RIBOSOME CONTENT ADAPTATIONS TO AEROBIC AND RESISTANCE EXERCISE TRAINING

THE ADAPTIVE RESPONSE OF RIBOSOME CONTENT TO AEROBIC AND RESISTANCE EXERCISE TRAINING

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LAY ABSTRACT

Ribosomes are essential in making proteins within the cell, and their content has been hypothesized to support the adaptive responses observed with exercise training. Ribosome content has previously been shown to increase following resistance training likely to support skeletal muscle growth. However as aerobic training also influences cellular adaptations, it is plausible that ribosome content also supports these training adaptations. We hypothesized that both aerobic and resistance training would increase ribosome content. Contrary to our hypotheses, no changes in ribosome content were observed following aerobic or resistance training despite previously observing adaptations characteristic of each respective training stimulus. However, those with the greatest increases in muscle mass had lower baseline ribosome content and less change in content following resistance training. These results suggest that baseline ribosome content is sufficient for aerobic adaptations and that ribosome's efficiency is likely more important than content to elicit resistance training adaptations.

ABSTRACT

Ribosomes are the essential machinery for cellular protein synthesis. Ribosome content is hypothesized to support muscle growth and is suggested that those with more ribosomes may better respond to resistance training. Aerobic training also elicits distinct physiological adaptations; however, no direct measures of ribosome content following aerobic training have been measured. Ribosomes interact with mitochondria for mitochondrial protein synthesis and import. Mitochondria may also provide cellular energy to ribosomes. We hypothesized that aerobic and resistance training would increase ribosome content and that ribosome content following aerobic training would correspond to changes in mitochondrial-related protein content and gene expression. Fourteen young men and women performed 6 weeks of single-legged aerobic followed by 10 weeks of bilateral resistance training. Muscle biopsies were taken following aerobic (Pre RT) and resistance training (Post RT) in the aerobically trained (EX) and control (CTL) legs. Pre RT, EX had greater COXIV staining intensity in Type 1 (1.17-fold; $p=0.020$) and Type 2 (1.22-fold; $p=0.015$) fibres compared to CTL; however, no differences in whole-muscle mitochondrial-related protein content or gene expression were observed ($p>0.05$). No differences in regulatory (UBF, Cyclin D1, TIF-1A, POLR-1B), cytosolic (45S, 5.8S, 18S, 28S rRNAs) or mitochondrial (12S rRNA) ribosome-related gene expression were observed ($p>0.05$), except for c-Myc (CTL>EX; $p=0.034$) and 5S rRNA (Pre RT CTL<Pre RT EX; $p=0.076$). When stratified for leg-lean soft tissue mass (LLSTM), legs with greater LLSTM had lower expression in 3/13 ribosome-related genes ($p<0.10$). When stratified for ΔLLSTM following resistance training, legs with the greatest ΔLLSTM had lower

expression in 11/13 ribosome-related genes prior to ($p<0.10$) and less change or decrease in expression in 9/13 genes following resistance training ($p<0.05$). These results indicate that baseline ribosome content was sufficient to support aerobic adaptations (capillarization, VO_2 peak) that were previously observed and that ribosome's efficiency, rather than content, is likely more important to support increases in muscle hypertrophy following resistance training.

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LIST OF ABBREVIATIONS AND SYMBOLS

AMPK	5’ AMP-activated protein kinase
ATP	Adenosine triphosphate
BMI	Body/mass index
Ca^{2+}	Calcium ion
CDK4	Cyclin-dependent kinase 4
c-Myc	Cellular myc
COX	Cytochrome oxidase subunits
CS	Citrate synthase
CSA	Cross-sectional area
eIF3	Eukaryotic initiation factor 3
eIF4	Eukaryotic initiation factor 4
ETS	External transcribed spacer
FBXO32	Muscle atrophy F-box protein 32
GTP	Guanosine triphosphate
ITS	Internal transcribed spacer
LLSTM	Leg-lean soft tissue mass
MAPK	Mitogen-activated protein kinase
MAX	Myc-associated factor X
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis

mRNA	Messenger RNA
mTORC1	Mechanistic target of rapamycin complex 1
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1α
PIC	Pre-initiation complex
POLR-1B	RNA polymerase 1B
rDNA	Ribosomal DNA
RNA	Ribonucleic Acid
rpS6	Ribosomal protein S6
rRNA	Ribosomal RNA
SL-1	Selective factor 1
S6K1	Ribosomal protein S6 kinase
TIF-1A	Transcription intermediary factor 1A
tRNA	Transfer RNA
TRRAP	Transcription domain-associated protein
UBF	Upstream binding factor
UCE	Upstream controller element
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1

DECLARATION OF ACADEMIC ACHIEVEMENT

A. Brown, S. Joanisse and G. Parise conceptualized the research questions, hypotheses, and experimental design. A. Brown and S. Joanisse performed experiments and analyzed the data. A. Brown, S. Joanisse and G. Parise interpreted the data.

INTRODUCTION

In humans, skeletal muscle is the largest organ contributing 40-50% of our total body weight (Frontera & Ochala, 2015; Schnyder & Handschin, 2015). It is essential for movement and locomotion and plays an indispensable role in metabolism and energy storage and acting as an endocrine organ. Skeletal muscle is therefore essential in maintaining our overall health, and lower muscle mass coupled with inactivity has been linked to several disease states including diabetes, obesity, cardiovascular disease and sarcopenia with aging (Frontera & Ochala, 2015; Iannuzzi-Sucich et al., 2002; Moon, 2014; Nocon et al., 2008; Schnyder & Handschin, 2015).

Skeletal muscle mass is maintained through a combination of physical activity and nutrition (Joanisse et al., 2020; McGlory et al., 2019; Rennie et al., 2004), which can impact several underlying molecular mechanisms known to contribute to the maintenance of muscle mass such as satellite cell transcriptional control (Bentzinger et al., 2013; Blaauw & Reggiani, 2014; Dumont et al., 2015) and muscle protein synthesis (MPS) (S. M. Phillips, 2004, 2014; Rasmussen & Richter, 2009; Rennie et al., 2004). More specifically, MPS must be greater than or equal to muscle protein breakdown (MPB) to have a net positive or neutral protein balance within the muscle to maintain or gain muscle mass (S. M. Phillips, 2004, 2014). Ribosomes play an essential role in protein translation and therefore can contribute to the adaptive responses to a variety of stimuli (Brook et al., 2019; Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Mayer & Grummt, 2006; P. B. Moore & Steitz, 2002; Wen et al., 2016).

1. Ribosome Overview

1.1. Ribosome structure and function

Ribosomes are essential cellular machinery responsible for translating mRNA into proteins (Brook et al., 2019; Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Khatter et al., 2015; P. B. Moore & Steitz, 2002; Piazzi et al., 2019; van Riggelen et al., 2010; Wen et al., 2016). They are composed of distinct small (40S) and large (60S) subunits, which join together upon translation initiation to form the mature 80S ribosome (Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Khatter et al., 2015; Kusnadi et al., 2015; Piazzi et al., 2019; van Riggelen et al., 2010; Wen et al., 2016). Mature ribosomes reside freely in the cytosol or bound to the endoplasmic reticulum, and the majority are found within the subsarcolemmal sarcoplasm and to a lesser extent in the myofibrillar sarcoplasm due to the tight packing of contractile proteins (Horne & Hesketh, 1990). Approximately 60% of the weight of each ribosomal subunit is composed of rRNA which are responsible for catalyzing and facilitating reactions between the ribosomes and mRNA, and the remainder is composed of ribosomal proteins which act as structural support (P. B. Moore & Steitz, 2002). Ribosomal proteins are highly organized and tightly linked during ribosome formation; the knockdown of specific ribosomal proteins in HeLa cells impairs ribosome formation through impaired production of almost all other proteins in that respective subunit (Robledo et al., 2008). The small subunit is made up of an 18S rRNA and 33 ribosomal proteins. It is responsible for facilitating the reaction between incoming mRNA and tRNA (which bring amino acids that correspond to the mRNA transcript) to order the amino acid sequence of the developing protein (**Figure 1**) (Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Khatter et al., 2015; P. B. Moore & Steitz, 2002; Piazzi et al., 2019; van Riggelen et al., 2010; Wen et al., 2016). The large subunit comprises 28S, 5.8S, and 5S rRNA and 47 ribosomal

proteins, catalyzes the formation of peptide bonds between amino acids in the growing polypeptide chain, which is the primary protein structure. At the polypeptide chain, tRNA transport and subsequently bind amino acids to the mRNA transcript using a 3-nucleotide anticodon sequence (Khatter et al., 2015; P. B. Moore & Steitz, 2002). In the large subunit, mRNA and tRNA are bound in the "acceptor" (A) site, move to the "peptidyl" (P) site once the previous tRNA is deacetylated and then moves to the "exit" (E) site and is removed. In the P site, amino acids are added one at a time to the polypeptide chain via aminoacyl tRNA synthetases, and tRNA molecules remain there until the next tRNA attaches. This interaction between mRNA and tRNA is required to form all proteins, and thus ribosome function and formation are indispensable for protein synthesis and cell growth. These processes are described graphically in **Figure 1**.

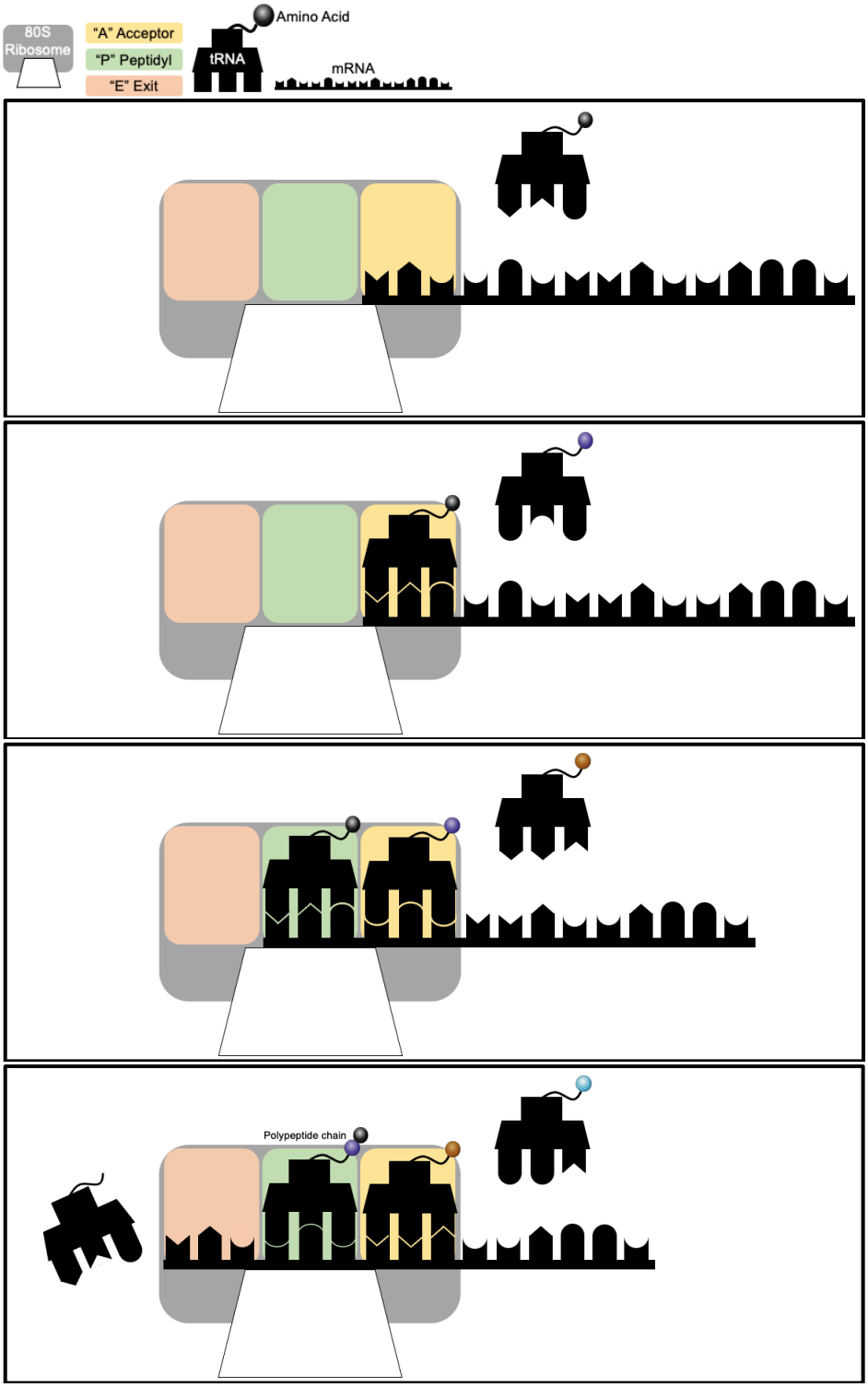

Figure 1. Pictorial representation of cellular protein translation. mRNA is entered into the acceptor site, where a tRNA with a corresponding sequence matches and binds to the mRNA with the respective amino acid. The mRNA and tRNA are then move to the peptidyl site, where the amino acid is added to the growing polypeptide chain, then removed in the exit site when a new tRNA is bound in the acceptor site.

1.2. Ribosomal biogenesis

Forming a functional protein first requires transcription, where a complementary sequence (mRNA) is made for genetic information on the DNA. The transcription step is then followed by translation where ribosomes "read" the mRNA sequence and match corresponding amino acids, thereby ordering and building a corresponding polypeptide chain which is eventually folded into mature functional protein structures (Brook et al., 2019; Figueiredo & McCarthy, 2019; Wen et al., 2016). Therefore, translational capacity refers to the number of ribosomes present within the cell to translate mRNA transcripts. Translational capacity (or "ribosome content") can be increased through ribosomal biogenesis, whereby the number of ribosomes within the cell is increased ("ribosomal biogenesis") and coincides with processes demanding an increase in protein synthesis such as feeding and resistance exercise (Brook et al., 2019; Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Wen et al., 2016). Ribosomal biogenesis is a highly regulated process involving the coordination between transcription factors and assembly units to join rRNA and ribosomal proteins into functional subunits and is rate-limiting for protein synthesis within the cell (Brook et al., 2019; Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Wen et al., 2016). It is estimated that over 2000 ribosomes are formed each minute in yeast, with rRNA accounting for approximately 80% of cellular transcription in all cells (Warner, 1999).

1.2.1. Pre-initiation complex formation

c-Myc is a transcription factor, a protein involved in initiating and regulating gene transcription, regulating cell growth and metabolism (Chaillou et al., 2014). It is the "master regulator" of ribosomal biogenesis as evidenced by its ability to induce it when overexpressed in mice in the absence of other growth stimuli (Mori et al., 2020). c-Myc acts by forming a heterodimer with Myc-associated factor X (MAX) to promote rRNA and ribosomal biogenesis regulator gene expression, such as the upstream binding factor (UBF) and transcription intermediary factor-1A (TIF-1A) through its interaction with RNA polymerase 2 (**Figure 2B**). UBF is then activated through mechanistic target of rapamycin (mTORC1)/mitogen-activated protein kinase (MAPK) signalling, where MAPK activates eukaryotic initiation factor (eIF) 4E to translate cyclin D1, which activates Cyclin-dependent kinase 4 (CDK4) to phosphorylate and activate UBF (**Figure 2C**) (Figueiredo & McCarthy, 2019). Along with RNA polymerase 2, c-Myc/MAX also activates transcription domain-associated protein (TRRAP), which together with UBF bind to the upstream controller element (UCE) region and open the histone 3 and 4 chromatin structure on rDNA promoters to allow for transcription of the rDNA genes, which contain genes that encode for the rRNAs, ribosomal proteins and tRNA (Figueiredo & McCarthy, 2019; Sanij et al., 2008; van Riggelen et al., 2010). Transcription intermediary factor-1A (TIF-1A) is transcribed by the c-Myc/MAX interaction with RNA polymerase 2 and binds to selective factor 1 (SL-1) upon activation by mTORC1 phosphorylation (**Figures 2B-C**) (Brook et al., 2019; Mayer & Grummt, 2006). SL-1 (now bound to TIF-1A) is phosphorylated by ribosomal protein S6 kinase (S6K1 – activated by mTORC1) which facilitates its interaction with UBF and stabilizes at the rDNA promoter region (**Figure 2D**) (Chauvin et al., 2014; Figueiredo & McCarthy, 2019;

Mayer & Grummt, 2006; van Riggelen et al., 2010; Wen et al., 2016). The interaction between SL-1 and UBF by S6K1 allows for RNA polymerase 1B (POLR-1B) recruitment. Once stabilized, POLR-1B is activated through mTORC1/MAPK, and c-Myc/MAX signalling and is bound to UBF and SL-1 through TIF-1A, which acts as a bridge between POLR-1B and SL-1 on UBF (Mayer & Grummt, 2006; van Riggelen et al., 2010; von Walden et al., 2016; Wen et al., 2016). POLR-1B, now bound to UBF at the UCE on the rDNA promoter region together are known as the pre-initiation complex (PIC), and once formed, allows POLR-1B to attach to rDNA and transcribe the 45S pre-rRNA in the nucleolus (Figueiredo & McCarthy, 2019; Mayer & Grummt, 2006; van Riggelen et al., 2010; Wen et al., 2016).

1.2.2. Subunit formation

Once the PIC is formed, 45S pre-rRNA is transcribed and is the rate-limiting step to ribosomal biogenesis (**Figure 2D**) (Brook et al., 2019; Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Kusnadi et al., 2015; Mayer & Grummt, 2006; Wen et al., 2016). c-Myc (independent of MAX) and mTORC1 interact with RNA polymerase 3 to transcribe tRNA and 5S rRNA in the nucleus (Mayer & Grummt, 2006; van Riggelen et al., 2010; von Walden et al., 2016; Wen et al., 2016). tRNA is then transported to the cytoplasm, and 5S rRNA is transported to the nucleolus for ribosome subunit formation. Ribosomal proteins and other accessory proteins for ribosomal biogenesis are transcribed through mTORC1 and c-Myc/MAX activation of RNA polymerase 2, are translated in the cytoplasm and shipped into the nucleolus (Mayer & Grummt, 2006; van Riggelen et al., 2010; von Walden et al., 2016; Wen et al., 2016). During subunit formation, 45S pre-rRNA is cleaved by nucleolin into mature 18S, 5.8S, and 28S rRNA (Figueiredo & McCarthy, 2019; van Riggelen et al., 2010; Wen et al., 2016). With

mature rRNA and ribosomal proteins, the 40S and 60S subunits are formed and exported into the cytoplasm and form the 80S mature ribosome upon translation initiation (**Figure 2F**).

1.2.3. Translation initiation

In the cytoplasm, the 40S subunit attaches to tRNA and GTP (energy for cellular reactions) (**Figure 2E**). When translation is initiated, mTORC1 phosphorylates and activates S6K1, which in turn activates eIF4B and causes eIF3 to associate with the 40S subunit to form the pre-initiation 43S complex (Brook et al., 2019; Chaillou et al., 2014; Goodman, 2019). eIF3 on the 43S complex binds to the eIF4G subunit on the eIF4F complex. mTORC1 inhibits the interaction of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) with eIF4, which allows eIF4 to bind to the 43S/eIF4F complex (Brook et al., 2019; Chaillou et al., 2014; Goodman, 2019; Thoreen et al., 2012). eIF4 is an RNA helicase, and once bound to the 43S/eIF4F complex, it can bind to the cap and unwind the secondary mRNA structure to begin translational scanning (Chaillou et al., 2014; Goodman, 2019). The 43S/eIF4F/eIF4E complex scans the mRNA until a start codon is located, after which the 60S subunit is recruited to form the mature 80S ribosome and begin protein translation (**Figure 2F**). The process of ribosomal biogenesis to increase translational capacity has recently been hypothesized to be essential for increasing and sustaining skeletal muscle hypertrophy following resistance exercise training.

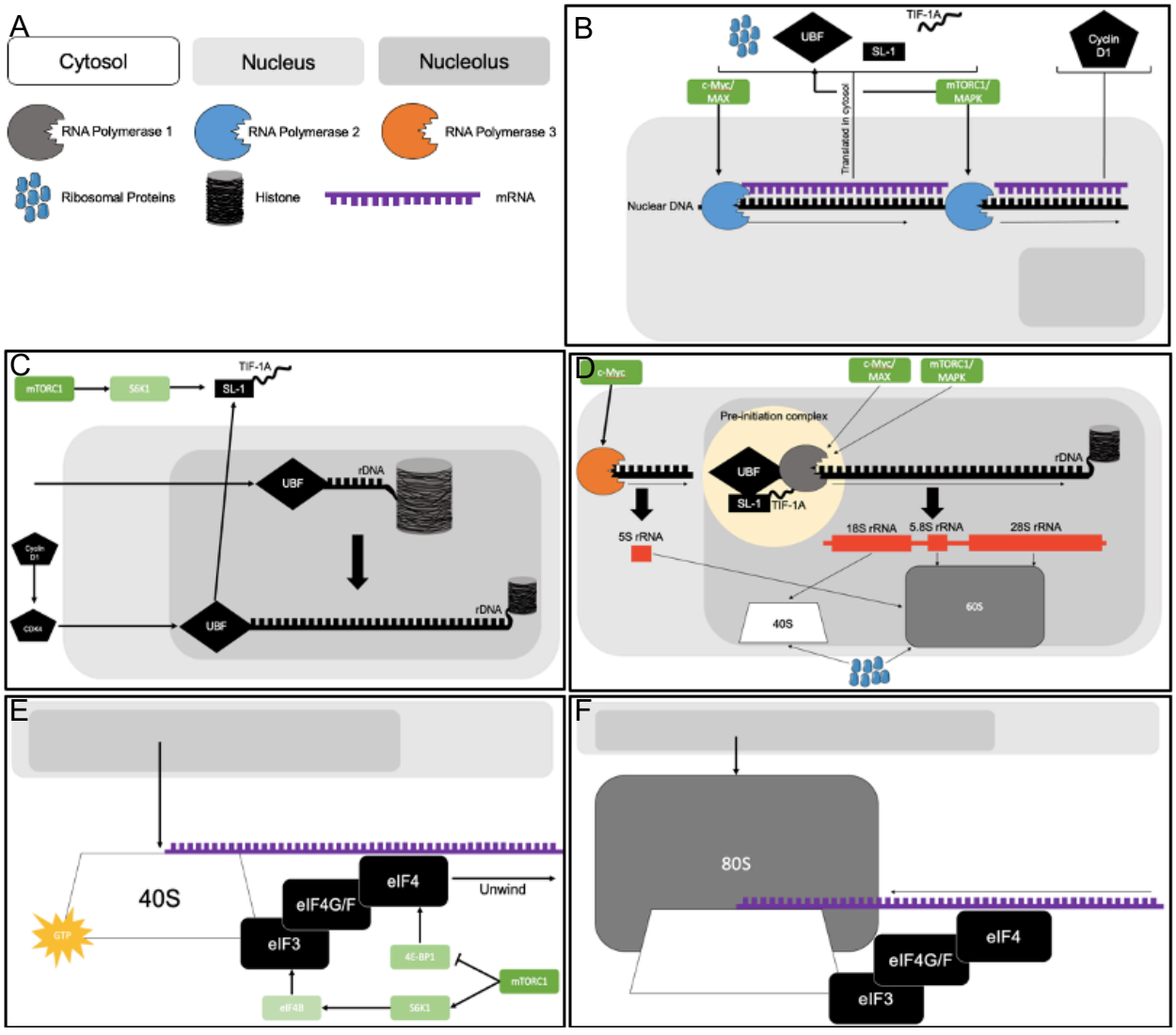

Figure 2. Schematic representation of translation initiation. *A*) Figure legend. *B*) mRNA transcription of ribosomal proteins, UBF, SL-1 and TIF-1A via c-Myc/MAX interaction with RNA polymerase 2, Cyclin D1 via mTORC1/MAPK interaction with RNA polymerase 2 in the nucleus, which are all translated in the cytosol. *C*) UBF binds to rDNA promoter region to unwind rDNA in the nucleolus, becomes activated through phosphorylation by CDK4 to recruit SL-1/TIF-1A, which interact via S6K1 phosphorylation. *D*) Pre-initiation complex formation, rRNA and ribosomal protein contribution to ribosome subunits. *E*) 43S complex binds to mRNA in the cytosol, eIF4 unwinds mRNA which 40S searches for a start codon. *F*) 43S recruits 60S subunit upon activation to form mature, 80S ribosome in the cytosol.

2. The role of ribosomes in muscle protein synthesis and hypertrophy

Protein synthesis within a cell is dictated by both translational capacity (ribosome content) and translational efficiency (activity of ribosomes) (Chaillou et al., 2014; Wen et al., 2016). Ribosomal biogenesis results in new ribosome formation, thus increasing translational capacity and is a rate-limiting characteristic of protein synthesis (Brook et al., 2019; Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Wen et al., 2016). Alternatively, acute growth stimuli can increase the amount of proteins synthesized per unit mRNA, or the ribosome's "translational efficiency" (Chaillou et al., 2014; Wen et al., 2016). This acute increase in translational efficiency is often assessed via S6K1 phosphorylation, which dictates the transcriptional program for ribosomal biogenesis (Chauvin et al., 2014). S6K1 phosphorylation is often used as a proxy measure for translational efficiency and has been correlated to strength and hypertrophy gains following resistance training in humans (Terzis et al., 2007). Translational efficiency is dictated by polyribosome complexes, which are clusters of mature ribosomes that form on individual mRNA molecules to increase the number of proteins formed per mRNA transcript. Identifying polyribosome fractions allows for the characterization of ribosome activity to determine the relative amount of proteins translated at a given time (Panda et al., 2017; Thoreen et al., 2012). Ribosome content and activity are important for all cell growth and have been of particular interest in exercise science for their role in skeletal muscle hypertrophy. Feeding and exercise are the best-known stimulators for MPS to support skeletal muscle maintenance and growth and are therefore highly reliant on ribosome content and activity.

2.1. Feeding

The intake of amino acids is a well-known stimulator of MPS. Sufficient ingestion of amino acids stimulates MPS and leads to an insulin response to reduce MPB, leading to net positive muscle protein balance (S. M. Phillips, 2014; Rasmussen & Richter, 2009; Rennie et al., 2004). MPS is stimulated approximately 30 minutes after sensing extracellular amino acids and is sustained for approximately 1.5 hours before returning to resting levels (Bohé et al., 2001, 2003). This spike in MPS corresponds with ribosome production and degradation, which are rapidly formed (likely to support MPS demands) and degraded approximately 1.5 hours following synthesis (Warner, 1999). While there is currently limited research examining direct markers of ribosome content and activity with feeding, the impact of protein restriction and starvation on ribosome content has garnered more attention. Work from the 1970's showed that rats fed a protein-free diet had a significant reduction in RNA concentration, signifying a decrease in ribosome content due to the absence of dietary protein (Millward et al., 1973). However, more recent literature has contradicted these findings. Ten weeks of calorie restriction in mice did not affect ribosome content; however, fewer ribosomes actively translating proteins were observed compared to mice fed a standard diet (Mathis et al., 2017). The calorically restricted mice also saw a trend for higher rates of rRNA and ribosomal protein turnover indicating higher rates of both ribosome biogenesis and breakdown compared to mice fed a standard diet. It is important to note that although calorie restricted, mice still had an intake of some dietary protein which may have ameliorated the ribosomal breakdown that was observed. Higher rates of ribosome breakdown is consistent with human embryonic kidney cell models in which an increase in ribosome degradation

("ribophagy") during periods of starvation is observed (Wyant et al., 2018). In a starved state, these cells inhibited mTORC1 signalling and upregulated the autophagosome, which interacts with ribosomes to degrade rRNA and ribosomal proteins to convert them to cellular energy. Therefore, with feeding, ribosome content has been shown to reflect rates of MPS and during periods of starvation increases in ribosome turnover are observed without a decrease in total ribosome content.

2.2. Acute resistance exercise

An acute bout of resistance exercise increases both MPS and MPB (elevated muscle protein turnover) but in a fed state, resistance exercise results in an overall net positive protein balance for up to 48 hours post-exercise (S. M. Phillips et al., 1997). An acute bout of resistance exercise increases MPS that is not mirrored by an increase in RNA concentration (Chesley et al., 1992). RNA concentration is used as an indirect measure of ribosome content and rates of protein synthesis as an indirect measure of translational efficiency, which suggests that acute rises in MPS are supported by increases in ribosome's translational efficiency rather than ribosome content (translational capacity).

The notion that acute resistance exercise increases translational efficiency but not capacity has recently been challenged. Aspects of resistance exercise have been recapitulated in rats using electrical stimulation, and despite several limitations, has demonstrated molecular signalling responses similar to an acute bout of resistance exercise (West et al., 2016). Electrical stimulation showed an increase in translational efficiency, as measured via S6K1 phosphorylation, which was elevated above resting level from 1.5-18

hours following stimulation. However, acute, significant increases in UBF phosphorylation, rRNA precursor (45S pre-rRNA) and c-Myc mRNA expression were observed in these rats, indicating increased ribosomal content in response to the electrical pulse stimulation. In agreement with electrical stimulation in rats, acute resistance exercise in healthy, recreationally active young men resulted in acute increases in phosphorylated TIF-1A 2 hours post-exercise and phosphorylated UBF and total c-Myc protein expression 24 and 48 hours following exercise (Figueiredo et al., 2016). Acute elevations in 45S pre-rRNA, 18S ETS and POLR-1B mRNA at 24 hours, 5.8S ITS, 28S ITS, and TIF-1A mRNA at 24 and 48 hours, and UBF mRNA expression were also reported 48 hours post-exercise, indicating that ribosomal biogenesis occurs to support the protein synthetic demands following a bout of resistance exercise.

Although some groups report acute increases in measures of translational capacity, results in human studies are inconsistent. Two studies had recreationally active, healthy young males undergo 8 weeks of resistance training at similar exercise intensities (Figueiredo et al., 2015; Fyfe et al., 2018). One took resting and 1 hour post-exercise biopsies before and after training (Figueiredo et al., 2015), whereas the other took resting biopsies before and after training in addition to 1 and 3 hour post-exercise biopsies following the 8 weeks of training (Fyfe et al., 2018). Both studies reported an acute increase in S6K1 phosphorylation following an acute bout of resistance exercise, both before and after training. These results are consistent with the hypothesis that translational efficiency increases in response to acute resistance exercise. Figueiredo et al. (2015) reported acute increases in total Cyclin D1 and UBF protein expression and TIF-1A phosphorylation 1 hour following resistance exercise in

both the trained and untrained states, but no differences in mRNA expression of UBF, TIF-1A or POLR-1B. No acute differences in 18S rRNA, 28S rRNA, 5.8S ITS, 18S ETS or 28S ITS expression was observed, however 45S pre-rRNA and 5.8S rRNA had significantly decreased expression 1 hour following acute resistance exercise in the trained state only (it is important to note that basal 45S pre-rRNA and 5.8S rRNA expression was elevated in the trained state). These findings contradict Figueiredo et al. (2016) and imply that acute resistance exercise does not increase translational capacity. Fyfe et al. (2018) demonstrated acute increases in the phosphorylation of TIF-1A and UBF 1- and 3-hours following resistance exercise. However, they also demonstrated a decrease in Cyclin D1 total protein expression 1 hour post resistance exercise and did not see any acute changes to the ribosomal precursor or other rRNA or transcribed spacer regions. The findings by Fyfe et al. (2018) in combination with results presented by Figueiredo et al. (2016) and Figueiredo et al. (2015) demonstrates highly variable and inconsistent findings as to whether acute resistance exercise augments translational capacity and may be a result of the limited number of timepoints used in their protocol. Following an acute bout of resistance exercise in young adult males and females, Figueiredo et al., (2021) reported a significant increase in S6K1 phosphorylation 8 hours post-, c-Myc mRNA expression 0.5 and 3 hours post- and 45S pre-rRNA expression 3 and 24 hours post-resistance exercise. These results signify an increase in both translational efficiency and capacity with an acute bout of resistance exercise. The discrepancies in the studies discussed may be due to different biopsy timepoints or failing to capture the full effect of ribosomal biogenesis.

When acute resistance exercise in young adult males was performed prior to, during and following 6 weeks of resistance training, c-Myc and TIF-1A protein content and S6K1 phosphorylation were elevated 1.5 hours post-acute resistance exercise only in the untrained state (Brook et al., 2016). The elevation of these markers in untrained individuals but not trained suggests that translational capacity and efficiency may only increase in response to an unaccustomed bout of exercise and skeletal muscle can adapt to have the appropriate cellular machinery, such as ribosomes, in place for any subsequent bouts. Therefore, it appears that both translational capacity and efficiency increase in response to acute resistance exercise, but training status may have an important role in the acute signalling following a bout of resistance exercise.

2.3. Chronic resistance exercise training

Resistance exercise training is the best-known stimulus to increase muscle mass and strength (Joanisse et al., 2020). Repeated transient spikes in MPS leads to an accumulation of sarcoplasmic and myofibrillar proteins within skeletal muscle that results in hypertrophy (Phillips, 2000). RNA concentration is often used as an indirect measure or ribosome content as rRNA accounts for approximately 85% of total RNA, and it has been found that RNA concentration increases following resistance training to support an increase in muscle mass (Chaillou et al., 2014; Chesley et al., 1992; Figueiredo & McCarthy, 2019; Wen et al., 2016).

Studies have described an increase in RNA concentration and 45S pre-rRNA expression as markers of ribosome content alongside myotube hypertrophy in cell models; however, the concept of increasing translational capacity to coincide with hypertrophy

becomes more complicated in rodent and human models (Nader et al., 2005; von Walden et al., 2016). Synergistic ablation (SA)-induced hypertrophy in rodent models has measured increases in ribosome content that subsequently return to baseline levels 7 and 14 days post-surgery (Nakada et al., 2016; von Walden et al., 2012). The increase in ribosome content within 7 days following SA alongside a sustained elevation in S6K1 phosphorylation after 7 days suggests that the ribosomal biogenesis response is either acute or increased early during supraphysiological muscle hypertrophy then later returns to baseline levels. Interestingly, no difference in translational capacity was observed in RNA concentration and c-Myc, UBF, RNA polymerase 1 and several ribosomal proteins when muscle hypertrophy was induced in rats via 6 weeks of progressive, resistance-loaded wheel running (Mobley, Holland, et al., 2018). However, a reduction in muscle atrophy F-box protein 32 (FBXO32) and poly-ubiquitinated protein marker of proteolysis was reported, which suggests that muscle hypertrophy seen with progressive resistance training in rodent models may be a result of reduced protein breakdown and not an increase in ribosome content or activity but is not necessarily translatable to humans.

The impact of chronic resistance training on translational capacity and efficiency has also been inconsistent in human studies. Resistance training for 8 weeks in recreationally active young men increased resting 45S pre-rRNA, 5.8S rRNA, 18S rRNA and UBF mRNA expression alongside an increase in resting UBF phosphorylation (Figueiredo et al., 2015). In a study where participants completed 12 weeks of resistance training consisting of one leg trained with a single set and the other trained with multiple sets (same load, greater volume in multiple set condition), both groups increased RNA concentration, 45S

pre-rRNA and 5.8S, 18S and 28S rRNA expression from baseline following resistance training (Hammarström et al., 2020). However, the multiple set condition had greater ribosome content than the single set after 2 weeks of training, whereas the single set condition had greater ribosome content after the 12 weeks. Similar to von Walden et al. (2012), this suggests that there may be an initial increase in ribosomal biogenesis early in the hypertrophic response to resistance training which is captured at the 2 week mark in the multiple set group and at 12 weeks in the single set group as they gained significantly less muscle mass compared to their counterparts and likely did not reach the same ribosome content/hypertrophy threshold as the multiple set group (Hammarström et al., 2020). Findings from these studies demonstrate an increase in ribosomal biogenesis following resistance training (Figueiredo et al., 2015; Hammarström et al., 2020). However, a conflicting study in young adult males reported no changes in resting markers of ribosome content (45S pre-rRNA, 5.8S rRNA, 18S rRNA, 28S rRNA, 18S ETS and TIF-1A, UBF, Cyclin D1 mRNA expression) following 8 weeks of resistance training and a decreased 5.8S rRNA, 28S rRNA, 5.8S ITS and POLR-1B mRNA expression, suggesting that ribosome content either does not change or decreases with resistance training (Fyfe et al., 2018). To date, the impact of resistance training on ribosome content in humans remains inconclusive, and although more research alludes to an increase in translational capacity to support muscle hypertrophy than not, more research should be conducted. It appears that there is a high degree of heterogeneity in how ribosome content is influenced between individuals and may impact how these individuals respond to exercise.

2.4. High and low responders to chronic resistance training

Researchers have reported a high degree of inter-individual variability with respect to gains in strength and muscle mass following resistance training in humans (Ahtiainen et al., 2016; Shrier, 2006). This variability is observed due to differences in nutrition, genetic predisposition (Clarkson et al., 2005; Pescatello et al., 2006), epigenetic and transcriptional regulation (Davidsen et al., 2011) and myonuclear accretion (Petrella et al., 2008) amongst other things. This inter-individual variability leads to what researchers have referred to as "high responders" or "low/non responders" to exercise training. High responders see the highest increase in strength and muscle size following resistance exercise training and low/non responders, accounting for up to 20% of the population, see little to no adaptive response to resistance training (Roberts et al., 2018a; Timmons, 2011). It has been hypothesized that differences in translational capacity may explain some of the variability between individuals observed with resistance training (Mobley, Haun, et al., 2018).

Untrained young adult males underwent 12 weeks of resistance training and were retrospectively divided into "Low," "Moderate" or "High" responders based on their change in *vastus lateralis* thickness using K-means cluster analysis (Mobley, Haun, et al., 2018). RNA concentration increased in all groups with training which led the authors to conclude that that ribosomal biogenesis occurred with resistance training. However, other markers of ribosome content such as c-Myc and POLR-1B protein content did not change with training. Additionally, a decrease in 45S pre-rRNA expression was reported with training. This shows that RNA concentration is likely a poor indicator of ribosome content and that perhaps rDNA transcription does not change or may even decrease with resistance training.

In another study, healthy older adults underwent 4 weeks of resistance training and were also retrospectively divided into "Low," "Moderate" or "High," responders based on their change in type 2 fibre cross-sectional area (CSA) (Stec et al., 2016). An increase in type 2 fibre CSA was only reported in the Moderate and High responder groups following training. RNA concentration (although Moderate tended to increase) and total rRNA abundance increased in the High responders only following resistance training. It therefore appears that ribosomal biogenesis is necessary for hypertrophy to occur following resistance training. However, it is important to note that RNA concentration may be a weak indicator of ribosome content and that 4 weeks of training was much shorter than the 8-12 weeks utilized in other studies, and they therefore may be observing the initial increase in ribosomal biogenesis that has been observed early following the start of resistance training (Hammarström et al., 2020; von Walden et al., 2012). Another important implication is the population used by Stec et al. (2016) as participants are approximately 40-50 years older than those observed in Mobley, Haun, et al. (2018). This is especially important as older adults have been shown to have higher baseline ribosome content compared to young and impaired ribosomal biogenesis (Stec et al., 2015). Therefore, while it appears that ribosomal biogenesis following resistance training may influence individual's responsiveness to training, more research needs to be conducted to limit variability of training interventions, sampling timepoints and be consistent across age groups.

2.5. Concurrent training

Concurrent training is the utilization of both aerobic and resistance exercise training techniques within a specified training regime and is used to increase endurance, strength and power outcomes, although exercise order and time between exercise is often inconsistent between studies (Baar, 2014). An early study showed an attenuation of muscle hypertrophy in individuals that underwent both aerobic and resistance training together compared to those who completed resistance training alone (Hickson, 1980). The researcher termed this as the interference effect, however they were not sure if this was due to training status, training volumes or conflicting oxidative and anabolic molecular signalling pathways.

The signalling responses to aerobic and resistance training result in the activation of different molecular signalling cascades. Aerobic exercise elicits a molecular response to increase oxidative capacity through AMP-activated protein kinase (AMPK) metabolic signalling (Russell et al., 2014), whereas resistance exercise increases mTORC1 signalling responses related to cell growth (Combes et al., 2015; Rasmussen & Richter, 2009). AMPK acts upstream of mTORC1 and therefore aerobic exercise can inhibit growth adaptations triggered by resistance exercise as measured through AMPK knock-out mouse models (Mounier et al., 2009). This contributes to the blunted hypertrophy observed with concurrent training while still eliciting the same gains in oxidative capacity as those who strictly undergo aerobic training (Fyfe et al., 2016; Hickson, 1980; Mounier et al., 2009). Conflicting findings regarding anabolic signalling following concurrent training are likely the result of varying exercise order and intensity

amongst other factors that may contribute to the interference effect (Apró et al., 2013; Jones et al., 2017).

Exercise training leads to the fine tuning of molecular signalling, where responses become more specific to the mode of exercise that is trained and the acute response becomes attenuated over time (Coffey et al., 2006). In fact, concurrent training in untrained young men has demonstrated an increase in muscle hypertrophy to the same extent as resistance training until after approximately 7 weeks of training, showing that concurrent training can elicit gains in muscle size until a certain point and signifies the importance of training status on the interference effect (Fernandez-Gonzalo et al., 2013; Lundberg et al., 2013).

A study comparing concurrent training to resistance training alone found that ribosome content, measured through rRNA, mRNA and protein expression of regulators of ribosomal biogenesis, decreased with both forms of training but to a greater extent with resistance training alone (Fyfe et al., 2018). Resistance training resulted in a greater degree of hypertrophy and acute ribosomal biogenesis signalling than concurrent training, which demonstrates that ribosome content may decrease alongside an increase in muscle hypertrophy following resistance training, but the signalling pathways involved in ribosomal biogenesis in response to acute exercise are enhanced. Therefore, this study demonstrates that resistance training, but not concurrent training, increases translational efficiency and acute translational capacity following an exercise bout. However, as concurrent training resulted in a lesser gain in muscle mass compared to resistance training, it again begs the question as to whether a certain hypertrophic threshold is required for ribosomes to stop increasing in content and become more efficient for protein translation.

2.6. Ribosomal DNA copy number

Recently, rDNA copy number has been used to help explain the high degree of inter-individual variability in ribosome content and biogenesis (Figueiredo et al., 2021; Gibbons et al., 2014, 2015). rDNA genes are arranged in palindromes on chromosomes 1 for 5S rRNA and 13, 15, 21 and 22 for 45S pre-rRNA, and contain hundreds of copies at each loci (Piazzi et al., 2019). The loci for 45S pre-rRNA form loops on the chromosome called nucleolar organizer regions, which allows for the recruitment of nucleolar-specific proteins for pre-rRNA transcription (Figueiredo & McCarthy, 2019). rDNA copy number is concerted between 5S and 45S pre-rRNAs and is similar between tissues within individuals but is highly variable between individuals (Gibbons et al., 2014, 2015; Kuo, 1996). This discrepancy in rDNA copy number between individuals has been hypothesized to impact individual's ribosome content and rDNA transcriptional response. Indeed, rDNA copy number was recently found to significantly and positively correlate to resting rRNA expression and the increase in 45S pre-rRNA expression 24 hours following a bout of resistance exercise (Figueiredo et al., 2021). They also reported a reduction in c-Myc enhancer site methylation following an acute bout of resistance exercise which resulted in an increase rDNA transcription. This was the first study to provide evidence that rDNA copy number influences ribosome content and biogenesis.

3. Evidence for a relationship between ribosomes and mitochondria

3.1. The impact of aerobic training on ribosome content and function

Ribosomal biogenesis and protein translation are typically studied in resistance training for their role in supporting muscle hypertrophy; however, less is known about the impact of aerobic exercise and training on ribosomal biogenesis. Recently, a single bout of aerobic exercise was demonstrated to have no impact on S6K1 phosphorylation or 45S pre-rRNA expression, whereas an increase was observed with a single bout of resistance exercise (Figueiredo et al., 2021). This signifies that neither translational efficiency nor capacity is increased following a single bout of aerobic exercise. No research to date has investigated the impact of aerobic training on direct markers of translational efficiency or ribosomal biogenesis.

Aerobic exercise training leads to a phenotypic change in skeletal muscle including a fibre-type shift from type 2X glycolytic to type 1, hybrid or type 2A fibres, mitochondrial biogenesis and microvascular perfusion (Hoier & Hellsten, 2014; Irrcher et al., 2003; Jornayvaz & Shulman, 2010). Acute aerobic exercise has been demonstrated to increase mitochondrial protein synthesis (Di Donato et al., 2014; Wilkinson et al., 2008) and in some cases, have demonstrated an increase in myofibrillar protein synthesis in addition to mitochondrial protein synthesis (Churchward-Venne et al., 2020). These remodelling processes involve the turnover and synthesis of new proteins, for example, myosin heavy chain proteins to dictate fibre type, DNA replication and formation of oxidative enzymes and phosphorylation complexes during mitochondrial biogenesis and proliferation and sprouting of endothelial cells through the extracellular matrix to form new capillary beds

(Hoier & Hellsten, 2014; Irrcher et al., 2003; Jornayvaz & Shulman, 2010). With the remodelling resulting from aerobic training, it is plausible that protein translation is altered to support the adaptive responses observed. Although no studies have directly examined markers of translational capacity with aerobic training, 12 weeks of high-intensity interval training in old individuals resulted in increased expression of both mitochondrial and ribosomal proteins (Robinson et al., 2017). The authors concluded that translational mechanisms were increased alongside mitochondrial content. Although ribosome content was not directly measured, the increase in ribosomal protein content shows that aerobic training may require an increase in translational machinery to support cellular remodelling. The mechanism of this phenomenon is unknown; however, it was recently found that peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), the master regulator of mitochondrial biogenesis that is highly upregulated following acute aerobic exercise, is essential for rDNA transcription and once again highlights the potential impact that aerobic training may have on ribosomal biogenesis (Jesse et al., 2017).

3.2. Fibre type-specific ribosome response

Skeletal muscle is composed of distinct fibre types which have individualized characteristics and functions. Type 1 fibres are slow-twitch oxidative fibres, which have a high mitochondrial content, are highly fatigue-resistant and are used for everyday tasks and endurance exercise (Schnyder & Handschin, 2015). Type 2 fibres are fast-twitch glycolytic fibres, which typically rely on non-oxidative energy sources for high power contractions. They have greater potential to hypertrophy following resistance training than type 1 fibres and

are classified as either type 2A (more oxidative) or 2X (more glycolytic) in humans. Due to their distinct properties and primary sources of energy, fibre types have varying rates of MPS and MPB, which are higher in type 1 fibres in humans (Mittendorfer et al., 2005). Along with rates of MPS, type 1 fibres have also been shown to have greater mRNA, rRNA and total RNA content than type 2 fibres, suggesting that they have the highest translational capacity among fibre types (Habets et al., 1999). In addition, mice subjected to SA were shown to have the greatest increase in phosphorylated ribosomal protein S6 (rpS6) in type 1 fibres compared to type 2, suggesting a greater capacity in type 1 fibres to increase ribosome content following this stimulus (Goodman et al., 2012). However, in young men who underwent a single bout of resistance exercise, translational efficiency, as measured by S6K1 phosphorylation, increased to a greater extent in type 2 fibres post-exercise (Koopman et al., 2006). As type 2 fibres are predominantly used during resistance exercise, greater S6K1 activation in these fibres suggest that ribosome activity may act in a fibre type-specific manner in response to exercise. It is therefore plausible to propose that translational mechanisms will respond to adapt to stimuli pertaining to aerobic training, as this form of exercise primarily targets type 1 muscle fibres.

3.3. Potential relationship between ribosomes and mitochondria

Ribosomal biogenesis and protein translation are very metabolically costly processes, and cellular energy is essential for their function. Ribosomes are constantly being synthesized at a high rate within the cell (Warner, 1999). rRNA synthesis accounts for approximately 60% of total cellular transcription, and an estimated 4 ATP molecules are

required per peptide bond during the formation of a polypeptide chain. Mitochondria are organelles responsible for oxidative energy production in the cell and are the primary producers of cellular energy (Bratic & Trifunovic, 2010). Due to their role in energy production, mitochondria likely contribute to the energy requirements for ribosomal biogenesis. We propose a relationship exists between ribosomes and mitochondria in which mitochondria aid in the supply of energy for ribosomal biogenesis and protein translation, and ribosomes translate mitochondrial proteins to increase oxidative capacity.

Mitochondria have been found to colocalize with cellular components requiring a high ATP supply. Chang et al. (2006) performed a study to observe the impact of mitochondria on neuronal energy supply to the synapse and Ca^{2+} concentrations. Active synapses require high levels of ATP to meet the metabolic demands of signal transduction through neurotransmission. It was found that mitochondria clustered around active neuronal synapses and had an altered shape to extend along the length of the axon to supply ATP to the neuron readily. Mitochondria have been shown to travel across the cytoskeleton to reside in cellular locations with the greatest energy demand (Yi et al., 2004). The ability to travel to different locations highlights mitochondria's dynamic nature and suggests that they may interact with other metabolically demanding cellular components, such as ribosomes for ribosomal biogenesis and protein translation. While mitochondria may assist in improving ribosome function, ribosomes may also play an important role in mitochondrial function.

As previously mentioned, aerobic training leads to an increase in the translation of several proteins linked to an adaptive response to increase oxidative capacity, the most

important of which being mitochondrial and ribosomal proteins (Robinson et al., 2017). Although they have yet to be evaluated in response to aerobic training, the increase in ribosomal proteins alongside mitochondrial proteins suggests an increase in translational capacity to support mitochondrial adaptations. A recent study in cells identified a relationship between the cytosolic and mitochondrial translational pathways (Molenaars et al., 2020). It was found that there was distinct, bilateral communication between translation in the cytosol and mitochondria, where altering one had a substantial impact on the other. To our knowledge, this is the first study to directly link mitochondria and translational mechanisms showing that it is likely that a relationship exists between ribosomes and mitochondria. It has also been previously shown that mitochondrial biogenesis occurs alongside muscle hypertrophy, likely to support the energetic demands of increasing muscle mass and further highlights the potential relationship between ribosomes and mitochondria (Kirby et al., 2015).

3.4. Mitochondrial ribosomes

Mitochondria contain their own structurally and functionally distinct ribosomes ("mitoribosomes") to translate the contents of the mitochondrial genome (O'Brien, 2003; Sharma et al., 2003). The mature 55S mitoribosome, like the cytosolic 80S ribosomes, are composed of a small and large subunit. The small subunit contains the 12S rRNA and 30 mitoribosome proteins, where the large subunit contains the 16S rRNA and 48 mitoribosome proteins. The genes that code for the mitoribosome proteins are on the nuclear genome, and are transcribed, translated, and transported into the mitochondria during mitoribosome synthesis. The 5S rRNA has also been shown to be shipped from the nucleus to the

mitochondria and become incorporated into the large subunit of the mitoribosome and is the only rRNA utilized by both ribosome species (Smirnov et al., 2008, 2010, 2011). The mitochondrial genome codes for 22 tRNAs, the 12S and 16S rRNAs and 13 proteins involved in oxidative phosphorylation within the mitochondria.

4. Objectives and hypotheses

The purpose of this study was to determine the impact of both aerobic and resistance training on ribosome content and to examine the impact of prior aerobic training on ribosome adaptations to resistance training. Both aerobic and resistance training lead to cellular adaptations, but ribosome content has not been fully explored in the context of aerobic training and there have been discrepancies in findings of ribosome content following resistance training. The primary objectives of this study were to examine the impact of 1) aerobic training, 2) resistance training, and 3) aerobic pre-training on ribosome content. We hypothesized that aerobic and resistance training would increase ribosome content to support the adaptive responses to respective exercise training. We also hypothesized that translational capacity would correspond to changes in mitochondrial-related protein content following aerobic training. If translational capacity was increased following aerobic training, then it would not increase further following resistance training as it is likely that sufficient translational machinery will already be in place.

METHODS

Experimental Outline

A visual depiction of the study design is in **Figure 3**. Each participant had one leg randomized to be aerobically trained (EX) via single-leg cycling on a cycle ergometer while the other leg acted as a sedentary control (CTL). Participants underwent aerobic training 3 times per week for 6 weeks starting at 50% work peak of their single-legged VO_2 peak tests and progressively increased their workload throughout training. Following this, participants underwent progressive bilateral, lower body resistance training 3 times per week for 10 weeks. The tissue used in the current study was taken from a larger study investigating the role of aerobic training prior to resistance training on satellite cell content and function, strength, and hypertrophy (Thomas, 2019).

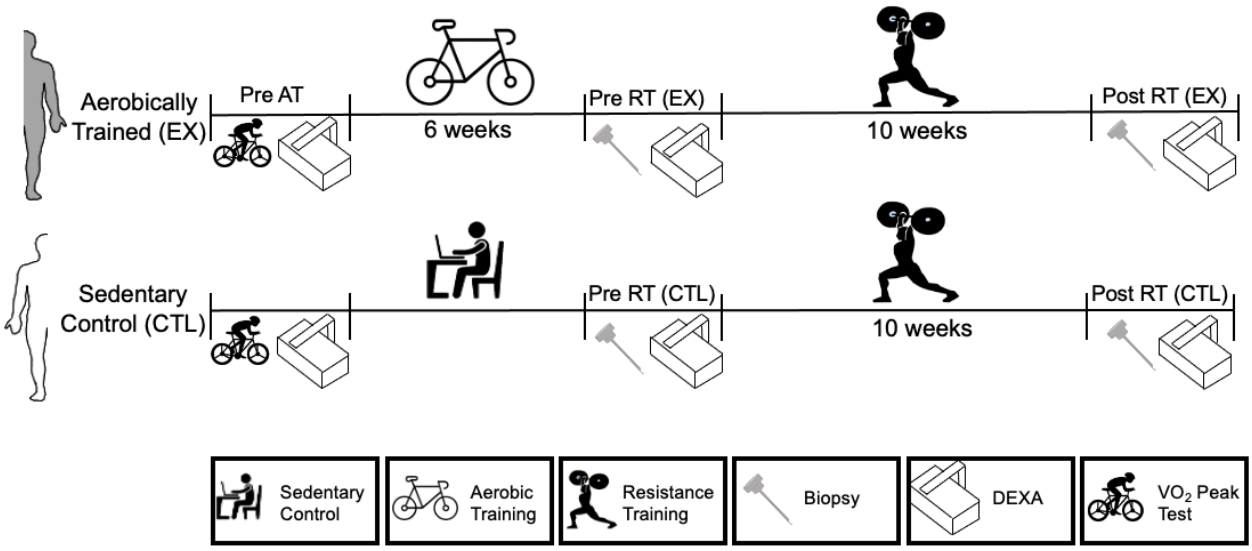

Figure 3. Overview of study design. Participants (n=14) underwent 6 weeks of single-legged aerobic training (MICT) followed by 10 weeks of bilateral resistance training. Testing and measures taken pre aerobic training, pre resistance training (Pre RT) and post resistance training (Post RT).

Subjects

A full list of baseline subject characteristics is included in **Table 1**. Briefly, fourteen, young (21 ± 1.69 years old) healthy (*BMI* 25.42 ± 4.65 kg/m^2), male (n=8) and female (n=6) participants were recruited to participate in this study. Participants had no formal aerobic or weight training experience 6 months prior to the study commencement. To qualify for participation, participant's VO_2 peak values were below 45 mL/kg/min for men, 40 mL/kg/min for women and were excluded from participation if they smoked, had diabetes, used non-steroidal anti-inflammatory drugs or statins, and had a history of respiratory disease and/or major orthopedic disability. The experiment and associated risks were explained to participants in full prior to obtaining their written consent to be included in the study. This study was approved by the Hamilton Health Sciences Integrated Research Ethics Board (HiREB #3885) and conformed to the guidelines outlined in the Declaration of Helsinki.

Table 1. Baseline subject characteristics.

Biological Sex	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	VO_2 Peak (mL/min/kg)	Average LLSTM (g)
Male (n=8)	21 ± 2	175 ± 5	84.2 ± 16.6	27.3 ± 5.1	42.3 ± 7.4	10950 ± 1577
Female (n=6)	21 ± 2	162 ± 8	60.6 ± 10.3	22.9 ± 2.5	34.8 ± 5.0	6714 ± 757
Total (n=14)	21 ± 2	**170 ± 9***	**74.1 ± 18.3***	25.4 ± 4.7 (p=0.073)	39.1 ± 7.4 (p=0.054)	**9135 ± 2509***

Values are means ± SD. ***p<0.05 significant difference between males and females**.

Pre-Training Procedures

Initially, whole-body peak oxygen uptake (VO_2 peak) and peak power output (W peak) were determined by an incremental double-legged test to exhaustion on an electronically braked cycle ergometer (Excalibur Sport, version 2.0; Lode, Groningen, The Netherlands). The test consisted of a 1-minute warm-up at 50 watts (W), after which the workload was increased 1 W every 2 seconds (s) until the participant's cadence fell below 60 revolutions per minute (rpm), which was defined as having reached volitional exhaustion. An online gas collection system (Moxus modular oxygen uptake system; AEI Technologies, Pittsburgh, PA, USA) was used to analyze expired gases and VO_2 peak was determined from the greatest 30 s average of the test.

For single-legged cycling, one pedal of an electronically braked cycle ergometer (Velotron; RacerMate, Seattle, WA, USA) was fitted with a custom-machined pedal with an 11.4 kg counterweight to assist with the upstroke phase of the revolution and was modelled on previous work (Abbiss et al., 2011; Burns et al., 2014; MacInnis, Zacharewicz, et al., 2017). One leg cycled using a standard pedal while the other remained on a stationary platform. At least 48 hours following the double-legged VO_2 peak test, subjects were familiarized with single-leg cycling and performed single-legged VO_2 peak tests with each leg to determine the initial training workload. Single-legged VO_2 peak tests were similar to the double-legged tests with the exception that the rate at which the workload was increased was reduced by half (increase of 1 W every 4 s). The order was randomized to determine which leg was tested first and was followed by the contralateral leg 10 minutes after the end of the first

test, as previous data has shown that there is no transfer of fatigue to the non-exercised leg (Elmer et al., 2013).

Aerobic Training

The leg that was randomized to be aerobically trained (EX) underwent three 45-minute sessions of single-legged cycling per week for 6 weeks (18 sessions total) with adherence of 94 ± 9% on the same cycle-ergometer that was used for baseline testing. The initial workload was set at 50% of the average work peak achieved in each participant's single-leg VO_2 tests and was incrementally increased by 2-4% every 4 sessions. Training sessions consisted of a 3-minute warm-up at 25 W, followed by 40 minutes of cycling at their prescribed workload and a 2-minute cool-down at 25 W. Participants were instructed to maintain a cadence of approximately 80 rpm throughout each session. Rating of perceived exertion and heart rate were recorded at the 2-, 7-, 40-, and 44-minute timepoints during each session.

Muscle Strength Measurements

Bilateral 1-repetition maximum (1RM) testing was performed for squat, leg press and leg extension exercises before and after resistance training. For each 1RM test, weight was increased incrementally until achieving a maximal weight at which 1 repetition could be completed. Squat and leg press repetitions were required to reach a depth of knee angle at 90 degrees, and leg extension required legs to be extended fully.

Resistance Exercise Training

Starting approximately 1 week after the last aerobic training session, participants performed progressive bilateral lower body resistance training 3 times per week for 10 weeks (30 sessions total) and had an adherence of 88.19 ± 10.35%. Resistance exercises were primarily geared to target muscle of the quadriceps. Participants completed 3 sets, 10-12 repetitions at 70-80% of their 1-repetition maximum (1RM) of squats, leg press, leg extension, hamstring curls, and calf extensions, with 2 minutes of rest between each set and the last set was completed to failure for each exercise. Weights for each exercise were increased for the following training session if the participant maintained 10-12 repetitions for each set, by the exercise supervisor's discretion.

Supplementation

Participants ingested 25 g of Ascent, whey protein isolate (Vanilla Bean or Chocolate) which included 2.7 g leucine immediately following each resistance exercise session.

Muscle Biopsy Sampling

A total of 9 percutaneous needle biopsies were taken from the mid portion of the *vastus lateralis* under local anesthetic (1% lidocaine (lignocaine)) using a 5-mm Bergstrom needle adapted for manual suction (Bergström, 1975). For the sake of the current study, only 4 muscle biopsy samples were used. Bilateral biopsies were taken following 6 weeks of aerobic training (Pre RT), one each from the EX and CTL legs. Bilateral biopsies were

taken once again after 10 weeks of resistance training (Post RT). The biopsies used for this study were obtained in resting conditions after a 10 to 12 hour overnight fast. Each biopsy collected approximately 150 mg of muscle tissue, which was dissected free of adipose and connective tissue and flash-frozen in liquid nitrogen, then stored at −80°C. Approximately 50 mg was dissected from the biopsies, orientated in cross-section, mounted in optimal cutting temperature compound (Tissue-Tek, Sakura Finetek, USA) and frozen in isopentane cooled with liquid nitrogen for immunohistochemical analysis. The mounted samples were sectioned with a thickness of 5 μm at −20°C, mounted on slides and stored at –80°C for subsequent analyses.

Body Composition

Dual X-ray absorptiometry (DXA – GE Lunar iDXA; Aymes Medical) scans were taken Pre RT, and Post RT after a 10-12 hour overnight fast to identify whole-body and leg-lean soft mass tissue (LLSTM).

Immunohistochemical Analysis

Mounted muscle tissue was stained using cytochrome c oxidase subunit IV (COXIV; Abcam ab110261, Mouse Anti-COX4+COX4L2) for mitochondrial-related protein content, alongside myosin heavy chain II (MHCII; Abcam ab91506, Rabbit Anti-Fast Myosin Heavy chain) for fibre-type determination and wheat-germ agglutinin (WGA Invitrogen™ W32466, Wheat Germ Agglutinin, Alexa Fluor™ 647 Conjugate) for identification of the sarcolemmal boarder. Slides were separated by participant and had all

4 samples on the same slide. Samples were initially fixed in 4% paraformaldehyde for 10 minutes, before being blocked with 2% bovine serum albumin (BSA), 5% fetal bovine serum, 2% Triton X-100, 1% sodium azide and 5% goat serum (GS) in 1X phosphate buffer solution (PBS) at room temperature for 90 minutes. Following the block, primary antibodies COXIV (1:800) and MHCII (1:500) were diluted in 1% BSA in 1X PBS and left overnight at 4ºC. The next day, secondary antibodies conjugated to 488 nm for COXIV (Invitrogen™ A11001, Goat Anti-Mouse IgG (H+L) Cross-Absorbed Secondary Antibody, Alexa Fluor™ 488, 1:500) and 647 nm for MHCII (Invitrogen™ A32733, Goat Anti-Rabbit IgG (H+L) Cross-Absorbed Secondary Antibody, Alexa Fluor™ 647, 1:500) were diluted in 1% BSA in 1X PBS and incubated with the muscle cross-sections for 2 hours at room temperature. After the incubation, WGA (1:300) diluted in 1% BSA in 1X PBS was added for 25 minutes at room temperature. After that, samples were washed, allowed to dry for 1 hour then coverslipped using DAKO fluorescent mounting medium. Negative controls for the primary and secondary antibodies were observed to validate the immunofluorescent staining signals.

Samples were imaged using a Nikon Fluorescent microscope under the 20X objective. Exposure times remained consistent for each participant to compare the intensity of signals between timepoints. Images were then blinded and analyzed by two researchers using NIS elements software. Muscle fibre type was determined using the CY5 channel, distinguishing between Type 1 (no MHCII signal) and Type 2 (MHCII signal). COXIV protein content was determined using an average of signal intensity from 50 fibres of each fibre type. Individual fibres were circled, and mean intensity in the 488 channel was

determined for each intact fibre devoid of freeze-fracture. A representative image of this stain is in **Figure 4A-B**.

Muscle homogenization, RNA and Protein Isolation

Approximately 20 mg flash-frozen skeletal muscle was homogenized by using 1 mL TRIzol® reagent in Lysing Matrix D tubes (MP Biomedicals, Solon, OH, U.S.A.), with FastPrep-24™ Tissue and Cell Homogenizer (MP Biomedicals, Santa Ana, California, U.S.A.) for 40 s at 6 m/s. RNA and protein were isolated using the TRIzol® manufacturer's protocol with simple modifications. During re-suspension of protein pellets, pellets were re-homogenized using the FastPrep-24™ instrument and were re-suspended in 1:1 SDS:8M urea solution. RNA yields were determined using a NanoDrop 2000™ Spectophotometer (Thermo Scientific, U.S.A.). Protein content was determined using a Pierce™ BCA Protein Assay Kit (microplate procedure) with a sample to working reagent ratio of 1:5 and absorbance measured using a SYNERGY Mx (BioTek®, Highland Park, Winooski, Vermont, U.S.A.) plate reader.

Reverse Transcription

Isolated RNA samples were reversed transcribed into cDNA using a RT^2 First Strand Kit (Qiagen, Toronto, Ontario, Canada) in 10 µL reaction volumes as per manufacturer's instructions and carried out using a SimpliAmp™ Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Mississauga, Ontario, Canada). cDNA was then

diluted to a final concentration of 10 ng/µL and stored at -20°C until subsequent analysis. RNA samples from 1 participant were excluded due to low RNA concentration yields.

Quantitative Real-time PCR (RT qPCR)

All RT qPCR reactions were run in a QuantStudio™ 5 – 384-Well Block (Applied Biosystems, Thermo Fisher Scientific) RT qPCR machine. Primers sequences are included in **Table 2**. All primers were re-suspended in 1X TE Buffer (10mM Tris-HCl and 0.11 mM EDTA) and stored alongside the commercially available gene expression assays (**Table 3**) at -20°C prior to use. RT qPCR reactions for individual primers and the 45S pre-rRNA assay (Qiagen) were prepared in triplicates of 12.5 µL reaction volumes containing RT^2 Sybr Green qPCR Master Mix (Qiagen, cat. #330500), forward and reverse primers (or 45S pre-rRNA assay) and 10 ng cDNA. 384-well PCR plates were prepared using the epMotion 5075 Eppendorf automated pipetting system (Eppendorf, Mississauga, Ontario, Canada). Taqman gene expression assays were prepared in triplicates of 10.0 µL reaction volumes containing Taqman™ Fast Advanced Master Mix (ThermoFisher, cat. #4444556), Taqman™ gene expression assays (ThermoFisher) and 10 ng cDNA. Taqman™ gene expression assays were conjugated to FAM reporter dye, NFQ-MGB quencher and ROX as a passive reference. The expression of the housekeeping gene ß2M was not affected by the intervention (**Appendix B5**). Samples were normalized to ß2M (ΔC_t; either respective SYBR or Taqman™ ß2M) and to the Pre RT (CTL) timepoint ($\Delta\Delta C_t$). Statistical analysis was performed on the $2^{-\Delta\Delta Ct}$ value (Livak & Schmittgen, 2001). Fold change was calculated for graphical purposes by relating all values to the average of Pre RT (CTL).

Missing data for gene expression analyses were replaced using a trend analysis considering the patterns of individual subjects, group means and specific timepoints. Outliers were determined at $2^{-\Delta\Delta Ct}$, removed and trend analyses were performed at ΔCt to account for outliers. If a subject had more than one timepoint missing during the trend analysis step, they were removed from the analysis.

Table 2. Primer sequences for quantitative real-time qPCR.

Target	Primer Concentration	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
ß2M	10 µM	TGCTGTCTCCATGTTTGATGTATCT	TCTCTGCTCCCCACCTCTAAGT
Cyclin D1	15 µM	GCTGCGAAGTGGAAACCATC	CCTCCTTCTGCACACATTTGAA
UBF	10 µM	CCTGGGGAAGCAGTGGTCTC	CCCTCCTCACTGATGTTCAGC
TIF-1A	10 µM	GTTCGGTTTGGTGGAACTGTG	TCTGGTCATCCTTTATGTCTGG
POLR-1B	10 µM	GCTACTGGGAATCTGCGTTCT	CAGCGGAAATGGGAGAGGTA
5.8S ITS span	10 µM	TCGCCAAATCGACCTCGTAC	AGCTGCGTTCTTCATCGACG
18S ETS span	15 µM	GCCCGTCCTCGCGAGGC	TGCATGGCTTAATCTTTGAGAC
28S ITS span	10 µM	CGGCGCGATTCCGTCCGT	GTTCACTCGCCGTTACTGAG
5.8S rRNA	10 µM	ACTCTTAGCGGTGGATCACTC	GACGCTCAGACAGGCGTAG
18S rRNA	10 µM	TGGCTCAGCGTGTGCCTAC	ACAAAGGGCAGGGACTTAATC
28S rRNA	10 µM	ACCTGGCGCTAAACCATTC	GTGTCGAGGGCTGACTTTC

ß2M, beta 2 microglobulin; *UBF*, upstream binding factor; *TIF-1A*, transcription intermediary factor 1A; *POLR-1B*, RNA polymerase IB; *ITS*, internal transcribed spacer; *ETS*, external transcribed spacer.

Table 3. Commercially available gene expression assays for quantitative real-time PCR.

Target	Company	Material/Catalogue Number	Catalogue Number/Assay ID
ß2M	ThermoFisher	4331182	Hs00187842_m
c-Myc	ThermoFisher	4331182	Hs00153408_m
45S pre-rRNA	Qiagen	330001	PPH82089A-200
5S rRNA	ThermoFisher	4426961	Hs03682751_gH
12S MT-rRNA	ThermoFisher	4331182	Hs02596859_g1
COXIVI1	ThermoFisher	4331182	Hs00266371_m1
TFAM	ThermoFisher	4331182	Hs01082775_m1

ß2M, beta 2 microglobulin; *c-Myc*, cellular Myc; *MT-rRNA*, mitochondrially-encoded rRNA; *COXIVI1*, cytochrome oxidase subunit IV-I1; *TFAM*, mitochondrial transcription factor A.

Western blotting

Isolated proteins were prepared at either 0.1 or 0.2 µL/µg (depending on the subject) in 4x Laemmli buffer (BioRad, Mississauga, Ontario, Canada) with 10% 2-mercaptoethanol (BME) and a total of 4.5 or 9 µg were loaded. Equal amounts of proteins were loaded into 1.5 mm, 10% (CS/α-Tubulin) or 12.5% (OXPHOS) gels and run using a BioRad PowerPac™ at 100 V for 3 hours (CS/α-Tubulin, 4°C) or 200 V for 2 hours (OXPHOS, room temperature). Each gel consisted of one subject (each timepoint) and two ladders (BioRad, Precision Protein™ Kaleidoscope™ Prestained Protein Standards, cat. #161-0375, 5 µL). Proteins were then transferred onto a 0.45 µm nitrocellulose membrane (BioRad) using a Trans-Blot® Turbo™ Transfer System (BioRad). Membranes were stained with Ponceau S (Sigma-Aldrich) and imaged using a ChemiDoc™ (BioRad) imaging machine to ensure equal loading for future normalization. The membranes were then blocked in 3% skim milk (Carnation® Fat-Free Instant Skim Milk Powder) in 1X tris-buffered saline with Tween-20 (TBST) at room temperature for 1 hour. Membranes were washed in 1X TBST prior to an overnight (~17 hours) primary antibody incubation at 4°C with either Citrate Synthetase (Abcam ab96600, Anti-Citrate synthetase antibody, 1:1000) and α-Tubulin (Cell Signalling Technology® #2125, α-Tubulin (11H10) Rabbit mAb, 1:1000), or an OXPHOS cocktail containing antibodies for COXI-V (Abcam ab110411, Total OXPHOS Human WB Antibody Cocktail, 1:1000) diluted in 5% BSA in 1X TBST. The next day, membranes were washed in 1X TBST and incubated for 1 hour at room temperature in secondary antibody (Cell Signalling®, Anti-rabbit IgG 7074S or Anti-mouse IgG 7076S, HRP-linked Antibody, 1:10 000) in 1X TBST. Following the secondary

incubation, membranes washed again in 1X TBST before antibody detection via Clarity Max™ Western ECL Substrate (BioRad) and imaged using the ChemiDoc™ Imaging System (BioRad) to determine mean pixel intensity for each band.

OXPHOS blot membranes were then stripped using a stripping buffer (15.0 g glycine, 1.0 g sodium dodecyl sulfate, 10.0 mL Tween 20, in 1.0 L double-distilled water, pH to 2.2) for 2x15 minutes, washed in 1X TBST, re-blocked in 3% skim milk, and the same steps as mentioned above were performed with the primary antibody COXIV (Abcam ab110261, COXIV + COXIVL2 mouse, 1:1000) in 1X TBST.

CS bands were normalized to respective housekeeping protein α-Tubulin on the same gel and at the same timepoint, where OXPHOS bands were normalized to the corresponding ponceau image. All blots were also normalized to a gel control (pooled sample).

Statistical Analysis

Statistical analysis was performed using Jamovi 1.6.23 analysis software. Outliers were determined using the means ± (2x standard deviation) and were replaced using trend analyses for RNA concentration and gene expression data but removed from immunoblot and immunohistochemical data as there were insufficient data to perform trend analyses for these measures. Differences in baseline subject characteristics between male and female participants were analyzed using independent t-tests. Differences in mitochondrial protein content via staining intensity and immunoblotting between CTL and EX at the Pre RT timepoint were analyzed using paired t-tests. Two-way repeated measures analysis of

variance (2-way RM-ANOVA), with factors of time (Pre RT and Post RT) and condition (EX and CTL) were used to determine differences in gene expression and Tukey's Honest Significant Difference Test was used to account for multiple post-hoc comparisons. Correlations were determined by using a correlation matrix for Pearson's Correlation Coefficient (binomial), with data representing the change in expression throughout RT being relative to Pre RT values of their respective condition.

For ribosome content and muscle size analyses, individual legs (CTL and EX) at different timepoints (Pre RT and Post RT) were treated independently. LLSTM and all corresponding ribosome-related gene expression were ranked from high to low based on LLSTM (13 participants with RNA data, CTL and EX, Pre RT and Post RT) (**Figure 9**). Ranked values were divided into tertiles, with the top LLSTM being categorized as HIGH (n=17) and bottom as LOW (n=17), where the middle group (n=18) was used to separate HIGH and LOW and was eliminated from the analyses. For baseline ribosome content and hypertrophy (**Figure 10**), and ribosome biogenesis and hypertrophy (**Figure 11**) "responder" analyses, legs were once again treated independently (CTL and EX) and ranked based on ΔLLSTM from Pre RT to Post RT, with corresponding ribosome values to respective legs. The top and bottom 10 values were taken from ΔLLSTM and classified as HIGH and LOW responders to RT, respectively, with a middle group (n=6) to separate HIGH and LOW and was eliminated subsequent analyses. Independent t-tests were used to determine differences between LOW and HIGH groups for LLSTM and the change in LLSTM (ΔLLSTM).

All data are expressed as means ± standard deviation (SD).

RESULTS

Subject Characteristics

Complete subject baseline characteristics are reported in **Table 1**. There was no significant difference in age (21 ± 1.85 years, 21 ± 1.67 years) between male and female participants. ($p>0.05$). Significant differences in height (175.38 ± 4.63 cm, 162.42 ± 7.91 cm; $p=0.002$), weight (84.15 ± 16.58 kg, 60.62 ± 10.32 kg; $p=0.010$) and average LLSTM (10950.63 ± 1576.95 g, 6713.92 ± 756.52 g; $p<0.001$) were observed between males and females, respectively. Trends for differences in BMI (27.33 ± 5.10 kg/m^2, 22.86 ± 2.45 kg/m^2; $p=0.073$) and relative VO_2 peak (42.27 ± 7.42 mL/min/kg, 34.77 ± 4.98 mL/min/kg; $p=0.054$) were observed between males and females, respectively.

Mitochondrial Content

Mitochondrial Protein Content Pre RT. Due to a lack of tissue availability, mitochondrial protein content was conducted at Pre RT only. COXIV staining intensity was significantly greater in EX compared to CTL in Type 1 fibres (**Figure 4C**; *EX* 3132.59 ± 1483.06 I.U., *CTL* 2495.05 ± 1042.95 I.U.; $p=0.020$), Type 2 fibres (**Figure 4D**; *EX* 2860.52 ± 1372.79 I.U., *CTL* 2176.23 ± 903.29 I.U.; $p=0.015$) and when Type 1 and Type 2 fibres were combined (**Figure 4E**; *EX* 2996.55 ± 1422.99 I.U., *CTL* 2335.64 ± 972.11 I.U.; $p=0.016$) following aerobic training (n=7). Immunoblots showed no differences between CTL and EX at Pre RT for COXI (**Figure 5A**; n=12) COXII (**Figure 5B**; n=12), COXIII (**Figure**

5C; n=12), COXIV (**Figure 5D**; n=12), COXV (**Figure 5E**; n=13) and CS (**Figure 5F**; n=9) protein content ($p>0.05$).

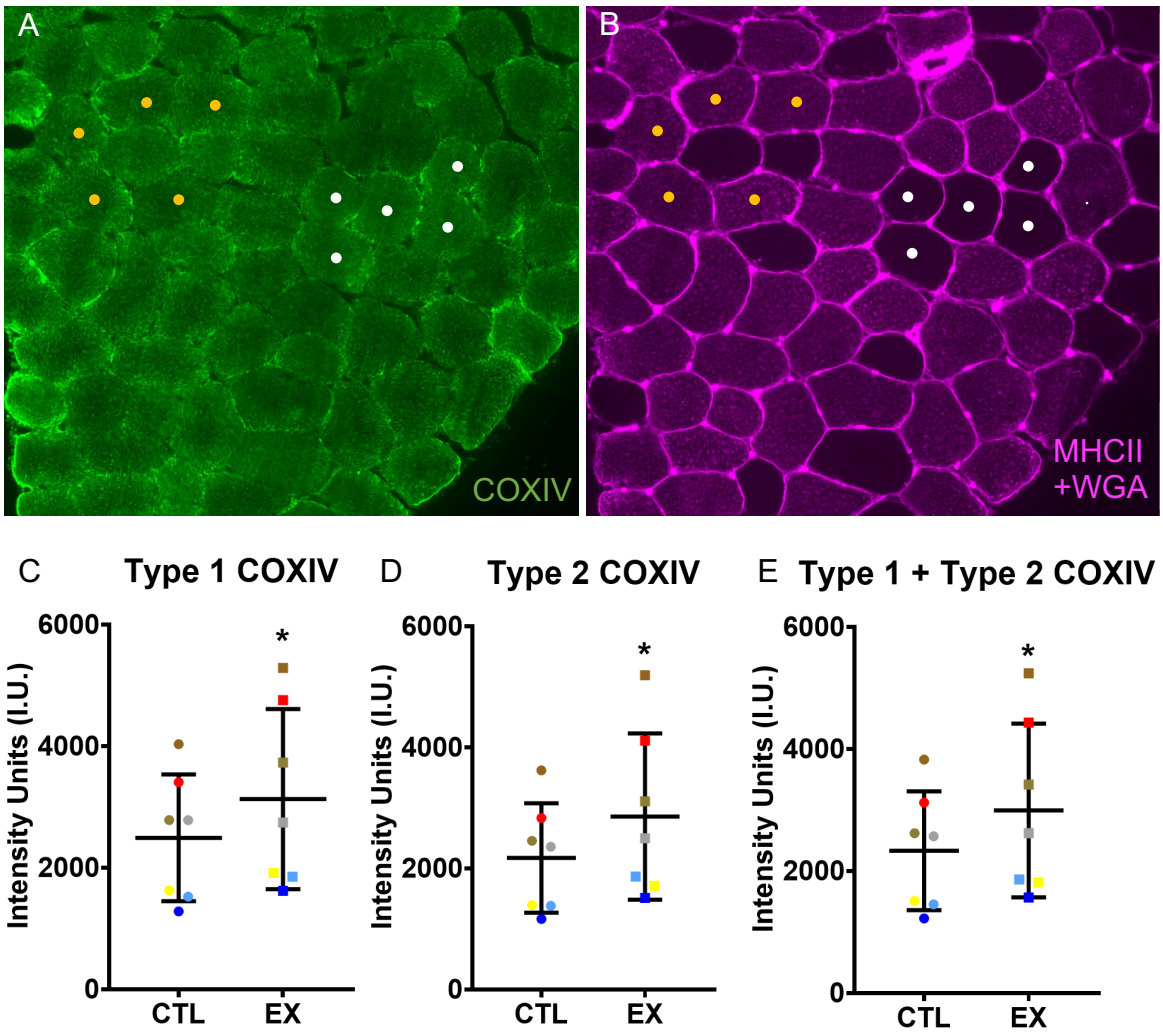

Figure 4. Fibre type-specific COXIV staining intensity. Representative images of an immunofluorescent stain containing *A*) COXIV (green) and *B*) MHCII and WGA (pink), with white dots highlighting a subset of Type 1 fibres and yellow highlighting a subset of Type 2 fibres. COXIV stain intensity Pre RT in CTL (•) and EX (■) for *C*) Type 1, *D*) Type 2 and *E*) Mixed-fibres (n=7). Values are individual data points (where each colour represents a different participant) overlayed on means (middle, horizontal line) ± SD (vertical line). *Significant difference from CTL ($p<0.05$).

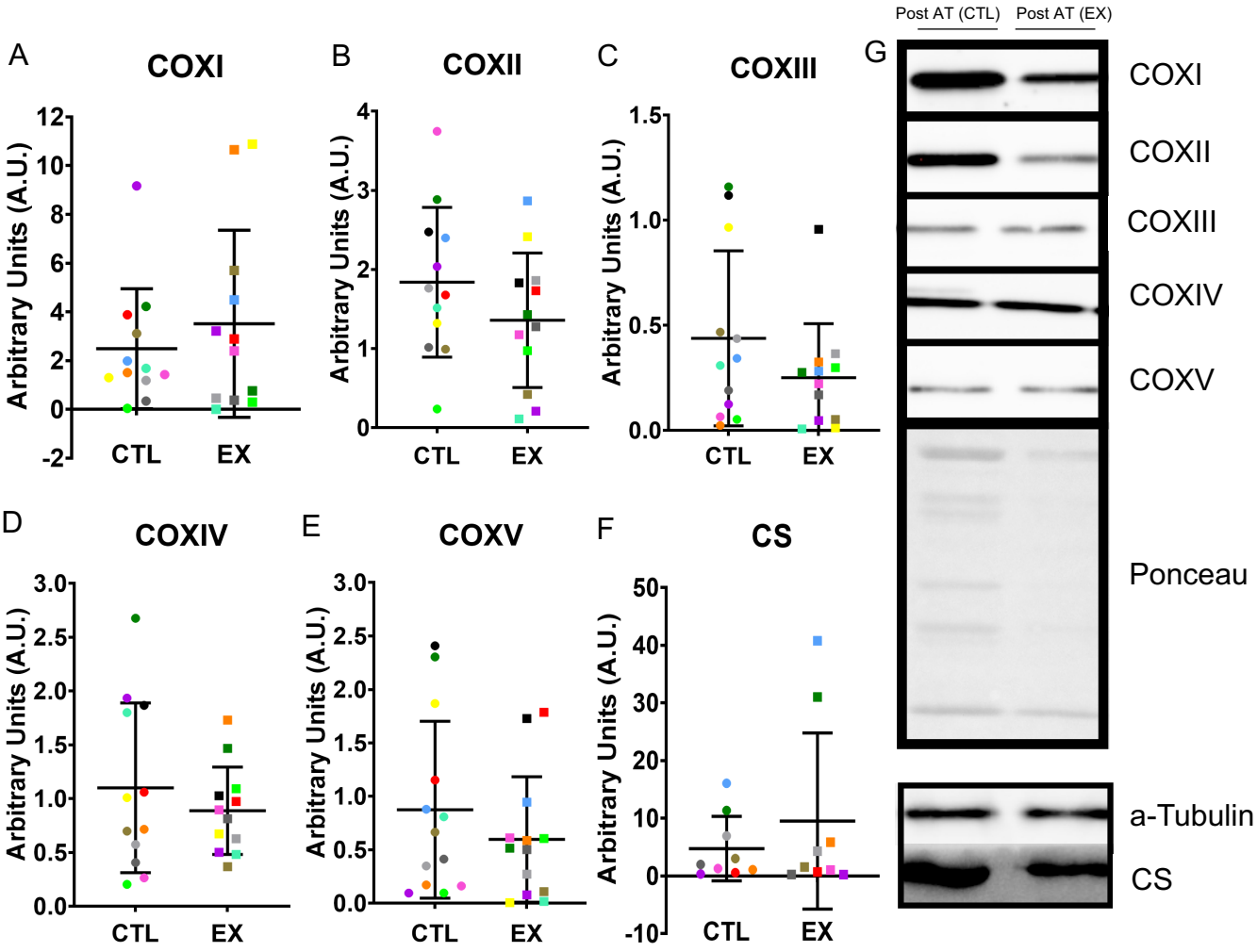

Figure 5. Immunoblot data for mitochondrial-related protein content. Protein content Pre RT in CTL (•) and EX (■) for *A*) COXI (n=12), *B*) COXII (n=12), *C*) COXIII (n=12), *D*) COXIV (n=12) and *E*) COXV (n=13), normalized to ponceau (lane) and a pooled sample, and *F*) CS (n=9) normalized to α-Tubulin. *G*) Representative images of immunoblots. Values are individual data points (where each colour represents a different participant) overlayed on means (middle, horizontal line) ± SD (vertical line).

Mitochondrial Gene Expression. No significant effects of time (Pre RT and Post RT) or condition (CTL and EX) were observed for COXIV mRNA (**Figure 6A**; n=12) and 12S mt-rRNA (**Figure 6C**; n=11) expression ($p>0.05$). A trend for a time x condition interaction was observed for TFAM mRNA (**Figure 6B**; n=12) expression from Pre RT (*CTL* 1.00 ± 0.39-fold, *EX* 0.92 ± 0.34-fold) to Post RT (*CTL* 0.80 ± 0.34-fold, *EX* 0.99 ± 0.35-fold)

(p=0.062), however no differences were observed using Tukey's post-hoc comparisons and no effects of time or condition were observed (p>0.05).

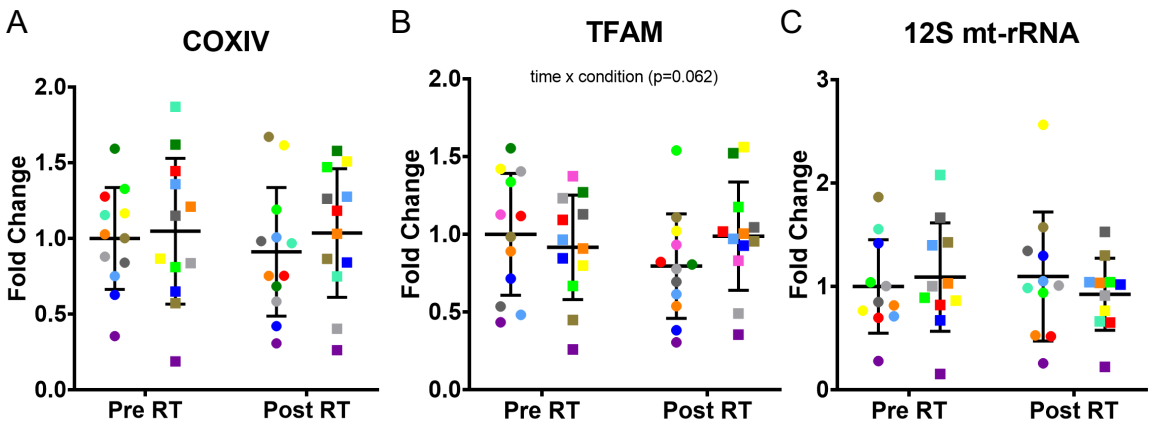

Figure 6. Mitochondrial-related gene expression. *A*) COXIV (n=12) and *B*) TFAM (n=12) mRNA expression, *C*) 12S mt-rRNA expression Pre RT and Post RT in CTL (•) and EX (■). Values are fold change relative to Pre RT (CTL), with individual data points (where each colour represents a different participant) overlayed on means (middle, horizontal line) ± SD (vertical line).

RNA Markers of Ribosomal Biogenesis

Ribosomal Biogenesis Regulators. A significant effect of condition (CTL and EX) was observed for c-Myc mRNA expression (**Figure 7B**; n=12), where CTL (*Pre RT* 1.00 ± 0.47-fold, *Post RT* 1.20 ± 0.55-fold) was greater than EX (*Pre RT* 0.78 ± 0.31-fold, *Post RT* 0.95 ± 0.40-fold) (p=0.034), however no effects of time or time x condition interaction were observed (p>0.05). No significant effects of time (Pre RT and Post RT) or condition (CTL and EX) were observed for Cyclin D1 (**Figure 7C**; n=11), UBF (**Figure 7D**; n=11), TIF-1A (**Figure 7E**; n=12) and POLR-1B (**Figure 7F**; n=11) mRNA expression (p>0.05).

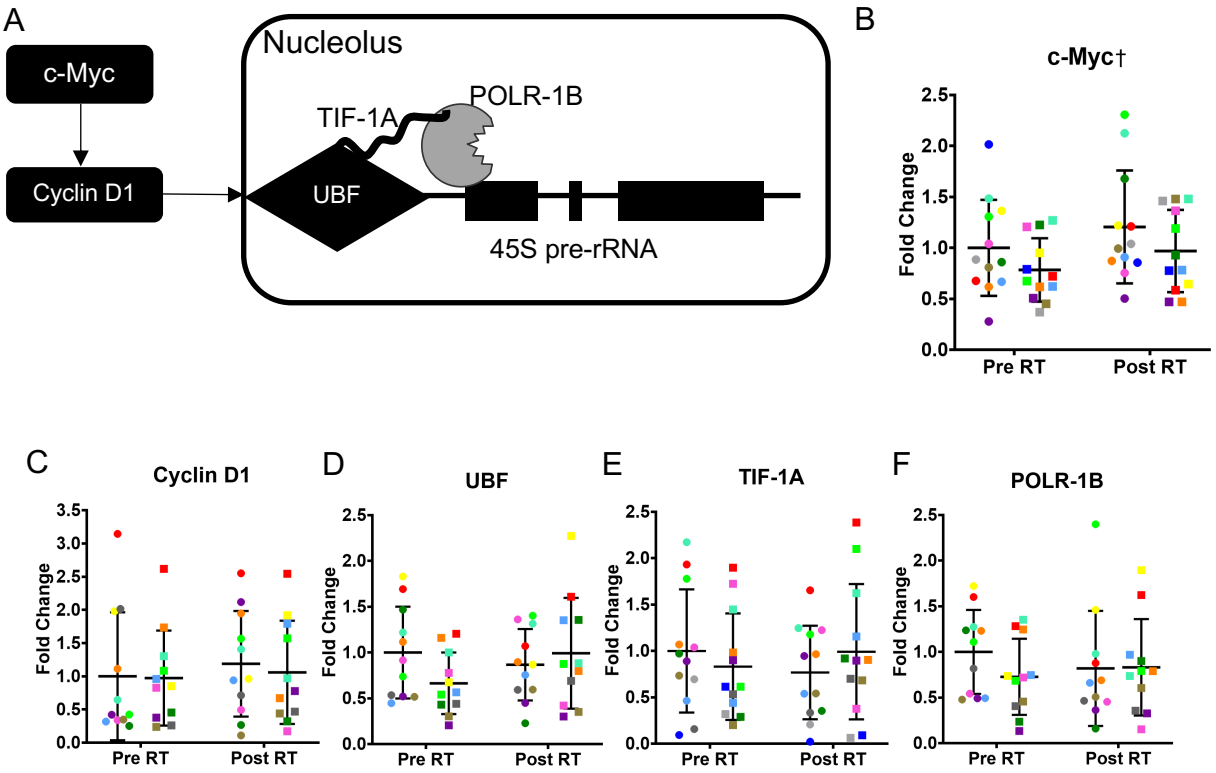

Figure 7. Ribosomal biogenesis regulator gene expression. *A*) Overview of upstream ribosomal biogenesis signaling. *B*) c-Myc (n=12), *C*) Cyclin D1 (n=11), *D*) UBF (n=11), *E*) TIF-1A (n=12) and *F*) POLR-1B (n=11) mRNA expression Pre RT and Post RT in CTL (•) and EX (■). Values are fold change relative to Pre RT (CTL), with individual data points (where each colour represents a different participant) overlayed on means (middle, horizontal line) ± SD (vertical line). †Significant effect of condition (CTL>EX) ($p<0.05$).

[RNA]. No significant effects of time (Pre RT and Post RT) or condition (CTL and EX) was observed for RNA concentration (**Figure 8B**; n=12) from Pre RT to Post RT or between CTL and EX ($p>0.05$).

Ribosomal RNAs. A significant time x condition interaction was observed for 5S rRNA (**Figure 8C**; n=12) expression from Pre RT (*CTL* 1.00 ± 0.75-fold, *EX* 1.42 ± 1.07-fold) to Post RT (*CTL* 1.56 ± 0.97-fold, *EX* 1.01 ± 0.59-fold) ($p=0.031$), where Tukey's post-hoc comparisons observed a trend for a difference between CTL and EX at Pre RT ($p=0.076$),

however no effects of time or condition were observed ($p>0.05$). No significant effects of time (Pre RT and Post RT) or condition (CTL and EX) were observed for 45S pre-rRNA (**Figure 8D**; n=12), 5.8S rRNA (**Figure 8H**; n=13), 18S rRNA (**Figure 8I**; n=13) and 28S rRNA (**Figure 8J**; n=12) expression ($p>0.05$).

Internal and External Transcribed Spacer Regions. A significant effect of condition (CTL and EX) was observed for 5.8S ITS (**Figure 8E**; n=12), where CTL (*Pre RT* 1.00 ± 0.58-fold, *Post RT* 0.82 ± 0.82-fold) was greater than EX (*Pre RT* 0.80 ± 0.67-fold, *Post RT* 0.64 ± 0.59-fold) ($p=0.029$) and for 18S ETS (**Figure 8F**; n=13) where CTL (*Pre RT* 1.00 ± 0.52-fold, *Post RT* 0.58 ± 0.39-fold) was greater than EX (*Pre RT* 0.75 ± 0.56-fold, *Post RT* 0.47 ± 0.31-fold) ($p=0.028$). 18S ETS also had a significant effect of time (Pre RT and Post RT) where Pre RT was greater than Post RT ($p=0.010$), but no significant effect of time was observed for 5.8S ITS expression ($p>0.05$). No significant effects of time or condition were observed for 28S ITS (**Figure 8G**; n=12) expression ($p>0.05$).

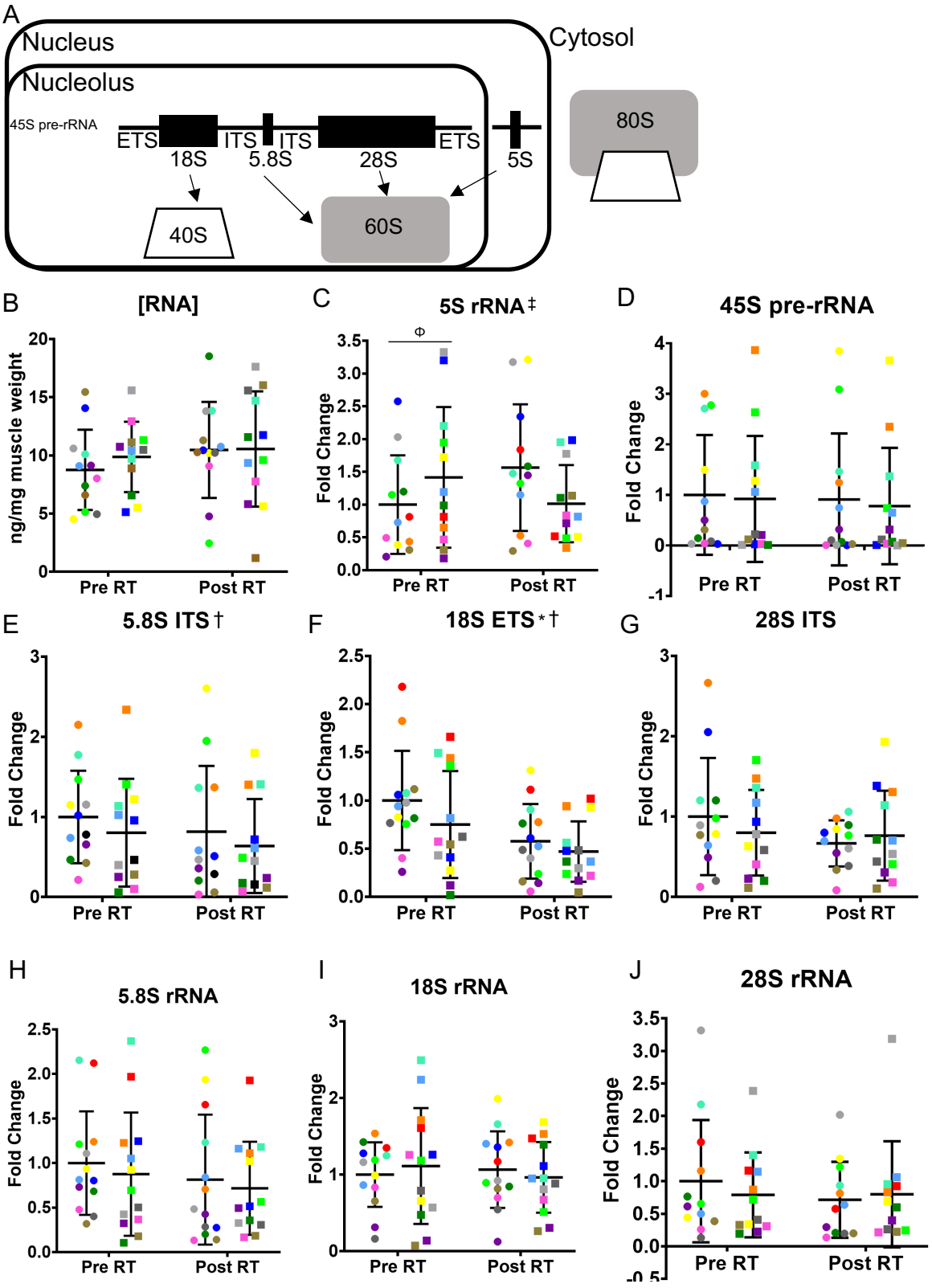
A
Nucleus
Nucleolus
45S pre-rRNA
ETS
18S
ITS
5.8S
ITS
28S
ETS
5S
40S
60S
Cytosol
80S
B
[RNA]
ng/mg muscle weight
Pre RT
Post RT
C
5S rRNA‡
Φ
Fold Change
D
45S pre-rRNA
E
5.8S ITS†
F
18S ETS*†
G
28S ITS
H
5.8S rRNA
I
18S rRNA
J
28S rRNA

Figure 8. Markers of ribosome content. *A*) Overview of rDNA contributions to ribosomal subunits. *B*) RNA concentration relative to muscle wet weight (n=12). *C*) 5S rRNA (n=12), *D*) 45S pre-rRNA (n=12), *E*) 5.8S ITS (n=12), *F*) 18S ETS (n=13), *G*) 28S ITS (n=12), *H*) 5.8S rRNA (n=13), *I*) 18S rRNA (n=13), *J*) 28S rRNA (n=12) expression Pre RT and Post RT in CTL (•) and EX (■). Values are fold change relative to Pre RT (CTL), with individual data points (where each colour represents a different participant) overlayed on means (middle, horizontal line) ± SD (vertical line). *Significant effect of time (Pre RT > Post RT), †significant effect of condition (CTL>EX), ‡significant time x condition interaction ($p<0.05$), Φtrend for a difference between timepoints within condition (Pre RT CTL < Pre RT EX) ($0.05<p<0.10$).

Ribosome-related Gene Expression and Muscle Size

Correlation Analysis. A correlation matrix was run to examine the relationship between ribosome-related gene expression and muscle size (**Table 4**). The most significant correlations coincided with LLSTM and therefore LLSTM was the focus for subsequent analyses. LLSTM had significant, negative correlations with 5.8S ITS (**Figure 9A**; r=-0.342, p=0.017) and 28S ITS (**Figure 9B**; r=-0.455, p=0.001) expression.

Table 4. Pearson's correlations between ribosome-related gene expression and muscle size. CTL (n=13) and EX (n=13) legs pooled at Pre RT (n=26; 13 participants) and Post RT (n=26; 13 participants) and total pooled CTL and EX legs and Pre RT and Post RT (n=52). Corresponds with **Figure 9**.

Timepoint	Marker	Sample Size (n)	Type 1 CSA	Type 2 CSA	Mixed-fibre CSA	LLSTM
Pre RT	c-Myc	24	r=0.162 p=0.451	r=-0.081 p=0.708	r=0.016 p=0.939	r=-0.290 p=0.170
	Cyclin D1	22	r=0.264 p=0.236	**r=0.426** **p=0.048***	*r=0.392* *p=0.072*	r=0.358 p=0.102
	UBF	22	r=0.276 p=0.214	r=0.303 p=0.171	r=0.317 p=0.151	r=0.240 p=0.282
	TIF-1A	24	r=0.280 p=0.185	r=0.132 p=0.540	r=0.209 p=0.327	r=0.076 p=0.725
	POLR-1B	22	r=0.192 p=0.392	r=0.136 p=0.546	r=0.173 p=0.442	r=0.033 p=0.883

	5S rRNA	24	r=-0.081 p=0.708	r=-0.239 p=0.261	r=-0.193 p=0.366	r=-0.275 p=0.193
	45S pre-rRNA	24	r=-0.127 p=0.555	r=-0.343 p=0.101	r=-0.273 p=0.196	**r=-0.447** **p=0.029***
	5.8S ITS	24	r=-0.064 p=0.766	r=-0.217 p=0.308	r=-0.165 p=0.440	**r=-0.468** **p=0.021***
	18S ETS	26	r=0.209 p=0.306	r=0.130 p=0.527	r=0.177 p=0.387	r=-0.149 p=0.467
	28S ITS	24	r=0.096 p=0.656	r=-0.173 p=0.419	r=-0.061 p=0.777	**r=-0.569** **p=0.004***
	5.8S rRNA	26	r=0.195 p=0.339	r=0.071 p=0.732	r=0.133 p=0.518	r=-0.199 p=0.329
	18S rRNA	26	r=0.109 p=0.595	r=-0.187 p=0.362	r=-0.071 p=0.731	**r=-0.404** **p=0.041***
	28S rRNA	24	r=0.040 p=0.853	r=0.073 p=0.734	r=0.065 p=0.761	r=-0.034 p=0.875
Post RT	c-Myc	24	r=-0.177 p=0.409	*r=-0.390* *p=0.060*	*r=-0.357* *p=0.087*	r=-0.255 p=0.229
	Cyclin D1	22	r=0.178 p=0.429	r=0.026 p=0.907	r=0.082 p=0.716	r=0.018 p=0.937
	UBF	22	r=0.339 p=0.122	r=0.202 p=0.366	r=0.263 p=0.237	r=0.071 p=0.754
	TIF-1A	24	r=-0.036 p=0.866	r=-0.363 p=0.081	r=-0.267 p=0.206	r=0.003 p=0.990
	POLR-1B	22	r=0.095 p=0.674	r=0.031 p=0.892	r=0.056 p=0.806	r=-0.014 p=0.950
	5S rRNA	24	r=0.044 p=0.839	r=0.049 p=0.820	r=0.050 p=0.817	r=0.055 p=0.800
	45S pre-rRNA	24	r=-0.011	r=-0.032	r=-0.027	r=-0.034

			p=0.958	p=0.880	p=0.902	p=0.874
	5.8S ITS	24	r=-0.085 p=0.692	r=-0.163 p=0.446	r=-0.144 p=0.502	r=-0.213 p=0.318
	18S ETS	26	r=0.045 p=0.826	r=-0.003 p=0.989	r=0.015 p=0.944	r=0.086 p=0.677
	28S ITS	24	r=0.111 p=0.607	r=-0.001 p=0.997	r=0.040 p=0.854	r=-0.287 p=0.174
	5.8S rRNA	26	r=-0.116 p=0.571	r=-0.251 p=0.216	r=-0.216 p=0.289	r=-0.051 p=0.803
	18S rRNA	26	r=0.081 p=0.693	r=0.031 p=0.879	r=0.051 p=0.803	r=-0.083 p=0.688
	28S rRNA	24	r=0.120 p=0.578	r=0.086 p=0.691	r=0.103 p=0.633	r=0.069 p=0.747
Combined Pre RT and Post RT	c-Myc	48	r=-0.024 p=0.871	r=-0.204 p=0.164	r=-0.151 p=0.304	r=-0.211 p=0.150
	Cyclin D1	44	r=0.228 p=0.136	r=0.202 p=0.187	r=0.266 p=0.140	r=0.204 p=0.184
	UBF	44	**r=0.322** **p=0.033***	*r=0.255* *p=0.095*	**r=0.297** **p=0.050***	r=0.164 p=0.288
	TIF-1A	48	r=0.113 p=0.445	r=-0.157 p=0.286	r=-0.062 p=0.674	r=0.032 p=0.829
	POLR-1B	44	r=0.127 p=0.410	r=0.055 p=0.723	r=0.086 p=0.577	r=0.001 p=0.996
	5S rRNA	48	r=-0.010 p=0.948	r=-0.055 p=0.709	r=-0.042 p=0.776	r=-0.110 p=0.457
	45S pre-rRNA	48	r=-0.072 p=0.628	r=-0.147 p=0.320	r=-0.128 p=0.387	r=-0.237 p=0.104
	5.8S ITS	48	r=-0.100 p=0.499	r=-0.211 p=0.150	r=-0.183 p=0.214	**r=-0.342** **p=0.017***

18S ETS	52	r=0.054 p=0.701	r=-0.060 p=0.670	r=-0.020 p=0.887	r=-0.101 p=0.476
28S ITS	48	r=0.062 p=0.675	r=-0.125 p=0.399	r=-0.061 p=0.682	**r=-0.455** **p=0.001***
5.8S rRNA	52	r=0.002 p=0.986	r=-0.156 p=0.269	r=-0.105 p=0.457	r=-0.142 p=0.317
18S rRNA	52	r=0.085 p=0.547	r=-0.073 p=0.608	r=-0.017 p=0.906	*r=-0.261* *p=0.062*
28S rRNA	48	r=0.060 p=0.688	r=0.047 p=0.751	r=0.055 p=0.710	r=-0.001 p=0.996

***p<0.05**.

Low and High LLSTM. To further examine the relationship between ribosome-related gene expression and muscle size, individual legs (CTL and EX) at Pre RT and Post RT were pooled and ranked based on LLSTM, then divided into tertiles for categorization into low (LOW) or high (HIGH) LLSTM (n=17), with the middle group (n=18) used to ensure separation between LOW and HIGH and was eliminated from analyses. HIGH (12484.41 ± 873.59 g) had significantly greater LLSTM compared to LOW (6490.76 ± 504.14 g) (**Figure 9C**; p<0.001). LOW had significantly greater 5.8S ITS (*LOW* 1.53 ± 0.65 A.U., *HIGH* 0.93 ± 0.92 A.U.; p=0.044) and 28S ITS (*LOW* 1.59 ± 0.70 A.U., *HIGH* 0.90 ± 0.60 A.U.; p=0.009) expression compared to HIGH and saw a trend for greater 18S rRNA (*LOW* 1.58 ± 0.56 A.U., *HIGH* 1.19 ± 0.59 A.U.; p=0.059) expression in LOW compared to HIGH (**Figure 9D**).

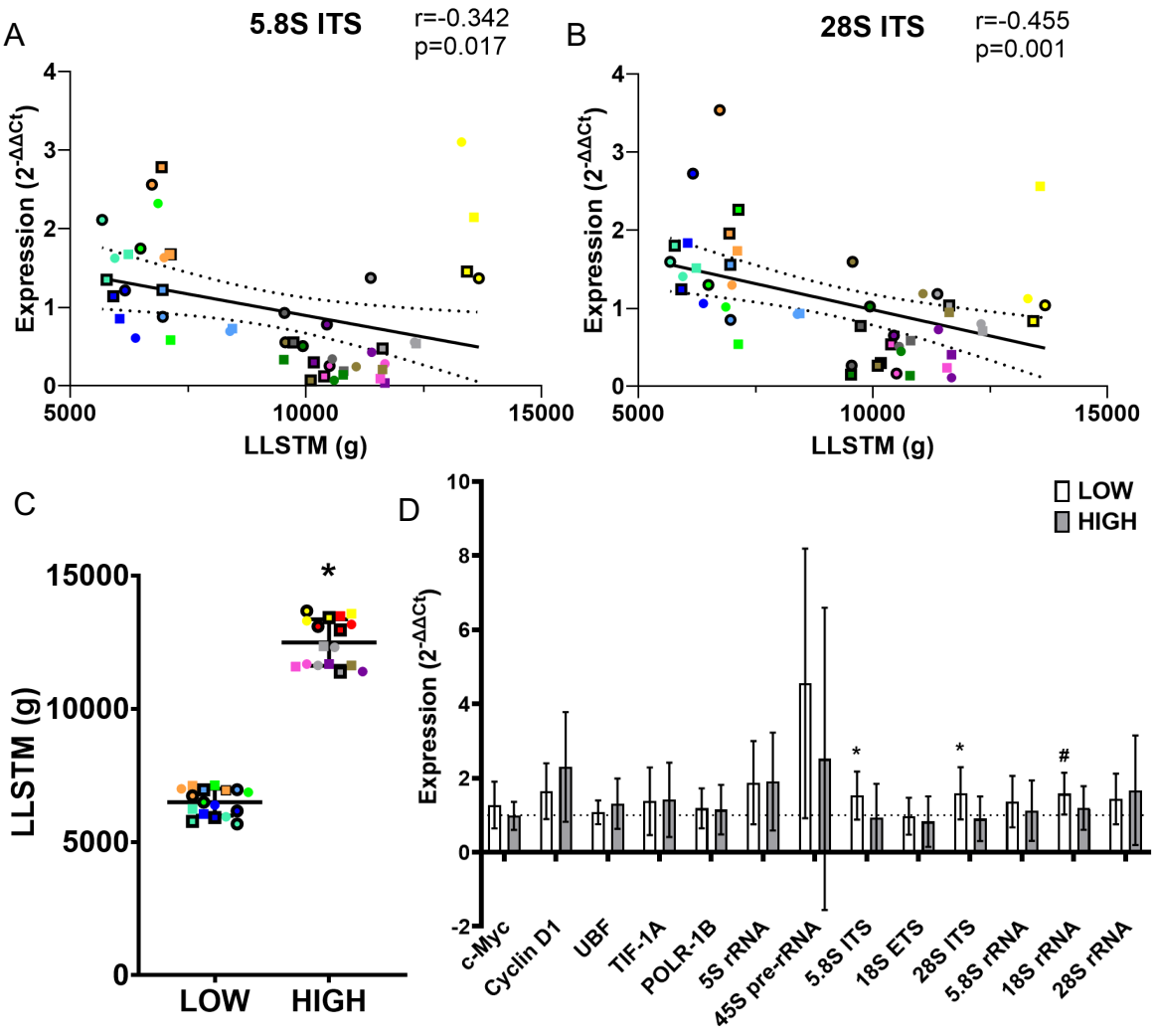

Figure 9. Ribosome-related gene expression and muscle size. Pearson's correlations between *A*) 5.8S ITS (n=48) and *B*) 28S ITS (n=48) expression and LLSTM. Legs at *C*) Pre RT CTL (○), Pre RT EX (□), Post RT CTL (•) and Post RT EX (■) were ranked independently based on LLSTM, divided using tertiles for LOW and HIGH LLSTM (n=17) with 1/3 of points in the middle (n=18) to ensure separation between groups. Values are individual data points (where each colour represents a different participant) overlayed on means (middle, horizontal line) ± SD (vertical line). *D*) Absolute ribosomal marker expression in LOW and HIGH LLSTM groups with values (means ± SD) relative to the average Pre RT in their respective condition. *Significant difference between LOW and HIGH ($p<0.05$), #trend for difference between LOW and HIGH ($0.05<p<0.10$).

Baseline Ribosome-related Gene Expression and Muscle Hypertrophy

Correlation Analysis. A correlation matrix was run to examine the relationship between ribosome-related gene expression at Pre RT and muscle hypertrophy from Pre RT to Post RT (**Table 5**). ΔLLSTM had significant, negative correlations with Cyclin D1 (**Figure 10A**; r=-0.564, p=0.006), UBF (**Figure 10B**; r=-0.583, p=0.004) and POLR-1B (**Figure 10C**; r=-0.695, p<0.001) mRNA, 45S pre-rRNA (**Figure 10D**; r=-0.585, p=0.003), 5.8S ITS (**Figure 10E**; r=-0.694, p<0.001), 18S ETS (**Figure 10F**; r=-0.503, p=0.009), 28S ITS (**Figure 10G**; r=-0.546, p=0.006) and 5.8S rRNA (**Figure 10H**; r=-0.533, p=0.005) expression.

Table 5. Pearson's correlations between ribosome-related gene expression Pre RT and the change in muscle size from Pre RT to Post RT. CTL (n=13) and EX (n=13) legs pooled at Pre RT and Post RT (n=26). Corresponds with **Figure 10**.

Marker	Sample Size (n)	Δ Type 1 CSA	Δ Type 2 CSA	Δ Mixed-fibre CSA	Δ LLSTM
c-Myc	24	r=0.050 p=0.817	r=0.108 p=0.616	r=0.094 p=0.663	r=-0.287 p=0.174
Cyclin D1	22	r=-0.178 p=0.427	r=-0.262 p=0.239	r=-0.250 p=0.263	**r=-0.564** **p=0.006***
UBF	22	**r=-0.445** **p=0.038***	*r=-0.401* *p=0.064*	**r=-0.488** **p=0.037***	**r=-0.583** **p=0.004***
TIF-1A	24	*r=-0.398* *p=0.054*	*r=-0.373* *p=0.073*	**r=-0.424** **p=0.039***	*r=-0.358* *p=0.086*
POLR-1B	22	r=-0.393 p=0.071	**r=-0.425** **p=0.048***	**r=-0.445** **p=0.038***	**r=-0.695** **p<0.001***
5S rRNA	24	*r=0.393* *p=0.058*	r=0.319 p=0.128	r=0.372 p=0.074	r=-0.303 p=0.150

45S pre-rRNA	24	r=-0.173 p=0.418	r=-0.269 p=0.205	r=-0.251 p=0.237	**r=-0.585** **p=0.003***
5.8S ITS	24	r=-0.170 p=0.426	r=-0.215 p=0.312	r=-0.214 p=0.316	**r=-0.694** **p<0.001***
18S ETS	26	*r=-0.353* *p=0.077*	**r=-0.482** **p=0.013***	**r=-0.469** **p=0.016***	**r=-0.503** **p=0.009***
28S ITS	24	r=-0.182 p=0.396	r=-0.189 p=0.375	r=-0.200 p=0.348	**r=-0.546** **p=0.006***
5.8S rRNA	26	r=-0.240 p=0.238	**r=-0.488** **p=0.011***	**r=-0.430** **p=0.029***	**r=-0.533** **p=0.005***
18S rRNA	26	r=-0.231 p=0.257	r=-0.279 p=0.168	r=-0.281 p=0.164	r=-0.300 p=0.136
28S rRNA	24	r=-0.023 p=0.915	r=-0.170 p=0.428	r=-0.125 p=0.559	r=-0.230 p=0.279

***p<0.05**.

Low and High ΔLLSTM. To further examine the relationship between ribosome-related gene expression at Pre RT and muscle hypertrophy, individual legs (CTL and EX) were ranked based on ΔLLSTM from Pre RT to Post RT and divided into bottom and top 10 for low (LOW) or high (HIGH) ΔLLSTM (n=10) respectively. Ribosome-related gene expression was examined at Pre RT only. HIGH (1314.20 ± 196.08 g) had a significantly greater ΔLLSTM from Pre RT to Post RT compared to LOW (128.40 ± 204.37 g) (**Figure 10I**; p<0.001). LOW had significantly greater Cyclin D1 (*LOW* 2.01 ± 1.30 A.U., *HIGH* 0.88 ± 0.81 A.U.; p=0.039), UBF (*LOW* 1.26 ± 0.53 A.U., *HIGH* 0.69 ± 0.41 A.U.; p=0.019) and POLR-1B (*LOW* 1.34 ± 0.41 A.U., *HIGH* 0.64 ± 0.35 A.U.; p=0.001) mRNA, 5S rRNA (*LOW* 1.91 ± 1.24 A.U., *HIGH* 0.91 ± 0.52 A.U.; p=0.049), 45S pre-rRNA (*LOW*

5.85 ± 3.98 A.U., *HIGH* 0.83 ± 1.10 A.U.; p=0.001), 5.8S ITS (*LOW* 1.79 ± 0.59 A.U., *HIGH* 0.52 ± 0.38 A.U.; p<0.001), 18S ETS (*LOW* 1.27 ± 0.68 A.U., *HIGH* 0.64 ± 0.35 A.U.; p=0.017), 28S ITS (*LOW* 1.83 ± 0.88 A.U., *HIGH* 0.65 ± 0.55 A.U.; p=0.002), 5.8S rRNA (*LOW* 1.46 ± 0.59 A.U., *HIGH* 0.57 ± 0.34 A.U.; p<0.001) and 28S rRNA (*LOW* 1.44 ± 0.91 A.U., *HIGH* 0.62 ± 0.45 A.U.; p=0.022) expression at Pre RT compared to HIGH and saw a trend for greater 18S rRNA (*LOW* 1.43 ± 0.33 A.U., *HIGH* 0.95 ± 0.78 A.U.; p=0.092) expression at Pre RT in LOW compared to HIGH (**Figure 10J**).

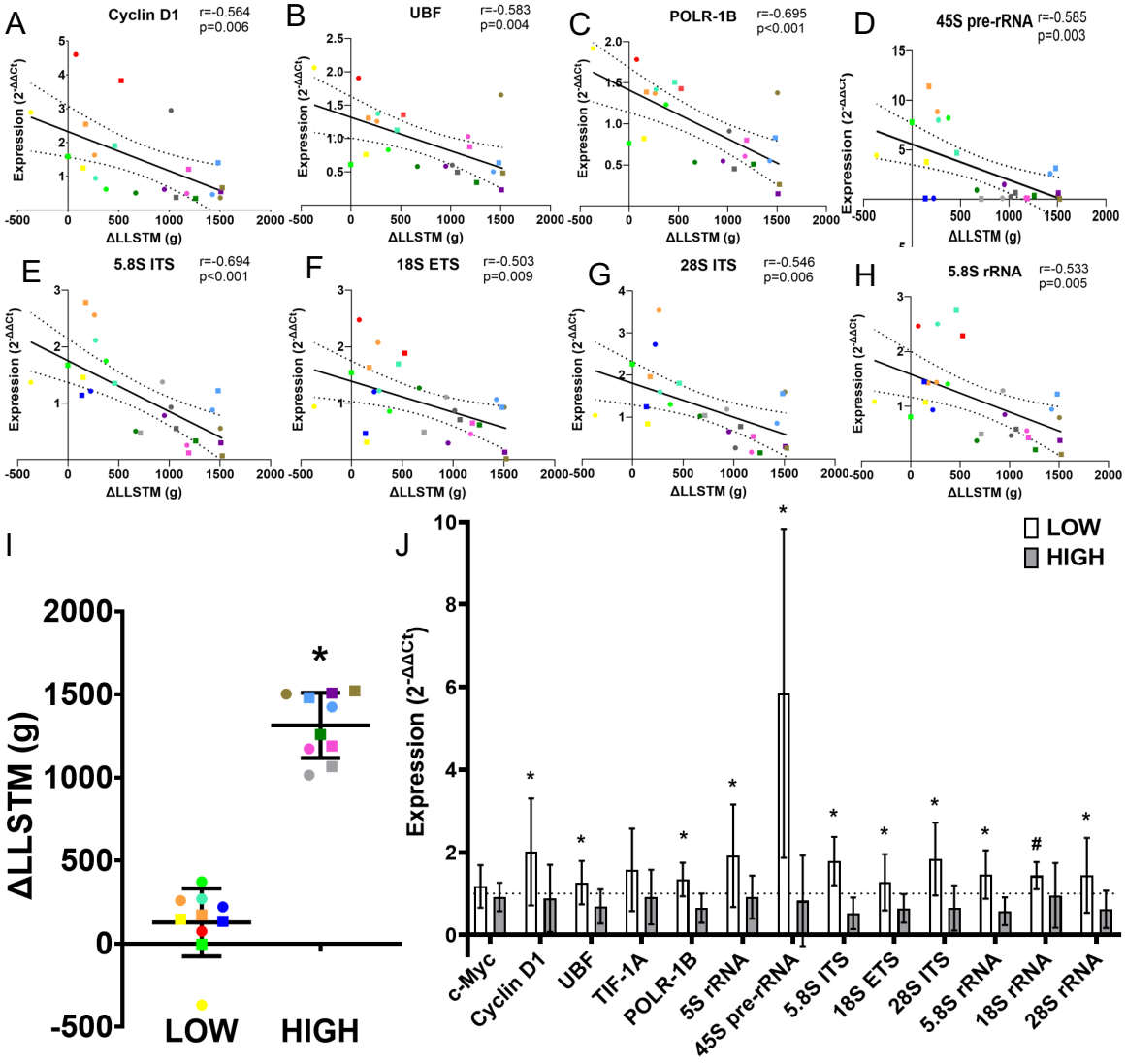

Figure 10. Ribosome-related gene expression at Pre RT and muscle hypertrophy from Pre RT to Post RT. Pearson's correlations between *A*) Cyclin D1 (n=22), *B*) UBF (n=22) and *C*) POLR-1B (n=22) mRNA, *D*) 45S pre-rRNA (n=24), *E*) 5.8S ITS (n=24), *F*) 18S ETS (n=26), *G*) 28S ITS (n=24) and *H*) 5.8S rRNA (n=26) at Pre RT and ΔLLSTM from Pre RT to Post RT. *I*) CTL (•) and EX (■) at Pre RT were ranked based on ΔLLSTM from Pre RT to Post RT, divided using bottom and top 10 for LOW and HIGH ΔLLSTM (n=10) respectively, with 6 in the middle (n=6) to ensure separation between groups. Values are individual data points (where each colour represents a different participant) overlayed on means (middle, horizontal line) ± SD (vertical line). *J*) Ribosome-related gene expression at Pre RT in LOW and HIGH ΔLLSTM groups with values (means ± SD) relative to the average Pre RT in their respective condition. *Significant difference between LOW and HIGH ($p<0.05$), #trend for difference between LOW and HIGH ($0.05<p<0.10$).

Ribosomal Biogenesis and Muscle Hypertrophy

Correlation Analysis. A correlation matrix was run to examine the relationship between ribosomal biogenesis (Δ ribosome-related gene expression) and muscle hypertrophy from Pre RT to Post RT (**Table 6**). ΔLLSTM had significant, negative correlations with ΔCyclin D1 (**Figure 11A**; r=-0.484, p=0.022) and ΔPOLR-1B (**Figure 11B**; r=-0.458, p=0.032) mRNA, Δ45S pre-rRNA (**Figure 11C**; r=-0.589, p=0.002), Δ5.8S ITS (**Figure 11D**; r=-0.707, p<0.001), Δ18S ETS (**Figure 11E**; r=-0.504, p=0.009), Δ28S ITS (**Figure 11F**; r=-0.489, p=0.013), Δ5.8S rRNA (**Figure 11G**; r=-0.483, p=0.012) and Δ18S rRNA (**Figure 11H**; r=-0.505, p=0.008) expression from Pre RT to Post RT.

Table 6. Pearson's correlations between the change in ribosome-related gene expression and the change in muscle size from Pre RT to Post RT. CTL (n=13) and EX (n=13) legs pooled at Pre RT (n=26). Corresponds with **Figure 11**.

Marker	Sample Size (n)	Δ Type 1 CSA	Δ Type 2 CSA	Δ Mixed-fibre CSA	Δ LLSTM
c-Myc	23	r=0.008 p=0.971	r=-0.043 p=0.841	r=-0.027 p=0.901	r=-0.076 p=0.724
Cyclin D1	22	r=0.093 p=0.681	r=-0.186 p=0.408	r=-0.092 p=0.683	**r=-0.484** **p=0.022***
UBF	22	**r=0.665** **p<0.001***	r=0.329 p=0.135	**r=0.483** **p=0.023***	r=-0.258 p=0.246
TIF-1A	24	r=0.053 p=0.804	**r=-0.433** **p=0.035***	r=-0.297 p=0.158	r=-0.277 p=0.190
POLR-1B	22	**r=0.532** **p=0.011***	r=0.167 p=0.459	r=0.320 p=0.146	**r=-0.458** **p=0.032***
5S rRNA	24	r=-0.104 p=0.628	r=-0.119 p=0.581	r=-0.122 p=0.571	r=-0.253 p=0.233
45S pre-rRNA	24	r=0.195	r=0.059	r=0.117	**r=-0.589**

		p=0.362	p=0.783	p=0.585	**p=0.002***
5.8S ITS	24	r=0.089 p=0.680	r=-0.080 p=0.710	r=-0.019 p=0.929	**r=-0.707** **p<0.001***
18S ETS	26	r=0.045 p=0.827	r=-0.055 p=0.791	r=-0.020 p=0.921	**r=-0.504** **p=0.009***
28S ITS	24	r=0.255 p=0.229	r=0.152 p=0.479	r=0.204 p=0.339	**r=-0.498** **p=0.013***
5.8S rRNA	26	r=-0.012 p=0.954	r=-0.301 p=0.135	r=-0.213 p=0.297	**r=-0.483** **p=0.012***
18S rRNA	26	r=0.105 p=0.610	r=0.039 p=0.849	r=0.067 p=0.743	**r=-0.505** **p=0.008***
28S rRNA	24	r=0.194 p=0.360	r=0.061 p=0.779	r=0.116 p=0.589	r=-0.214 p=0.315

***p<0.05**.

Low and High ΔLLSTM. To further examine the relationship between ribosomal biogenesis and muscle hypertrophy, individual legs (CTL and EX) were once again ranked based on ΔLLSTM as previously described. LOW had a significantly greater ΔCyclin D1 (*LOW* 2.23 ± 0.87 A.U., *HIGH* 0.88 ± 0.63 A.U.; p=0.001) and ΔPOLR-1B (*LOW* 1.62 ± 0.85 A.U., *HIGH* 0.75 ± 0.49 A.U.; p=0.015) mRNA, Δ45S pre-rRNA (*LOW* 6.66 ± 5.77 A.U., *HIGH* 0.85 ± 1.03 A.U.; p=0.006), Δ5.8S ITS (*LOW* 1.99 ± 0.99 A.U., *HIGH* 0.40 ± 0.33 A.U.; p<0.001), Δ18S ETS (*LOW* 1.10 ± 0.57 A.U., *HIGH* 0.55 ± 0.31 A.U.; p=0.015), Δ28S ITS (*LOW* 1.58 ± 0.81 A.U., *HIGH* 0.68 ± 0.42 A.U.; p=0.006), Δ5.8S rRNA (*LOW* 1.45 ± 0.73 A.U., *HIGH* 0.60 ± 0.51 A.U.; p=0.007), Δ18S rRNA (*LOW* 1.60 ± 0.51 A.U.,

HIGH 0.97 ± 0.48 A.U.; p=0.011) and Δ28S rRNA (*LOW* 1.26 ± 0.49 A.U., *HIGH* 0.64 ± 0.50 A.U.; p=0.017) expression from Pre RT to Post RT compared to HIGH (**Figure 11I**).

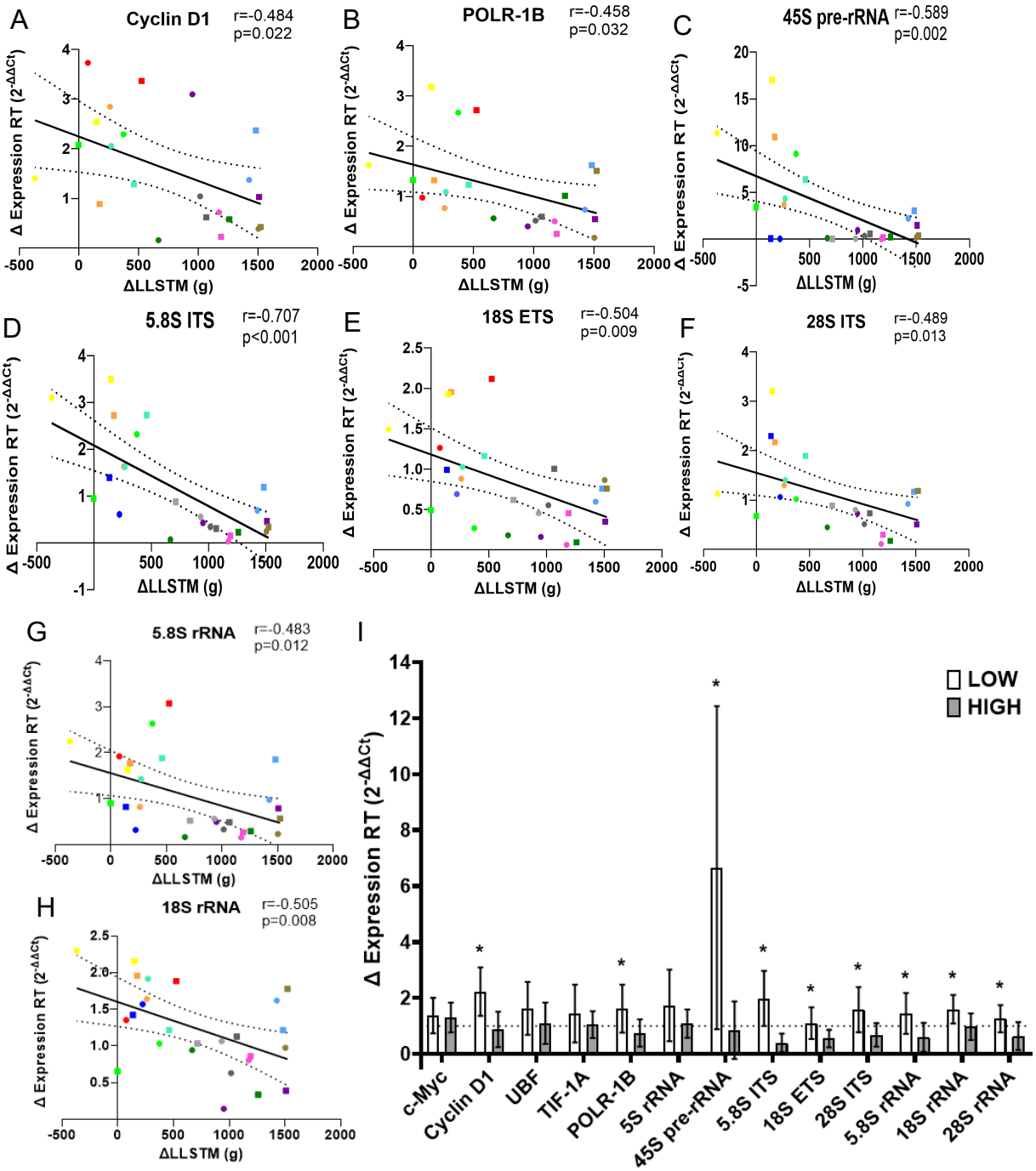

Figure 11. Ribosomal biogenesis and muscle hypertrophy from Pre RT to Post RT. Pearson's correlations between *A*) ΔCyclin D1 (n=18) and *B*) ΔPOLR-1B (n=18) mRNA, *C*) Δ45S pre-rRNA (n=19), *D*) Δ5.8S ITS (n=19), *E*) Δ18S ETS (n=20), *F*) Δ28S ITS (n=19), *G*) Δ5.8S rRNA (n=20) and *H*) Δ18S rRNA (n=20). CTL (•) and EX (■) previously ranked based on ΔLLSTM from Pre RT to Post RT, divided using bottom and top 10 for LOW and HIGH ΔLLSTM (n=10) respectively, with 6 in the middle (n=6) to ensure separation between groups. *I*) ΔRibosome-related gene expression from Pre RT to Post RT in LOW and HIGH ΔLLSTM groups with values (means ± SD) relative to the average Pre RT in their respective condition. *Significant difference between LOW and HIGH ($p<0.05$).

DISCUSSION

This study was the first to investigate the impact of chronic aerobic training and aerobic training before resistance training on direct markers of ribosome content. Contrary to our hypotheses, no change in ribosome content (assessed via ribosome-related gene expression and RNA concentration) was detected following aerobic training or resistance training (with and without prior aerobic training). Although COXIV staining intensity was greater in the EX versus the CTL leg following aerobic training, other mitochondrial-related protein and mRNA markers were not affected suggesting that the aerobic training protocol was not sufficient to induce mitochondrial biogenesis to increase mitochondrial content. Despite observing no changes in mitochondrial content following aerobic training and no change in ribosome content following aerobic or resistance training, we observed a relationship between ribosome content and muscle size (assessed via LLSTM) where a lower ribosome content was associated with a greater LLSTM. In addition, lower ribosome content before resistance training and no change or a decrease in ribosome content following resistance training corresponded to a greater increase in muscle size (ΔLLSTM) following resistance training. These findings suggest that translational efficiency, rather than capacity, may be more important to support muscle size and growth and that individuals able to increase translational efficiency with exercise training are better able to adapt to resistance training.

The original study in which the muscle tissue was collected contained a biopsy in the EX leg before aerobic training, representing a baseline muscle sample, however, this timepoint was omitted for the present analysis. We think it is more appropriate to directly

compare the CTL and EX legs prior to resistance training as our main hypotheses were concerned with how aerobic "pre-training" affected adaptation to resistance training. Omitting the biopsy of the EX leg prior to aerobic training from analyses also allowed us to conduct what we think were more appropriate statistical analyses to capture the effects of the within-subject design. The unilateral exercise model assumes no crosstalk between the exercised and non-exercised limb (MacInnis, McGlory, et al., 2017) and therefore the impact of aerobic training on the EX leg should be accounted for by the CTL leg following aerobic training.

Ribosomal Biogenesis with Aerobic Training

Aerobic training is the most effective stimulus for increasing oxidative capacity through shifts in muscle fibre-type, mitochondrial biogenesis and microvascular perfusion (Hoier & Hellsten, 2014; Irrcher et al., 2003; Jornayvaz & Shulman, 2010). This phenotypic shift coincides with an increase in protein content of factors influencing the adaptive response to exercise training, including endothelial and mitochondrial-related protein expression. Despite observing no change in mitochondrial-related protein content, we have previously demonstrated that these individuals increased VO_2 peak and skeletal muscle capillarization in the EX compared to the CTL leg (Thomas et al., 2019). As the synthesis of proteins for these adaptive responses requires ribosomes for the protein translation process, our findings suggest that the basal ribosome pool is likely sufficient. Therefore, ribosomal biogenesis to increase translational capacity is not necessary to support adaptations to aerobic training.

To our knowledge, only one other study has examined markers of ribosome content with aerobic training. Proteomic analysis revealed that 12 weeks of high-intensity interval training resulted in increased expression of proteins involved in translational pathways similar to that observed following resistance training, in addition to an increase in mitochondrial-related protein abundance in both young and old individuals (Robinson et al., 2017). Increased expression of ribosomal proteins was also reported in old individuals only. The increased expression of proteins involved in protein translation suggests an increase in translational capacity following aerobic training. However, as the increased expression of proteins involved in the translational pathway also coincided with an increase in fat-free mass, the increase in translational capacity may have occurred to support the increase in muscle mass in addition to supporting the increase in mitochondrial protein abundance (Figueiredo et al., 2015; Hammarström et al., 2020; Nader et al., 2005; Nakada et al., 2016; Robinson et al., 2017; von Walden et al., 2012, 2016). In our study, the lack of muscle growth and mitochondrial biogenesis following aerobic training, both of which require protein translation and synthesis (Holloszy, 1967; Short et al., 2003), could partly explain why we report no impact of aerobic training on ribosome-related gene expression.

Mitochondrial Content

Mitochondrial content was indirectly measured via protein (immunofluorescence and immunoblotting) and mRNA expression following aerobic training. Six weeks of aerobic training resulted in greater COXIV staining intensity in the EX than CTL leg; however, mitochondrial-related gene expression and protein content were not affected by

training. COXIV stain intensity has previously been demonstrated to increase following 6 weeks of aerobic training, albeit at a higher exercise intensity than our protocol and with a slightly larger sample size of 10 individuals compared to 7 in the current analysis (Tan et al., 2018). The COXIV stain intensity data are inconsistent with our mitochondrial-related protein and gene expression which were not affected by the aerobic training intervention. This is in disagreement with previous work describing an increase in mitochondrial-related protein and gene expression following aerobic training (Egan et al., 2013; Holloszy, 1967; Short et al., 2003). The discrepancies observed in our findings are likely due to analyses being performed at fibre-specific compared to a whole-muscle level, where whole muscle analyses may not have been specific enough to detect any differences (MacInnis, Zacharewicz, et al., 2017; Wyckelsma et al., 2017). It is possible that our aerobic training protocol was not intense enough to elicit mitochondrial adaptations, as intermittent bouts at 65% work peak has been shown to elicit greater mitochondrial adaptations compared to 50% work peak (which was the intensity of our study) (MacInnis, Zacharewicz, et al., 2017). Both 2 weeks of continuous or intermittent training at either 50% or 65% work peak respectively elicited increases in whole-muscle mitochondrial-related protein content; however, only the protocol training at 65% work peak increased maximal CS activity and mitochondrial respiration (MacInnis, Zacharewicz, et al., 2017). Taken together the mitochondrial-related protein and gene expression data suggest that aerobic training did not increase mitochondrial content. Even though our aerobic training intervention did not result in mitochondrial biogenesis, we have previously reported an increase in VO_2 peak and skeletal muscle capillarization, indicating that the stimulus was indeed sufficient to drive some aerobic adaptations (Thomas et al., 2019). These data

indicate that although the aerobic training stimulus was sufficient to induce certain training adaptations, no mitochondrial adaptations were observed. This suggests that ribosome content either does not change with aerobic training or perhaps that ribosome content may only increase when mitochondrial biogenesis occurs (Robinson et al., 2017).

Ribosomal Biogenesis with Acute Aerobic Exercise

The expression of 45S pre-rRNA is routinely used as a marker of ribosome content as it is the precursor for 5.8S, 18S and 28S rRNAs and its transcription is rate-limiting for ribosomal biogenesis (Figueiredo & McCarthy, 2019; Wen et al., 2016). Expression of 45S pre-rRNA has been shown to decrease within 30 minutes of an acute bout of aerobic exercise and then return to baseline levels after 3 hours (Figueiredo et al., 2021; Hansson et al., 2019). This suggests that either an initial decrease in ribosomal content occurs immediately following aerobic exercise or that the basal pre-rRNA transcripts are processed into mature rRNAs resulting in a decreased expression of the full pre-rRNA transcript. The later would suggest that the mature rRNA are contributing to ribosomal subunit formation following aerobic exercise, thereby contributing to ribosomal biogenesis. The addition of rRNAs to ribosomal subunits are consistent with the observation that c-Myc mRNA expression increases 8 hours following aerobic exercise, as c-Myc, in addition to rRNA transcription, is involved in processing rRNA and ribosomal subunit assembly (Figueiredo et al., 2021; van Riggelen et al., 2010). Although it is difficult to conclude without examining mature rRNA or ribosomal protein expression, the lack of change in mRNA expression of ribosomal biogenesis regulators UBF, TIF-1A and POLR-1B following a bout of aerobic exercise with a concomitant

decrease in 45S pre-rRNA and increase in c-Myc mRNA expression suggests that rDNA transcription is temporarily impaired, but resting pre-rRNA transcripts can still be processed and contribute to ribosome subunit formation (Figueiredo et al., 2021; Hansson et al., 2019; West et al., 2016). The decrease in 45S pre-rRNA and increase in c-Myc mRNA expression is only observed acutely and returns to baseline within 24 hours following aerobic exercise. Therefore, it is possible that an acute ribosomal biogenesis response, characterized by an acute increase in ribosome content, occurred in our study following aerobic exercise however, we were unable to measure it due to our sampling timepoints.

Ribosome Content with Resistance Training

The resistance training intervention used in the current study increased muscle strength (1RM) and hypertrophy (type 2 CSA, mixed-fibre CSA and LLSTM) (Thomas et al., 2019), however this was not accompanied by a change in ribosome-related gene expression, contradicting our hypotheses. There was the exception of c-Myc, 5S rRNA and the transcribed spacer regions of rRNA that demonstrated a change in expression following resistance training and/or were affected by aerobic pre-training where c-Myc was greater in the CTL compared to the EX leg, 5S rRNA tended to be greater following aerobic training in the EX compared to the CTL leg and the rRNA transcribed spacer regions decreased with exercise training. It is generally accepted in the literature that ribosome content increases alongside muscle hypertrophy in both rodents and humans to support the increase in protein synthetic demands (Figueiredo et al., 2015; Hammarström et al., 2020; Nader et al., 2005; Nakada et al., 2016; von Walden et al., 2016). However, recent studies in humans have contradicted these

findings and reported either no change, a decrease, or an initial increase followed by a decrease in ribosome content following an intervention resulting in hypertrophy, suggesting that ribosome content may not be the most important factor in protein translation to support increases in muscle mass (Fyfe et al., 2018; Hammarström et al., 2020).

The results of the present study demonstrate a high degree of inter-individual variability with respect to ribosome-related gene expression, which is in line with several other studies in humans and may help to discern why no differences were observed following aerobic or resistance training (Fyfe et al., 2018; Hammarström et al., 2020; Mobley, Haun, et al., 2018). The variability observed between individuals may be explained in part by rDNA copy number, in which there are hundreds of copies throughout the genome, and these copy numbers are vastly different between individuals (Gibbons et al., 2014; Piazzi et al., 2019). It has recently been discovered that both basal rRNA expression and the change in expression following resistance exercise are positively associated to rDNA copy number in humans (Figueiredo et al., 2021). The relationship between rDNA copy number and rRNA expression indicates that those with more rDNA genes have more ribosomes at baseline and can increase ribosome content to a greater extent following an exercise stimulus.

Previous work has demonstrated that the expression of ribosome-related genes is associated with their gene copy number, where rDNA copy number (Gibbons et al., 2015) and their basal expression (Figueiredo et al., 2021) is related. Therefore, to ascertain the reliability of our ribosome-related gene expression data, we correlated all ribosome-related markers to each other. **Table 7** demonstrates that for each marker, there are either trends or significant positive relationships between 65 ± 25% of ribosome markers (excluding 12S mt-rRNA),

therefore signifying their relationship with one another and suggesting that the variability observed in our data is valid. However, c-Myc and 5S rRNA expression seem to be less related compared to the others, correlating with only 17% and 25% of the other markers, respectively. The lesser association between c-Myc and the other markers of ribosome-related gene expression is likely because c-Myc is highly involved in regulating a number of other cellular processes in addition to ribosomal biogenesis, including metabolism, angiogenesis, DNA repair, cell growth, proliferation and apoptosis amongst other processes (van Riggelen et al., 2010). c-Myc has been suggested to regulate components of up to 15% of the entire genome and it is not surprising that its expression is not as tightly coupled as some of the other markers that were measured. The role of c-Myc in various other processes may also explain why its expression changed with resistance training and the other regulators of ribosomal biogenesis did not. The expression of c-Myc was greater in the CTL compared to the EX leg, which is likely due to the CTL leg being naïve to exercise (Coffey & Hawley, 2017). In addition to c-Myc, 5S rRNA was also poorly associated with other markers of ribosome-related gene expression (**Table 7**). Although 5S rDNA copy number is similar to 45S within an individual (Gibbons et al., 2015), the two genes are transcribed independently (Mayer & Grummt, 2006; van Riggelen et al., 2010; von Walden et al., 2016; Wen et al., 2016) and 5S has also been found to contribute to the mitochondrial ribosome (Smirnov et al., 2008, 2010, 2011). Being independently transcribed and having additional contributions to the mitochondrial ribosome likely explains why 5S rRNA is not as highly associated to other markers of ribosome-related gene expression when compared to some of the other markers. It could also explain the disconnect between rRNA expression following exercise training where 5S rRNA showed a trend for greater

expression in the EX compared to the CTL leg following aerobic training, however no differences in expression of 5.8S, 18S or 28S rRNA were observed following aerobic or resistance training.

Table 7. Pearson's correlations between markers of ribosome-related gene expression.

Marker	c-Myc	Cyclin D1	UBF	TIF-1A	POLR-1B	5S rRNA	45S pre-rRNA	5.8S ITS	18S ETS	28S ITS	5.8S rRNA	18S rRNA	28S rRNA
Cyclin D1	r=-0.046												
	p=0.777												
UBF	r=0.110	**r=0.563**											
	p=0.500	**p<0.001***											
TIF-1A	r=0.061	**r=0.526**	**r=0.667**										
	p=0.693	**p<0.001***	**p<0.001***										
POLR-1B	r=0.256	**r=0.582**	**r=0.816**	**r=0.595**									
	p=0.111	**p<0.001***	**p<0.001***	**p<0.001***									
5S rRNA	*r=0.269*	r=0.076	r=-0.036	r=0.076	r=0.189								
	p=0.064	p=0.639	p=0.827	p=0.639	p=0.243								
45S pre-rRNA	r=0.091	**r=0.431**	**r=0.485**	**r=0.493**	**r=0.749**	r=-0.032							
	p=0.555	**p=0.006***	**p=0.002***	**p<0.001***	**p<0.001***	p=0.836							
5.8S ITS	r=0.159	**r=0.490**	**r=0.456**	**r=0.347**	**r=0.733**	r=0.200	**r=0.908**						
	p=0.303	**p=0.001***	**p=0.003***	**p=0.021***	**p<0.001***	p=0.194	**p<0.011***						
18S ETS	r=-0.066	**r=0.494**	**r=0.394**	**r=0.346**	**r=0.508**	r=0.055	**r=0.612**	**r=0.697**					
	p=0.654	**p<0.001***	**p=0.008***	**p=0.016***	**p<0.001***	p=0.713	**p<0.001***	**p<0.001***					
28S ITS	r=0.037	**r=0.336**	**r=0.470**	r=0.102	**r=0.541**	r=0.220	**r=0.590**	**r=0.693**	**r=0.771**				
	p=0.810	**p=0.034***	**p=0.002***	p=0.512	**p<0.001***	p=0.151	**p<0.001***	**p<0.001***	**p<0.001***				
5.8S rRNA	r=0.184	**r=0.587**	**r=0.594**	**r=0.654**	**r=0.787**	r=0.182	**r=0.726**	**r=0.808**	**r=0.641**	**r=0.511**			
	p=0.209	**p<0.001***	**p<0.001***	**p<0.001***	**p<0.001***	p=0.215	**p<0.001***	**p<0.001***	**p<0.001***	**p<0.001***			
18S rRNA	r=0.109	*r=0.291*	**r=0.525**	**r=0.292**	**r=0.561**	r=0.227	**r=0.529**	**r=0.591**	**r=0.648**	**r=0.664**	**r=0.626**		
	p=0.460	*p=0.056*	**p<0.001***	**p=0.044***	**p<0.001***	p=0.120	**p<0.001***	**p<0.001***	**p<0.001***	**p<0.001***	**p<0.001***		
28S rRNA	r=0.073	**r=0.390**	**r=0.490**	r=0.035	**r=0.661**	**r=0.578**	r=0.159	**r=0.347**	**r=0.309**	**r=0.326**	**r=0.418**	**r=0.339**	
	p=0.637	**p=0.009***	**p<0.001***	p=0.821	**p<0.001***	**p<0.001***	p=0.302	**p=0.021***	**p=0.033***	**p=0.031***	**p=0.003***	**p=0.018***	
12S mt-rRNA	*r=0.304*	**r=-0.362**	r=-0.072	r=-0.180	r=0.094	*r=0.305*	r=0.133	r=0.135	r=0.209	r=0.062	r=0.099	**r=0.361**	r=0.102
	p=0.056	**p=0.030***	p=0.676	p=0.266	p=0.586	*p=0.055*	p=0.414	p=0.405	p=0.173	p=0.704	p=0.522	**p=0.016***	p=0.530

***p<0.05 significant correlation**.

Interestingly, the transcribed spacer regions in the 45S pre-rRNA decreased with exercise training, where the expression of 5.8S ITS and 18S ETS was greater in the CTL than the EX leg and expression of 18S ETS decreased with resistance training. Previously, little emphasis has been placed on determining the expression transcriber regions of rRNA, with the measures either not being included (Figueiredo et al., 2020, 2021; Hammarström et al., 2020; Mobley, Haun, et al., 2018), showing no change (Figueiredo et al., 2015) or decreasing (Fyfe et al., 2018) in expression following resistance training. These transcribed spacer regions are transcribed alongside 5.8S, 18S and 28S rRNA as 45S pre-rRNA and are cleaved out by nucleolin as mature rRNAs are formed (Figueiredo & McCarthy, 2019; Kusnadi et al., 2015; Wen et al., 2016). As the transcribed

spacer regions are non-coding and transcription of the whole 45S pre-rRNA is required to synthesize new rRNA, it is likely that once 45S pre-rRNA is processed, the transcribed spacer regions are degraded. We suggest that although no change in 45S pre rRNA, 5.8S, 18S or 28S rRNA were observed following resistance training in this study, a decrease in transcribed spacer region expression suggests that there may be an increased contribution of rRNA from basal pre-rRNA transcripts, which would then, in turn, result in the degradation of the ITS and ETS regions. The increased rRNA contribution may also suggest an increase in ribosomal turnover as no change in 5.8S, 18S or 28S rRNA were observed following resistance training.

We report no change in ribosome-related gene expression following aerobic or resistance training. Additionally, completing 6 weeks of aerobic training prior to resistance training did not impact ribosome-related gene expression following the resistance training intervention. The lack of change in RNA concentration supports these observations; however, it is important to note that although it is one of the most popular measures of ribosome content, it is likely not a good indicator due to methodological inconsistencies. Some studies choose to omit measuring RNA concentration (Nakada et al., 2016), while others have observed no change or a decrease (Fyfe et al., 2018) following resistance training. The lack of change in ribosome-related gene expression is likely due to the high variability between individuals and requires further examination.

Ribosome Content and Muscle Size

Legs were pooled from conditions (CTL and EX) and timepoints (Pre RT and Post RT), then stratified into groups (LOW and HIGH) based on LLSTM as an indicator of muscle size. When comparing the LOW and HIGH LLSTM groups, expression of 5.8S ITS and 28S ITS were significantly greater and 18S rRNA tended to have greater expression in the LOW compared to the HIGH group. To our knowledge, this is the first study to compare ribosome-related gene expression between legs classified as having a "high" and "low" LLSTM in humans. Our results suggest that those with the most muscle mass have the lowest ribosome content. However, it is important to note that all muscle samples (i.e. time, pre and post-training; legs, aerobically pre-trained and control) were treated individually and therefore participants and training status were not taken into consideration. The analysis was performed in this manner because the sample size was too small to perform cluster analyses.

Typically, ribosome content is measured following an intervention that results in skeletal muscle hypertrophy, such as SA in rodents and resistance training in humans (Brook et al., 2016; Figueiredo et al., 2015; Fyfe et al., 2018; Hammarström et al., 2020). However one study in rats found a significant, positive relationship between muscle size and ribosome content (Nakada et al., 2016). A positive relationship between muscle size and ribosome content contradicts our findings, which may be a result of discrepancies between rodent and human models and the types of measurements that were used. The study by Nakada et al. (2016) related 18S+28S rRNA to muscle weight/body weight in rats, whereas we related and compared the expression of a panel of ribosome-related genes to LLSTM. We think that our approach is appropriate

and gives a more wholistic representation than what was reported in the rats (Nakada et al., 2016). Although DXA does not directly measure muscle mass, the majority of LLSTM is composed of skeletal muscle and is highly correlated to the gold standard measure of muscle mass, magnetic resonance imaging (Haun et al., 2019). LLSTM also showed significant, positive correlations to both type 2 and mixed-fibre CSA (**Appendix D**) and therefore we believe that LLSTM is a good indicator of muscle size. In addition, the HIGH group had significantly greater LLSTM compared to the LOW group and therefore the comparisons made between the HIGH and LOW groups are between groups with significantly different muscle size.

As 5.8S ITS, 28S ITS and 18S rRNA expression were greater in the LOW compared to the HIGH group, this could suggest that larger muscles have fewer ribosomes but those present may be more efficient. However, as this study focused solely on translational capacity, we were unable to measure translational efficiency and therefore these findings warrant further investigation. Another explanation as to why the HIGH group has greater ribosome-related gene expression compared to the LOW group could be a result of less basal pre-rRNA transcript expression within the muscle. As the two markers that were significantly greater in the LOW compared to the HIGH group were ITS regions, this suggests that the HIGH group may have fewer circulating pre-rRNA transcripts and are more efficient in contributing rRNA to ribosomal subunits.

Ribosome Content Pre RT and Muscle Hypertrophy

Following resistance training, individual legs were stratified into two groups (HIGH and LOW) based on their change in LLSTM. Ribosome-related gene expression was greater in the legs with the lowest change in LLSTM (LOW) compared to those with the greatest change in LLSTM (HIGH) in 11 of the 13 markers measured. To our knowledge, this is the first study to determine how markers of ribosome content prior to resistance training are related to muscle hypertrophy. Like with the ribosome content and muscle size analyses, these analyses operate under the assumption that each leg within an individual are independent of each other. Despite observing no significant relationship between the change in LLSTM and type 2 and mixed-fibre CSA following resistance training, each measure (on average) increased in the complete sample population following resistance training (**Appendix E**).

Legs which had the lowest expression of ribosome-related genes prior to resistance training observed the greatest increase in LLSTM following resistance training. Anabolic resistance partly contributes to the reduced muscle mass observed in older adults (D. R. Moore et al., 2015). Previous work has reported that although older adults have a greater baseline expression of ribosome-related genes, they have a blunted response to acute increases in ribosome content following resistance exercise compared to young adults (Stec et al., 2015). Rodent models have observed elevated nucleolin and nucleophosmin mRNA expression in old compared to young, both of which are involved in splicing pre-rRNA into mature rRNAs, following acute electrical stimulation (West et al., 2019). As ribosomes are formed and degraded as a unit it is likely that elevated nucleolin and nucleophosmin signify impaired

rRNA processing in old which prevents acute increases in ribosome content following resistance exercise (Mathis et al., 2017). An attenuated acute increase in ribosome content likely impairs protein synthesis and contributes to anabolic resistance observed in older adults.

If those who experience an attenuated anabolic response to resistance exercise have greater ribosome content at baseline and a dysregulated ribosomal biogenesis response to acute resistance exercise, this could indicate that individuals who demonstrate the smallest increase in LLSTM following resistance training have a lower anabolic response compared to those who had the greatest increase in LLSTM. It is therefore likely that individuals who have the lowest ribosome content prior to resistance training have ribosomes that are more efficient than those with greater ribosome content which can therefore support the greater increase in LLSTM. Not surprisingly, the largest discrepancy between the LOW and HIGH groups was 45S pre-rRNA expression as these transcripts are transcribed through the activity of the ribosomal biogenesis regulators (c-Myc, Cyclin D1, UBF, TIF-1A, POLR-1B) and account for both the transcribed spacer regions and rRNAs (with the exception of 5S rRNA) (Brook et al., 2019; Chaillou et al., 2014; Figueiredo & McCarthy, 2019; Kusnadi et al., 2015; Wen et al., 2016). The expression of 45S pre-rRNA was approximately 7 times greater in the LOW compared to the HIGH group (the next highest marker, 5.8S ITS, was 3 times greater in LOW compared to HIGH). Having such a large difference in 45S pre-rRNA expression between the LOW and HIGH groups suggests that more ribosomes need to be formed to support protein translation within the cell and that more 45S pre-rRNA transcripts are formed than are perhaps required for ribosomal biogenesis in resting conditions. The discrepancy in ribosome-related gene expression between the LOW and the HIGH groups further indicates

an impaired anabolic response in those with a high ribosome content, and that translational efficiency is likely more important than capacity for dictating muscle hypertrophy following resistance training.

Ribosomal Biogenesis and Muscle Hypertrophy

When individual legs were once again stratified based on the change in LLSTM, the change in 9/13 markers of ribosome-related gene expression were significantly greater following resistance training in the LOW compared to HIGH group. This may suggest that those who increase muscle size to the greatest extent have the lowest change or even have a decrease in ribosome content following resistance training. The notion that muscle hypertrophy decreases ribosome content directly contradicts numerous conclusions in both rodent (Kirby et al., 2015; Nakada et al., 2016) and human (Figueiredo et al., 2015; Hammarström et al., 2020; Mobley, Haun, et al., 2018; Stec et al., 2016) studies. However, when the evidence is further explored it appears as though ribosomal biogenesis occurs following an acute bout of resistance exercise and in response to a novel stimulus (Figueiredo et al., 2016, 2021; Nakada et al., 2016; von Walden et al., 2012) but then decreases thereafter following exercise training (Brook et al., 2016; Fyfe et al., 2018; Hammarström et al., 2020; Mobley, Haun, et al., 2018), likely as a result of ribosomes becoming more efficient (Joanisse et al., 2020) or to preserve metabolic energy (Warner, 1999).

Figueiredo et al. (2015) demonstrated an increase in ribosome-related gene expression alongside muscle hypertrophy in young men following 8 weeks of resistance training. However, other studies in young individuals show either no change or a decrease in markers of ribosome-related gene expression following 8 and 12 weeks of resistance

training (Fyfe et al., 2018; Mobley, Haun, et al., 2018) in addition to an attenuated increase in ribosomal content following acute resistance exercise after 3 and 6 weeks of resistance training (Brook et al., 2016). The discrepancy between these studies may be due to the fact that both the muscle hypertrophic response following resistance training (Ahtiainen et al., 2016; Shrier, 2006) and ribosomal gene expression (Figueiredo et al., 2021; Gibbons et al., 2014) is highly variable between individuals. When young men underwent 12 weeks of resistance training completing either single or multiple sets, the multi-set group increased markers of rRNA content above the single set group after 2 weeks and it remained elevated above baseline after 12 weeks (Hammarström et al., 2020). No change in rRNA content was observed in the single-set group until after 12 weeks, where rRNA content was elevated above the multi-set group. Following training, the multi-set group also had significantly greater muscle mass compared to the single-set group, which suggests that as muscle hypertrophy occurs, there is an initial increase in ribosome content but over time, the translational machinery becomes more refined and allows for a decrease in ribosome content whilst likely increasing translational efficiency. The initial increase in ribosome content following a hypertrophic stimulus is in part consistent with what is observed following SA in mice, where an initial rise in ribosome-related gene expression is observed but returns to baseline levels within 1 or 2 weeks post-surgery (von Walden et al., 2012). The refinement of translational machinery is consistent with observations found following resistance training in young adults, where young individuals demonstrate a reduction in acute S6K1 phosphorylation following 3 and 6 weeks resistance training, where older adults (who were unable to increases muscle mass following training) saw no difference following training (Brook et al., 2016). It appears that translational capacity increases

initially, then decreases with resistance training and therefore the timing of muscle sampling is important to capture these observations.

The concept of high and low responders has recently gained traction in the scientific literature (Roberts et al., 2018b; Timmons, 2011). One study found that high responders to resistance training, based on changes in type 2 CSA, increased ribosome content following resistance training where moderate and low responders did not (Stec et al., 2016). The authors hypothesized that those with a greater ability to undergo ribosomal biogenesis would elicit a greater muscle hypertrophic response following resistance training. However, this study was performed in old individuals, who have been shown to have dysregulated ribosome function (Stec et al., 2015) and perhaps needed to increase translational capacity because they were unable to increase translational efficiency (Stec et al., 2016). When comparing global gene expression analysis in individuals who had undergone 20 weeks of resistance training, there was a downregulation in rDNA gene expression consistent in almost all of those who presented the greatest increase in lean mass in addition to a greater expression in those who showed the least change in lean mass (B. E. Phillips et al., 2013). Low ribosome-related gene expression in high-responders to resistance exercise is consistent with our results and further indicates that translational efficiency rather than capacity may be more important in dictating muscle hypertrophy following resistance training.

Taken together, our findings indicate that those who increase muscle mass following resistance training either do not change or decrease translational capacity, likely due to either having more efficient ribosomes prior to resistance training or making the translational machinery more efficient following resistance training. Our findings are

consistent with the hypotheses presented by McGlory et al., (2017) and Joanisse et al. (2020) and likely also offers a mechanism to conserve cellular energy (Warner, 1999).

Mitochondrial Ribosomes

The expression of 12S mt-rRNA, a marker of the mitochondrial ribosome, did not change following aerobic or resistance training. It is not surprising that there was no change in 12S mt-rRNA expression, as no difference in mitochondrial gene expression or protein content was observed between the aerobically trained (EX) and the control (CTL) legs. Mitochondrial DNA codes for 13 proteins involved in oxidative phosphorylation, the 12S and 16S rRNAs involved in the mitochondrial ribosomes, and their own subset of tRNAs, therefore mitochondrial ribosomes function exclusively to translate more mitochondrial ribosomes and mitochondrial proteins (Bonawitz et al., 2006). However, numerous mitochondrial proteins, including all of those which make up the mitochondrial ribosome, are encoded in the nucleus and therefore are also dependent on cytosolic ribosomes to accumulate proper machinery within the mitochondria (Bonawitz et al., 2006; O'Brien, 2003). Interestingly, a recent study in both *c. elegans* and human K562 cells determined that the translational pathways between cytosolic and mitochondrial ribosomes are maintained in balance, where impacting one has a similar impact on the other (Molenaars et al., 2020). In addition, cytosolic ribosomes have been shown to localize on the outer membrane of mitochondria, likely to aid in mitochondrial protein translation and import (Gold et al., 2017). The relationship between translational pathways and cytosolic and mitochondrial ribosomes has not been studied *in vivo* in humans.

Within the confines of this study, it is impossible to directly measure the relationship between cytosolic and mitochondrial ribosomes. However, 12S mt-rRNA showed a significant, positive relationship to 18S rRNA and showed similar trends for a relationship with c-Myc and 5S rRNA expression (**Table 7**). Importantly, 5S rRNA has been shown to associate with mitochondrial ribosomes and may help to explain both the relationship observed with the mitochondrial ribosome and the discrepancy between the other cytosolic rRNAs (Smirnov et al., 2008, 2010, 2011). Several markers of mitochondrial-related gene expression also showed significant, positive correlations to other markers of ribosome-related gene expression (**Appendix F**). Observing significant relationships between mitochondrial and ribosome-related gene expression suggest that there is indeed a relationship between the cytosolic and mitochondrial translational pathways, however more research should be conducted to directly measure the interaction between them.

Conclusions

When analysing the complete subject population as a single cohort, no changes in ribosome-related gene expression was observed with aerobic or resistance training, which is likely a testament of the high inter-individual variability in ribosome-related gene expression (Figueiredo et al., 2021; Gibbons et al., 2014). Although there may have been an increase in mitochondrial content following aerobic training as observed using immunohistochemical analyses, whole-muscle measurements observed no change in mitochondrial content. Therefore, more research should be conducted to ribosomal adaptations alongside changes in mitochondrial content. Low ribosome content was associated with greater LLSTM and

increase in LLSTM following resistance training. A lack of change or a reduction in ribosome content following resistance training was also associated with a greater change in LLSTM following resistance training. In agreement with the hypothesis put forth by Joanisse et al. (2020), we propose that translational capacity increases acutely and temporarily in response to a novel stimulus and then decreases following exercise training as ribosomes become more efficient. It is therefore likely that translational efficiency rather than capacity is most important in dictating cellular adaptations to exercise training.

Limitations

This study was performed with several limitations and considerations. First, as the tissue collected was part of a larger study (Thomas et al., 2019), there was limited tissue availability for some of our measures (primarily for immunofluorescence, n=7) that decreased the number of samples used in an already small sample size (14 participants total, 13 for gene expression analyses). In addition, there is not sufficient evidence to support that the aerobic training stimulus induced mitochondrial adaptations, which was initially an important consideration in our study and may in part contribute to the lack of change in ribosome content following aerobic training. However, it is possible that even in the presence of mitochondrial adaptations that there would not be any change in translational capacity as other aerobic adaptations (improved oxygen uptake, increased skeletal muscle capillarization) were observed without the change in ribosome content (Thomas et al., 2019). The lack of mitochondrial biogenesis with our aerobic training intervention also limited the ability to examine the relationship between ribosomes and mitochondria. Due to the chronic

time course of the study, only translational capacity was measured. Since translational capacity decreases while maintaining the adaptive response to resistance training, it appears that translational efficiency may have increased in individual's who gained the most muscle mass following resistance training, although no direct measure of translational efficiency was performed. Lastly, our HIGH versus LOW analyses operate under the assumption that each condition (leg) and timepoint are independent of each other, which is an important consideration as each participant has multiple variables in each analysis.

Future Directions

Recent work has identified rDNA gene copy as a potential mechanism explaining inter-individual variability in ribosome-related gene expression (Figueiredo et al., 2021), however future research should continue to measure how rDNA gene copy influences ribosomal adaptation to both acute and chronic exercise stimuli. Research in humans should also place more focus on measuring translational efficiency in addition to capacity and to use better, more accurate measures of translational efficiency. More timepoints should be added in future studies measuring translational capacity to capture the initial increase and progressive decrease following the initial onset of exercise training. Finally, more research should continue to explore the relationship between mitochondria and ribosomes and more specifically in the context of exercise adaptations.

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APPENDIX

Appendix A. Raw Data.

Appendix A1. Baseline subject characteristics raw data.

	Baseline Subject Characteristics						
Subject	Sex	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	DL V02 Relative	Average LLSTM
S01	Male	22	177.0	110.70	35.33	30.61	12898
S02	Female	21	163.0	64.40	24.24	39.78	6837
S03	Male	23	174.0	104.20	34.42	43.07	13581
S04	Female	23	172.0	60.60	20.48	36.57	6813
S05	Female	20	152.0	50.21	21.73	31.24	5727
S06	Female	19	162.5	62.40	23.63	29.29	7004
S07	Female	23	155.0	49.00	20.40	40.91	6040
S09	Male	21	176.5	71.20	22.86	44.71	10645
S10	Male	19	167.0	74.90	26.86	38.83	9425
S11	Male	21	177.5	71.12	22.57	44.28	10560
S12	Male	18	178.0	75.90	23.96	43.71	9386
S13	Male	23	182.0	95.25	28.76	36.75	11381
S14	Male	22	171.0	69.90	23.90	56.2	9730
S15	Female	20	170.0	77.10	26.68	30.84	7865
AVERAGE		21.07	169.82	74.06	25.42	39.06	9134.89
SD		1.69	8.94	18.30	4.65	7.35	2508.61

Appendix A2. COXIV staining intensity raw data.

	COXIV Stainin Intensity (I.U.)					
	Type 1		Type 2		Type 1 + Type 2	
Subject	Pre RT (CTL)	Pre RT (EX)	Pre RT (CTL)	Pre RT (EX)	Pre RT (CTL)	Pre RT (EX)
S01	3410.55	4759.97	2836.67	4113.51	3123.61	4436.74
S03	1631.93	1916.53	1395.65	1716.56	1513.79	1816.55
S06	1527.49	1856.40	1386.51	1870.56	1457.00	1863.48
S07	1285.63	1623.75	1168.82	1516.14	1227.22	1569.94
S12	2788.20	3734.76	2459.69	3110.77	2623.94	3422.76
S13	2785.73	2747.05	2362.02	2501.16	2573.88	2624.10
S15	4035.82	5289.71	3624.24	5194.93	3830.03	5242.32
AVERAGE	2495.04857	3132.59357	2176.22607	2860.51571	2335.63732	2996.55464
SD	1042.94536	1483.06205	903.290869	1372.78609	972.11164	1422.99365

Appendix A3. Immunoblot, mitochondrial-related protein content raw data.

	Protein Content (A.U.)											
	COXI		COXII		COXIII		COXIV		COXV		CS	
Subject	Pre RT (CTL)	Pre RT (EX)	Pre RT (CTL)	Pre RT (EX)	Pre RT (CTL)	Pre RT (EX)	Pre RT (CTL)	Pre RT (EX)	Pre RT (CTL)	Pre RT (EX)	Pre RT (CTL)	Pre RT (EX)
S01	3.88	2.89	1.68	1.73			1.06	0.97	1.15	1.79	0.57	0.68
S02	1.51	10.65			0.02	0.33	0.72	1.73	0.17	0.59	1.08	5.85
S03	1.30	10.89	1.32	2.41	0.97	0.01	1.01	0.67	1.87	0.01		
S04	0.04	0.30	0.24	0.97	0.05	0.30	0.20	1.09	0.10	0.61		
S05	1.68	0.00	1.52	0.11	0.31	0.01	1.80	0.48	0.81	0.02		
S06	1.99	4.49	2.40	2.87	0.34	0.28			0.88	0.95	16.06	40.75
S09	9.17	3.22	2.04	0.21	0.12	0.05	1.94	0.50	0.10	0.08	0.32	0.23
S10	0.34	0.37	1.02	1.28	0.19	0.17	0.41	0.81	0.41	0.50	1.99	0.25
S11	1.43	2.40	3.75	1.18	0.07	0.22	0.26	0.90	0.16	0.61	1.28	1.01
S12	3.11	5.70	0.99	0.42	0.47	0.05	0.70	0.37	0.67	0.11	3.01	1.55
S13	1.19	0.46	1.76	1.86	0.44	0.37	0.57	0.63	0.35	0.27	6.94	4.28
S14	4.22	0.76	2.89	1.43	1.16	0.28	2.68	1.47	2.30	0.52	11.35	31.02
S15			2.48	1.83	1.12	0.96	1.87	1.03	2.41	1.73		
AVERAGE	2.49	3.51	1.84	1.36	0.44	0.25	1.10	0.89	0.88	0.60	4.73	9.51
SD	2.46	3.84	0.95	0.85	0.42	0.26	0.79	0.41	0.83	0.59	5.58	15.27

Appendix A4. RNA concentration raw data.

	[RNA] ng/mg			
Subject	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)
S03	4.54	5.53	10.21	5.63
S04	5.14	11.32	2.46	9.60
S05	10.11	9.72	13.84	14.71
S06	9.10	10.43	10.76	9.36
S07	14.07	5.13	10.44	11.75
S09	9.14	10.75	4.77	5.82
S10	4.96	10.48	10.23	15.59
S11	8.03	12.93	9.09	7.75
S12	7.39	6.57	18.53	11.58
S13	10.60	15.59	13.81	17.62
S14	15.45	11.13	11.30	16.04
S15	6.60	8.89	10.27	1.19
AVERAGE	8.76	9.87	10.47	10.55
SD	3.45	3.02	4.12	4.95

Appendix A5. Housekeeper gene expression raw data.

Gene	Subject	Housekeeper Gene Expression (1/Cq)			
		Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)
	S01	0.050	0.051	0.052	0.051
	S02	0.051	0.051	0.053	0.053
	S03	0.052	0.052	0.050	0.053
	S04	0.051	0.049	0.047	0.053
	S05	0.052	0.050	0.051	0.053
	S06	0.053	0.050	0.051	0.050
	S07	0.050	0.052	0.053	0.051
	S09	0.057	0.060	0.055	0.057
	S10	0.038	0.053	0.047	0.049
	S11	0.051	0.052	0.051	0.054
	S12	0.051	0.049	0.055	0.053
	S13	0.052	0.052	0.053	0.053
	S14	0.053	0.052	0.051	0.053
	AVERAGE	0.051	0.052	0.051	0.052
ß2M	SD	0.004	0.003	0.002	0.002
	S01	0.041	0.042	0.041	0.042
	S02	0.040	0.040	0.041	0.041
	S03	0.042	0.041	0.038	0.042
	S04	0.039	0.038	0.038	0.040
	S05	0.039	0.039	0.040	0.040
	S06	0.041	0.039	0.040	0.040
	S07	0.037	0.040	0.037	0.037
	S09	0.045	0.045	0.043	0.044
	S10	0.032	0.040	0.036	0.037
	S11	0.039	0.040	0.040	0.039
	S12	0.039	0.037	0.039	0.038
	S13	0.039	0.039	0.039	0.038
	S14	0.040	0.039	0.038	0.039
ß2M	AVERAGE	0.039	0.040	0.039	0.040
(Taqman)	SD	0.003	0.002	0.002	0.002

Appendix A6. Mitochondrial-related gene expression raw data.

Gene	Subject	Mitochondrial Gene Expression (2^-ΔΔCt)			
		Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)
	S01	1.36	1.54	0.80	1.26
	S02	1.10	1.29	0.80	1.10
	S03	1.24	0.92	1.72	1.61
	S04	1.42	0.86	1.27	1.57
	S05	1.23	1.99	1.03	0.80
	S06	0.80	1.45	1.07	1.36
	S07	0.67	0.69	0.45	0.90
	S09	0.38	0.20	0.33	0.28
	S10	0.90	1.23	1.05	1.35
	S11				
	S12	1.70	1.73	0.73	1.68
	S13	0.94	0.89	0.62	0.43
	S14	1.07	0.61	1.78	0.92
	AVERAGE	1.07	1.12	0.97	1.10
COXIV	SD	0.36	0.51	0.45	0.45
	S01	1.22	1.19	0.89	1.11
	S02	0.97	0.99	0.58	1.09
	S03	1.55	0.87	1.11	1.70
	S04	1.45	0.73	1.67	1.28
	S05				
	S06	0.52	1.05	0.67	1.06
	S07	0.78	0.92	0.42	1.01
	S09	0.47	0.28	0.33	0.39
	S10	0.58	1.23	0.76	1.14
	S11	1.23	1.49	1.01	0.90
	S12	1.69	1.38	0.88	1.65
	S13	1.53	1.34	0.85	0.53
	S14	1.07	0.49	1.21	1.04
	AVERAGE	1.09	1.00	0.86	1.07
TFAM	SD	0.43	0.37	0.37	0.38
	S01	0.77	0.91	0.57	0.72
	S02	0.91	1.14	0.59	1.15
	S03	0.85	0.96	2.85	0.85
	S04	1.16	0.99	1.04	1.16
	S05	1.73	2.31	1.09	0.73
	S06	0.79	1.55	1.17	1.16
	S07	1.57	0.75	1.44	1.13
	S09	0.31	0.17	0.29	0.25
	S10	0.94	1.85	1.49	1.70
	S11				
	S12				
	S13	1.11	1.11	1.12	1.01
	S14	2.07	1.58	1.75	1.44
	AVERAGE	1.11	1.21	1.22	1.03
12S mt-rRNA	SD	0.50	0.58	0.69	0.39

Appendix A7. Ribosomal biogenesis regulator gene expression raw data.

	Ribosomal Biogenesis Regulator Gene Expression (2^-ΔΔCt)				
Gene	Subject	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)
	S01	0.75	0.80	1.34	0.65
	S02	0.73	0.68	0.97	0.52
	S03	1.51	1.05	1.35	0.71
	S04	1.45	0.75	2.55	1.32
	S05	1.64	1.41	2.35	1.65
	S06	0.74	0.69	1.06	0.87
	S07	2.23	0.93	0.95	0.86
	S09	0.31	0.56	0.56	0.52
	S10				
	S11	1.21	1.33	0.83	1.51
	S12	0.95	1.36	1.86	0.87
	S13	0.98	0.41	1.20	1.62
	S14	0.89	0.50	1.10	1.64
	AVERAGE	1.12	0.87	1.34	1.06
c-Myc	SD	0.52	0.34	0.61	0.45
	S01	4.60	3.83	3.73	3.72
	S02	1.63	2.54	2.85	0.98
	S03	2.89	1.25	1.41	2.81
	S04	0.62	1.58	2.29	2.30
	S05	0.94	1.91	2.06	1.42
	S06	0.47	1.40	1.37	2.62
	S07				
	S09	0.62	0.55	3.10	1.14
	S10	2.95	0.38	1.04	0.68
	S11	0.50	1.21	0.72	0.25
	S12	0.37	0.66	0.39	0.47
	S13				
	S14	0.51	0.35	0.16	0.64
	AVERAGE	1.46	1.42	1.74	1.55
Cyclin D1	SD	1.41	1.05	1.16	1.14
	S01	1.91	1.36	1.21	1.81
	S02	1.26	1.31	1.01	0.90
	S03	2.06	0.76	0.98	2.56
	S04	0.83	0.61	1.58	0.99
	S05	1.37	1.13	1.48	1.00
	S06	0.50	0.64	0.85	1.52
	S07				
	S09	0.59	0.23	0.51	0.34
	S10	0.60	0.50	0.67	0.78
	S11	1.03	0.88	1.53	0.47
	S12	1.65	0.49	0.26	1.53
	S13				
	S14	0.58	0.34	0.67	0.40
	AVERAGE	1.13	0.75	0.98	1.12
UBF	SD	0.56	0.38	0.44	0.68
	S01	2.63	2.58	2.25	3.24
	S02	1.45	1.34	1.31	1.23
	S03				
	S04	2.42	0.84	1.60	2.85
	S05	2.95	1.97	1.69	2.21
	S06	0.63	0.60	0.73	1.57
	S07	0.13	0.84	0.03	0.12
	S09	1.21	1.22	1.29	1.22
	S10	0.21	0.72	0.45	0.95
	S11	1.41	2.34	1.66	0.51
	S12	1.32	0.39	0.48	1.25
	S13	0.94	0.43	0.28	0.09
	S14	1.00	0.27	0.74	0.93
	AVERAGE	1.36	1.13	1.04	1.35
TIF-1A	SD	0.90	0.78	0.68	0.99
	S01	1.78	1.43	0.98	1.81
	S02	1.37	1.39	0.77	0.88
	S03	1.92	0.82	1.63	2.11
	S04	1.23	0.76	2.67	0.88
	S05	1.42	1.51	1.09	0.82
	S06	0.55	0.83	0.74	1.08
	S07				
	S09	0.55	0.15	0.40	0.36
	S10	0.91	0.45	0.52	0.40
	S11	0.60	0.80	0.51	0.17
	S12	1.38	0.26	0.18	1.00
	S13				
	S14	0.53	0.51	0.56	0.67
	AVERAGE	1.11	0.81	0.91	0.93
POLR-1B	SD	0.51	0.46	0.70	0.59

Appendix A8. Ribosomal RNA gene expression raw data.

		Ribosomal RNA Expression (2^-ΔΔCt)			
Gene	Subject	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)
	S01	1.07	1.07	2.42	0.68
	S02	0.57	0.85	0.69	0.45
	S03	0.51	2.27	4.23	0.67
	S04	1.51	2.56	1.74	0.64
	S05	2.21	2.89	1.94	2.57
	S06	0.96	1.57	1.51	1.07
	S07	3.39	4.21	3.08	2.61
	S09	0.27	0.24	1.90	0.94
	S10				
	S11	0.65	0.61	0.53	1.10
	S12	1.58	1.30	2.09	1.45
	S13	2.67	4.38	4.18	2.34
	S14	0.41	0.40	0.39	1.49
	AVERAGE	1.32	1.86	2.06	1.33
5S rRNA	SD	0.99	1.41	1.27	0.78
	S01				
	S02	8.87	11.42	3.68	6.94
	S03	4.42	3.77	11.36	10.82
	S04	8.21	7.78	9.13	2.18
	S05	8.00	4.68	4.32	4.04
	S06	2.58	3.13	2.20	1.92
	S07	0.09	0.07	0.02	0.03
	S09	1.48	0.62	0.94	0.93
	S10	0.25	0.65	0.32	0.35
	S11	0.12	0.09	0.02	0.10
	S12	0.43	0.03	0.22	0.22
	S13	0.10	0.03	0.02	0.01
	S14	0.92	0.36	0.08	0.14
45S pre-rRNA	AVERAGE	2.96	2.72	2.69	2.31
	SD	3.50	3.68	3.86	3.40
	S01	2.47	2.29	1.92	2.24
	S02	1.44	1.43	0.82	1.29
	S03	1.09	1.07	2.25	1.19
	S04	1.41	0.81	2.64	0.66
	S05	2.50	2.75	1.43	1.37
	S06	0.94	1.22	0.98	1.35
	S07	0.93	1.45	0.32	0.60
	S09	0.85	0.38	0.50	0.57
	S10	0.47	0.58	0.33	0.36
	S11	0.56	0.43	0.15	0.19
	S12	0.79	0.12	0.23	0.42
	S13	1.29	0.49	0.56	0.38
	S14	0.37	0.21	0.16	0.21
	AVERAGE	1.16	1.02	0.95	0.83
5.8S rRNA	SD	0.67	0.80	0.85	0.61
	S01	1.56	1.86	1.35	1.70
	S02	1.78	1.98	1.64	1.77
	S03	1.14	0.76	2.30	1.95
	S04	1.37	1.37	1.03	0.59
	S05	1.44	2.88	1.92	1.10
	S06	1.00	2.59	1.62	1.10
	S07	1.48	1.45	1.57	1.28
	S09	0.36	0.16	0.14	0.35
	S10	0.19	0.91	0.63	1.02
	S11	0.96	1.45	0.81	0.78
	S12	1.65	0.55	0.98	1.61
	S13	1.34	0.66	1.06	0.93
	S14	0.75	0.08	0.94	0.30
	AVERAGE	1.16	1.28	1.23	1.11
18S rRNA	SD	0.49	0.87	0.58	0.53
	S01	2.32	1.68	0.83	1.34
	S02	1.68	1.26	1.18	1.20
	S03	0.64	0.49	1.95	1.00
	S04	0.95	1.04	1.76	0.35
	S05	3.15	2.03	1.35	1.38
	S06	0.72	1.66	0.92	1.54
	S07				
	S09	0.89	0.32	0.43	0.58
	S10	0.19	0.59	0.28	0.38
	S11	0.37	0.44	0.19	0.31
	S12	1.11	0.28	0.30	0.87
	S13	4.80	3.45	2.92	4.61
	S14	0.56	0.47	0.29	0.32
	AVERAGE	1.45	1.14	1.03	1.16
28S rRNA	SD	1.36	0.94	0.84	1.18

Appendix A9. Ribosomal transcribed spacer region gene expression raw data.

		Ribosomal Transcribed Spacer Expression (2^-ΔΔCt)			
Gene	Subject	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)
	S01				
	S02	2.56	2.79	1.63	1.67
	S03	1.37	1.46	3.10	2.15
	S04	1.75	1.67	2.32	0.58
	S05	2.11	1.35	1.63	1.68
	S06	0.88	1.22	0.70	0.73
	S07	1.22	1.14	0.61	0.86
	S09	0.78	0.30	0.43	0.28
	S10	0.93	0.55	0.34	0.19
	S11	0.26	0.12	0.04	0.09
	S12	0.56	0.07	0.25	0.21
	S13	1.38	0.48	0.56	0.54
	S14	0.51	0.33	0.07	0.14
	AVERAGE	1.19	0.96	0.97	0.76
5.8S ITS	SD	0.69	0.80	0.98	0.70
	S01	2.48	1.89	1.26	1.16
	S02	2.07	1.64	0.88	1.07
	S03	0.94	0.31	1.49	1.05
	S04	0.86	1.54	0.27	0.27
	S05	1.22	1.70	1.03	0.64
	S06	1.07	0.93	0.60	0.42
	S07	1.20	0.47	0.69	0.54
	S09	0.29	0.14	0.16	0.19
	S10	0.87	0.71	0.55	0.55
	S11	0.46	0.65	0.06	0.25
	S12	0.93	0.02	0.87	0.42
	S13	1.11	0.49	0.46	0.34
	S14	1.27	0.62	0.18	0.05
	AVERAGE	1.14	0.85	0.65	0.53
18S ETS	SD	0.59	0.63	0.44	0.36
	S01				
	S02	3.54	1.96	1.30	1.74
	S03	1.04	0.84	1.12	2.56
	S04	1.30	2.26	1.02	0.54
	S05	1.60	1.80	1.41	1.52
	S06	0.85	1.56	0.92	0.93
	S07	2.73	1.24	1.06	1.84
	S09	0.65	0.30	0.73	0.41
	S10	0.27	0.78	0.51	0.59
	S11	0.17	0.54	0.11	0.24
	S12	1.60	0.27	1.19	0.95
	S13	1.18	1.04	0.80	0.71
	S14	1.02	0.15	0.45	0.14
	AVERAGE	1.33	1.06	0.89	1.01
28S ITS	SD	0.97	0.71	0.38	0.74

Appendix A10. Gene expression at Post RT relative to respective Pre RT raw data.

		Expression (2^-ΔΔCt) at Post RT Relative to Respective Pre RT															
Condition	Subject	COXIV	TFAM	12S mt-rRNA	c-Myc	Cyclin D1	UBF	TIF-1A	POLR-1B	5S rRNA	45S pre-rRNA	5.8S rRNA	18S rRNA	28S rRNA	5.8S ITS	18S ETS	28S ITS
	S01	0.80	0.89	0.57	1.34	3.73	1.21	2.25	0.98	2.42		1.92	1.35	0.83		1.26	
	S02	0.80	0.58	0.59	0.97	2.85	1.01	1.31	0.77	0.69	3.68	0.82	1.64	1.18	1.63	0.88	1.30
	S03	1.72	1.11	2.85	1.35	1.41	0.98		1.63	4.23	11.36	2.25	2.30	1.95	3.10	1.49	1.12
	S04	1.27	1.67	1.04	2.55	2.29	1.58	1.60	2.67	1.74	9.13	2.64	1.03	1.76	2.32	0.27	1.02
	S05	1.03		1.09	2.35	2.06	1.48	1.69	1.09	1.94	4.32	1.43	1.92	1.35	1.63	1.03	1.41
	S06	1.07	0.67	1.17	1.06	1.37	0.85	0.73	0.74	1.51	2.20	0.98	1.62	0.92	0.70	0.60	0.92
	S07	0.45	0.42	1.44	0.95			0.03		3.08	0.02	0.32	1.57		0.61	0.69	1.06
	S09	0.33	0.33	0.29	0.56	3.10	0.51	1.29	0.40	1.90	0.94	0.50	0.14	0.43	0.43	0.16	0.73
	S10	1.05	0.76	1.49		1.04	0.67	0.45	0.52		0.32	0.33	0.63	0.28	0.34	0.55	0.51
	S11		1.01		0.83	0.72	1.53	1.66	0.51	0.53	0.02	0.15	0.81	0.19	0.04	0.06	0.11
	S12	0.73	0.88		1.86	0.39	0.26	0.48	0.18	2.09	0.22	0.23	0.98	0.30	0.25	0.87	1.19
	S13	0.62	0.85	1.12	1.20			0.28		4.18	0.02	0.56	1.06	2.92	0.56	0.46	0.80
	S14	1.78	1.21	1.75	1.10	0.16	0.67	0.74	0.56	0.39	0.08	0.16	0.94	0.29	0.07	0.18	0.45
	AVERAGE	0.97	0.86	1.22	1.34	1.74	0.98	1.04	0.91	2.06	2.69	0.95	1.23	1.03	0.97	0.65	0.89
CTL	SD	0.45	0.37	0.69	0.61	1.16	0.44	0.68	0.70	1.27	3.86	0.85	0.58	0.84	0.98	0.44	0.38
	S01	1.29	1.21	0.70	0.80	3.37	2.76	3.61	2.72	0.51		3.07	1.88	1.58		2.12	
	S02	1.13	1.20	1.11	0.64	0.89	1.37	1.37	1.32	0.33	10.93	1.77	1.96	1.42	2.73	1.95	2.17
	S03	1.65	1.86	0.82	0.88	2.54	3.90		3.18	0.50	17.03	1.63	2.16	1.18	3.50	1.93	3.21
	S04	1.61	1.40	1.12	1.63	2.08	1.50	3.18	1.33	0.48	3.44	0.90	0.65	0.42	0.95	0.49	0.68
	S05	0.82		0.71	2.04	1.28	1.52	2.46	1.23	1.92	6.36	1.88	1.22	1.63	2.73	1.16	1.90
	S06	1.40	1.16	1.12	1.07	2.37	2.32	1.75	1.62	0.80	3.03	1.85	1.21	1.81	1.19	0.76	1.17
	S07	0.92	1.10	1.09	1.06			0.14		1.95	0.05	0.82	1.42		1.40	0.99	2.30
	S09	0.29	0.42	0.24	0.64	1.03	0.52	1.36	0.55	0.70	1.46	0.79	0.39	0.68	0.46	0.35	0.51
	S10	1.38	1.24	1.64		0.62	1.18	1.06	0.60		0.55	0.49	1.13	0.44	0.31	1.00	0.73
	S11		0.99		1.86	0.23	0.72	0.57	0.26	0.82	0.16	0.27	0.86	0.37	0.15	0.45	0.30
	S12	1.73	1.81		1.08	0.43	2.32	1.39	1.51	1.08	0.34	0.57	1.78	1.02	0.34	0.76	1.19
	S13	0.44	0.58	0.98	1.99			0.10		1.74	0.01	0.52	1.03	5.43	0.88	0.62	0.89
	S14	0.95	1.14	1.39	2.02	0.58	0.60	1.03	1.01	1.12	0.22	0.29	0.34	0.38	0.23	0.09	0.17
	AVERAGE	1.13	1.18	0.99	1.31	1.40	1.70	1.50	1.39	0.99	3.63	1.14	1.23	1.36	1.24	0.98	1.27
EX	SD	0.46	0.41	0.37	0.56	1.03	1.04	1.10	0.88	0.58	5.35	0.83	0.59	1.39	1.14	0.65	0.93

Appendix A11. CSA and LLSTM raw data. Taken from Thomas et al. (2019).

	Type 1 CSA (μm^2)				Type 2 CSA (μm^2)				Mixed-fibre CSA (μm^2)				LLSTM (g)					
Subject	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)	Pre AT (CTL)	Pre AT (EX)	Pre RT (CTL)	Pre RT (EX)	Post RT (CTL)	Post RT (EX)
S01	6619.50	6162.00	6076.82	6638.00	8459.50	8251.00	6621.07	7593.50	7539.50	7206.50	6348.95	7115.75	12964	12831	13089	12953	13164	13477
S02	5834.00	5906.36	4633.62	4450.57	5465.50	5302.64	4793.81	5051.93	5649.75	5604.50	4713.71	4751.25	6734	6939	6734	6939	6994	7112
S03	5432.65	4616.57	4906.07	8846.61	7403.74	5875.41	7278.53	12936.76	6418.19	5245.99	6092.30	10891.68	13523	13639	13670	13417	13301	13565
S04	3787.12	3486.89	4717.98	5985.83	3311.64	2760.19	4764.99	6734.22	3549.38	3123.54	4741.49	6360.02	6492	7133	6492	7133	6865	7130
S05	5334.54	6173.26	4920.87	6188.23	3224.12	4742.36	2740.50	1897.52	4279.33	5457.81	3830.69	4042.87	5678	5775	5678	5775	5948	6235
S06	4840.08	3738.41	5438.80	4557.33	3967.19	3060.59	6356.40	4347.69	4403.63	3399.50	5897.60	4452.51	7094	6913	6964	6960	8389	8441
S07	7192.86	4646.00	7767.08	4797.71	7051.46	3746.00	9398.70	6421.55	7122.16	4196.00	8582.89	5609.63	6164	5916	6164	5916	6386	6051
S09	6108.38	6162.42	6502.13	5259.20	7621.29	7121.13	8182.71	6966.52	6864.83	6641.78	7342.42	6112.86	10661	10629	10447	10168	11397	11676
S10	4820.26	7579.94	5241.50	8937.34	4854.01	7172.49	7687.06	10014.28	4837.13	7376.22	6464.28	9475.81	9425	9425	9546	9737	10561	10803
S11	5610.19	7324.18	5104.82	7225.75	6817.79	5049.80	6882.72	11168.63	6213.99	6186.99	5993.77	9197.19	10548	10572	10503	10389	11676	11579
S12	4744.88	3385.00	3828.15	6147.70	4255.04	3976.50	4855.33	7091.60	4499.96	3680.75	4341.74	6619.65	9561	9210	9565	10103	11068	11625
S13	4536.85	5674.58	5782.34	7327.00	6617.88	6024.69	9136.09	9271.50	5577.37	5849.64	7459.22	8299.25	11545	11217	11375	11626	12306	12339
S14	4283.51	4315.11	4952.54	4134.04	5411.29	4415.18	6271.72	3022.54	4847.40	4365.15	5612.13	3578.29	9721	9739	9935	9533	10601	10792
S15	2949.29	2933.00	3508.42	5573.10	3595.37	3199.00	4011.34	6764.02	3272.33	3066.00	3759.88	6168.56	7817	7912	8112	7942	9014	8744
AVERAGE	5149.58	5150.26	5241.51	6147.74	5575.41	5049.78	6355.78	7091.59	5362.50	5100.02	5798.65	6619.66	9137.64	9132.14	9162.43	9185.07	9833.57	9969.21
SD	1117.72	1483.23	1072.71	1526.53	1756.71	1680.52	1943.32	3041.49	1332.87	1479.34	1421.11	2179.34	2543.30	2479.88	2558.27	2477.84	2546.88	2628.64

Appendix B. Statistical outputs.

Appendix B1. Baseline subject characteristics statistical output between males and females.

Baseline Subject Characteristics

Independent Samples T-Test

		Statistic	df	p
Age (years)	Student's t	0.132	12.0	0.897
Height (cm)	Student's t	3.860	12.0	0.002
Weight (kg)	Student's t	3.045	12.0	0.010
BMI (kg/m^2)	Student's t	1.969	12.0	0.073
VO2 peak (mL/min/kg)	Student's t	2.132	12.0	0.054
Average LLSTM (g)	Student's t	6.036	12.0	<.001

Group Descriptives

	Group	N	Mean	Median	SD	SE
Age (years)	Male	8	21.1	21.5	1.81	0.639
	Female	6	21.0	20.5	1.67	0.683
Height (cm)	Male	8	175.4	176.8	4.63	1.639
	Female	6	162.4	162.8	7.91	3.231
Weight (kg)	Male	8	84.1	75.4	16.58	5.862
	Female	6	60.6	61.5	10.32	4.213
BMI (kg/m^2)	Male	8	27.3	25.4	5.10	1.804
	Female	6	22.9	22.7	2.45	1.001
VO2 peak (mL/min/kg)	Male	8	42.3	43.4	7.42	2.622
	Female	6	34.8	33.9	4.98	2.033
Average LLSTM (g)	Male	8	10950.8	10602.5	1576.97	557.544
	Female	6	6714.3	6825.0	756.61	308.884

Appendix B2. COXIV staining intensity statistical output.

COXIV Staining Intensity

Paired Samples T-Test

			statistic	df	p
T1 Pre RT (CTL)	T1 Pre RT (EX)	Student's t	-3.12	6.00	0.020
T2 Pre RT (CTL)	T2 Pre RT (EX)	Student's t	-3.38	6.00	0.015
Mixed Pre RT (CTL)	Mixed Pre RT (EX)	Student's t	-3.31	6.00	0.016

Descriptives

	N	Mean	Median	SD	SE
T1 Pre RT (CTL)	7	2495	2786	1043	394
T1 Pre RT (EX)	7	3133	2747	1483	561
T2 Pre RT (CTL)	7	2176	2362	903	341
T2 Pre RT (EX)	7	2861	2501	1373	519
Mixed Pre RT (CTL)	7	2336	2574	972	367
Mixed Pre RT (EX)	7	2997	2624	1423	538

Appendix B3. Mitochondrial-related protein content statistical output.

Mitochondrial-related Protein Content

Paired Samples T-Test

			statistic	df	p
COXI Pre RT (CTL)	COXI Pre RT (EX)	Student's t	-0.776	11.00	0.454
COXII Pre RT (CTL)	COXII Pre RT (EX)	Student's t	1.472	11.00	0.169
COXIII Pre RT (CTL)	COXIII Pre RT (EX)	Student's t	1.623	11.00	0.133
COXIV Pre RT (CTL)	COXIV Pre RT (EX)	Student's t	0.865	11.00	0.406
COXV Pre RT (CTL)	COXV Pre RT (EX)	Student's t	1.218	12.00	0.246
CS Pre RT (CTL)	CS Pre RT (EX)	Student's t	-1.411	8.00	0.196

Descriptives

	N	Mean	Median	SD	SE
COXI Pre RT (CTL)	12	2.488	1.595	2.461	0.7105
COXI Pre RT (EX)	12	3.510	2.642	3.840	1.1085
COXII Pre RT (CTL)	12	1.839	1.721	0.946	0.2732
COXII Pre RT (EX)	12	1.359	1.354	0.850	0.2455
COXIII Pre RT (CTL)	12	0.438	0.326	0.416	0.1202
COXIII Pre RT (EX)	12	0.251	0.249	0.257	0.0741
COXIV Pre RT (CTL)	12	1.101	0.863	0.788	0.2276
COXIV Pre RT (EX)	12	0.888	0.855	0.406	0.1173
COXV Pre RT (CTL)	13	0.875	0.665	0.827	0.2295
COXV Pre RT (EX)	13	0.598	0.515	0.586	0.1626
CS Pre RT (CTL)	9	4.731	1.988	5.584	1.8612
CS Pre RT (EX)	9	9.513	1.549	15.268	5.0894

Appendix B4. RNA concentration statistical output.

[RNA]

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	17.22	1	17.22	1.294	0.279
Residual	146.35	11	13.30		
Condition	4.24	1	4.24	0.337	0.573
Residual	138.50	11	12.59		
Time ✻ Condition	3.21	1	3.21	0.334	0.575
Residual	105.71	11	9.61		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	297	11	27.0		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	0

Appendix B5. Housekeeper gene expression statistical outputs.

ß2M

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	5.53e-6	1	5.53e-6	1.88366	0.195
Residual	3.53e-5	12	2.94e-6		
Condition	1.14e-5	1	1.14e-5	1.50828	0.243
Residual	9.09e-5	12	7.58e-6		
Time ✻ Condition	2.63e-8	1	2.63e-8	0.00428	0.949
Residual	7.36e-5	12	6.13e-6		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	2.19e-4	12	1.82e-5		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
13	0

ß2M Taqman

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	2.26e-8	1	2.26e-8	0.0266	0.873
Residual	1.02e-5	12	8.49e-7		
Condition	2.81e-6	1	2.81e-6	1.3187	0.273
Residual	2.56e-5	12	2.13e-6		
Time ✻ Condition	9.32e-8	1	9.32e-8	0.0420	0.841
Residual	2.67e-5	12	2.22e-6		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	1.91e-4	12	1.59e-5		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
13	0

Appendix B6. Mitochondrial-related gene expression statistical outputs.

COXIV

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.0345	1	0.0345	0.211	0.655
Residual	1.7992	11	0.1636		
Condition	0.1013	1	0.1013	0.879	0.368
Residual	1.2674	11	0.1152		
Time ✻ Condition	0.0201	1	0.0201	0.283	0.605
Residual	0.7821	11	0.0711		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	4.98	11	0.452		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	1

TFAM

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.0628	1	0.0628	0.619	0.448
Residual	1.1164	11	0.1015		
Condition	0.0418	1	0.0418	0.374	0.553
Residual	1.2283	11	0.1117		
Time ✻ Condition	0.2714	1	0.2714	4.296	0.062
Residual	0.6949	11	0.0632		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	3.47	11	0.315		

Note. Type 3 Sums of Squares

Post Hoc Tests

Post Hoc Comparisons - Time ✻ Condition

Comparison									
Time	Condition		Time	Condition	Mean Difference	SE	df	t	p_{tukey}
Pre RT	CTL	-	Pre RT	EX	0.0914	0.1284	11.0	0.711	0.890
		-	Post RT	CTL	0.2227	0.0985	11.0	2.260	0.167
		-	Post RT	EX	0.0133	0.1175	11.0	0.114	0.999
	EX	-	Post RT	CTL	0.1314	0.1474	11.0	0.891	0.809
		-	Post RT	EX	-0.0780	0.1332	11.0	-0.586	0.934
Post RT	CTL	-	Post RT	EX	-0.2094	0.1124	11.0	-1.862	0.298

Group Summary

N	Excluded
12	1

12S mt-rRNA

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.0163	1	0.0163	0.0719	0.794
Residual	2.2693	10	0.2269		
Condition	0.0224	1	0.0224	0.1116	0.745
Residual	2.0072	10	0.2007		
Time ✻ Condition	0.2335	1	0.2335	1.5608	0.240
Residual	1.4964	10	0.1496		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	6.43	10	0.643		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
11	2

Appendix B7. Ribosomal biogenesis regulator gene expression statistical outputs.

c-Myc

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.52192	1	0.52192	2.7779	0.124
Residual	2.06666	11	0.18788		
Condition	0.82835	1	0.82835	5.8634	0.034
Residual	1.55402	11	0.14127		
Time ✻ Condition	0.00447	1	0.00447	0.0286	0.869
Residual	1.71812	11	0.15619		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	5.26	11	0.478		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	1

Cyclin D1

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.4426	1	0.4426	0.7884	0.395
Residual	5.6142	10	0.5614		
Condition	0.1435	1	0.1435	0.2900	0.602
Residual	4.9476	10	0.4948		
Time ✻ Condition	0.0624	1	0.0624	0.0800	0.783
Residual	7.8056	10	0.7806		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	38.9	10	3.89		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
11	2

UBF

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.133	1	0.1328	1.50	0.249
Residual	0.884	10	0.0884		
Condition	0.155	1	0.1549	1.85	0.204
Residual	0.837	10	0.0837		
Time ✻ Condition	0.742	1	0.7422	2.11	0.177
Residual	3.515	10	0.3515		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	5.96	10	0.596		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
11	2

TIF-1A

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.0281	1	0.0281	0.150	0.706
Residual	2.0542	11	0.1867		
Condition	0.0164	1	0.0164	0.165	0.692
Residual	1.0926	11	0.0993		
Time ✻ Condition	0.8557	1	0.8557	2.189	0.167
Residual	4.3007	11	0.3910		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	24.2	11	2.20		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	1

POLR-1B

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.0197	1	0.0197	0.113	0.743
Residual	1.7391	10	0.1739		
Condition	0.2322	1	0.2322	1.583	0.237
Residual	1.4665	10	0.1466		
Time ✻ Condition	0.2756	1	0.2756	1.227	0.294
Residual	2.2455	10	0.2245		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	7.70	10	0.770		

Note. Type 3 Sums of Squares

Appendix B8. Ribosomal RNA gene expression statistical outputs.

5S rRNA

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.1372	1	0.1372	0.313	0.587
Residual	4.8227	11	0.4384		
Condition	0.0946	1	0.0946	0.374	0.553
Residual	2.7809	11	0.2528		
Time ✻ Condition	4.8551	1	4.8551	6.149	0.031
Residual	8.6854	11	0.7896		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	40.8	11	3.71		

Note. Type 3 Sums of Squares

Post Hoc Tests

Post Hoc Comparisons - Time ✻ Condition

Comparison									
Time	Condition		Time	Condition	Mean Difference	SE	df	t	p_{tukey}
Pre RT	CTL	-	Pre RT	EX	-0.5473	0.198	11.0	-2.760	0.076
		-	Post RT	CTL	-0.7430	0.335	11.0	-2.221	0.177
		-	Post RT	EX	-0.0181	0.167	11.0	-0.109	1.000
	EX	-	Post RT	CTL	-0.1957	0.296	11.0	-0.662	0.909
		-	Post RT	EX	0.5291	0.304	11.0	1.738	0.351
Post RT	CTL	-	Post RT	EX	0.7249	0.367	11.0	1.977	0.254

Group Summary

N	Excluded
12	1

45S pre-rRNA

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	1.3722	1	1.3722	0.1840	0.676
Residual	82.0413	11	7.4583		
Condition	1.1559	1	1.1559	0.5122	0.489
Residual	24.8245	11	2.2568		
Time ✻ Condition	0.0682	1	0.0682	0.0554	0.818
Residual	13.5317	11	1.2302		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	454	11	41.3		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	1

5.8S rRNA

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.52161	1	0.52161	2.2398	0.160
Residual	2.79465	12	0.23289		
Condition	0.21567	1	0.21567	1.0720	0.321
Residual	2.41426	12	0.20119		
Time ✻ Condition	0.00318	1	0.00318	0.0299	0.866
Residual	1.27903	12	0.10659		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	19.8	12	1.65		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
13	0

18S rRNA

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.0300	1	0.0300	0.13510	0.720
Residual	2.6629	12	0.2219		
Condition	5.15e-4	1	5.15e-4	0.00356	0.953
Residual	1.7353	12	0.1446		
Time ✻ Condition	0.1961	1	0.1961	0.73126	0.409
Residual	3.2188	12	0.2682		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	11.8	12	0.983		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
13	0

28S rRNA

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.4827	1	0.4827	1.779	0.209
Residual	2.9841	11	0.2713		
Condition	0.0997	1	0.0997	0.630	0.444
Residual	1.7393	11	0.1581		
Time ✻ Condition	0.5464	1	0.5464	1.561	0.237
Residual	3.8506	11	0.3501		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	44.7	11	4.06		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	1

Appendix B9. Ribosome transcribed spacer region gene expression statistical outputs.

5.8S ITS

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.51956	1	0.51956	1.9669	0.188
Residual	2.90562	11	0.26415		
Condition	0.60036	1	0.60036	6.2804	0.029
Residual	1.05152	11	0.09559		
Time ✻ Condition	0.00139	1	0.00139	0.0102	0.921
Residual	1.49517	11	0.13592		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	22.7	11	2.06		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	1

18S ETS

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	2.0859	1	2.0859	9.31	0.010
Residual	2.6891	12	0.2241		
Condition	0.5326	1	0.5326	6.20	0.028
Residual	1.0305	12	0.0859		
Time ✻ Condition	0.0865	1	0.0865	1.55	0.237
Residual	0.6699	12	0.0558		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	8.35	12	0.696		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
13	0

28S ITS

2-way Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	0.7257	1	0.7257	2.414	0.149
Residual	3.3068	11	0.3006		
Condition	0.0589	1	0.0589	0.291	0.600
Residual	2.2284	11	0.2026		
Time ✻ Condition	0.4685	1	0.4685	1.441	0.255
Residual	3.5763	11	0.3251		

Note. Type 3 Sums of Squares

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Residual	14.5	11	1.31		

Note. Type 3 Sums of Squares

Group Summary

N	Excluded
12	1

Appendix B10. Muscle size and ribosome-related gene expression, statistical output between HIGH and LOW LLSTM.

Muscle Size and Ribosome-related Gene Expression

Independent Samples T-Test

		Statistic	df	p
LLSTM	Student's t	24.5013 [a]	32.0	<.001
c-Myc	Student's t	-1.6442 [a]	32.0	0.110
Cyclin D1	Student's t	1.4237 [a]	24.0	0.167
UBF	Student's t	1.1188 [a]	24.0	0.274
TIF-1A	Student's t	0.1098	28.0	0.913
POLR-1B	Student's t	-0.1580	24.0	0.876
5S rRNA	Student's t	0.0719	32.0	0.943
45S pre-rRNA	Student's t	-1.4411	28.0	0.161
5.8S rRNA	Student's t	-0.9362	32.0	0.356
18S rRNA	Student's t	-1.9546	32.0	0.059
28S rRNA	Student's t	0.5304 [a]	28.0	0.600
5.8S ITS	Student's t	-2.1068	28.0	0.044
18S ETS	Student's t	-0.7143	32.0	0.480
28S ITS	Student's t	-2.8124	28.0	0.009

[a] Levene's test is significant ($p < .05$), suggesting a violation of the assumption of equal variances

Group Descriptives

	Group	N	Mean	Median	SD	SE
LLSTM	HIGH	17	12484.412	12339.000	873.589	211.8765
	LOW	17	6490.765	6492.000	504.138	122.2714
c-Myc	HIGH	17	0.980	0.874	0.379	0.0920
	LOW	17	1.274	0.966	0.631	0.1531
Cyclin D1	HIGH	13	2.302	2.808	1.479	0.4101
	LOW	13	1.646	1.627	0.753	0.2089
UBF	HIGH	13	1.310	1.357	0.680	0.1887
	LOW	13	1.076	1.011	0.320	0.0886
TIF-1A	HIGH	13	1.413	1.248	1.001	0.2777
	LOW	17	1.374	1.336	0.914	0.2216
POLR-1B	HIGH	13	1.148	1.003	0.668	0.1853
	LOW	13	1.185	1.091	0.538	0.1492
5S rRNA	HIGH	17	1.905	1.451	1.320	0.3201
	LOW	17	1.875	1.741	1.123	0.2723
45S pre-rRNA	HIGH	13	2.519	0.217	4.075	1.1302
	LOW	17	4.552	4.042	3.634	0.8814
5.8S rRNA	HIGH	17	1.122	1.072	0.814	0.1975
	LOW	17	1.365	1.371	0.695	0.1685
18S rRNA	HIGH	17	1.194	1.141	0.588	0.1426
	LOW	17	1.580	1.476	0.563	0.1366
28S rRNA	HIGH	17	1.671	0.999	1.475	0.3578
	LOW	13	1.437	1.352	0.683	0.1894
5.8S ITS	HIGH	13	0.929	0.539	0.916	0.2539
	LOW	17	1.529	1.625	0.648	0.1571
18S ETS	HIGH	17	0.827	0.489	0.681	0.1652
	LOW	17	0.973	0.928	0.497	0.1206
28S ITS	HIGH	13	0.903	0.839	0.600	0.1664
	LOW	17	1.588	1.516	0.704	0.1708

Appendix B11. Ribosome-related gene expression at Pre RT and muscle hypertrophy, statistical output between HIGH and LOW ΔLLSTM.

Ribosome-related Gene Expression Pre RT and Muscle Hypertrophy

Independent Samples T-Test

		Statistic	df	p
ΔLLSTM	Student's t	13.24	18.0	<.001
c-Myc	Student's t	-1.19	16.0	0.251
Cyclin D1	Student's t	-2.25	16.0	0.039
UBF	Student's t	-2.61	16.0	0.019
TIF-1A	Student's t	-1.69	16.0	0.111
POLR-1B	Student's t	-3.87	16.0	0.001
5S rRNA	Student's t	-2.13 [a]	16.0	0.049
45S pre-rRNA	Student's t	-3.84 [a]	17.0	0.001
5.8S rRNA	Student's t	-4.16	18.0	<.001
18S rRNA	Student's t	-1.78	18.0	0.092
28S rRNA	Student's t	-2.53	16.0	0.022
5.8S ITS	Student's t	-5.63	17.0	<.001
18S ETS	Student's t	-2.63	18.0	0.017
28S ITS	Student's t	-3.57	17.0	0.002

[a] Levene's test is significant (p < .05), suggesting a violation of the assumption of equal variances

Group Descriptives

	Group	N	Mean	Median	SD	SE
ΔLLSTM	HIGH	10	1314.200	1342.000	196.078	62.005
	LOW	10	128.40	160.500	204.369	64.627
c-Myc	HIGH	8	0.917	0.846	0.346	0.122
	LOW	10	1.17	0.990	0.516	0.163
Cyclin D1	HIGH	10	0.884	0.524	0.811	0.257
	LOW	8	2.01	1.606	1.295	0.458
UBF	HIGH	10	0.686	0.553	0.413	0.131
	LOW	8	1.26	1.282	0.525	0.186
TIF-1A	HIGH	10	0.913	0.677	0.661	0.209
	LOW	8	1.57	1.395	0.998	0.353
POLR-1B	HIGH	10	0.645	0.577	0.354	0.112
	LOW	8	1.34	1.379	0.406	0.144
5S rRNA	HIGH	8	0.912	0.802	0.521	0.184
	LOW	10	1.91	1.859	1.241	0.392
45S pre-rRNA	HIGH	10	0.825	0.394	1.098	0.347
	LOW	9	5.85	7.782	3.983	1.328
5.8S rRNA	HIGH	10	0.570	0.511	0.336	0.106
	LOW	10	1.46	1.417	0.587	0.185
18S rRNA	HIGH	10	0.954	0.938	0.784	0.248
	LOW	10	1.43	1.448	0.329	0.104
28S rRNA	HIGH	10	0.616	0.459	0.451	0.143
	LOW	8	1.44	1.148	0.906	0.320
5.8S ITS	HIGH	10	0.522	0.443	0.381	0.121
	LOW	9	1.79	1.674	0.586	0.195
18S ETS	HIGH	10	0.638	0.680	0.347	0.110
	LOW	10	1.27	1.213	0.680	0.215
28S ITS	HIGH	10	0.648	0.420	0.547	0.173
	LOW	9	1.83	1.596	0.882	0.294

Appendix B12. Ribosomal biogenesis and muscle hypertrophy, statistical output between HIGH and LOW ΔLLSTM.

Ribosomal Biogenesis and Muscle Hypertrophy

Independent Samples T-Test

		Statistic	df	p
ΔLLSTM	Student's t	-13.240	18.0	<.001
c-Myc	Student's t	0.245	16.0	0.809
Cyclin D1	Student's t	3.836	16.0	0.001
UBF	Student's t	1.342	16.0	0.198
TIF-1A	Student's t	1.082	16.0	0.296
POLR-1B	Student's t	2.724	16.0	0.015
5S rRNA	Student's t	1.357 [a]	16.0	0.194
45S pre-rRNA	Student's t	3.136 [a]	17.0	0.006
5.8S rRNA	Student's t	3.034	18.0	0.007
18S rRNA	Student's t	2.835	18.0	0.011
28S rRNA	Student's t	2.655	16.0	0.017
5.8S ITS	Student's t	4.811 [a]	17.0	<.001
18S ETS	Student's t	2.683	18.0	0.015
28S ITS	Student's t	3.108	17.0	0.006

[a] Levene's test is significant (p < .05), suggesting a violation of the assumption of equal variances

Group Descriptives

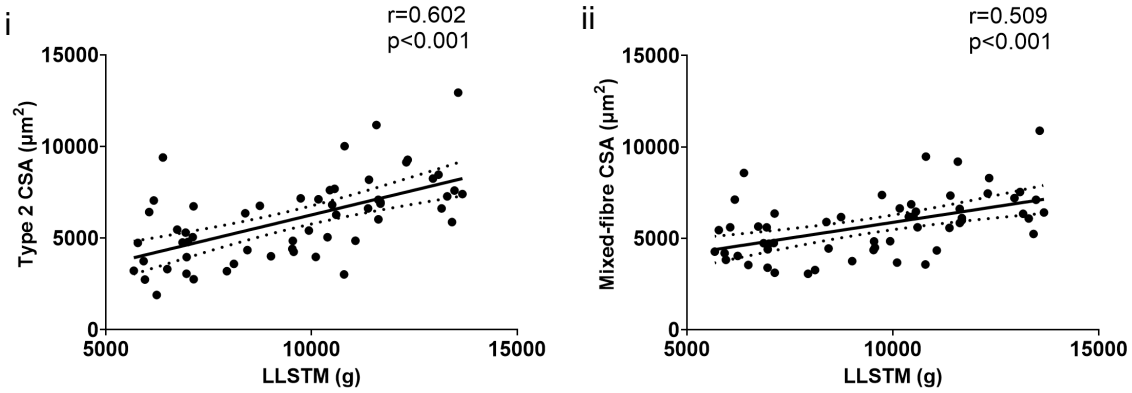
	Group	N	Mean	Median	SD	SE
ΔLLSTM	LOW	10	128.40	160.50	204.369	64.627
	HIGH	10	1314.200	1342.000	196.078	62.0052
c-Myc	LOW	10	1.37	1.20	0.636	0.201
	HIGH	8	1.303	1.074	0.529	0.1870
Cyclin D1	LOW	8	2.23	2.19	0.867	0.307
	HIGH	10	0.878	0.671	0.630	0.1992
UBF	LOW	8	1.63	1.43	0.945	0.334
	HIGH	10	1.098	0.786	0.736	0.2328
TIF-1A	LOW	8	1.45	1.48	1.033	0.365
	HIGH	10	1.050	1.048	0.482	0.1523
POLR-1B	LOW	8	1.62	1.32	0.855	0.302
	HIGH	10	0.749	0.572	0.490	0.1549
5S rRNA	LOW	10	1.74	1.84	1.280	0.405
	HIGH	8	1.081	0.951	0.505	0.1785
45S pre-rRNA	LOW	9	6.66	4.32	5.774	1.925
	HIGH	10	0.852	0.329	1.030	0.3256
5.8S rRNA	LOW	10	1.45	1.53	0.728	0.230
	HIGH	10	0.596	0.410	0.513	0.1623
18S rRNA	LOW	10	1.60	1.61	0.510	0.161
	HIGH	10	0.974	0.917	0.478	0.1511
28S rRNA	LOW	8	1.26	1.26	0.488	0.172
	HIGH	10	0.640	0.411	0.497	0.1571
5.8S ITS	LOW	9	1.99	1.63	0.985	0.328
	HIGH	10	0.400	0.324	0.329	0.1041
18S ETS	LOW	10	1.10	1.01	0.566	0.179
	HIGH	10	0.550	0.575	0.314	0.0994
28S ITS	LOW	9	1.58	1.30	0.808	0.269
	HIGH	10	0.680	0.622	0.421	0.1333

Appendix C. RNA concentration and gene expression. Corresponds with **Figures 6, 7 and 8**.

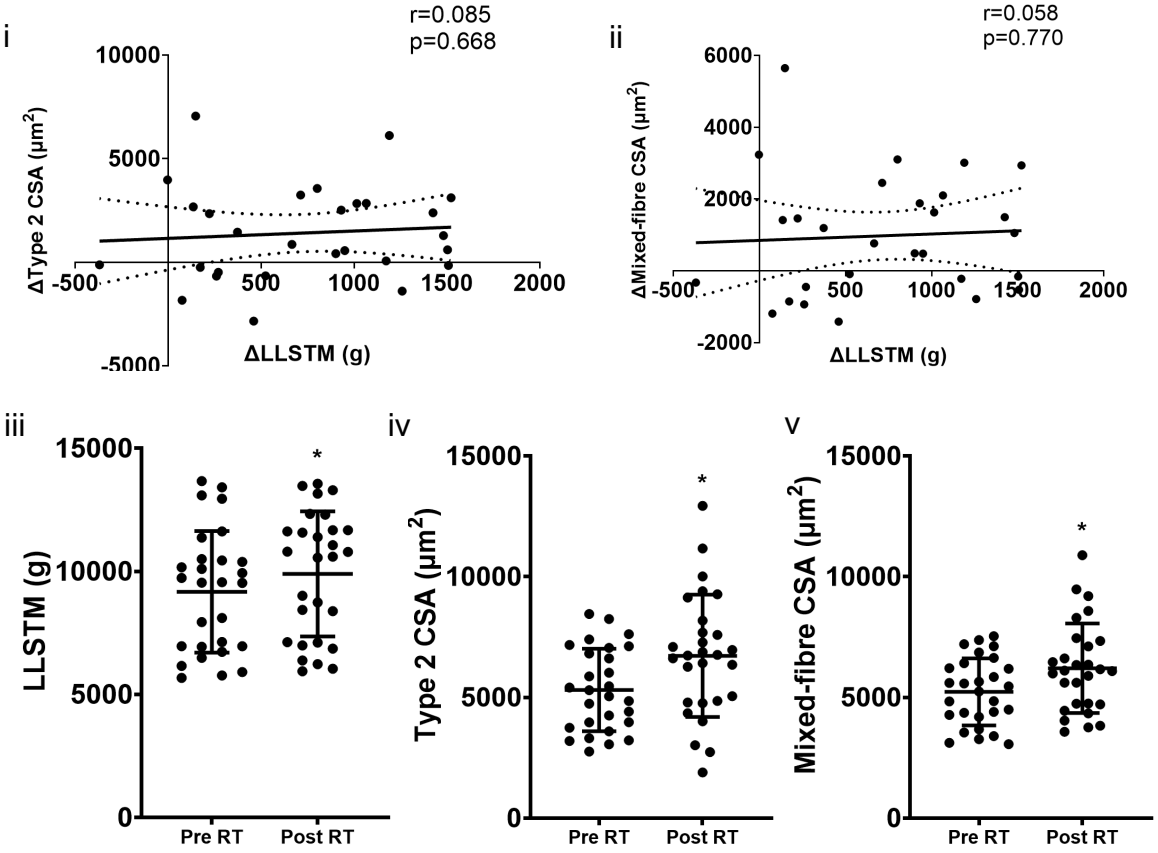
Category	Marker	n	Pre RT		Post RT				
			CTL	EX	CTL	EX	p time	p condition	p time x condition
Mitochondria	COXIV	12	1.00 ± 0.34	1.05 ± 0.48	0.91 ± 0.42	1.04 ± 0.42	0.655	0.368	0.605
	TFAM	12	1.00 ± 0.39	0.92 ± 0.34	0.80 ± 0.34	0.99 ± 0.35	0.448	0.553	0.062
	12S mt-rRNA	11	1.00 ± 0.45	1.09 ± 0.52	1.10 ± 0.62	0.92 ± 0.35	0.794	0.745	0.240
Ribosomal biogenesis regulators	c-Myc	12	1.00 ± 0.47	0.78 ± 0.31	1.20 ± 0.55	0.95 ± 0.41	0.124	**0.034** †	0.869
	Cyclin D1	11	1.00 ± 0.96	0.97 ± 0.72	1.19 ± 0.80	1.06 ± 0.78	0.395	0.602	0.783
	UBF	11	1.00 ± 0.50	0.66 ± 0.34	0.87 ± 0.39	0.99 ± 0.61	0.249	0.204	0.177
	TIF-1A	12	1.00 ± 0.66	0.83 ± 0.57	0.77 ± 0.50	0.99 ± 0.73	0.706	0.692	0.167
	POLR-1B	11	1.00 ± 0.46	0.73 ± 0.42	0.82 ± 0.63	0.83 ± 0.53	0.743	0.237	0.294
Ribosome content	[RNA]	12	8.76 ± 3.45	9.87 ± 3.02	10.47 ± 4.12	10.55 ± 4.95	0.279	0.573	0.575
	5S rRNA	12	1.00 ± 0.75	1.42 ± 1.07	1.56 ± 0.97	1.01 ± 0.59	0.587	0.553	**0.031** ‡
	45S pre-rRNA	12	1.00 ± 1.18	0.92 ± 1.25	0.91 ± 1.30	0.78 ± 1.15	0.676	0.489	0.818
	5.8S ITS	12	1.00 ± 0.58	0.80 ± 0.67	0.82 ± 0.82	0.64 ± 0.59	0.188	**0.029** †	0.921
	18S ETS	13	1.00 ± 0.52	0.75 ± 0.56	0.58 ± 0.39	0.47 ± 0.31	**0.010** *	**0.028** †	0.237
	28S ITS	12	1.00 ± 0.73	0.80 ± 0.53	0.67 ± 0.29	0.76 ± 0.56	0.149	0.600	0.255
	5.8S rRNA	13	1.00 ± 0.58	0.88 ± 0.69	0.81 ± 0.73	0.72 ± 0.52	0.160	0.321	0.866
	18S rRNA	13	1.00 ± 0.42	1.11 ± 0.76	1.06 ± 0.50	0.96 ± 0.46	0.720	0.953	0.409
	28S rRNA	12	1.00 ± 0.94	0.79 ± 0.65	0.71 ± 0.58	0.80 ± 0.81	0.209	0.444	0.237

Values for RNA concentration are ng/mg and for gene expression are fold change. Values for CTL and EX are expressed as means ± SD. ***Significant effect of time, †significant effect of condition, ‡significant time x condition interaction (p<0.05)**.

Appendix D. Pearson's correlations between LLSTM and *i*) Type 2 CSA and *ii*) Mixed-fibre CSA (n=56).



Appendix E. ΔLLSTM and ΔCSA from Pre RT to Post RT (n=28). Pearson's correlations between ΔLLSTM and *i*) ΔType 2 CSA and *ii*) Mixed-fibre CSA. Muscle size for *iii*) LLSTM (p<0.001), *iv*) Type 2 CSA (p=0.003) and *v*) Mixed-fibre CSA (p=0.005) at Pre RT and Post RT. Values are individual data points overlayed on means (middle, horizontal line) ± SD (vertical line). *Significant difference from Pre RT (p<0.05).



Appendix F. Significant correlations between ribosome-related and mitochondrial-related gene expression ($2^{-\Delta\Delta Ct}$). Correlations between *i*) Cyclin D1 and 12S mt-rRNA (n=36), *ii*) UBF and COXIV (n=40), *iii*) UBF and TFAM (n=40), *iv*) TIF-1A and COXIV (n=44), *v*) TIF-1A and TFAM (n=44), *vi*) POLR-1B and COXIV (n=40), *vii*) POLR-1B and TFAM (n=40), *viii*) 45S pre-rRNA and COXIV (n=44), *ix*) 18S ETS and COXIV (n=48), *x*) 5.8S rRNA and COXIV (n=48), *xi*) 5.8S rRNA and TFAM (n=48), *xii*) 18S rRNA and COXIV (n=48), *xiii*) 18S rRNA and TFAM (n=48) and *xiv*) 18S rRNA and 12S mt-rRNA (n=44) expression.