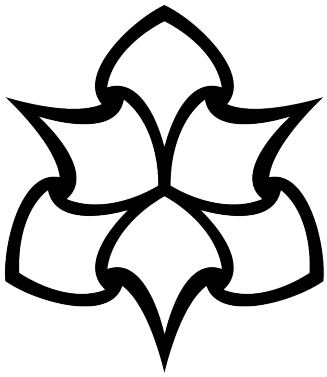

# Aerobic conditioning alters the satellite cell and ribosome response to acute eccentric contractions in young men and women.

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ABSTRACT

Satellite cells (SC) and ribosomes are key determinants of the skeletal muscle adaptive response. Both are thought to increase acutely after resistance exercise and chronically with resistance training. However, the acute SC and ribosome exercise response with prior aerobic conditioning is unknown. Fourteen young men and women underwent 6 weeks of single-legged aerobic conditioning followed by an acute bout of 300 eccentric contractions on each leg. Muscle biopsies were taken from the *vastus lateralis* of the aerobically conditioned (AC) and the control (CTL) legs before (Pre), 24 (24h) and 48 (48h) hours post-contractions. Pre-eccentric contractions, 45S pre-rRNA and 5.8S ITS expression were lower in the AC leg compared to the CTL leg. SC content ($PAX7^+$ cells/100 fibres) in type I and mixed fibres showed a main effect of condition, where values were greater in the AC leg compared to the CTL. A main effect of condition for *Pax7* and *MyoD1* mRNA expression was observed where expression was greater in the AC leg compared to the CTL. AC had greater RNA concentration and mRNA expression of *Ubf* and *Tif-1a* compared to CTL. Only the AC leg increased (Pre-24h) *45S* pre-rRNA, *5.8S ITS* and *28S ITS* following eccentric contractions. We discovered that aerobic conditioning increased type-I SC abundance, and the acute increase in ribosome content following eccentric contractions.

# 1 INTRODUCTION

In humans, eccentric contractions lead to skeletal muscle damage resulting in the subsequent activation of cellular processes to support repair (1, 2). Muscle-specific stem cells, commonly referred to as satellite cells (SC) are particularly important for skeletal muscle repair (3–5). Following various stimuli, such as exercise or damage-inducing eccentric contractions, SC are activated, proliferate and either fuse to existing myofibres to support repair and remodelling or return to quiescence to replenish the SC pool (4–7).

We have previously reported that young individuals with greater skeletal muscle capillarization showed an augmented SC expansion/activation following a single bout of eccentric contractions, resulting in an accelerated recovery of muscle function (8). Therefore, aerobic conditioning (a well-known stimulus to induce skeletal muscle capillarization) preceding an acute damaging stimulus may heighten the SC response and support muscle adaptation and repair (9, 10).

Ribosomes play a key role in protein translation (11–13) and recent work in rodents has demonstrated that SC may supply certain ribosomal proteins to muscle fibres to support adaptation (14). Following an acute bout of resistance exercise, ribosome content increases to support the synthesis of proteins involved in cellular remodeling (15–18) and muscle contractions (19–21). Increases in ribosome content likely precede muscle protein synthesis, which is stimulated following aerobic (22, 23) and resistance exercise (24, 25). Additionally, damage-inducing eccentric contractions increases the expression of genes associated with the regulation of muscle protein synthesis (2). Although ribosomes are essential for regulating protein translation, changes in ribosome content following an acute bout of eccentric contractions have been measured in rodents (26, 27) but not in humans.

The purpose of this study was to determine the impact of aerobic conditioning on the acute SC and ribosome response to eccentric contractions. We hypothesized that ribosome content would increase following acute eccentric contractions and that aerobic conditioning would augment both the SC and ribosome response to eccentric damage.

# 2 MATERIALS AND METHODS

## 2.1 Ethics Approval

Participants were informed about the nature and risks of the study and gave written consent prior to enrollment. 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.

## 2.2 Participants

Baseline participant characteristics have previously been described by (28) and are summarized in **Table 1**.

## 2.3 Study design

Participants underwent 6 weeks of single-legged aerobic conditioning on a randomized leg (Aerobically Conditioned, "AC") where the other acted as a non-conditioned control (CTL) (28). Participants underwent resting (Pre) skeletal muscle biopsies from the *vastus lateralis* of both the AC and CTL legs according to Tarnopolsky et al., (2011) at least 1 week following the last AC bout. Participants then underwent 300 isokinetic, eccentric contractions of the quadriceps muscles at 180

degrees/second using a Biodex dynamometer (Biodex-System 4, Biodex Medical Systems, Shirley, NY, USA) with each leg, a protocol used frequently in our laboratory to elicit skeletal muscle damage (8, 30–32). Participants returned to the laboratory 24 and 48 hours following eccentric contractions and underwent biopsies from both the CTL and AC legs. Samples were either mounted in OCT and frozen in pre-cooled isopentane or frozen in liquid nitrogen and stored at –80°C.

### 2.4 Immunohistochemical analyses

Immunofluorescent staining for fibre-specific SC content ($PAX7^+$ cells) and activation status (quiescent $PAX7^+/MYOD^-$, activated $PAX7^+/MYOD^+$, differentiating $PAX7^-/MYOD^+$) are described previously (8, 33–36) and expressed per 100 fibres. All staining procedures were verified for specificity using negative controls for primary (primary only) and secondary (secondary only) antibodies. For quantification, PAX7 (anti-PAX7 Mouse, DHSB, neat; Alexa Fluor 594 goat anti-mouse, 1:500) and/or MYOD (anti-MYOD 5.8A Mouse, DAKO, 1:100; goat anti-mouse biotin, 1:200, and streptavidin 488, 1:200) was overlayed with DAPI (Sigma-Aldrich, 1:20000) and examined with laminin (anti-Laminin Rabbit, Abcam ab11575, 1:500; Alexa Fluor 647 goat anti-rabbit 1:500) or wheat germ agglutinin (Wheat Germ Agglutinin, Invitrogen W32466, 1:200) to determine the appropriate SC location, myosin heavy chain I (anti-MHCI Mouse, DHSB A4.951, neat; Alexa Fluor 488 goat anti-mouse, 1:500) and II (anti-MHCII Rabbit, Abcam ab51263, 1:1000; Alexa Fluor 647 goat anti-rabbit, 1:500) to determine fibre type-specific associations and expressed per 100 fibres. Images were taken on a Nikon Eclipse Ti Microscope (Nikon Instruments, USA) with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA) at a 20X objective. Analyses were performed in a blinded fashion.

### 2.5 RNA isolation and reverse transcription

RNA was isolated from muscle homogenate using the TRIzol® and reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, cat. #4368814) according to the manufacturer's protocol and stored at -20°C until subsequent analysis. Samples from 3 participants were excluded due to low RNA concentration yield (see **Table 1**).

### 2.6 Quantitative real-time PCR (RT-qPCR)

RT-qPCR reactions were run using 10 ng cDNA in a QuantStudio™ 5 – 384-Well Block (Applied Biosystems, Thermo Fisher Scientific) RT qPCR machine. Primer sequences (5'-3' forward, reverse; concentration) for *Gapdh* (CCACCCATGGCAAATTC, TGGGATTTCCATTGATGACAA; 15 µM), *Cyclin D1* (GCTGCGAAGTGGAAACCATC, CCTCCTTCTGCACACATTTGAA; 15 µM), *Ubf* (CCTGGGGAAGCAGTGGTCTC, CCCTCCTCACTGATGTTCAGC; 10 µM), *Tif-1a* (GTTCGGTTTGGTGGAACTGTG, TCTGGTCATCCTTTATGTCTGG; 10 µM), *Polr-1b* (GCTACTGGGAATCTGCGTTCT, CAGCGGAAATGGGAGAGGTA; 10 µM), *5.8S* rRNA (ACTCTTAGCGGTGGATCACTC, GACGCTCAGACAGGCGTAG; 10 µM), *18S* rRNA (TGGCTCAGCGTGTGCCTAC, ACAAAGGGCAGGGACTTAATC; 10 µM), *28S* rRNA (ACCTGGCGCTAAACCATTC, GTGTCGAGGGCTGACTTTC; 10 µM), *5.8S ITS* (TCGCCAAATCGACCTCGTAC, AGCTGCGTTCTTCATCGACG; 10 µM), *18S ETS* (GCCCGTCCTCGCGAGGC, TGCATGGCTTAATCTTTGAGAC; 15 µM) and *28S ITS* (CGGCGCGATTCCGTCCGT, GTTCACTCGCCGTTACTGAG; 10 µM) and assays for *Gapdh* (ThermoFisher, Hs00187842_m), *Pax7* (ThermoFisher, Hs00242962_m1), *MyoD1* (ThermoFisher, Hs00159528_m1), *Myf5* (ThermoFisher, Hs00929416_g1), *c-Myc* (ThermoFisher, Hs00153408_m), *45S* pre-rRNA (Qiagen, ID PPH82089A-200) and *5S* rRNA (ThermoFisher, Hs03682751_gH) were

used. Reactions for individual primers and the *45S* pre-rRNA assay were run with $RT^2$ Sybr Green qPCR Master Mix (Qiagen, #330500) and all other assays using Taqman™ Fast Advanced Master Mix (ThermoFisher, #4444556). The housekeeping gene (*Gapdh*) expression was not impacted by the intervention. Samples were normalized to *Gapdh* ($\Delta C_t$; either respective SYBR or Taqman™ *Gapdh*) and to Pre eccentric contractions in the CTL leg ($\Delta\Delta C_t$).

### 2.7 Statistical analyses

Jamovi 1.6.23 was used to run statistical analyses. Outliers were determined using means ± 2 x standard deviation (SD) and removed from analyses. Trend analyses for missing and removed data were used for participants with 1 or less missing data point. A paired t-test was used to determine the change (Δ) in $VO_2$ peak between CTL and AC following aerobic conditioning. An independent t-test was used to compare the Total and RNA group characteristics. SC content and activation and gene expression data were analyzed using a two-way repeated measure analysis of variance with factors of time (Pre, 24h and 48h) and condition (CTL and AC), where Tukey's Honest Significant Difference Test was used to analyze multiple post-hoc comparisons.

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

## 3 RESULTS

### 3.1 Participant characteristics

Due to tissue availability, only 11 participants (n=6 males, n=5 females) were included in the gene expression analyses compared to 14 (n=8 males, n=6 females) in the immunohistochemical analyses (**Table 1**). Both the "Total (n=14)" and "Gene expression analyses (n=11)" groups had a similar age (21 ± 2 years), BMI (n=14, 25.4 ± 4.7; n=11, 25.8 ± 5.2 $kg/m^2$) and $\Delta VO_2$ peak (n=14, 3.9 ± 3.6; n=11, 3.7 ± 3.0 mL/min/kg) from pre-AC to post-AC ($p>0.05$).

### 3.2 Satellite cell content

A significant time effect was observed for type-I ($p=0.000232$), type-II ($p<0.0001$), and mixed fibre ($p<0.0001$) SC content.

A significant effect of condition was observed for type-I SC content (**Figure 1D**; $Pax7^+$ cells) where the AC leg was greater than the CTL leg ($p=0.0184$) and tended to have greater mixed-fibre SC content (**Figure 1F**; $p=0.0546$).

A significant time x condition interaction was observed for type-II SC content (**Figure 1E**; $p=0.00228$), where the CTL leg significantly increased type-II-specific SC content from Pre to 24h ($p=0.00702$) and 48h ($p=0.00616$) post-eccentric contractions. The AC leg increased from Pre to 48h ($p=0.00319$) but was not different at 24h ($p>0.05$).

No time x condition interactions were observed for type-I or mixed-fibre SC content ($p>0.05$).

### 3.3 Myogenic gene expression

A significant effect of time was observed for *Pax7* ($p=0.0105$), *MyoD1* ($p<0.0001$) and *Myf5* ($p=0.00217$) mRNA expression.

A significant effect of condition was observed for *Pax7* mRNA expression (**Figure 1G**; fold-change), where the AC leg was greater than the CTL (p=0.00419) and tended to have greater *MyoD1* (**Figure 1H**; p=0.0952) but not *Myf5* (**Figure 1I**; p>0.05) mRNA expression. No time x condition interactions were observed (p>0.05).

### 3.4 Satellite cell activation status

A significant effect of time was observed for type-I activated ($PAX7^+/MYOD^+$; p=0.00489), type-II quiescent ($PAX7^+/MYOD^-$; p=0.0220) and type-II activated (p=0.00256) SC content.

A significant effect of condition was observed for type-I quiescent SC content (**Figure 2G**) where the AC leg was greater than the CTL leg (p=0.00427). No differences were observed for activated or differentiating SC content between legs (p>0.05). No time x condition interactions were observed (p>0.05).

### 3.5 [RNA]

A significant effect of condition was observed for [RNA] (**Figure 3A**; ng/mg muscle) where the AC leg was greater than the CTL leg (p=0.00982).

### 3.6 Ribosomal biogenesis regulators

A significant effect of time was observed for *c-Myc* (**Figure 3B**; p=0.0134), *Tif-1a* (**Figure 3E;** p<0.0001) and *Polr-1b* (**Figure 3F;** p<0.0001) mRNA expression.

*C-Myc* mRNA expression (fold change) tended to increase from Pre (1.12 ± 1.07) to 48h post-damage (3.31 ± 1.81) in the AC leg (p=0.0733) (**Figure 3B**).

Significant effects of condition were observed for *Ubf* (**Figure 3D**; p=0.0489) and *Tif-1a* (**Figure 3E**; p=0.00436) mRNA expression where the AC leg was greater than the CTL leg. No effects of condition were observed for *Cyclin D1* (**Figure 3C**) or *Polr-1b* (**Figure 3F**) mRNA expression (p>0.05). No time x condition interactions were observed (p>0.05).

### 3.7 Ribosomal RNAs

A significant effect of time (p=0.00392) and time x condition interaction (p=0.0117) was observed for 45S pre-rRNA expression (**Figure 3H**). The AC leg tended to increase *45S* pre-rRNA expression from Pre to 24h post-eccentric contractions (p=0.0825), where the CTL leg had significantly greater *45S* pre-rRNA expression Pre eccentric contractions compared to the AC leg (p=0.00297) and decreased at 24h (p<0.0001).

No effects of time, condition or time x condition interactions were observed for *5S* rRNA (**Figure 3G**), *5.8S* rRNA (**Figure 3I**), *18S* rRNA (**Figure 3J**) or *28S* rRNA (**Figure 3K**) expression (p>0.05).

### 3.8 Internal and external transcribed spacer regions

Significant effects of time (p=0.00145, p=0.00173) and time x condition interactions (p=000530, p=0.000507) were observed for *5.8S ITS* (**Figure 3L**) and *28S ITS* (**Figure 3N**) expression, respectively. The AC leg significantly increased *5.8S ITS* expression from Pre to 24h (p=0.0347), then decreased from 24h to 48h (p=0.0412). The CTL leg tended to have greater *5.8S ITS* expression Pre eccentric contractions compared to the AC leg (p=0.0571). The AC leg significantly increased

188 *28S ITS* expression from Pre to 24h (p=0.0151), then decreased from 24h to 48h (p=0.0418). No
189 effects of time, condition or time x condition interactions were observed for *18S ETS* expression
190 (**Figure 3M**; p>0.05).

191 **4 DISCUSSION/CONCLUSIONS**

192 We report that the type-I fibre-associated SC content, and that the acute increase in ribosome content
193 were greater following acute eccentric contractions preceded by AC compared to the CTL.
194 Nonetheless, no differences between conditions were observed for SC activation, differentiation, or
195 type-II-associated SC expansion. This study is the first to characterize the acute SC and ribosome
196 response with AC and to determine the impact of eccentric contractions on the change in ribosome
197 content in humans.

198 We have previously demonstrated that individuals with greater skeletal muscle capillarization have a
199 greater SC response to damage-inducing exercise (8) suggesting that muscle capillarization may be a
200 key factor governing SC function. In addition, studies in both humans (33, 34) and mice (37, 38)
201 have demonstrated that aerobic conditioning alters SC dynamics to break quiescence and increase the
202 number of activated SC at rest (humans) and following a damaging stimulus (mice). Work in middle-
203 aged women has also demonstrated that endurance training is able to alter the acute SC response to a
204 bout of resistance exercise (40). The participants in the present study experienced an increase in $VO_2$
205 peak and skeletal muscle capillarization following single-legged AC (28) which was associated with
206 an augmented type-I SC content, further supporting the notion that training status and specifically
207 capillary content can impact SC function.

208 The muscle damaging protocol that we used in the current study has been used on numerous
209 occasions by our group (8, 30, 31, 35) and others (1, 2, 39). We report an effect of condition for a
210 greater type-I-specific $Pax7^+$ and quiescent SC content in the AC leg compared to the CTL. As total
211 type-I $Pax7^+$ cells appear similar between legs before eccentric contractions, this may indicate that
212 type-I SC were primed to respond to stimuli as aerobic conditioning primarily targets type-I fibres
213 (40). This finding is consistent with the AC leg having greater mRNA expression of *Pax7* and
214 tending to have a greater mRNA expression of *MyoD1*, but in contrast with a previous study in which
215 middle-aged women completed 12 weeks of aerobic training and an increase in type-I SC content
216 was reported (41). However, it is important to note that there were differences in both study
217 populations (young men and women compared to middle-aged women) and an increase in type-I
218 fibre CSA following the aerobic stimulus was reported in middle-aged women-which may explain the
219 increase in type-I associated SC content. Another study in sedentary middle-aged individuals that
220 completed 12 weeks of aerobic conditioning also reported an increase in type-I SC content, however
221 this was also accompanied by an increase in type-I fibre CSA (42). While the participants in our
222 study did not increase type-I CSA following aerobic conditioning (28), previous work by our lab has
223 demonstrated an increase in activated SC following aerobic conditioning which may act as an
224 anticipatory response for future stimuli (34).

225 Although type-I and mixed-fibre-specific SC content was greater in the AC leg compared to the CTL,
226 only type-II-specific SC content increased following eccentric contractions. Both the AC and CTL
227 legs increased type-II SC content to a similar extent, however the CTL leg increased total $PAX7^+$
228 cells at 24h, whereas the AC leg showed delayed $PAX7^+$ cell accumulation, peaking at 48h. The
229 number of activated ($PAX7^+/MYOD^+$) SC increased in both type-I and -II fibres following eccentric
230 contractions with no difference between conditions. Therefore, AC augmented type-I-specific SC

content and appeared to delay the acute increase in type-II SC content, but did not appear to influence the type-I or -II activation or differentiation status.

Ribosome ~~a~~l biogenesis increases acutely following resistance exercise to synthesize new ribosome complexes; however, following resistance training, the acute increase in ribosome content following a bout of resistance exercise may be blunted (15–18). Aerobic conditioning resulted in greater expression of several ribosome-related genes. Expression of ribosomal biogenesis regulators upstream binding factor (*Ubf*) and transcription intermediary factor 1A (*Tif-1a*) were greater in the AC leg. *C-Myc*, the master regulator of ribosomal biogenesis (43), has previously been demonstrated to peak at 8 hours and return to baseline 24 hours following an acute bout of exercise (44). In the current study we report an increase in *C-Myc* 48 hours post-eccentric contractions in the AC leg. The differences in this increase could be due to the differences in exercise stimulus, where perhaps a more damaging stimulus could delay the spike in *c-Myc* expression. These observations (alongside the other ribosome biogenesis markers) indicate a greater capacity for the AC leg to increase ribosome content following eccentric contractions. Ribosomal RNAs did not increase following eccentric contractions and were similar between conditions, likely due to the high degree of inter-individual variability previously observed in their expression (44, 45). However, *45S* pre-rRNA, *5.8S ITS* and *28S ITS* increased expression 24h post-eccentric contractions in the AC leg and returned to baseline after 48h, which aligns with previous work (44). The increase and subsequent decrease in *45S* pre-rRNA and *ITS* expression suggests that ribosome content increases following eccentric contractions and that the increase is greater in the AC leg.

The impacts of exercise training on the acute changes in ribosome content are not well understood. The only studies to measure acute changes in ribosome content following resistance training reported either no change or an increase (15) and no change or a decrease (46) in markers of ribosomal biogenesis. Our study is the first to measure the change in ribosome content following an acute bout of exercise (any type) after a period of AC and the first to measure these acute changes beyond the 1h-acute timepoint. It appears that AC augments the acute increase in ribosome content following eccentric contractions and therefore, suggests that AC may "prime" ribosomes to respond to a novel stimulus.

We discovered that AC augments type-I and mixed-fibre SC content and the acute increase in ribosome content following eccentric contractions. The greater SC content and markers of ribosome biogenesis, and acute increases in ribosome content following eccentric contractions in the AC leg indicates a more efficient transcription and translational control in exercise-accustomed muscle to better support repair and adaptation to damaging stimuli. Future work should measure protein synthesis and specific sub-fractions (i.e. myofibrillar, sarcoplasmic, mitochondrial) in response to eccentric damage and markers of translational efficiency, another important determinant in protein synthesis (13, 43).

## 4.1 Conflict of Interest

The authors declare no conflicts of interest.

## 4.2 Disclosures

Dr. Phillips reports grants from US National Dairy Council, Dairy Farmers of Canada, Roquette Freres, National Science and Engineering Research Council, Canadian Institutes for Health Research during the conduct of the study; personal fees from US National Dairy Council, non-financial support from Enhanced Recovery, outside the submitted work; In addition, Dr. Phillips has a patent Canadian

3052324 issued to Exerkine, and a patent US 20200230197 pending to Exerkine but reports no financial gains.

### 4.3 Author Contributions

AB, AT, GP and SJ contributed to the conceptualization and design of the study. ACQT, CM, SMP and DK collected tissue. AB, ACQT, AAH and SJ analysed data. AB, ACQT and SJ interpreted results. AB, ACQT, AH, CM, SMP, DK, GP and SJ revised the manuscript and approved the final, submitted version.

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## 6 TABLE

**Table 1**: Participant characteristics.

| Characteristic | Males (n=8) | Females (n=6) | Overall (n=14) | RNA Analyses (n=11) |
|---|---|---|---|---|
| Age (years) | 21.0 ± 1.7 | 21.0 ± 1.5 | 21.1 ± 1.6 | 21.3 ± 1.6 |
| Body Mass (kg) | 82.2 ± 15.5* | 60.0 ± 9.4 | 74.1 ± 17.6 | 75.2 ± 20.6 |
| BMI ($kg/m^2$) | 27.3 ± 4.8 | 22.9 ± 2.2 | 25.4 ± 4.5 | 25.8 ± 5.2 |
| $VO_2$ Relative (mL/min/kg) | 42.3 ± 6.9 | 34.8 ± 4.6 | 39.1 ± 7.1 | 37.0 ± 5.8 |

Independent t-test, *significant difference between males and females ($p<0.05$). No difference between the "Overall" and "RNA Analyses" groups.

## 7 FIGURE LEGENDS

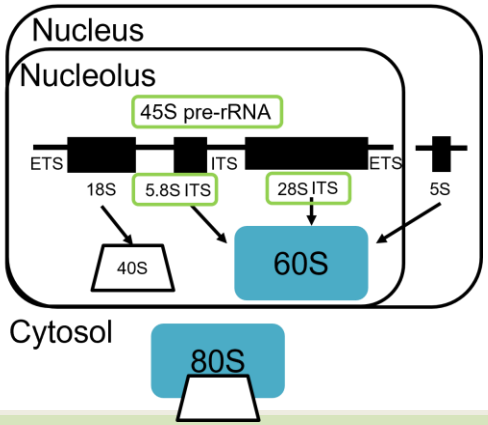
**Figure 1**. SC content and myogenic gene expression. (**A**) Schematic of the study design. Representative images of immunofluorescent stains for (**B**) MHCI, Laminin, MHCII and PAX7

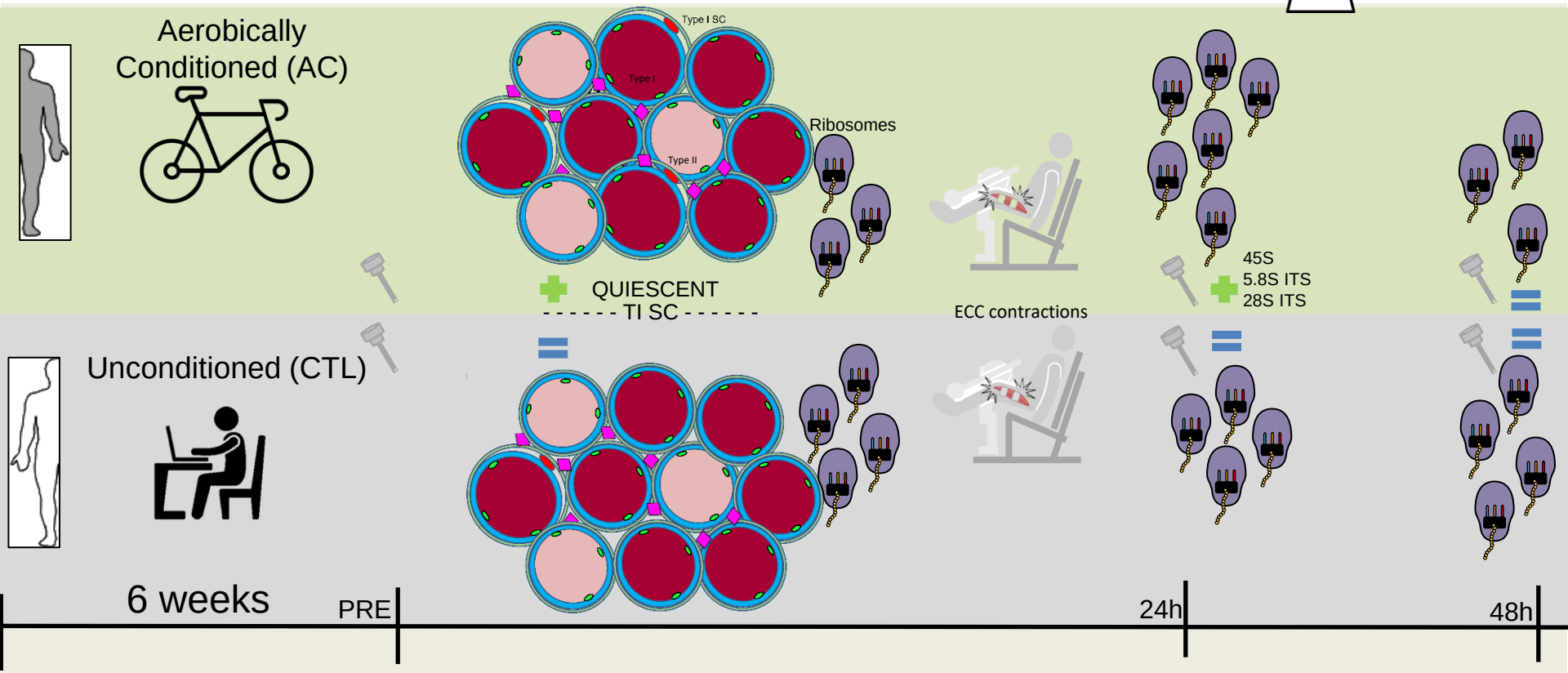
overlayed, (**C**) PAX7 and (**D**) PAX7 and DAPI. The white arrows indicate $PAX7^+/DAPI^+$ cells and the scale bar is 100 μm. SC per 100 fibres for SC located to (**E**) type-I, (**F**) type-II and (**G**) mixed-fibres (n=14). Myogenic genes (**H**) *Pax7* (n=10), (**I**) *MyoD1* (n=10) and (**J**) *Myf5* (n=10) mRNA expression Pre, 24 and 48 hours following eccentric contractions. All values are individual data points for the CTL (•) and AC (■) legs, where each colour represents a different participant and is overlayed on means (middle, horizontal line) ± SD (vertical line). 2-way repeated measures of variance, *significant effect of time, †significant effect of condition (AC>CTL) ‡significant time x condition interaction, Φsignificant difference between means (Tukey's Honest Significant Difference Test, $p<0.05$).

**Figure 2**. SC activation status. Representative images of immunofluorescent stains for (**A**) PAX7, MYOD, MHCII and WGA, and DAPI overlayed, (**B**) MYOD, (**C**) MYOD and DAPI, (**D**) PAX7, MYOD and DAPI, (**E**) PAX7, and (**F**) PAX7 and DAPI. The red arrows indicate $PAX7^+/MYOD^-$ cells, yellow arrows indicate $PAX7^+/MYOD^+$ cells and green arrows indicate $PAX7^-/MYOD^+$ cells, and the scale bar is 100 μm. Type-I-specific (**G**) quiescent ($PAX7^+/MYOD^-$; n=12), (**H**) activated ($PAX7^+/MYOD^+$; n=11) and (**I**) differentiating ($PAX7^-/MYOD^+$; n=12) SC. Type-II-specific (**J**) quiescent (n=11), (**K**) activated (n=12) and (**L**) differentiating (n=11) SC Pre, 24 and 48 hours following eccentric contractions. All values are individual data points for the CTL (•) and AC (■) legs, where each colour represents a different participant and is overlayed on means (middle, horizontal line) ± SD (vertical line). 2-way repeated measures of variance, *significant effect of time, †significant effect of condition (AC>CTL), Φsignificant difference between means (Tukey's Honest Significant Difference Test, $p<0.05$).

**Figure 3**. Markers of ribosomal biogenesis and ribosome content. (**A**) RNA concentration relative to muscle wet weight (n=9). Ribosomal biogenesis markers (**B**) *c-Myc* (n=9), (**C**) *Cyclin D1* (n=11), (**D**) *Ubf* (n=10), (**E**) *Tif-1a* (n=10) and (**F**) *Polr-1b* (n=10) mRNA expression, ribosomal RNA markers (**G**) *5S* rRNA (n=9), (**H**) *45S* pre-rRNA (n=8), (**I**) *5.8S* rRNA (n=10), (**J**) *18S* rRNA (n=11), (**K**) *28S* rRNA (n=9), (**L**) *5.8S ITS* (n=9), (**M**) *18S ETS* (n=9), (**N**) *28S ITS* (n=9) expression Pre, 24 and 48 hours following eccentric contractions. All values are individual data points for CTL (•) and AC (■), where each colour represents a different participant and is overlayed on means (middle, horizontal line) ± SD (vertical line). 2-way repeated measures of variance, *significant effect of time (Pre RT > Post RT), †significant effect of condition (AC>CTL), ‡significant time x condition interaction, Φsignificant difference between means (Tukey's Honest Significant Difference Test, $p<0.05$).

# The acute satellite cell and ribosome response to eccentric contractions following aerobic conditioning




**Aerobic conditioning resulted in increased type-I SC content and a greater acute increase in ribosome content after eccentric contractions.**