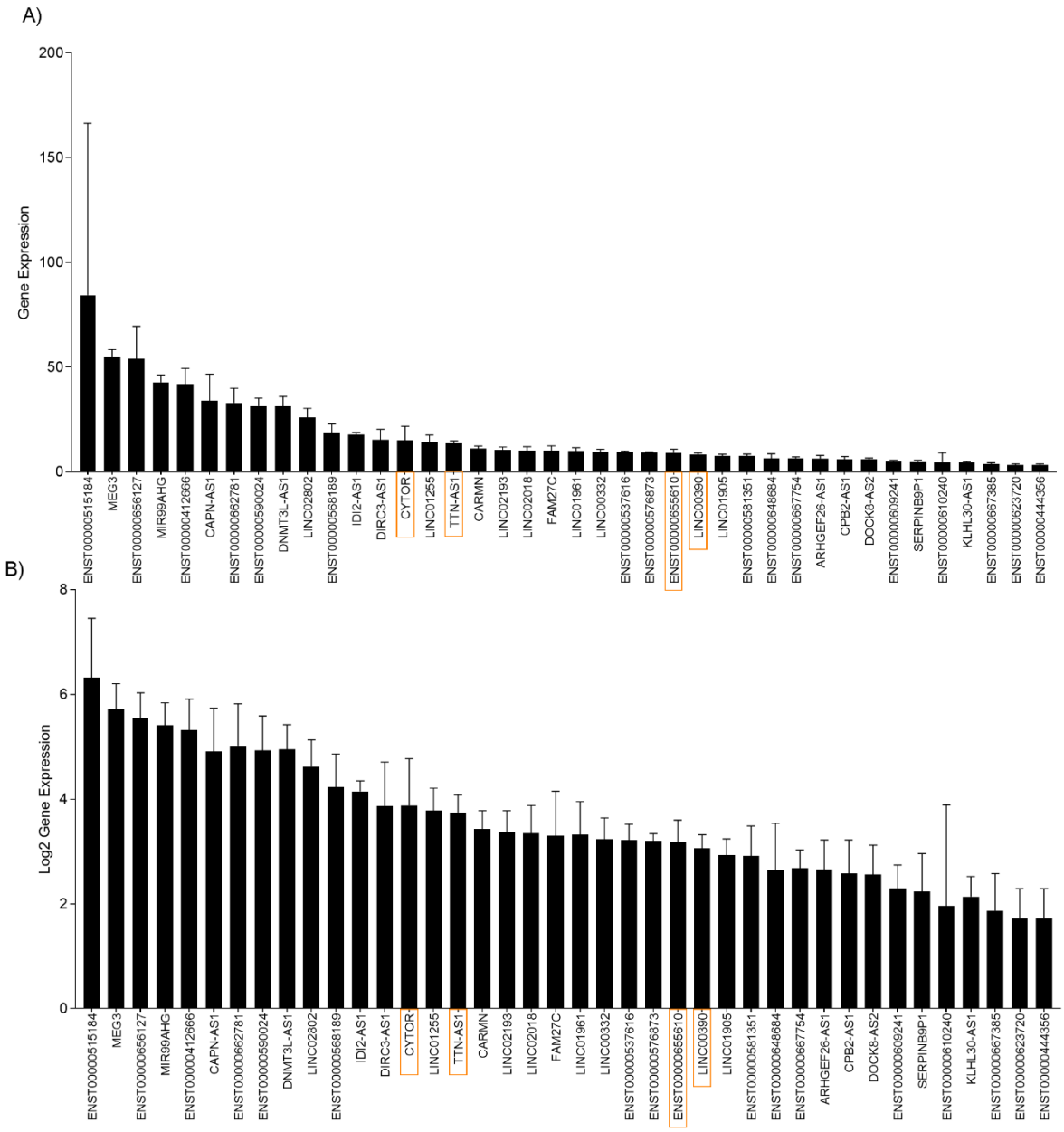
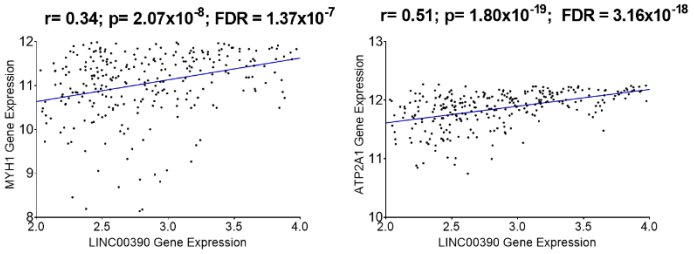


Figure 6. > 2.5% dLM lncRNA genes contain a wide dynamic range in mean gene expression. A) Mean gene expression and B) Mean Log_2 gene expression**.** Data represent 144 baseline samples from our 5 exercise trials. Orange rectangles highlight lncRNA genes interrogated for association with cell-type-specific gene markers (Supplementary Data S6). Data are means and SD.

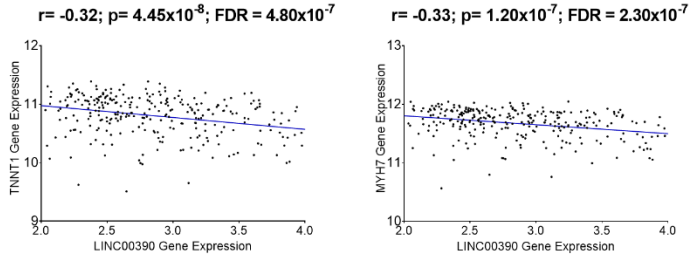
A) Type II Fibre Gene Markers

MYH1*
ATP2A1*
MYH2



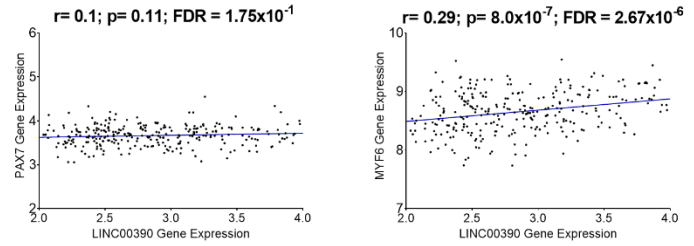
B) Type I Fibre Gene Markers

MYH7*
TNNT1*
PDLIM1
TPM3



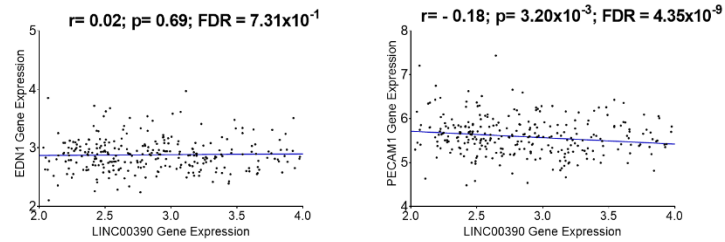
C) Satellite Cell Gene Markers

PAX7*
MYF6*
MYF5
MYOG



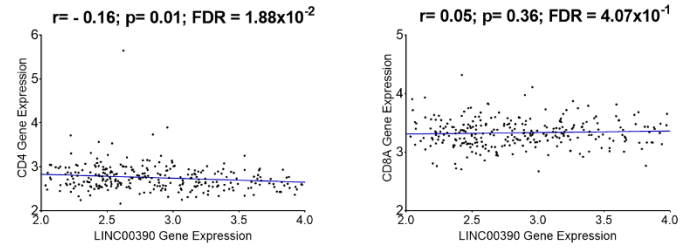
D) Endothelial Cell Gene Markers

PECAM1*
EDN1*
ECSCR
ENG



E) T-Cell Gene Markers

CD4*
CD8A*
CD8B



F) B-Cell Gene Markers

CD79A*
MS4A1*

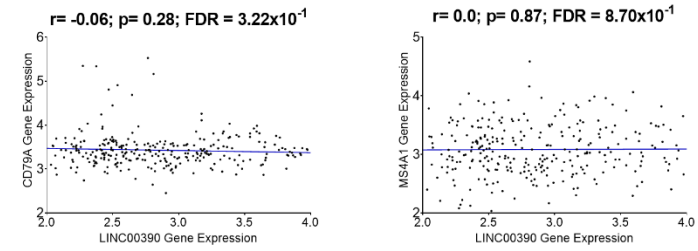


Figure 7. LINC00390 is positively correlated with type II fibre gene markers and negatively correlated with Type I fibre gene markers. Linear regression plots between LINC00390 mean gene expression and mean gene expression for A) type II fibre, B) Type I fibre, C) satellite cell, D) endothelial cell, E) T-cell, and F) B-cell gene markers. * Represent cell-type specific gene marker correlations displayed on the right. Cell-type specific gene markers without an asterisk are not displayed but can be found in Supplementary Data S6. P values were adjusted according to the number of cell-specific gene markers interrogated using Benjamini Hochberg (40). Absolute r values ≥ 0.3 and an FDR of $\leq 5.0x10^{-3}$ were considered meaningful.

Independent Validation of lncRNA Genes Using Skeletal Muscle Disuse Studies. Our two smallest *in vivo* studies (Fernadez-Gonzalo et al., (16); Mcleod et al., (unpublished)) did not significantly alter changes in gene expression of our dLM lncRNA genes, yet both studies were used to identify trends in changes in gene expression across all studies. There were several lncRNA genes with directionally consistent changes in gene expression across all 4 *in vivo* studies but they failed to reach statistical significance (Figure 8a). Notably, *CAPN-AS1* – lncRNA gene antisense to calpain1 – was significantly reduced following two weeks of immobilization (Figure 8b), and this change in gene expression was directionally consistent across all 4 *in vivo* studies (Figure 8a). In our largest disuse trial (n=18; Lim et al., (17)), *LINC00390* gene expression was significantly reduced following immobilization; however, the change in gene expression was not directionally consistent with the other 3 *in vivo* studies (Figure 8a).

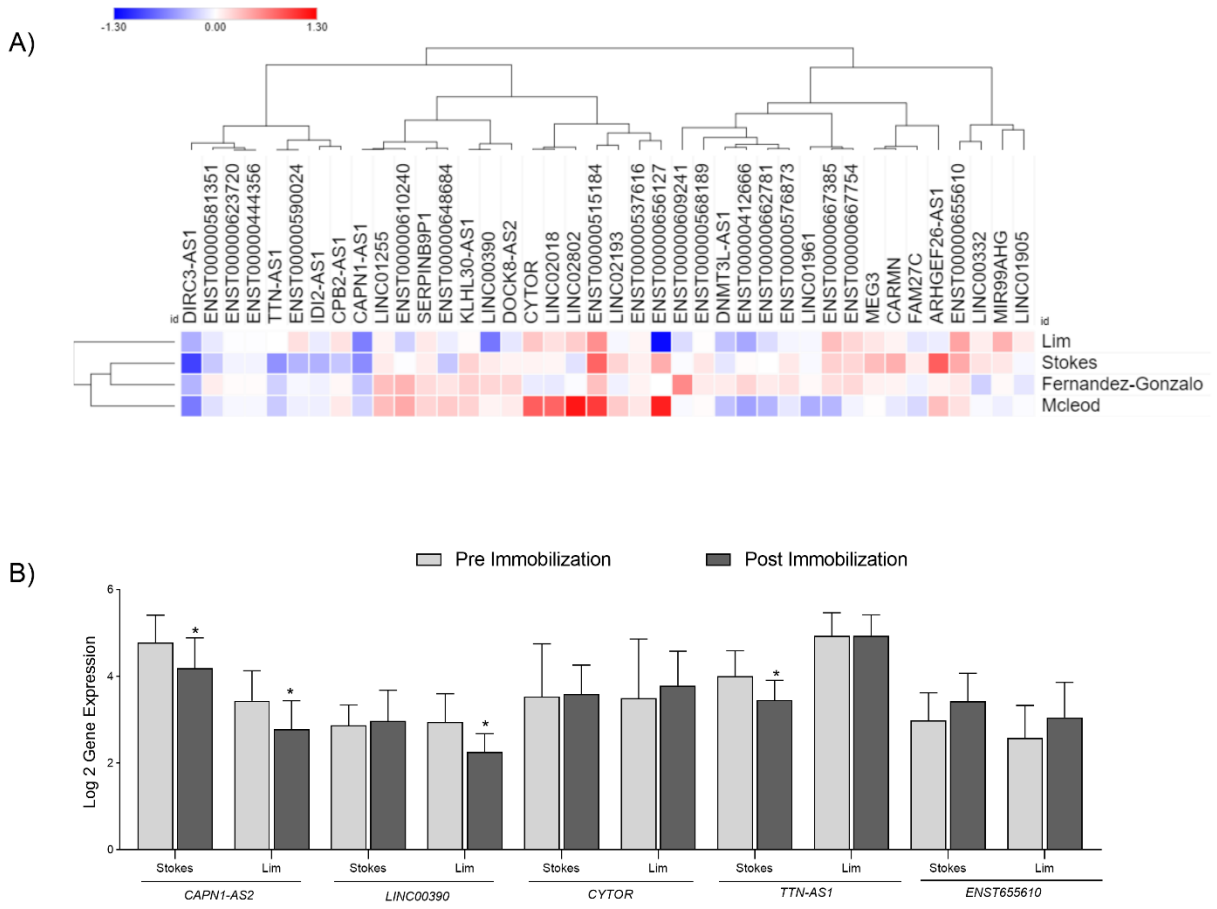


Figure 8. Independent Validation of lncRNA Genes Using Skeletal Muscle Disuse Studies. A) Heat plot depicting the changes in lncRNA gene across four skeletal muscle disuse trials (6, 16, 17) (Mcleod et al., unpublished), and B) represents pre and post-RT Log_2 gene expression for several lncRNA genes pre- and post-immobilization, from our two largest included studies (6, 17). These additional studies were all profiled on the same gene chip technology (HTA 2.0) and subjected to the same pre-processing, quality control, and normalization procedures described previously. For each gene, data were analyzed using a paired t-test per study. * Denotes a significant adjusted p-value ($p \leq 0.05$) at post- vs pre-immobilization, within the same study.

DISCUSSION:

We profiled the lncRNA transcriptome using microarray technology in 144 participants before and after supervised resistance exercise training. We found a core set of lncRNA genes uniquely regulated in individuals that accrued LM beyond the precision error of the DXA. Although we identified several lncRNA genes with known functions, most of the LM lncRNA genes are novel genes that have not been previously characterized. Several of our identified lncRNA genes were nested within gene networks encoding proteins involved in biological processes central to skeletal muscle physiology, such as angiogenesis, extracellular remodelling, and mitochondrial translation. We also found that some lncRNA genes were low expressed in skeletal muscle tissue, and by assessing their linear relationship with several gene markers representative of fibre-and mononuclear cell-type populations, we demonstrated that some of our lncRNA genes could be expressed in a cell-specific manner. Interestingly, human muscle disuse did not significantly regulate most dLM lncRNA genes. To our knowledge, ours is the first clinical transcriptomics study to interrogate the lncRNA transcriptome and leverage heterogeneous exercise-induced changes in LM to identify lncRNA genes uniquely associated with LM accrual.

The current understanding of transcriptomics in skeletal muscle biology is mostly limited to protein-coding genes (6, 15). Conversely, lncRNA have garnered much attention as regulators of cellular processes, such as translation and transcription (55); however, the lncRNA transcriptome is poorly characterized, especially within skeletal muscle biology. One reason for the poor characterization of lncRNAs is the popularized use of short-read RNA sequencing (41) and the concomitant use of Poly-A enrichment strategies (which some lncRNAs lack) that result in a median count of 5000 genes across various tissue types. In the current study, we used high-density microarrays processed with a GENCODE CDF, which enabled us to quantify ~45,000

lncRNA ENSTs (corresponding to ~12,000 lncRNA genes) in human skeletal muscle. However, it should be acknowledged that we did not utilize a tissue-specific CDF, which may provide more extensive coverage of the lncRNA transcriptome (~16,000 lncRNA genes; (10))

Using next-generation sequencing, Lavin and colleagues recently measured aspects of the lncRNA transcriptome in response to one bout of endurance exercise and high-intensity interval training (11). Although this study (11) provided insight into the temporal dynamics of the lncRNA transcriptome following a bout of exercise, it is difficult to reconcile whether the acute changes in lncRNA gene expression reflect physiological adaptations conferred through repeated exercise exposure. Further, there is considerable inter-individual variability to exercise training regarding the physiological outcome of interest, which can provide a unique opportunity to examine the relationship between transcriptomic responses to exercise and the magnitude of a physiological response (6). We leveraged interindividual variability in exercise-induced LM gains to identify a set of 91 lncRNA genes uniquely regulated in individuals with > 2.5% changes in LM; interestingly, most of these genes have never been characterized.

We identified *CYTOR* as a differentially expressed lncRNA gene, and recent work supports the importance of *CYTOR* in skeletal muscle. Specifically, Wohlwend and colleagues (12) showed that *CYTOR* expression was upregulated following an acute bout of leg extension. Further, the same group demonstrated that overexpression of *CYTOR* in mice promoted muscle growth (12). *CYTOR* may promote myogenesis by sequestering the *TEAD1* transcription factor, which prevents the binding of *TEAD1* to its target genes (12). *TEAD1* overexpression is implicated in the fast-to-slow skeletal muscle fibre type transition (56), and therefore its inhibition by *CYTOR* may be important for the maturation of type II skeletal muscle fibres.

Collectively, the results of the current study demonstrate that CYTOR may be an important regulator of LM accretion.

Most of the > 2.5% dLM lncRNA genes were novel or intergenic genes that have not been previously characterized (Table 2). Category enrichment analysis using GO is a widely used strategy that takes a set of differentially expressed protein-coding genes and statistically identifies enriched themes, and when biases are minimized (57), these themes may reflect biological processes regulated in a clinical experiment (6). However, functional labels have yet to be assigned to lncRNAs, so lncRNA gene sets cannot be mined for biological interpretation (58). Therefore, to overcome this limitation, we first used quantitative, data-driven (i.e., independent of any knowledge of gene function) network analysis (26) to identify if any of our > 2.5% dLM lncRNA genes were co-regulated with protein-coding genes in significant network structures. We then submitted the network structures to category enrichment analysis using GO categoriesfollowed by submitting a gene set comprising the network structure to GO analyses. This strategy allowed us to associate our lncRNA genes with biological processes. We found that novel or intergenic genes were embedded in significant network structures associated with biological processes such as angiogenesis, adaptive immune response, mitochondrial translation, and extracellular matrix remodelling. This finding is well aligned with recent work from our group (6) demonstrating that a core set of 141 genes correlated with the muscle growth response to chronic muscle loading in humans (n = 100), and these activated genes form functional networks that were observed to be associated with extracellular matrix remodelling, angiogenesis, and mitochondrial function. Therefore, the results of the current study demonstrate that lncRNA genes may be involved in cellular processes important in loading-induced skeletal muscle adaptation.

We detected that most lncRNA genes associated with exercise-induced LM change contain a low mean expression in human skeletal muscle biopsy samples. As skeletal muscle is a heterogenous tissue comprising multinucleated muscle fibres and mononuclear cell types, we hypothesized that several lncRNA genes might be cell-specific. *LINC00390* is a novel, uncharacterized lncRNA gene nested in biological processes related to skeletal muscle contraction, such as sarcomere organization, the regulation of skeletal muscle contraction, and the transition of fast to slow fibre. Interestingly, we found that *LINC00390* was positively correlated with type II fibre markers, yet negatively correlated with type I fibre gene markers and an endothelial cell gene marker. It is tempting to speculate that *LINC00390* may be related to fibre-type shifting following exercise training, but we cannot elucidate this from the current study. Nonetheless, the current data suggest that *LINC00390* may be a type II fibre-specific gene. An exciting future direction will be to determine if the change in type II fibre gene markers is correlated with the change in gene expression of *LINC00390*, following supervised exercise training.

We also found that *ENST00000655610* is low expressed in human skeletal muscle samples and human primary myotubes (data not shown), and its expression *in vivo* was positively associated with CD4 expression ($r = 0.37$; FDR= 2.52×10^{-08}), a T-cell gene marker. Strikingly, *ENST00000655610* was nested within a network structure associated with the biological processes: adaptive immune system and positive regulation of the T-cell population. *ENST00000655610* was not associated with CD8 expression, suggesting that this lncRNA may be expressed in $CD4^+$ T helper cells rather than CD8+ cytotoxic cells. CD4+ T-cells release inflammatory cytokines that act on skeletal muscle cells to support growth and regeneration (59). Collectively, we were able to identify two uncharacterized lncRNA genes (*LINC00390* and

ENST00000655610) that may be expressed in a cell-specific manner; these lncRNA genes were linked to biological processes pertinent to exercise-induced skeletal muscle adaptation. We only interrogated the association of our lncRNA genes with several cell type populations, while there are several other cell types in human skeletal muscle, such as fibroblasts, fibro-adipogenic progenitor cells, and pericytes (28). We also used the literature to select 2 – 4 genes to represent a cell type population; however, single-cell transcriptional profiles in human skeletal muscle revealed more than 20 gene markers per cell type (28). A future direction for bulk transcriptomic studies is to implement cellular deconvolution (60), a computational methodology that uses the bulk tissue expression of a gene and creates a linear combination of its expression levels with a cell type-specific expression matrix.

At the molecular level, skeletal muscle mass is primarily dictated by the balance of muscle protein synthesis and muscle protein breakdown. Increases in skeletal muscle mass may only precede when muscle protein synthesis rates exceed that of muscle protein breakdown. Compared with resistance training-induced increases in muscle protein synthesis, there is limited research describing the effects of resistance exercise training on muscle protein breakdown and the molecular mechanisms governing the response (61). However, calpains are one of the major molecular mechanisms regulating muscle protein breakdown (61). Specifically, calpains are calcium-dependent cysteine proteases responsible for degrading proteins that maintain the structural integrity of the sarcomere, including nebulin, titin, a-actinin, and desmin (50). We found that the expression of *CAPN-AS1*, a lncRNA antisense to calpain 1 (CAPN1), was significantly elevated in individuals who accrued LM. As an independent validation, CAPN-AS1 expression levels were significantly reduced following a period of reduced contractile activity (e.g., single leg immobilization, voluntary bed rest) across 4 different trials. This finding is

interesting, considering that *CAPN1* is inconsistently regulated in unloaded skeletal muscle (62). Indeed, data demonstrate reduced, unchanged or increased expression of calpains in response to muscle disuse (62). However, we did not measure gene expression of *CAPN1* (i.e., the sense strand, protein-coding gene), so we are unaware if the changes in expression of *CAPN-AS1* following different loading regimes result in changes in gene expression of *CAPN1*. Also, studies are needed to determine whether the sequence pairs between *CAPN1* and *CAPN-AS1* hybridize. Nonetheless, these findings demonstrate that *CAPN-AS1* is sensitive to contractile stimuli and may be an important lncRNA regulator of the skeletal muscle phenotype.

Despite the novel findings of this study, some limitations must be acknowledged. We relied on DXA to quantify changes in LM. It should be recognized that DXA does not measure skeletal muscle mass directly, and we used DXA-derived LM as a proxy for muscle; however, LM includes tissue from organs and connective tissue and is subject to the hydration status of the participant (63). A more direct and accurate measurement of muscle mass should be used, such as magnetic resonance imaging (64), ultrasonography (64) or D_3-creatine dilution (63). Surprisingly, most of our exercise-induced lncRNA genes did not statistically differ following a skeletal muscle disuse event. Although the aforementioned findings could be attributed to the low sample size used, a more likely explanation – and limitation of the current study – is that similar to exercise-induced heterogeneity in LM change, skeletal muscle disuse events also lead to highly heterogenous atrophic losses in LM (30, 31). Using microarray, Chen and colleagues (65) demonstrated that following a disuse event, individuals who lost more muscle mass had a transcriptomic response distinct from individuals less suspectable to atrophic losses. Our small sample size limited us from using heterogenous atrophic losses to validate our lncRNA genes. Although we used gene-gene co-expression networks to infer biological processes that our

lncRNAs may be associated with, we did not directly explore the biological features of our differentially expressed lncRNA gene sets. Novel tools such as *lnCompare* (58) are being developed to explore features directly, and gene sets directly related to lncRNA genes, and validating these tools is needed for their implementation in clinical transcriptomics.

In 144 participants across 5 independent studies, we leveraged interindividual heterogeneity in exercise-induced changes in LM following supervised exercise training to identify 91 lncRNA genes uniquely associated with LM accrual. We also showed that many of our lncRNA genes were embedded in co-expression networks associated with well-established biological processes necessary for loading-induced skeletal muscle adaptation. Further, although some of the lncRNA genes identified contain antisense function, most are novel genes that are not characterized and have a low mean expression. In particular, we identified two lncRNA genes, *LINC00390* and *ENST655610*, associated with cell-specific markers of type II fibres and CD4 T-cells, suggesting that these novel genes may be cell-specific. Notably, the gene expression of *CAPN-AS1*, a lncRNA gene antisense to Calpain 1, was reduced in individuals undergoing skeletal muscle disuse, demonstrating that *CAPN-AS1* is sensitive to loading stimuli. To our knowledge, this is the first study to measure the lncRNA transcriptome and its association with exercise-induced changes in LM. Future work is warranted to identify how several of our identified novel lncRNA genes mechanistically affect transcription and translation of protein-coding genes.

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CHAPTER 5: STUDY 4

Changes in Protein Synthesis During Short-term Unloading and Loading in Human Skeletal Muscle.

ABSTRACT

Purpose: We aimed to characterize changes in integrated rates of myofibrillar fractional synthetic rate (MyoFSR) in skeletal muscle under a variety of scenarios in young, healthy males.

Methods: Healthy young males (n=7) were recruited for the study. Within an individual, one limb was randomized to undergo single-leg immobilization, via a knee brace, for 14 days. The contralateral, non-immobilized leg was first used as a short-term control (CTRL) for 6 days, followed by 4 sessions of unilateral resistance exercise (RE; 8 days). Isometric muscle strength, leg lean mass (LLM), and *vastus lateralis* cross-sectional area (VL_{CSA}) were measured on day 1 (baseline), day 6 (short-term immobilization and CTRL), and day 14 (long-term immobilization and RE). Bilateral *vastus lateralis* skeletal muscle biopsies were collected throughout to measure myofibrillar fractional synthetic rate (MyoFSR).

Results: At day 6 (short-term immobilization and CTRL), LLM, muscle strength, and MyoFSR were significantly depressed in the IMMOB leg, and these measures remained decreased at day 14. Short-term RE was sufficient to increase measures of MyoFSR. There were no significant changes in any measures of the non-immobilized leg during the first 6 days (short-term immobilization and CTRL).

Conclusions: This study highlights that single-leg immobilization is sufficient to induce rapid decrements in muscle mass, strength and MyoFSR measures. In contrast, short-term RE increases MyoFSR. This study also highlights that a contralateral non-immobilized limb, at least for the measures here, is an appropriate internal comparator compared to the immobilized limb.

INTRODUCTION

Aside from its obvious role in locomotion, skeletal muscle plays a paramount role in various metabolic processes and maintaining muscle is important for human health. Skeletal muscle is a highly plastic tissue that can adapt to environmental stimuli by altering its metabolic and contractile properties. Reducing skeletal muscle contractile activity [1] or unloading muscle [2] results in skeletal muscle fibre atrophy. In contrast, increased skeletal muscle loading through resistance exercise training [3] leads to muscle fibre hypertrophy. Identifying molecular targets sensitive to changes in contractile activity may be important for maintaining skeletal muscle size and could yield therapeutic targets.

At the proteostatic level, skeletal muscle is regulated by the balance between muscle protein synthesis and muscle protein breakdown, with net protein balance being the algebraic difference between synthesis and breakdown. Altering rates of protein turnover changes protein abundance, and skeletal muscle proteins are constantly being turned over to maintain a robustly functioning proteome. Acute, periodic bouts of resistance exercise and sufficient dietary protein intake, increase muscle protein synthesis rates, resulting in a net positive turnover of skeletal muscle proteins [4, 5] and muscle fibre hypertrophy [6].

Compared to hypertrophy, atrophy during muscle disuse represents a state of net muscle protein loss; however, the etiology of atrophy and its underpinning mechanisms is debated [7]. Work from our group [1, 8-10] and others [11-14] attribute the loss of skeletal muscle mass with uncomplicated (i.e., disease-free) muscle disuse, for the most part, to a reduction in rates of muscle protein synthesis, rather than elevated muscle proteolysis [15]. Nonetheless, a more detailed investigation of the mechanisms leading to disuse atrophy is required to achieve clarity

as rates of protein synthesis decline and, as some suggest, rates of proteolysis may also be elevated [16].

The changes in skeletal muscle size observed following unloading or resistance exercise training are highly heterogenous across individuals [17, 18]. For example, in five hundred eighty-five subjects undergoing 12 weeks of progressive resistance exercise training, the relative percent change in biceps brachii cross-sectional area ranged from -2 to 59% [18]. The heterogeneity across humans provides a novel opportunity to examine the relationship between molecular targets and the magnitude of physiological change [10, 17, 19, 20]. Properly modelling physiological heterogeneity in molecular "omic" human studies requires utilizing large sample sizes [10, 19, 21]; however, this is not pragmatic for small-scale human physiology studies, which often entail invasive procedures and expensive analyses, thereby placing restraints on sample sizes [22]. Leveraging innovative human models are required to increase statistical power and reduce between-person heterogeneity. Our group recently utilized a within-person unilateral differential loading strategy, estimated to reduce heterogeneity for changes in muscle mass by ~40% [10]. Specifically, one leg performed resistance training for 10 weeks to induce skeletal muscle hypertrophy, whereas the other leg underwent single-leg immobilization for 2 weeks to induce skeletal muscle atrophy [23].

A common criticism of unilateral disuse models is that skeletal muscle adaptations in one limb can transfer to the contralateral limb, invalidating the contralateral limb as a control. A recent meta-analysis in healthy adults demonstrated that single-leg disuse did not impact knee extensor size in the contralateral, non-immobilized limb [24]. Further, Kilroe and colleagues [12] showed that 7 days of single-leg immobilization was sufficient to depress myofibrillar muscle protein synthesis rates, with no statistically significant change in the non-immobilized limb.

Comparison of differences in individual protein kinetics between the immobilized and non-immobilized limb would further validate the use of unilateral human models in skeletal muscle metabolism research.

The aim of this study was to characterize the changes in integrated rates of myofibrillar fractional synthetic rate (MyoFSR) in skeletal muscle under a variety of scenarios in young, healthy men. We adopted a paired model, such that, within an individual, one limb was exposed to unloading to induce atrophy (14 days), whereas the contralateral, non-immobilized limb was first used as a short-term *bona fide* control (6 days), followed by exposure to short-term loading (8 days). We aimed to determine if short-term (6 days) unilateral skeletal muscle unloading was sufficient to alter MyoFSR of the immobilized and contralateral, non-immobilized limbs. We hypothesized that 6 days of unloading would alter MyoFSR; in contrast, we hypothesized that MyoFSR would remain largely unchanged in the *bona fide* control limb. The second objective of this study was to contrast the MyoFSR of muscle subjected to single-leg immobilization with the contralateral limb subjected to a short-term hypertrophic stimulus (i.e., short-term resistance exercise). We hypothesized that there would be differential regulation of MyoFSR by both loading and unloading.

METHODS

Experimental Design

For an overview of the experimental design, please see Figure 1. The total duration of the trial was 3 weeks. The first week was a lead-in phase, whereas the remaining two weeks were the experimental trial phase. Immediately following baseline data collection on day 1, participants

had one of their limbs randomized (www.randomization.org; the allocation list was concealed and handled by a third party with no knowledge of the trial) to undergo 14 days of immobilization employing a knee brace [9, 10] (IMMOB leg), whereas the contralateral, non-immobilized leg was first used as a short-term control for 6 days; thereafter, the non-immobilized leg performed 4 unilateral resistance exercise sessions (leg extension and leg press), dispersed over 8 days, with 24 hours rest in between each session (RE leg). During the experimental trial phase, body composition, *vastus lateralis* cross-sectional area, and maximal isometric voluntary knee extension were assessed on days 1, 6, and 14. Nine skeletal muscle biopsies were obtained from the *vastus lateralis* and used, in conjunction with deuterium oxide ingestion, to quantify integrated rates of muscle protein synthesis. Participants reported to the laboratory following an overnight fast for study visits requiring skeletal muscle biopsies. This study was registered on clinicaltrials.gov (NCT04514744).

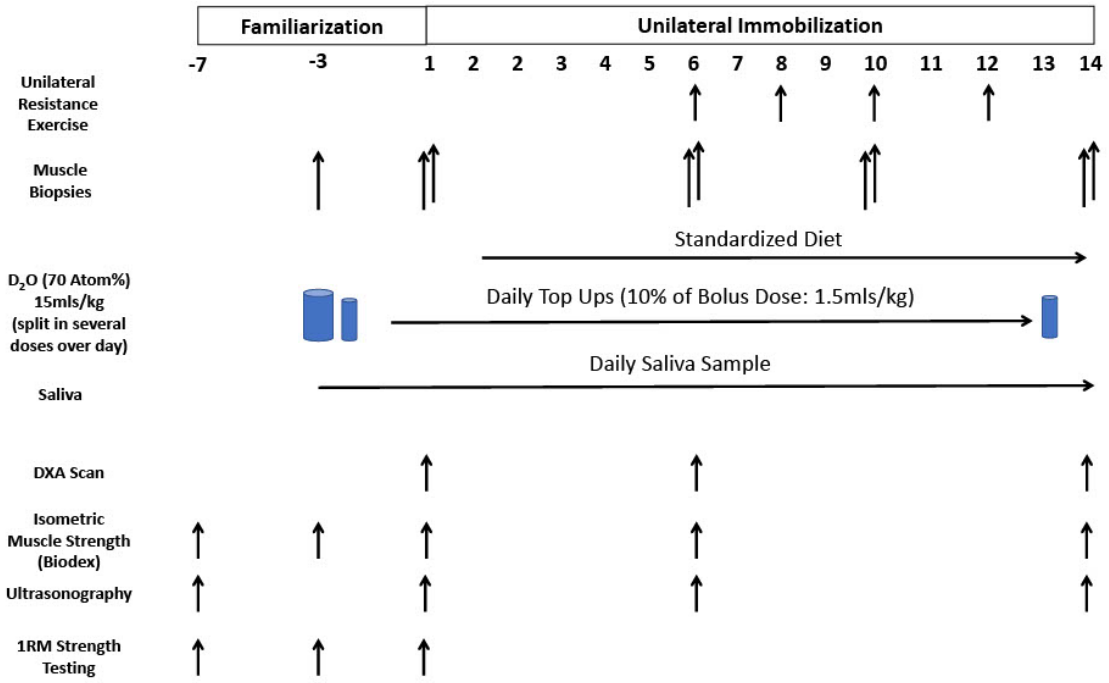

Figure 1. Overview of Experimental Design.

Single-leg Immobilization

Immobilization was accomplished by applying an X-ACT ROM knee brace (DonJoy, Vista, CA, USA). The brace angle was adjusted to permit toe clearance during ambulation with crutches without active hamstring flexion (~60° of flexion). The angle was subsequently locked into place with tabs provided by the manufacturer and secured with ties to prevent angle unlocking. The tape was wrapped around the brace, and an investigator's signature was written such that if the brace were removed, the tape would be damaged. An investigator briefly removed the brace at each study visit, and the immobilized leg was inspected for signs of deep vein thrombosis by checking for a robust dorsal pedal pulse. There were no reports of adverse events, and only minor skin chaffing, which required minor brace adjustments, was reported.

Standardized Diet

For the entire experimental trial phase, participants were provided with all foods that consisted largely of flash-frozen and prepackaged foods (Heart to Home, Brampton, ON, Canada) and various snacks and fruits. Protein intake was controlled at 1.0 g/kg body mass/d, fat comprised 30% of daily energy requirements, and carbohydrates comprised the remainder of the diet [9, 10]. The daily energy requirements of each participant were calculated using the Harris-Benedict equation [25], adjusted with an activity factor based on the results of the International Physical Activity Questionnaire [26].

Activity Monitoring

Throughout the entire study (lead-in phase and experimental trial phase), participants wore an ActiGraph wGT3X-BT activity monitor (ActiGraph, Pensacola, FL, USA) on their wrist to track daily step count as an indicator of physical activity levels. Data were downloaded from the activity monitors and analyzed using ActiLife version 6 13.2 software (ActiGraph, Pensacola, FL, USA).

Unilateral Resistance Exercise

The contralateral, non-immobilized limb underwent resistance exercise sessions on days 6, 8, 10, and 12. On days 6 and 10, resistance exercise sessions were performed after primary data collection. Each resistance exercise session was supervised by a strength and conditioning coach and consisted of 4 sets of 8 - 12 repetitions (corresponding to ~80% one-repetition maximum) of unilateral leg press and leg extension. 2 mins of recovery were provided between sets and exercises. Participants were instructed to go to volitional fatigue, defined as an inability to complete a repetition with a full range of motion. The load was adjusted for the subsequent bout if the participant completed more than 12 or less than 8 repetitions.

1-Repetition Maximum

To determine initial resistance exercise working loads, unilateral leg extension and unilateral leg press one-repetition maximum were performed on 2 separate occasions during the familiarization phase (days -7 and -2) and then tested on day 1. Proper performance of unilateral leg extension and leg press contractions was demonstrated, after which participants performed one set of non-exhaustive contractions while critiquing and adjusting their form. On day -7, before one-

repetition maximum determination, participants completed 1 submaximal set of 8 – 12 repetitions on the leg press and leg extension machine (~ 20 – 60% of their estimated one-repetition maximum). Participants then attempted to lift 90% of their predicted one-repetition maximum. Weight was progressively increased until the participant could complete a full repetition with an adequate range of motion. Participants were provided ~ 5mins of rest in between each attempt. One-repetition maximum load on both the leg press and leg extension was re-tested on days -3 and 1 verified or adjusted accordingly and was used for subsequent calculation of unilateral resistance exercise working loads.

Dual Energy X-Ray Absorptiometry

Body composition and leg lean mass (LLM) were assessed using a dual-energy absorptiometry scan (GE Healthcare). Measurements were determined at the same time of day (0600–0900) in the fasting state following a urinary void.

Isometric Maximal Voluntary Contraction

Unilateral isometric maximal voluntary contractions were performed on days 1, 6, and 14 using a Biodex dynamometer (Biodex System 3, Biodex Medical Systems, Shirley, NY, USA) to assess peak knee extensor torque. During each session, both legs were assessed for peak knee extensor torque in a randomized fashion. Prior to baseline, participants underwent two familiarization sessions (days -7 and -2) during the lead-in phase to avoid practice effects. Peak torque (in Nm) was recorded. During familiarization sessions, chair settings were adjusted for each participant such that the knee joint was aligned with the axis of rotation of the machine. All chair settings

were recorded for replication during subsequent testing sessions. During each session, participants performed four maximal isometric knee extensor contractions at 60 degrees (from the resting 90 degrees neutral positions). Each contraction was held for 5s, and participants were given 120s rest between each contraction. Researchers provided standardized verbal encouragement during each contraction to limit a motivational influence on trial performance.

Ultrasonography

The mid-thigh cross-sectional area of the *vastus lateralis* (VL_{CSA}) was measured by B-mode ultrasonography using an 18L5 probe (BK Medical North America, Peabody, MA, USA), on days 1, 6 and 14. Our group has demonstrated that ultrasonography is a suitable alternative to magnetic resonance imaging for quantifying changes in VL_{CSA} [23]. Participants laid supine on an exam bed for at least 10 minutes to normalize any potential fluid shifts in the body prior to images being acquired. Participants' feet were placed in a custom apparatus that prevented depression of the thigh against the bed. Ultrasound images were recorded from the mid-thigh of the *vastus lateralis.* We determined the mid-thigh of the *vastus lateralis* by marking equidistant between the head of the greater trochanter and the lateral epicondyle of the femur. The distance of the vastus lateralis mid-thigh from the femur's greater trochanter was recorded to ensure repeatable probe placement for subsequent sessions. A straight line was drawn down the leg perpendicular to the beds surface with horizontal markings made every 2 cm, which served as a guideline for ultrasound probe placement. Images were captured 2cm apart for 7 – 9 images. The same investigator collected images during each visit to reduce variability between image acquisition. Acquired images were stitched together using Gnu Image Manipulation Program (GIMP, version 2.9.22, Mountain View, CA, USA) to create a successive image of the *vastus*

lateralis by aligning subcutaneous fat, superficial and deep aponeuroses, and intramuscular fat deposits between images. VL_{CSA} was analyzed using the Polygon tracing tool in ImageJ. The same investigator conducted these procedures but was blinded to both group and time during VL_{CSA} determination.

Isotope Protocol: Deuterated Water Ingestion

Oral consumption of deuterated water (70 atom %; Cambridge Isotope Laboratories, Tewksbury, MA, USA) was used to label newly synthesized skeletal muscle proteins as previously described [27]. Based on work from our lab [1, 9, 23], low-loading doses of deuterated water (~ 0.65mL/kg of body mass) are adequate to maintain the body water pool at ~0.5% atom percent excess (APE), which is sufficient for bulk measurements of skeletal muscle protein synthesis. However, to ensure adequate labelling for individual protein kinetics (not described in the current thesis), highly enriched body water pools are required (~ 1 - 2 % APE, depending on the sensitivity of the instrument) [28]. Participants reported to the laboratory in the fasting state on day -2, and following the collection of a saliva sample and skeletal muscle biopsy, participants began to consume a loading dose of deuterated water, totalling 15mL/kg of body mass, to increase the body water pool to ~2% APE. To our knowledge, ours was the first study to administer larger loading doses of deuterated water, and thus, we opted to split the loading dose into 15 aliquots of 1.5mL/kg body mass over 48 hours (days -2 and -1). All participants successfully consumed the loading dose, and no adverse events were reported aside from minor sensations of nausea and dizziness. Thereafter, participants consumed 1 maintenance dose of deuterated water (1 aliquot of 1.5mL/kg of body mass) daily to preserve body water enrichment at ~2% APE throughout the experiment. Daily saliva samples were obtained using a cotton swab until completely saturated

with saliva. Saliva samples were centrifuged at 4000 rpm for 10 min at 4°C, after which aliquots of each were snap-frozen in liquid nitrogen and stored at -80°C for subsequent analysis, as previously described [10].

Measurement of Body Water Enrichment

Each saliva sample was centrifuged at 1500 g for 10 minutes at 4 °C, and the supernatant (water phase) was collected for further analysis. The sample was then diluted using double-distilled water to a ratio of 1:400 and analyzed for deuterium enrichment utilizing a cavity ring-down spectroscopy and liquid isotope analyzer (Picarro L2130-I, Santa Clara, CA) in express mode. Six wet flushes were performed per sample, followed by four injections; data generated from the first injection was discarded, and the final three injections were used to determine the average deuterium isotopic concentration. Internal standards containing low, medium, and high deuterium enrichments were tested in parallel before and following each participant's samples to account for drift in enrichment. The deuterium enrichments were initially expressed as changes in deuterium % relative to Vienna Standard Mean Ocean Water and were converted to APE using standard equations, as previously described [27].

Vastus Lateralis Skeletal Muscle Biopsies

During the study, nine muscle biopsies were collected (unilateral biopsy: days -2; bilateral biopsies: days 1, 6, 10, 14) from the *vastus lateralis*. All muscle biopsies were obtained using a 5-mm Bergstrom needle, custom modified for manual suction under 1% xylocaine local anesthesia. Through manual dissection, muscle tissues were carefully freed from visible

connective tissue, fat, and blood. The muscle tissue was snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

Isolation of Myofibrillar Protein Fraction

Between 20 and 30 mg of muscle was used. The muscle tissue was homogenized with scissors in an Eppendorf tube using 10 μL/mg−1 of ice-cold homogenization buffer (50 mmol Tris–HCl, 1 mmol EDTA, 1 mmol EGTA, 10 mmol β-Glycerophosphate, 50 mmol NaF, 0.5 mmol of activated sodium orthovanadate, pH 7.4 (all from Sigma Aldrich, Poole, UK) containing a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK). The homogenate was mixed at 1000 rpm for 10 min before being centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant (sarcoplasmic fraction) was then collected and stored at -80 °C until further analysis. The myofibrillar pellet was resuspended in 500 μL mitochondrial extraction buffer (MEB), and then Dounce homogenized and centrifuged at 11,000 g at 4°C for 5 min. The myofibrillar pellet was washed with 500 μL of MEB, vortexed, and centrifuged at 1000g. The myofibrillar pellet was then stored at -80°C until further analysis.

Bulk Integrated Myofibrillar Fractional Synthetic Rate (MyoFSR):

The myofibrillar pellet was solubilized using 1 mL of 0.3M NaOH incubated for 30 min at 37°C, then centrifuged for 10 min, at 4°C and 10,000 rpm. The myofibrillar fraction (supernatant) was separated from the insoluble collagen pellet, and precipitated by adding 1 mL of 1M perchloric acid, following incubation at 4 °C for 20 min. The myofibrillar protein was pelleted at 4000 rpm for 20 min, separated from the supernatant containing the free amino acids, then washed twice

with 70% ethanol. After adding 1 mL of 0.1M HCl and 1 mL of a Dowex H+ resin slurry, the myofibrillar samples were incubated overnight at 110 °C to release the protein-bound amino acids [20, 31, 32].

As previously described, the myofibrillar amino acids (AA) were derivatized as their N-methoxycarbonyl methyl esters (MCME) [33]. Briefly, the AA were resuspended in 60 μL of distilled H2O and vortex mixed before adding 32 μL of methanol and 10 μL of pyridine. 8 μL of methylchloroformate (MCF) was carefully added directly into the aqueous mix, and immediately vortexed for 30 s. The solution was left at room temperature for 5 min to react. After adding 100 μL of chloroform and 100 μL of 0.001 M NaHCO3, the samples were vortexed mixed to extract the MCME amino acids. The sample was transferred to an autosampler vial, ready for mass spectrometric analysis. Deuterium enrichment into protein-bound alanine was measured using gas-chromatography-pyrolysis-isotope-ratio-mass spectrometry (GC-pyrolysis-IRMS, Delta V Advantage, Thermo Scientific) [34]. The calculation of MyoFSR were based on the following product-precursor equation:

$$FSR\ \left(\frac{\%}{day}\right) = \left[\frac{(\Delta E_{ALA})}{E_{BW}\ x\ t}\right]\ x\ 100$$

where E_{Ala} is deuterium enrichment of protein-bound alanine, E_{BW} is mean alanine enrichment corrected from D2O, and t is the time between muscle biopsies.

Statistical Analyses

All analyses were performed in R version 4.0.4, using the following R packages: '*ez'*, '*emmeans'*, '*ggpubr'*, '*car'*, and '*rstatix'*. Before undertaking statistical analyses, data were

checked for normality (visually assessed using histograms and qqplots; statistically evaluated using the Shapiro Wilk Test), and homogeneity of variance was assessed using Levene's test. Step count data were compared using a paired t-test. Peak torque, lean mass, ultrasound cross-sectional area, and myofibrillar fractional synthetic rate were analyzed using a two-way repeated measures analysis of variance [29], with time (3) and leg (2) as within-subjects factors. For this thesis, data reported on myofibrillar synthetic rate are derived from 4 out of 7 participants. Therefore, to determine the effect of our intervention on myofibrillar protein fractional synthetic rate, independent of sample size restrictions [30], we also calculated an effect size for the group x time interaction, using partial eta squared ($\eta 2p$) and the effect size was reported as small, .02; medium, 0.13; or large, 0.26 [31]. Body water enrichment (i.e., APE) was assessed using a one-way repeated measures ANOVA with time as a within-subjects factor. Sphericity was assessed, and the Greenhouse-Geisser correction was applied to the ANOVA if the sphericity assumption was violated. If the ANOVA test revealed significant interactions or main effects, a Tukey's HSD (we used Fisher's LSD for MyoFSR) post hoc test was employed to interrogate pairwise differences between legs and across time points. Data are reported as means±standard deviation [32], and results were considered statistically significant when $p \leq 0.05$.

RESULTS

Participant Characteristics and Activity Monitoring

Briefly, 7 male participants (age, 21 ± 1 years; weight, 79 ± 8 kg; height, 1.8 ± 0.1 m; LLM, 19 ± 3 kg) completed the study. The average number of steps taken per day was significantly ($p <$

0.05) lower during the experimental trial phase (4868 ± 1073 steps/d) compared with the lead-in phase (9039 ± 3150 steps/day).

Knee Extensor Peak Torque, LLM, and VL_{CSA}*:*

A two-way repeated measures ANOVA revealed a significant ($p < 0.05$) time x leg interaction for knee extensor peak torque, LLM and VL_{CSA} (Table 1). At day 1 (baseline), there were no differences in knee extensor peak torque, LLM and VL_{CSA} across legs. Over the first 6 days (short-term immobilization and CTRL) of the experimental trial, knee extensor peak torque and LLM of the CTRL leg was not statistically different from day 1 (baseline), whereas the IMMOB leg showed a 16% and 3% significant reduction ($p < 0.05$) in peak torque and LLM, respectively. At day 14 (long-term immobilization and RE), compared with the RE leg, knee extensor peak torque, LLM and VL_{CSA} of the IMMOB leg were significantly depressed. Compared with day 1 (baseline), VL_{CSA} at day 14 (long-term immobilization and RE) decreased by 11% in the IMMOB leg, whereas the RE leg showed a 6% increase.

Table 1. Knee Extensor Torque, LLM and VL_{CSA} for the IMMOB Leg and RE Leg.

	IMMOB			RE		
	Day 1	Day 6	Day 14	Day 1	Day 6	Day 14
Peak Torque (Nm)	189 ± 23	158 ± 23 *	159 ± 32 *	189 ± 27	193 ± 21‡	212 ± 20 ‡
LLM (kg)	10.6 ± 1.7	10.3 ± 1.7 *	10.1 ± 1.8 *	10.7 ± 1.5	10.5 ± 1.6	10.7 ± 1.7 ‡
VL_{CSA} (cm^2)	26.8 ± 5.4	26.1 ± 5.9	23.9 ± 5.1 * †	26.5 ± 5.2	26.6 ± 4.1	28.1 ± 5.3 * ‡

Data are means ± SD. Day 1 = baseline; Day 6 = short-term immob and CTRL; Day 14 = long-term immob and RE. * Denotes a statistical difference ($p < 0.05$) from day 1 value in the respective leg. † Denotes a significant difference from day 6 value in the respective leg. ‡ Denotes a significant difference between legs at a given time point. LLM, leg lean mass; VL_{CSA}, *vastus lateralis* cross-sectional area.

Body Water Enrichment

APE values of ^{2}H enrichment in saliva is presented in Figure 2. The mean ^{2}H enrichment throughout the experiment was 2.30 ± 0.43.

MyoFSR:

An ANOVA revealed a significant ($p \leq 0.05$) time x leg interaction, with a large reported effect size ($\eta^2p=0.28$). In the IMMOB leg, MyoFSR was significantly depressed at day 6 (short-term immobilization and CTRL), and day 14 (long-term immobilization and RE), compared with day 1 (baseline). In the CTRL leg, MyoFSR was unchanged at day 6 (short-term immobilization and CTRL), compared with day 1 (baseline). At day 14 (long-term immobilization and RE), MyoFSR was significantly increased in the RE leg, compared with day 6 (short-term immobilization and CTRL). At day 14 (long-term immobilization and RE), MyoFSR was significantly depressed in the IMMOB leg, compared with the RE leg (Figure 3).

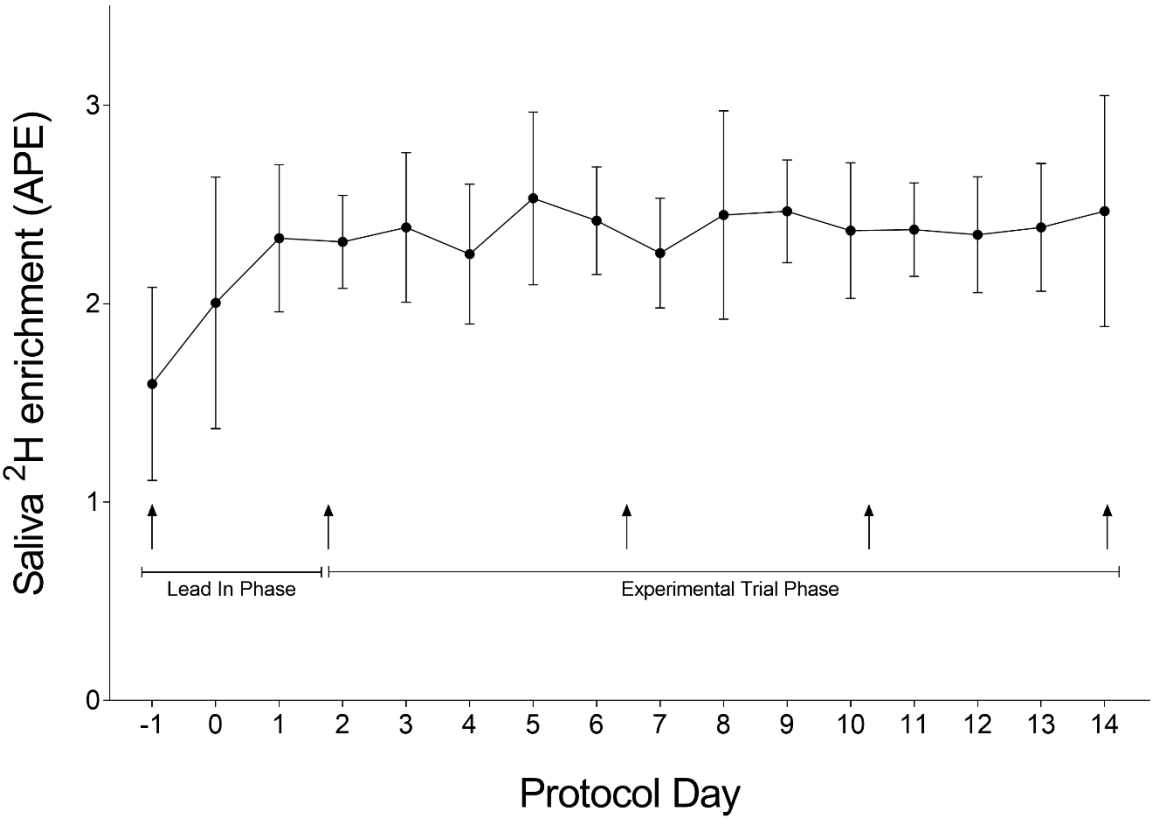

Figure 2. Saliva ^{2}H enrichment (APE) throughout the duration of the study. APE, atom percent excess.

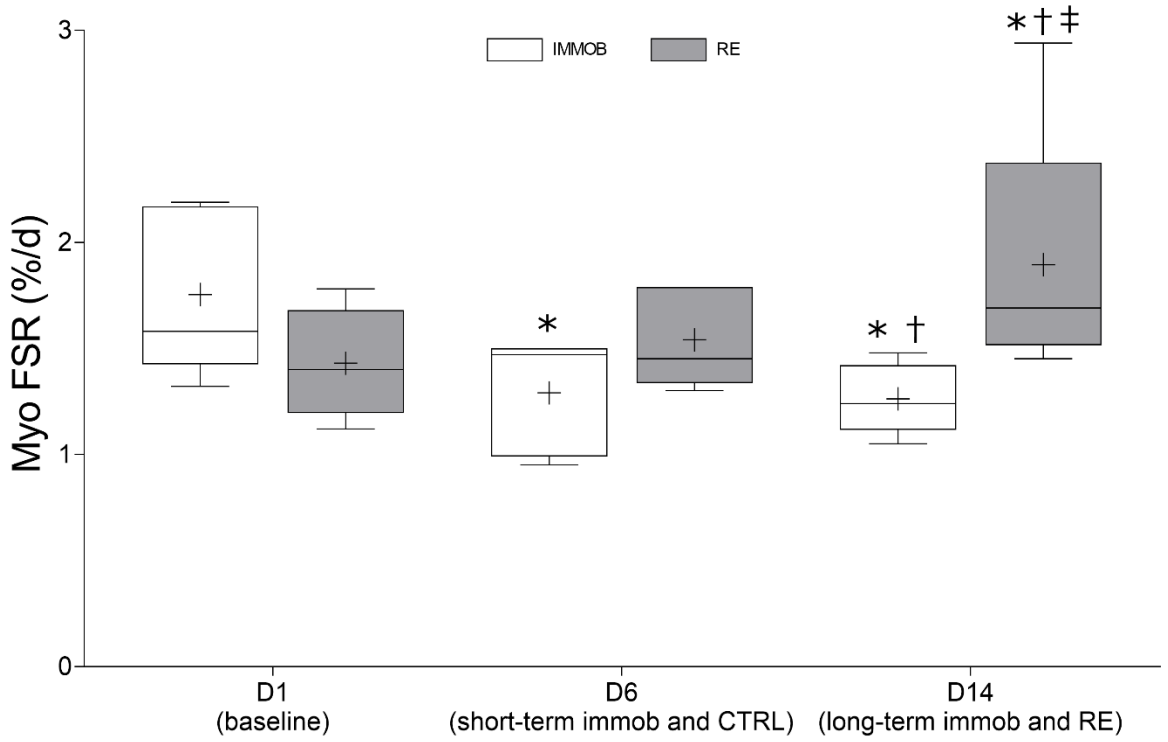

Figure 3. Integrated rates of MyoFSR throughout the intervention. The data are displayed as box and whisker plots; the cross represents the median, whereas the horizontal lines represent quartile responses, and the upper and lower error bars depict the maximum and minimum values, respectively. The data represent MyoFSR values derived from 4 out of 7 participants. The effect size is considered large ($\eta^2p=0.28$) for the leg x time interaction. * Denotes a statistical difference ($p \leq 0.05$) from day 1 value in the respective leg. † Denotes a significant difference between from day 6 value in the respective leg. ‡ Denotes a significant difference between legs at a given time point.

DISCUSSION

The aim of this study was to characterize the changes in strength, skeletal muscle mass, and integrated rates of myofibrillar fractional synthetic rate (MyoFSR) in skeletal muscle under a variety of loading scenarios in young, healthy males. During the first 6 days (short-term immobilization and CTRL), neither muscle strength, LLM, VL_{CSA}, nor MyoFSR changed in the RE leg. These findings highlight that a contralateral non-immobilized limb, at least for the measures here, is an appropriate internal comparator compared to the immobilized limb. In contrast, we found that just 6 days of unloading was sufficient to decrease peak torque, MyoFSR, and LLM, and this remained depressed at 14 days of unloading. We also found that 14 days of immobilization significantly reduced VL_{CSA}. Short-term RE increased $VL_{CSA.}$ Further, MyoFSR was significantly lower at 14 days in the immobilized leg compared with the RE leg.

We observed significant decrements in DXA-derived LLM throughout 14 days of single-leg immobilization. Indeed, previous studies have reported significant decrements in skeletal muscle mass after 5 days [15], 7 days [12], and 14 [9, 10, 33] days of single-leg immobilization. It should be noted that DXA does not directly quantify skeletal muscle mass; rather, DXA-derived LLM includes tissue from, as well as fibrotic and other lean tissue [34]. A more direct and accurate measurement of muscle mass should be used, such as magnetic resonance imaging [23], or ultrasonography [23]. Using ultrasonography to quantify VL_{CSA}, a method shown to have good reliability with MRI [23] we report a significant reduction in VL_{CSA} with a 4% and 11% decrease in VL_{CSA} at days 6 and 14, respectively. The findings of our study align well with reported decrements of 3.9% [13] and 9% [23] in VL_{CSA}, after 5 and 15 days of single-leg immobilization, respectively. We also observed that just 4 sessions of RE were sufficient to increase VL_{CSA}. Previous work [35] has demonstrated that measurable muscle growth –the

addition of contractile machinery – only occurs after prolonged periods of RET, and the attenuation of skeletal muscle damage. Based on the work of Damas and colleagues [35], we hypothesize that the early increase in VL_{CSA} observed with short-term RE results from muscle edema. Exercised muscle experiences a fluid shift proportional to the metabolic demand of repetitive skeletal muscle contractions [36]. For example, Hirono and colleagues demonstrated an increase in cross-sectional area following just 1 bout of RE [37]. Nonetheless, our DXA-derived LLM and ultrasound data agree with previous data showing that skeletal muscle mass is dependent upon the presence of contractile activity, and removal of contractile activity – as in the case of single-leg immobilization – induces rapid muscle atrophy that eventually slows once muscle mass reaches nadir [2].

Skeletal muscle atrophy can arise from a variety of uncomplicated models of disuse, including single-leg immobilization [10], microgravity [38], step reduction (i.e., voluntary increase in sedentary behaviour) [39], and bed rest [40]. However, these models are not without their limitations. A common criticism of the single-leg immobilization model is that the contralateral, non-immobilized leg is not a valid internal control. A primary finding of the current study was that 6 days of single-leg immobilization was insufficient to affect indices of muscle strength, mass and MyoFSR in the contralateral, non-immobilized limb. While we cannot rule out a small-sample size [41] for the observed null effects on the contralateral limb (especially for MyoFSR), previous data is in concordance with the results of the current study that the non-immobilized limb is generally unaffected during the contralateral limb disuse. For example, a recent meta-analysis of 15 studies and 194 participants undergoing single-leg immobilization demonstrated that single-leg disuse did not impact knee extensor size and marginally impacted knee extensor strength in the contralateral, non-immobilized limb [24].

Further, Kilroe and colleagues [12] demonstrated that 7 days of single-leg immobilization was sufficient to depress MyoFSR, with no measurable change in the non-immobilized limb. Brook and colleagues demonstrated no differences in muscle mass, strength or integrated rates of MyoFSR in a non-immobilized limb during contralateral limb immobilization [15]. Collectively, the results of the current study support the utility of the contralateral, non-immobilized leg as a valid control that is unaffected during a period of single-leg immobilization.

At the molecular level, skeletal muscle mass is dictated by the difference in MPS and MPB. MyoFSR was significantly depressed following just 6 days of single-leg immobilization and remained depressed following 14 days. Further, just 4 sessions of RE were sufficient to increase integrated rates of MyoFSR. Indeed, a wealth of literature demonstrates that immobilization [1, 9, 10, 12-14], and short-term RE [35, 42, 43] are sufficient to decrease and elevate integrated rates of MyoFSR, respectively. We also observed that MyoFSR was depressed in the IMMOB leg, compared with the RE leg. Similarly, Stokes and colleagues showed that MyoFSR was depressed in the immobilized leg, compared with the contralateral limb that underwent 10 weeks of RET [10]. It is important to acknowledge that the data for MyoFSR are presented for 4 of 7 participants, and because of this current limitation with sample size, we opted to use a less conservative post-hoc test (Fisher's LSD). Nonetheless, our preliminary MyoFSR data nicely align with trends reported in the literature that MyoFSR are susceptible to the presence – or absence – of contractile activity.

There are several limitations of the current work that must be addressed. Firstly, only young, healthy males were included in the study. Females are frequently excluded from exercise physiology research to avoid the potential influence of varying ovarian hormones across the menstrual cycle [44]. However, recent work from our group (published after data collection of

the present study) reported no effect of menstrual cycle changes in acute hormonal fluctuations on RT-induced adaptations [44]. Future work is needed to elucidate whether menstrual cycle hormonal fluctuations influence measures of MyoFSR during paired loading and unloading. Secondly, we reported MyoFSR that is an average of thousands of proteins in the whole muscle or sub-cellular protein fractions (e.g., myofibrillar, sarcoplasmic, mitochondrial) [45]. Dynamic proteomic profiling (DPP) is a novel technique combining the fractional synthetic rate of individual proteins with measurements of protein abundance [28, 46]. By performing proteomics in combination with stable isotope tracers, the relative abundance of labelled to unlabelled peptides could be determined using liquid chromatography-mass spectrometry, and the turnover rates of these individual proteins can be determined [28]. Changes in protein abundance that are not matched by changes in protein synthesis may be regulated by protein degradation [47]. Therefore, dynamic proteomic profiling has the potential to provide unprecedented insight regarding the potential regulatory events driven by skeletal muscle unloading and resistance exercise training that underpin changes in protein abundance before they are detected. Future work using paired loading and unloading should interrogate the dynamic proteome.

We adopted a paired model, such that, within an individual, one limb was exposed to unloading to induce atrophy (14 days), whereas the contralateral, non-immobilized limb was first used as a short-term *bona fide* control (6 days), followed by exposure to short-term loading (8 days). Single-leg immobilization can induce significant atrophy, strength loss, and decrements in MyoFSR. In contrast, in the contralateral non-immobilized leg, muscle mass, MyoFSR, and strength remain unchanged during the first 6 days of the immobilization protocol, suggesting that the contralateral leg may serve as an adequate control for future studies. Short-term RE did not

induce significant increases in skeletal muscle mass or strength, albeit increases in MyoFSR were observed.

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CHAPTER 6 – GENERAL DISCUSSION

6.1 Introduction:

Skeletal muscle is important for metabolic health and locomotion and is required to maintain independence in later life. Concerted efforts to maintain skeletal muscle throughout life and minimize health and catabolic crises are important for human health. Skeletal muscle is in part, a functional reserve and should be protected to a maximum degree possible. Importantly, skeletal muscle is a plastic tissue that can adapt to several stimuli by changing its metabolic and contractile properties. Skeletal muscle can be increased by loading through resistance exercise training (2), which leads to muscle fibre hypertrophy. In contrast, reducing skeletal muscle contractile activity (3) or unloading (4) results in skeletal muscle fibre atrophy. Identifying variables that may be important determinants of skeletal muscle size could yield therapeutic targets for metabolic disease and musculoskeletal frailty.

The algebraic difference between muscle protein synthesis and muscle protein breakdown regulates the molecular-level control of skeletal muscle mass and size. Rates of muscle protein synthesis can be influenced by several factors broadly categorized as external or internal system variables. External system variables are environmental perturbations indispensable for activating internal system variables. Internal system variables are local, skeletal muscle-specific, biological processes that mechanistically underpin skeletal muscle adaptations (5). The overarching objective of the studies conducted as part of this thesis was to discover the influence of external variables (RET program variables) and internal variables (lncRNA) on skeletal muscle adaptations and characterize changes in skeletal muscle protein synthesis under various scenarios.

In study 1, we conducted a systematic umbrella review to summarize the current state of the evidence on the influence of individual RET program variables on skeletal muscle adaptations. In study 1, we discovered that RET load, weekly frequency, and RET volume (number of sets) were the most investigated RET program variables and were also important determinants of RET-induced skeletal muscle adaptations. The results of study 1 were used as a preface to study 2, where we conducted an innovative Bayesian NMA to statistically determine the influence of several combinations of RET program variables (i.e., resistance training prescriptions) on resistance training-induced skeletal muscle adaptations. As an internal variable, the lncRNA transcriptome is poorly characterized in skeletal muscle biology, and for study 3, we used five independent exercise studies to identify a novel lncRNA signature associated with resistance exercise-induced changes in lean mass. Lastly, in study 4, we characterized integrated rates of bulk MPS under distinct loading states in young, healthy men. We found that 14 days of single-leg immobilization was sufficient to induce rapid declines in MPS, whereas 4 sessions of

RE increased MPS. For a schematic overview of the main contributions as part of this thesis, please refer to Figure 1.

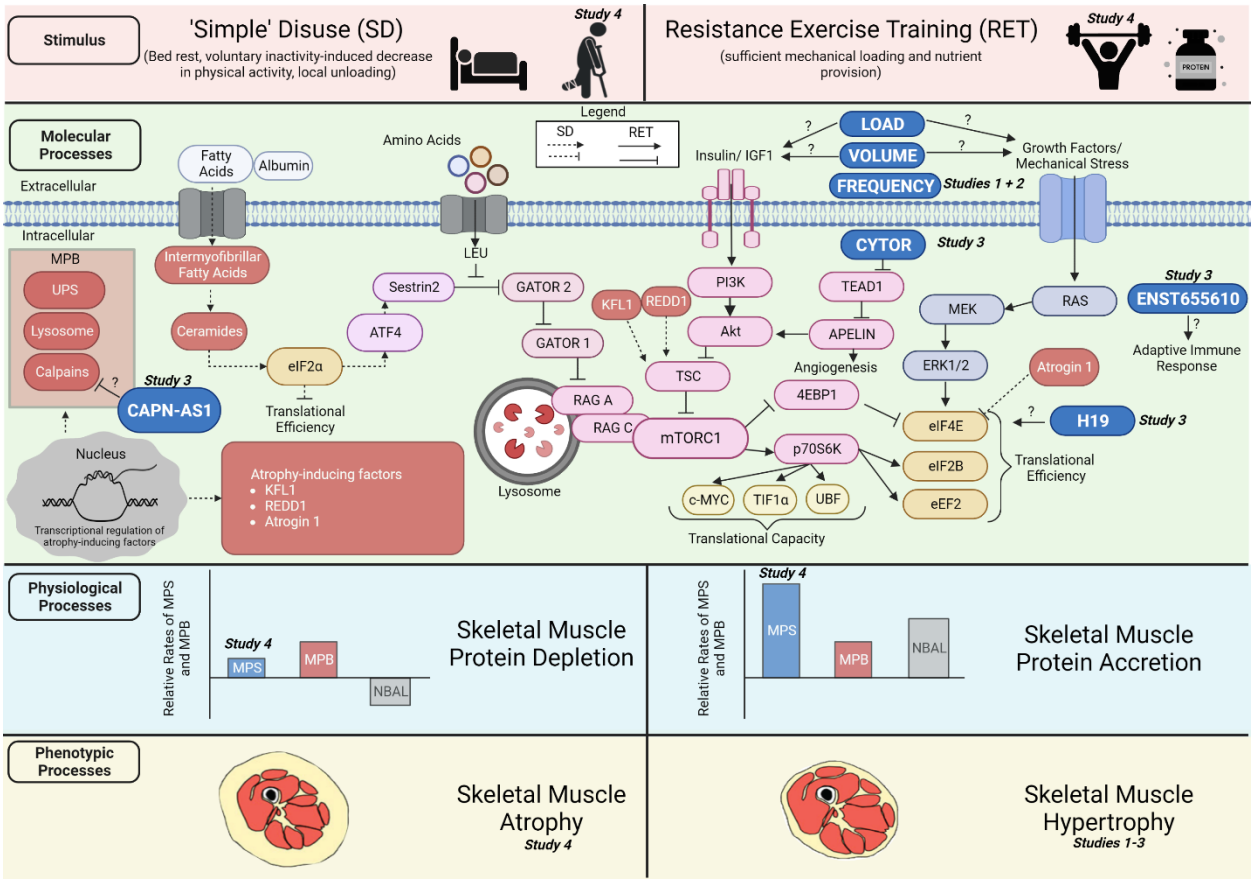

Figure 1. An updated simplified overview contrasting simple disuse atrophy and resistance training induced skeletal muscle hypertrophy, highlighting primary thesis contributions. Highlighted in blue are several internal and external variables studied in this thesis.

6.2 Leveraging individual response heterogeneity to study skeletal muscle physiology:

There is growing interest in personalized or precision medicine (6), and within the context of skeletal muscle biology, this was largely inspired by the seminal HERITAGE family study that demonstrated highly heterogeneous changes in VO_{2max} following supervised exercise training (7). In study 3, we reported a 30%-fold difference (-4.9% to 24.7%) in DXA-derived changes in LM following supervised exercise training. Although many variables might explain heterogeneous skeletal muscle responses to exercise (such as diet, biological sex, age, and length and timing of exercise) (8), using the observed heterogeneity presents an exciting opportunity to identify variables driving skeletal muscle adaptation. For example, Petrella and colleagues showed a greater satellite cell population number and myonuclear addition in individuals that achieved 'robust' skeletal muscle growth, compared with individuals achieving little-to-no measurable hypertrophy (9). Further, our group recently demonstrated that the magnitude of resistance training-induced muscle growth was in part modulated by a greater resting intramuscular androgen receptor content (10). In contrast, not considering physiological heterogeneity or using means-based data analysis (11, 12) could obscure our ability to improve understanding of internal variables driving skeletal muscle adaptation (13).

Extending beyond internal system variables, acknowledging individual heterogeneity might also help elucidate external variables' role on skeletal muscle adaptations. A limitation of our NMA in study 2 is that we relied on summary (or aggregate) data to generate estimated effects; this does not consider individual heterogeneity. In contrast, individual participant data (IPD) meta-analyses involve the analysis of individual data from eligible clinical trials (14). Riley and colleagues (15) demonstrated that including IPD in an NMA improves the quality and robustness of findings, allows adjustment for prognostic factors, and enables treatment effects to

scale more accurately with participant-level covariates, such as age. However, given the large number of included trials in study 2, collecting IPD from all trials was not feasible. Interestingly, there are novel NMA methods being explored that combine aggregate and IPD (16).

Accounting for physiological heterogeneity, by considering individual differences (17) or implementing a within-person design (13) has heightened our understanding of the molecular responses driving skeletal muscle remodelling. For example, our group used a within-person differential loading design that combined RET-induced hypertrophy and disuse-induced atrophy (HypAt) to reduce heterogeneity of changes in muscle mass by ~40% and found a greater number of differentially expressed genes with loading (13) compared with similar-sized studies (12). In studies 3 and 4, we attempted to account for physiological heterogeneity; In study 3, we considered differences in exercise-induced changes in LM to interrogate the lncRNA transcriptome; In study 4, we utilized our previously described (13) within-person differential loading design to characterize changes in MPS.

6.3 Changes in muscle protein synthesis during loading and unloading:

At the molecular level, skeletal muscle mass is largely dictated by the difference between MPS and MPB. Therefore, before characterizing the influence of external and internal system variables on exercise-induced skeletal muscle adaptations, the aim of study 4 was to characterize integrated rates of MPS in skeletal muscle under a variety of scenarios in young, healthy males.

In study 4, MPS significantly declined following just 6 days of single-leg immobilization and remained depressed following 14 days of unloading. The observed decrement in muscle protein synthesis coincided with reduced muscle mass and strength. Indeed, work from our group (3, 13, 18, 19) and others (20-23) have attributed the loss of skeletal muscle mass with muscle disuse,

mostly, to a reduction in rates of MPS. However, a limitation of study 4 is that we did not measure MPB. Recently, Brook and colleagues (24) showed MPB as playing a minimal role during short-term immobilization-induced skeletal muscle atrophy. Despite these findings, there is still conflicting evidence suggesting that MPB is the dominant mechanism underpinning proteostatic changes of human skeletal muscle during disuse (25). Calpains are calcium-dependent cysteine proteases and are one of the major molecular mechanisms regulating MPB (26). Interestingly, in study 3 we discovered that the expression of *CAPN-AS1*, a lncRNA gene that is anti-sense to *CAPN1*, was significantly reduced following a period of single-leg immobilization in young, healthy adults. This novel finding is interesting, considering that *CAPN1* is inconsistently regulated in unloaded skeletal muscle (27). However, we did not measure gene expression of *CAPN1* (i.e., protein-coding gene), so we are unaware if the changes in expression of *CAPN-AS1* following disuse reflect changes in gene expression of *CAPN1*. Nonetheless, decrements in MPS are the primary mechanism driving skeletal muscle atrophy, and changes in rates of MPB may or may not be a contributing factor.

We also found that short-term resistance exercise was sufficient to elevate MPS. Confirming our findings is a wealth of literature showing that short-term RE increases MPS (26). However, given that previous research has shown a poor correlation between early exercise-induced increases in MPS and long-term hypertrophy (28, 29), the current data are unlikely to reflect the addition of contractile machinery. Indeed, we observed no exercise-induced increases in DXA-derived LLM, but we did find increased VL_{CSA}, and we propose this may be related more to exercise-induced edema (30) and not true hypertrophy. At the molecular level, the RE-induced stimulation of MPS may occur owing to two mechanisms; Increased translational capacity, with a greater pool of ribosomes available to translate mRNA into protein, or increased translational

efficiency, with more mRNAs being translated for a fixed pool of ribosomes. In study 1, we did not measure markers of translational efficiency or capacity; however, Chesley and colleagues were the first to demonstrate that following a bout of RE, the acute rise in MPS was attributed to translation efficiency, as a rise in MPS occurred without increases in RNA (31). In a series of papers, Brook and colleagues (32, 33) demonstrated that rates of RNA synthesis were increased above basal rates over the 0-6wk period with RET; however, this showed discordance with long-term measures of MPS and muscular adaptations. Notably, Phillips and colleagues (34) demonstrated individuals who belonged to the highest quartile of changes in leg lean mass had greater *reductions* in gene expression of ribosomal associated proteins. In agreement, in study 3 we discovered that *H19* was upregulated following chronic resistance exercise training, and Liang and colleagues (35) demonstrated that *H19* regulates the expression of eukaryotic initiation factors. Collectively, this work suggests that translational capacity and efficiency may be dominant mechanisms driving resistance exercise induced increases in MPS observed in study 4.

A limitation of study 4 is that we reported fractional synthetic rates that are an average of thousands of proteins in the whole muscle or sub-cellular protein fractions (e.g., myofibrillar, sarcoplasmic, mitochondrial) (36). However, it can now be appreciated that thousands of proteins with different functions may turnover at considerably different rates (37, 38). Dynamic proteomic profiling (DPP) is a novel technique combining the fractional synthetic rate of individual proteins with protein abundance measurements. By performing proteomics in combination with the incorporation of labelled amino acids, the relative abundance of labelled to unlabelled peptides could be determined using liquid chromatography-mass spectrometry, and the turnover rates of these individual proteins could be determined (39). Further, changes in

protein abundance that are not matched by changes in protein synthetic rates may be attributed to protein degradation (38). The dynamic proteome was first quantified in yeast cells by deuterium labelled LEU (40). Although the previous work (40) interrogated the dynamic proteome, this was captured *in vitro*, requiring large amounts (up to 100%) of labelled amino acids, which is not feasible for human work. Using D_2O, Price and colleagues (41) measured the enrichment of peptides and extracted protein abundance data for 114 unique proteins, representing one of the first measures of the dynamic proteome in human plasma. Camera and colleagues (37) subjected middle-aged men to 3 bouts of RE over the course of 9 days. Camera and colleagues found that only 17/90 identified proteins demonstrated changes in abundance. Further, several proteins were identified that increased in turnover rate, with no change in abundance (37). Lastly, this analysis demonstrated that while the synthesis of certain proteins was the key determinant of changes in abundance, others were significantly modulated by changes in their rates of breakdown (37), which has been previously reported in rat fast-twitch extensor digitorum longus undergoing chronic-low frequency stimulation (38). Therefore, the ability to measure the synthesis and degradation rates of individual proteins is a cutting-edge and novel methodology that would be highly informative in advancing our understanding of the changes in translational regulation that occur with skeletal muscle loading and unloading and is a future endeavour for study 4.

6.4 External system variables:

External system variables are environmental perturbations indispensable for activating internal system variables. In studies 1 and 2, we leveraged systematic methodologies and Bayesian NMA methodologies and found that, compared with a control group (i.e., non-exercising individuals), RET is a potent external system variable for skeletal muscle mass and strength accrual (43). This consistent finding across both of our studies confirms the results of

previous investigations (44, 45) and provides the most robust form of evidence to date on the inclusion of strength-training activities in physical activity guidelines (46).

As RET is a potent stimulator of hypertrophy, much attention has been given to optimizing RET program variables (47). Therefore, in study 1, we reviewed 44 systematic reviews (i.e., umbrella review) – the largest umbrella review on RET program variables to date (48) – to identify the influence of manipulating individual RET program variables. We investigated a plethora of individual RET program variables, such as load, set-end point, contraction velocity/time under tension, volume (number of sets), set configuration, inter-set rest, periodization, weekly frequency, muscle action type, time of day, and exercise order. We found that load, volume (number of sets), and weekly frequency were the most well-studied RET program variables. There was a paucity of evidence on other RET program variables, and future well-controlled randomized controlled trials (and ensuing systematic reviews and meta-analyses) are needed to draw firm conclusions on the effects of other RET program variables on skeletal muscle adaptations.

6.4.1 Univariate resistance exercise training program variables:

RET load is the amount of resistance undertaken during a RE set. RET load is commonly expressed as a percentage of maximum strength achieved on a strength test (e.g., maximum voluntary contraction [MVC] or 1-repetition maximum [1RM]). In study 1, we found that 8 out of 44 systematic reviews investigated the role – or lack thereof – of RT load on skeletal muscle adaptations. Specifically, we found that six out of eight systematic reviews contained some (49-51) or sufficient (52-54) evidence supporting the notion that higher-load RET is pertinent for maximizing muscular strength gains. Similarly, in study 2, we found that RET prescriptions with higher loads (≥ 80% 1RM) were more likely to rank in the top three for strength than all lower-

load (< 80% 1RM) prescriptions. The superiority of higher-load RET for muscular strength gains can be attributed to the principle of specificity (i.e., participants in higher-load groups regularly train using loads that are closer to the test of maximal [1RM] strength) (54) and neural adaptations with exercising at higher relative loads (55).

In contrast to muscular strength gains, we also showed that the current evidence provided little support for the importance of RET load on skeletal muscle hypertrophy (study 1). In study 2, we demonstrated that a high-load RET prescription and lower load RET prescription were most likely to rank in the top three for skeletal muscle hypertrophy. The lack of importance of RET load for hypertrophy is not a novel finding and is well-reported throughout the literature (50, 56-58), however performing RET to momentary muscular failure (fatigue) has been posited as a key component for RT-induced hypertrophy, particularly for lower loads (50). Interestingly, in study 2, we performed a network meta-regression for exercise fatigue, and we found that accounting for exercise fatigue did not improve model fit nor substantially alter network estimates, suggesting that lifting to fatigue does not suitably explain the observed hypertrophic response. Indeed, previous work has demonstrated that exercising to momentary muscle failure/fatigue is not mandatory for RET-induced adaptations (59) but may help to eliminate some degree of interindividual variability across the amount of effort exerted (60). Therefore, the results of study 3 suggest that exercising until volitional fatigue is not necessary for RET-induced skeletal muscle hypertrophy.

It must be acknowledged that our initial objective for study 2 was to categorize load beyond 2 classes (i.e., high-load, moderate-load, low-load), but this yielded an unstable network, NMA assumptions being violated (e.g., transitivity), and resulted in imprecise network estimates (61). A recent NMA comprising 24 studies and 747 participants aimed to characterize the impact of

low (<60% 1RM), moderate (60% - 79% 1RM), and high loads (≥80% 1RM) on skeletal muscle mass and strength. The work of Lopez and colleagues supports our findings of studies 1 and 2, suggesting that high loads are needed for superior strength gains, whereas skeletal muscle hypertrophy is load independent.

RET volume is the total amount of work performed in a resistance exercise session, and for the context of this thesis was defined as the number of sets performed per exercise session (or week). Weekly frequency is closely related to RET volume, defined as the number of times a muscle group is trained per week. It was evident from the results of study 1 that RET volume positively impacts skeletal muscular strength gains and hypertrophic gains. In contrast, in study 1, we found discrepancies in the effect of weekly training frequency on skeletal muscle adaptations, which can be attributed to whether volume-equated studies were included in systematic reviews. Further, our network meta-analysis demonstrated that the top-ranked resistance training prescriptions involved performing multiple sets/exercise, compared with single sets per exercise, while weekly frequency appeared less important for maximizing adaptations. While the current work of this thesis demonstrated that increasing resistance exercise training volume confers an increasingly additive benefit, previous work suggests that this advantage eventually plateaus, after which there are diminishing returns (less gain per volume increase) for skeletal muscle adaptations and possibly detrimental outcomes (47).

It is challenging to tease apart the effects of weekly training frequency on skeletal muscle adaptations because training frequency is related to training volume, and future studies are needed to determine the impact of splitting weekly RT volume across additional weekly training sessions maximizes skeletal muscle adaptations. A limitation of study 2 is that the continuous resistance training program variables investigated herein (load, sets, frequency) were classified

categorically, so future work could utilize dose-response/model-based NMA methods to explore these resistance training program variables as continuous predictors of skeletal muscle adaptations (62, 63).

6.4.2 From univariate resistance exercise training program variables to multivariate resistance exercise training prescriptions:

Study 1 provided foundational knowledge on the impact of univariate RET program variables on skeletal muscle adaptations. But a limitation of study 1, and pairwise meta-analyses in general, is that guideline developers and clinicians do not prescribe RET variables independent of other RET variables; several variables are collectively a part of any resistance training prescription (46). Further, among pairwise meta-analyses in exercise science, there are differences in inclusion criteria, statistical modelling, search strategy and RET variable categorization that is likely to lead to inconsistent findings in the literature. Therefore, rather than comparing univariate RET program variables, comparing different resistance training prescriptions might allow for coherent and complete decision-making across the totality of available evidence, and is likely to be of greater relevance for guideline developers and clinicians.

To overcome the aforementioned limitations with pairwise meta-analyses, we used NMA (64), a novel extension of pairwise meta-analyses, which allowed for coherent and complete decision-making across all possible resistance training prescriptions created in study 2. Another advantage of using a NMA is that it allowed us to produce estimates between resistance training prescriptions, even when some have never been tested in randomized trials (65). To our knowledge, the current network-meta analysis represents the largest synthesis of RET from randomized trials (strength: 178 studies, n=5,097 participants; hypertrophy: 119 studies, n=3,364

participants), and a more comprehensive search strategy that was previously implemented (2,629 (57) versus 16,880 records identified). The results of our network meta-analysis suggest that higher load and multiple sets/exercise are required for optimizing strength gains and hypertrophy gains, regardless of weekly training frequency.

Analysts and decision-makers need to be made aware of the robustness of findings and conclusions drawn from meta-analyses. Indeed, the popularized Grading of Recommendations Assessment, Development and Evaluation (GRADE) scale was developed to assess the quality of evidence for a given meta-analysis. However, GRADE relies on qualitative judgements to determine the confidence we have in recommendations of evidence and cannot evaluate the credibility of a network meta-analysis (66). As an alternative to GRADE, in study 2, we implemented thresholding, a novel methodology to assess the confidence in guideline recommendations derived from our NMA (66). Threshold analysis quantifies precisely how much the evidence could change (for any reason, such as potential biases or simply sampling variation) before the recommendation changes and what the revised recommendation would be. We used thresholding in study 3 to directly inform guideline developers and clinicians of the robustness of our top-ranked resistance training prescriptions (66). Specifically, 65 direct comparisons were identified that could potentially impact the recommendation of the top-ranked strength treatment; however, the revised treatment recommendation was resistance exercise training prescriptions with higher loads, supporting the strong recommendation of higher-load RET for strength training. Further, 80% of the direct comparisons for skeletal muscle hypertrophy would need to change by more than four standard deviations to displace the top-ranked resistance training prescription for hypertrophy, suggesting that our top-ranked prescription skeletal muscle hypertrophy is robust.

It is common for meta-analyses in exercise science (2, 44, 45, 57, 67) to follow a frequentist framework whereby parameters are objectively estimated from the empirical evidence to generate a true or real change (68). Further, within a frequentist framework, confidence intervals are commonly misinterpreted as representing the range of the true difference, when in fact, confidence intervals are a percentage of similarly-sized intervals obtained from repeatedly completing the trial that will contain the true mean change (68). A fundamental advantage of study 2 is that we implemented a Bayesian framework, which allowed us to generate a probability distribution containing an actual parameter value distribution. Unlike frequentist statistics that rely on only observed evidence to generate estimates, Bayesian statistics considers *both* observed evidence and prior knowledge about the intervention effects to generate distributions. In essence, Bayesian statistics allows for a "continuous update of knowledge" (68). Therefore, rather than testing a null hypothesis of multivariate resistance training prescriptions repeatedly, study 2 serves as a Bayesian foundation for future investigators wishing to update our findings with new evidence.

6.5 Internal variables:

Although external system variables are important for skeletal muscle adaptations, it is becoming more apparent that internal system variables are paramount for skeletal muscle adaptations (69). Internal system variables are local, skeletal muscle-specific, biological processes that mechanistically underpin skeletal muscle adaptations. Internal system variables primarily encompass genomic, epigenomic, transcriptomic and proteomic variables, which can ultimately be affected by external system variables (69).

6.5.1 The lncRNA transcriptome: a novel driver in resistance exercise training-induced skeletal muscle adaptations.

Our current understanding of transcriptomics in skeletal muscle biology is mostly limited to protein-coding genes. Most of the mammalian genome is transcribed into RNAs with little-to-no protein-coding potential (70), and there is a strong and statistically significant correlation between the proportion of the genome that is non-coding and organismal complexity (71). Long non-coding RNAs (lncRNA) are a class of non-coding transcripts that are >200 nucleotides long and have no protein-coding potential. The lncRNA transcriptome is poorly characterized in skeletal muscle, with only a handful of studies published to date (72, 73). In study 3, we profiled the lncRNA transcriptome using human transcriptome 2.0 (HTA2.0) microarrays in 144 participants, from 5 independent studies, before and after supervised resistance exercise training. To our knowledge, this is the first, and largest, clinical transcriptomics study to characterize the lncRNA transcriptome following resistance exercise training. Further, we are the first to associate a core set of lncRNA genes regulated in individuals that accrued LM – beyond the precision error of the DXA.

We discovered *CYTOR* as a lncRNA gene uniquely regulated in individuals who displayed exercise-induced increases in LM. Recent work supports the importance of *CYTOR* in skeletal muscle. Wohlwend and colleagues (73) showed that *CYTOR* expression was upregulated following an acute bout of leg extension. Further, the same group demonstrated that overexpression of *CYTOR* in mice promoted muscle growth (73). *CYTOR* may promote myogenesis by sequestering the *TEAD1* transcription factor, which prevents the binding of *TEAD1* to its target genes (73). *TEAD1* overexpression induces a switch to a slow contractile phenotype *in vivo* (74), and the knock-down of *TEAD1* stimulates *apelin* secretion *in vivo*, stimulating endothelial cell expansion via the apelin receptor (75). The apelin receptor is predominately expressed in endothelial cells (76) and was recently identified as a gene that

correlated with muscle growth in human skeletal muscle (13). In study 3 we found that baseline expression of *CYTOR* was moderately associated with the endothelial cell gene marker, *PECAM1* (r=0.28; FDR= 5.20 x 10^{-4}). Our work suggests that *CYTOR* may be an important lncRNA regulator of muscle growth and might mechanistically exert its effects via the *TEAD1-Apelin* axis.

Interestingly, in study 3, we also identified a set of lncRNA genes that were differentially expressed, regardless of changes in LM, such as *H19*. Specifically, we found that *H19* was significantly up-regulated following exercise training. *H19* expression is strongly repressed after birth, yet significant transcription persists only in skeletal muscle (77). Mechanistically, *H19* gives rise to several microRNAs that regulate post-transcriptional gene expression. *H19*-encoded miRNAs are differentially expressed in several muscle-wasting diseases (such as COPD) (78). At a molecular level, muscle-wasting diseases are characterized by a diminished MPS, elevated MPB, and elevated inflammatory burden (27). Liang and colleagues (35) recently demonstrated that *H19* was shown to directly regulate protein translation by modulating the expression of eukaryotic initiation factors. This work suggests that miRNAs encoded by *H19* appear to be related to skeletal muscle dysfunction, and future therapeutic interventions should be explored. Nonetheless, results from study 4 highlight that a subset of lncRNA genes may set or limit the potential physiological adaptation to exercise training; in contrast, we also detected a subset of lncRNA genes differentially expressed – regardless of changes in LM – suggesting that these genes may be important for a healthy functioning skeletal muscle proteome.

6.5.2 Data-driven networks identify biological associations of lncRNA genes:

Differential expression (DE) is the most common transcriptomic analysis, and a large number of differentially regulated genes (>1000) (13) following resistance exercise training makes it

difficult to reconcile the mechanistic biological underpinnings. Fortunately, category enrichemnet using GO is a widely used strategy that takes a set of differentially expressed protein-coding genes. Statistically, it identifies enriched biological themes, and minimizes biases (79), these themes may reflect true biological processes regulated in a clinical experiment (13). However, functional labels have yet to be assigned to lncRNA genes, making it difficult to determine true biological interpretations (80). Indeed, tools such as *lnCompare* (80) are being developed to explore biological features directly related to lncRNA genes, and further validation of such a tool is needed before their implementation in clinical transcriptomics.

The emergence of data-driven network modelling has recently provided an unbiased and global opportunity to interrogate the complex transcriptional control of cells. There are numerous data-driven network models; however, most of these methods first evaluate an association strength between each gene pair (i.e., Pearson correlation) with statistical significance testing, followed by graph theory applications to identify "communities" of co-expressed genes. Indeed, co-expressed gene communities enable the identification of unique functional pathways under an array of scenarios, such as skeletal muscle loading (13), insulin resistance (81), and other disease states (82). Therefore, a novel aspect of study 3 is that to overcome the lack of biological annotations of lncRNA genes; we used a data-driven network analysis (83) to identify if any of our lncRNA genes were co-regulated with protein-coding genes in significant network structures, followed by submitting a gene set comprising the network structure to gene ontology analyses. This innovative strategy allowed us to associate our lncRNA genes with biological processes.

We found that lncRNA genes were embedded in network structures associated with biological processes such as angiogenesis, the adaptive immune response, mitochondrial translation, and extracellular matrix remodelling. These findings are well-aligned with recent

work from our group (13) demonstrating that a core set of 141 protein-coding genes correlated with the muscle growth response to chronic muscle loading in humans (n = 100), and these activated genes form functional networks that were observed to be associated with extracellular matrix remodelling, angiogenesis, and mitochondrial function. Therefore, the results of the current study demonstrate that lncRNA genes may be involved in cellular processes paramount to loading-induced skeletal muscle adaptation.

6.5.3 Sensitive coverage of low abundance lncRNA genes

Most skeletal muscle transcriptomics experiments are derived from "bulk" muscle samples containing multinucleated muscle fibres and several mononuclear cell types. From a bulk tissue sample, such as skeletal muscle, there is a wide range in basal gene expression across genes, suggesting that low(er) expressed genes may reflect changes in cell-type specific biology. Indeed, single-cell profiling has emerged as a method to resolve cell-type specific gene expression changes; unfortunately, these methods are costly and still in their infancy. Short-read RNA sequencing and microarray are the two most common profiling methods implemented with bulk tissue (1). Due to the expression-dependent bias of short-read RNA sequencing, more abundant genes in a bulk skeletal muscle sample (e.g., PGC1-α, mean expression: 537.6) makes it nearly impossible (unless the transcript is "jackpotted") to detect low(er)-expressed genes, such as several lncRNA genes (e.g., *LINC00390*, mean expression: 6.72). In contrast, microarray relies on a non-competitive quantification strategy and enables the detection of low-expressed genes (1). Indeed, in study 3, we showed that most lncRNA genes associated with resistance exercise-induced changes in lean mass contained a low mean expression. Therefore, low-expressed genes with potentially significant cell-specific, biological roles can be detected with modern microarrays.

As an example, in study 4, we found that *ENST00000655610* is low expressed in human skeletal muscle samples and human primary myotubes (data not shown), and its expression *in vivo* was positively associated with CD4 expression ($r = 0.37$; FDR= 2.52×10^{-08}), a T-cell gene marker. Strikingly, *ENST00000655610* was nested within a network structure associated with the biological processes of the adaptive immune system and positive regulation of the T-cell population. *ENST00000655610* was not associated with CD8 expression, suggesting that this lncRNA may be expressed in $CD4^+$ T helper cells rather than CD8+ cytotoxic cells. CD4+ T-cells release inflammatory cytokines that act on skeletal muscle cells to support growth and regeneration (84). Collectively, we were able to identify two uncharacterized lncRNA genes (*LINC00390* and *ENST00000655610)* that may be expressed in a cell-specific manner; these lncRNA genes were linked to biological processes pertinent to exercise-induced skeletal muscle adaptation. A future direction for bulk transcriptomic studies is to implement cellular-level deconvolution (85). This computational methodology uses the bulk tissue expression of a gene and creates a linear combination of its expression levels with a cell type-specific expression matrix (85).

6.6 Conclusions and main contributions to thesis

Skeletal muscle is a plastic tissue important for metabolic health, locomotion, and for functional independence in later life. Discovering variables that may be an important determinant of skeletal muscle size could yield therapeutic targets. Fundamentally, changes in muscle size are dictated by a chronic imbalance between MPS and MPB, leading to either skeletal muscle hypertrophy or skeletal muscle atrophy. Indeed, in study 4, we demonstrated that 2 weeks of unloading decreased MPS rates, which coincided with marked atrophy. In contrast, short-term loading increased rates of MPS. As RET is a potent stimulator of MPS, and thus skeletal muscle

hypertrophy, much attention has been given to manipulating RET variables. In study 1 we conducted the largest umbrella review to date and found that several RET variables – namely load, sets (volume), and weekly frequency) – influenced skeletal muscle adaptations. In study 2, we used NMA, a cutting-edge extension of pairwise meta-analyses to compare several unique resistance training prescriptions, and to date, represents the largest synthesis of randomized trials in resistance training. We discovered that high load and multiple sets/exercise are required to optimize skeletal muscle adaptations, regardless of weekly training frequency. Study 2 provides an unprecedented amount of knowledge surrounding resistance training prescriptions and will undoubtedly inform clinicians and physical-activity guidelines developers. Lastly, in study 3, we are the first to discover a unique set of lncRNA genes associated with exercise-induced changes in LM in 144 individuals across five independent supervised resistance exercise training studies. At an attempt to associate our discovered lncRNA genes with skeletal muscle biology, we used innovative data-driven network analysis and an extensive literature search. We also found some novel lncRNA genes to be correlated with cell-type specific gene markers, suggesting possible cell-specific transcriptional specificity for some of these lncRNA genes. The findings of study 3 provide the foundation for researchers to generate mechanistic-based data on the effects of lncRNA genes in skeletal muscle biology. (Figure 1).

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