Part 2: Does baseline myonuclear density in type-I and type-II myofibres predict the hypertrophic response following a 6-week RT-protocol?

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1 Abstract

Background: The aim of this study was to re-analyze data from two previous studies [1,2]. We aimed to assess whether baseline myonuclear density could predict the change in fCSA for both type-I and type-II myofibres.

Methods: Using a linear-mixed effect model, we assessed whether baseline myonuclear density (myonuclei per fibre CSA) predicted the rate of fCSA change over a 6-week progressive volume RT-protocol in 30 previously trained men. Separate models were fit for type-I and type-II myofibres, with baseline myonuclear density as our predictor and fCSA measured at three timepoints (baseline, week 3 and week 6) as our outcome variable.

Results: Our main finding was a positive association between higher baseline myonuclear density and hypertrophy of fCSA in type-II myofibres (estimate: 53.6 μm^2, SE: $\pm$ 23.7 μm^2/week, [95% CI: 7.1 to 100]). We did not find an interaction effect for baseline myonuclear density in type-I myofibres (-11 μm^2, $\pm$ 29.1 μm^2/week, [-67.9 to 45.9])

Conclusion: Baseline myonuclear density in type-II myofibres may contribute to the individual responses in hypertrophy to RT. This may reflect greater transcriptional capacity, which may lead to improved protein synthesis. The results should be interpreted cautiously, given our wide confidence intervals. Sampling bias, small sample size and a lack of an outcome variable at a whole-muscle level warrants further research.

2 Introduction

Resistance training (RT) is an effective method to induce skeletal muscle hypertrophy [3]. However, individual responses to the same RT-protocol can vary greatly. Research has shown a wide array of responses in to improvements in skeletal muscle hypertrophy, maximal strength and metabolic markers of health [4,5]. Several mechanisms have been proposed, and includes but is not limited to genetics, epigenetics and environmental factors [6].

Within genetics, myonuclei are thought to play a major role in skeletal muscle hypertrophy [7]. Skeletal myofibres are multinucleated cells, each containing hundreds to thousands of myonuclei. Each myonucleus governs a finite amount of cytoplasm and serves an important function for gene transcription and protein synthesis [7]. In the early stages of hypertrophy, myonuclei can expand their domains to accommodate the initial growth, known as myonuclear expansion [8]. Beyond a certain threshold, quiescent satellite cells (SC) must proliferate and differentiate into new myonuclei to facilitate further skeletal muscle hypertrophy. This is known as myonuclear accretion. However, the research is equivocal as to whether this threshold exists [9].

There has been extensive research on myonuclei accretion and expansion for skeletal muscle hypertrophy. A study in 2008 found that differences in hypertrophy between high and low responders to RT can be explained by the degree of myonuclear accretion [8]. However, Haun and colleagues found no group differences in pre- to post-measurements in myonuclear density after a 6-week RT-protocol, despite significant differences in measured hypertrophy [1]. Litterature also challenges the notion that myonuclear accretion is associated with hypertrophy [10].

On myonuclear expansion, Petrella and their group suggested a maximum ceiling for myonuclear domain expansion [8]. This led to the suggestion that a fibre size increase of more than ~26-27% must be accompanied by myonuclear accretion [11]. The research is divering, as another group of researchers found significant myonuclear accretion without expansion, despite type-II growing more than 40% [7].

Whether baseline myonuclear density in type-I and type-II myofibres predicts the hypertrophic response following RT remains unclear. Previous research found no differences in myonuclear accretion between low, moderate and high hypertrophic responders following a 12-week RT protocol in young, untrained men [10]. Another study found a moderate correlation between satellite cell count and myofibre growth [8]. Haun's group found in previously trained males that pre-training type-II fibre size was the strongest predictor of hypertrophy, while changes in type-I myonuclear number showed a positive but non-significant effect on hypertrophy. Baseline myonuclear number was used in the regression model, but it did not emerge as a predictor of hypertrophy based on their composite hypertrophy score.

The present study re-examines this using linear-mixed models to assess whether baseline myonuclear density, i.e the amount of myonuclei per cross-sectional area, is associated with the rate of functional cross-sectional area (fCSA) change over time. A logical assumption is that greater baseline myonuclear density reflects increased transcriptional capacity, allowing for a superior hypertrophic response.

We re-analysed the data from the experimental studies performed by Haun and colleagues [1,2] to determine whether baseline myonuclear density in type-I and type-II fibres is predictive of hypertrophy following a 6-week resistance-training protocol, using an observational study design. We hypothesized a positive association between myonuclear density in fibre types and changes in vastus lateralis fCSA, as greater myonuclear density may reflect increased transcriptional capacity and protein synthesis.

3 Materials and methods

The data in the study were obtained from Haun and colleagues study in 2018 [2]. They recruited 34 young, trained males (aged 21.48 ± 2.13) with previous RT-experience (5 ± 3 years). See table 1 for more information on the participants, retrieved from the studies [1,2]. Briefly, the participants performed a six week RT-protocol with progressive volume increases. The

program was performed 3d/week, with each session consisting of two upper-body and two lower-body exercises. The starting volume for each exercise was 10 sets/exercise in week one, and was progressively increased each week until 32 sets/exercise in week six. Both functional cross-sectional area (fCSA) and myonuclear density of both type-I and type-II fibres was assessed via immunohistochemistry from vastus lateralis biopsies at three different time points: baseline (T1), midway through the intervention (T2) and post-intervention (T3). Myonuclear density was determined for both type-I and type-II fibres. The reader is referred to the original study for full details on the methodology [1].

After accounting for participant drop-out and missing values, 30 participants were included in their final dataset, which were used in our study's analysis.

3.1 Baseline characteristics of participants

Table 1: Participant characteristics at baseline. Data from [1].

Variable	Mean $\pm$ SD
n	31
Age (years)	21.5 ± 2.1
Height (cm)	179.8 ± 7.9
Body mass (kg)	82.9 ± 11.5
Training age (years)	5.4 ± 2.6
Baseline Type II fCSA (μm^2)	4103 ± 836
Baseline Type II myonuclei density (nuclei/fiber CSA)	2.63 ± 1.07
Baseline Type I fCSA (μm^2)	3837 ± 1004
Baseline Type I myonuclei density (nuclei/fiber CSA)	2.49 ± 0.99

3.2 Statistical analysis

All statistical analyses were performed using R (version 4.5.1) in Rstudio (version 2025.09.2) using a frequentist framework. The *lme4* package was used for statistical modeling. For complete information regarding packages, see Del2.qmd and the README files in the GitHub repository.

Statistical approach

The primary estimand of interest was the **time x myonuclear density** interaction effect, determined by fitting the data to a linear-mixed effect model (LMM). We fit separate LMMs for type-II fibres (FAST) and type-I fibres (SLOW). The rationale for choosing an LMM was threefold. Firstly, an LMM allows us to handle dependent data points with more flexibility than models such as ANOVA or ANCOVA. In the dataset, fCSA was measured at three different time points (T1, T2 and T3). This also allows us to model the trajectory of change over time. The second key advantage of the LMM, is the ability to assign a random intercept for each participant. This is important, since baseline fCSA in the vastus lateralis is not equal

across the participants. And thirdly, it allows the model to leverage shrinkage. This pulls each individual participants estimate closer towards the group mean, allowing for more accurate predictions by accounting for outliers. Time was treated as a numeric factor given the expected linear relationship over the six-week intervention.

Several alternative statistical approaches were considered. A change score in pre- to post-measurements was considered, but this option does not consider differing baseline values of fCSA impacting our outcome variable. It also does not allow us to incorporate multiple measurement points. Repeated measures ANOVA could handle multiple measurements but would require categorizing participants into arbitrary groups based on myonuclear density. An ANCOVA model was also an option, seeing as we could control for different baseline values of fCSA with a covariate. However, this model would also require us to discard the T2-timepoint measurement or employing multiple models. The LMM satisfies all three requirements: the three different measurements points of fCSA (T1 – T3), a continuous predictor variable in baseline myonuclear density and the model can account for differences in baseline values of fCSA with random intercepts for each participant.

We chose to fit our statistical analysis into two separate models for each fibre type, seeing as the baseline densities were different and that type-II fibres have a much larger growth potential than type-I fibres [1]. Both models used fCSA measured at three different time points (T1, T2 and T3) as the outcome variable, while our predictor variable was only baseline myonuclear density (T1). The predictor variable was centered around the mean value of baseline myonuclear density for both models, to aid with interpretability of the coefficients of the model.

Our first model, FAST, examined type-II myofibres. The primary estimand of interest was the interaction between time and baseline myonuclear density in type-II myofibres to predict the rate of change in fCSA. Our second model, SLOW, examined type-I myofibres. The primary estimand of interest was the interaction between time and baseline myonuclear density in type-I myofibres to predict the rate of change in fCSA.

We chose fCSA as our hypertrophy outcome because our hypothesis is at the cellular level. We acknowledge that there exist limitations to this approach compared to whole-muscle outcomes such as lean mass measured by DEXA or muscle thickness measured by B-mode ultrasound. See section 5 for further discussion regarding the outcome variable.

Statistical power

Haun reported that with n = 29 participants, 2 predictors, $\alpha = 0.05$ and 1 - $\beta = 0.8$, their minimum detecable effect size was f^2 = 0.35. The design was acknowledged as underpowered to detect small effects [1]. Since this power calculation does not directly apply to our LMM, we focused on effect estimates, standard errors (SE) and confidence intervals (CIs) rather than relying on statistical significance to aid with interpretation. This allows us to interpret the results with varying degrees of confidence, rather than a dichotomous verdict of significance vs. non-significance.

Testing our model assumptions

Our LMMs assume that our residuals are normally distributed around zero and do not differ across the fitted values. Figure 1 shows the residual vs. fitted values for both models. The type-II plot appeared randomly scattered around zero, suggestion homoscedasticity. The type-I plot suggested heteroscedasticity, prompting a sensitivity analysis reported in section 4. Figure 2 indicated that our residuals were approximately normally distributed and that the relationship between predictor and outcome variables are approximately linear.

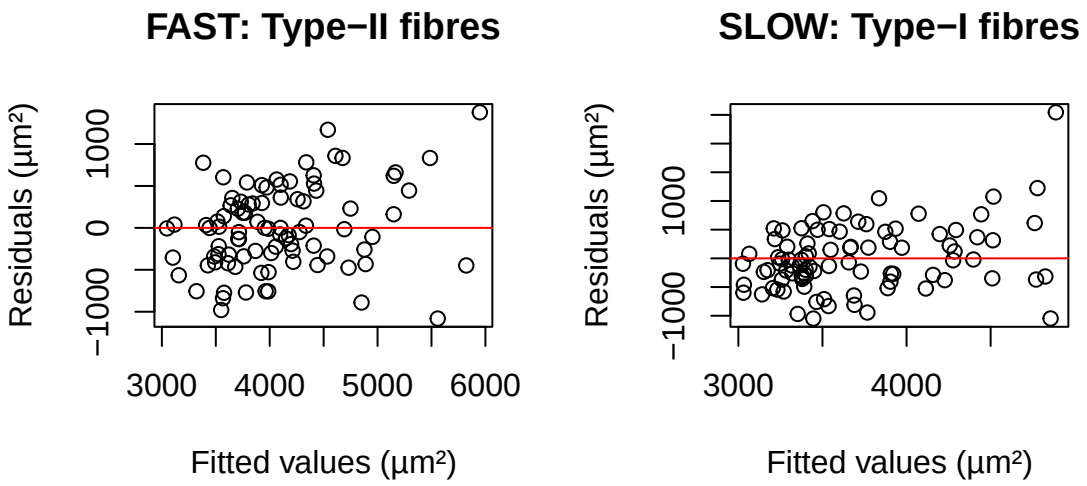

Figure 1: Residuals versus fitted values for FAST (Type-II fibres, left) and SLOW (Type-I fibres, right).

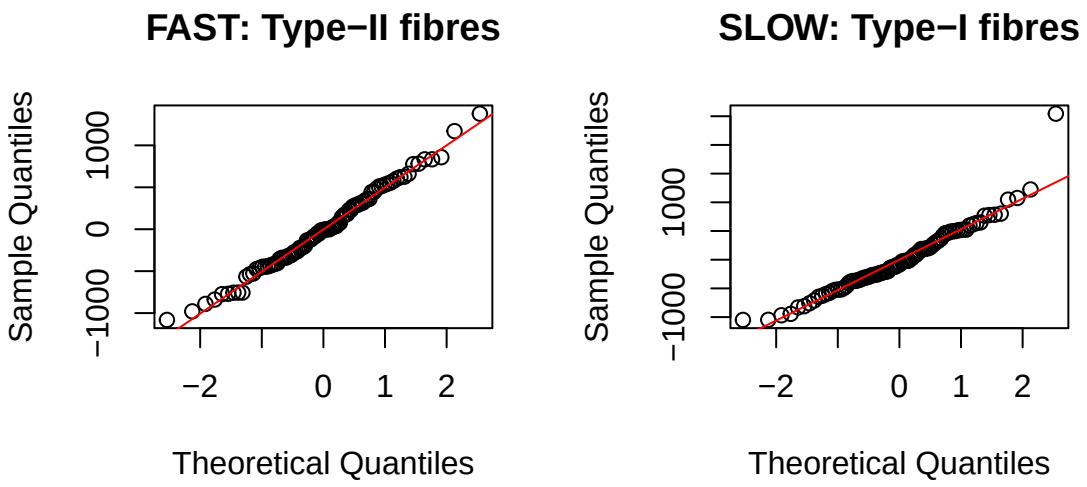

Figure 2: Q-Q plots for FAST (Type-II fibres, left) and SLOW (Type-I fibres, right)

4 Results

Descriptive statistics

Table 2: Fixed effects and random effects of type-I and type-II models

	Type II (fast)	Type I (slow)
Fixed effects		
Intercept	3972.8 ± 140.7 [3700.2 to 4245.4]	3679.9 ± 147.2 [3394.9 to 3965]
Time (weeks)	38.5 ± 25 [-10.5 to 87.4]	6.2 ± 28.2 [-49.1 to 61.5]
Myonuclear density	178.8 ± 133.6 [-80.0 to 437.6]	270.1 ± 151.6 [-23.4 to 563.7]
Time × myonuclear density	53.6 ± 23.7 [7.1 to 100.0]	-11 ± 29.1 [-67.9 to 45.9]
Random effects		
Intercept)	SD = 559.2	SD = 540.1

Fixed effects shown as estimate ± SE [95% CI]. All values in μm^2.

The interaction effect time x baseline myonuclear density is our primary estimand for both models (see Table 2). All values in this section are reported as estimate SE ±, [95% CI] unless stated otherwise. In type-II myofibres, the interaction estimates between time x myonuclear density was 53.6 (± 23.7, [7.1 to 100.0]). For each additional nucleus per type-II myofibre at baseline, there was a weekly increased area of 53.6 μm^2 per type-II myofibre. However, the large SE and wide CI means that there is a great degree of uncertainty tied to this estimate. The CI does not cross zero, which suggests a positive effect of having higher myonuclear density

for hypertrophy outcomes in type-II myofibres. In type-I myofibres, the interaction estimate between time x myonuclear density was -11 ($\pm$ 29.1, [-67.9 to 45.9]). For each increase in nucleus per type-I myofibre at baseline, we observed a weekly decrease of 11 μm^2. The CI crosses zero and the SE is larger than the estimate itself, which indicates that there is no clear association between baseline myonuclear density and hypertrophy in type-I myofibres.

Sensitivity analysis excluding one extreme observation (n = 1) from our residuals yielded a positive estimate for the type-I myofibre interaction of 20.82 ($\pm$25.2, [-28.4 to 70.1] compared to our original estimate (see Table 2). This suggested that the single observation significantly influenced the results.

Figure 3 shows us the predicted trajectories of fCSA in both type-I and type-II myofibres, at different baseline values of myonuclear density (mean $\pm$ SD). We chose to split the trajectories into three categories for easier interpretation by the reader. In type-II myofibres, we observed a steep slope for changes in fCSA with higher baseline values. In type-I myofibres, however, the slopes are relatively parallel for changes in fCSA for all three categories.

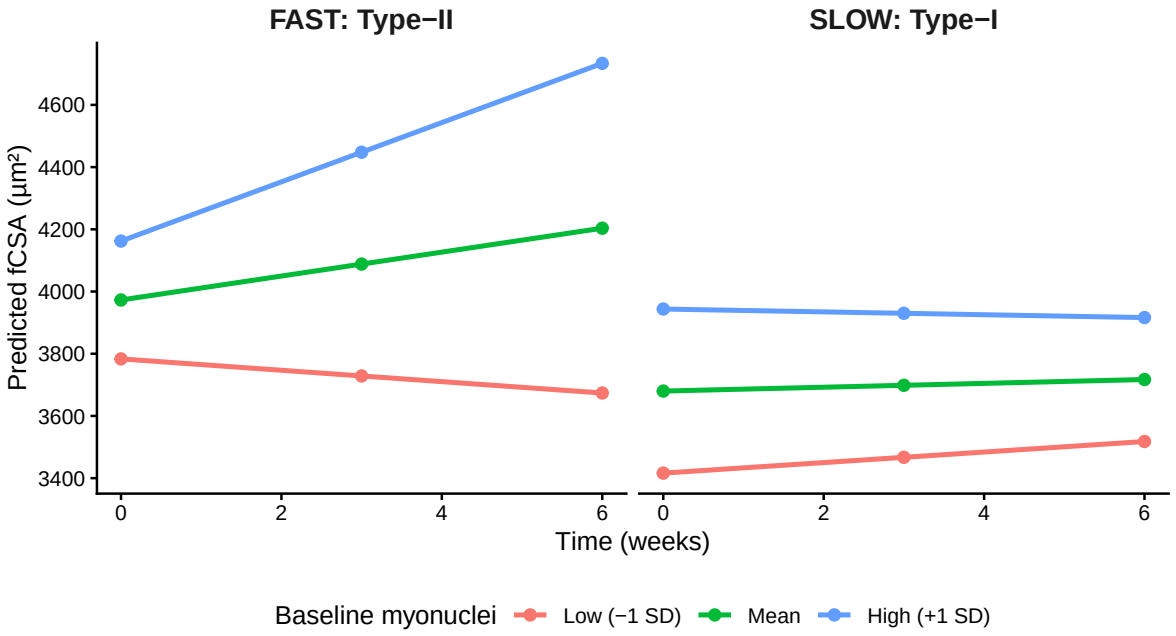

Figure 3: Line plots showing predictions at population level for changes in fCSA at different baseline myonuclear densities.

5 Discussion

The present study aimed to explore if baseline myonuclear density could predict changes in fCSA in both type-I and type-II myofibres. Our main finding was a positive association between higher baseline myonuclear density and hypertrophy of fCSA in type-II myofibres, when baseline values were at or above the mean ($\geq$ 2.63 nuclei per fibre CSA). The results must be interpreted with caution given our wide confidence intervals. For the type-I model, we found no interaction effect between baseline myonuclear density and changes in fCSA. However, our sensitivity analysis excluding one extreme outlier revealed a positive but uncertain effect for our type-I model, indicating that the small sample size may limit our ability to detect a true effect.

5.1 Interpretation

Our findings deviate from Haun and colleagues, who did not find that baseline myonuclear density predicted the hypertrophic response [1]. However, we had distinct methodological approaches. Haun used a composite hypertrophy outcome score using four different measurement methods, while we only used fCSA. Similarly, we also deployed different statistical approaches (two-way ANOVA and stepwise regression vs. LMM). Our approach may have been more sensitive to predict changes between predictor-outcome at the cellular level.

Our results suggest that greater baseline myonuclear density reflects greater transcriptional capacity in type-II myofibres, which may contribute to superior hypertrophy outcomes. This is in agreement with a review from 2018 that highlighted the fact that type-II myofibres have a greater myonuclear domain flexibility and transcriptional reserve compared to type-I [9]. The authors specifically highlighted that there has not been identified an upper ceiling to a specific myonuclear domain size, implying that myonuclei can support substantial hypertrophy without myonuclear accretion. Conversely, it is possible that type-I myofibres are more dependent on myonuclear accretion for muscle growth, making baseline values a weaker predictor for hypertrophy. Nonetheless, this remains highly speculative, and future research should specifically assess the relationship between myonuclear density and type-I myofibre hypertrophy.

Petrella's group found that total myonuclear per fibre did not predict the hypertrophic response following a 16-week intervention [8], which is in contrast to our findings. An important distinction is that they measured total myonuclei per fibre, while our predictor variable was nuclei per fibre CSA. Our divergent findings could be explained by the fact that myofibres with greater myonuclear density have greater transcriptional capacity relative to their size and cellular demands, promoting greater hypertrophy. Another potential confounder is also the fact that Petrella's group did not distinguish between type-I and type-II myofibres.

5.2 Limitations

We chose a cellular outcome variable of fCSA to examine the predictor-outcome relationship at the cellular level. However, our divergent results with Haun's study suggest that our findings do not generalize to whole-muscle measurements such as muscle thickness or thigh circumference. Additionally, fCSA was based on a small number of fibres were sampled per biopsy, and individuals vary considerable when it comes to total fibre number within the vastus lateralis [12]. This inter-individual variation means that changes at fibre-level do not correspond to changes at whole muscle-level, introducing a potential confounding variable.

The present study also has limited ecological validity, based on the highly homogenous sample size and RT-protocol. All the recruited participants were previously trained young males, introducing sampling bias. Past research has shown us that young males exhibit different biomarkers responses and greater hypertrophy in response RT-training compared to older adults and females [11], limiting extrapolation to other populations.

Lastly, our study is limited in establishing causality based on our observational study design. An observational study can be used to generate hypotheses and identify patterns, but unmeasured biomarkers that independently influenced the hypertrophic response could have been potential confounding variables.

5.3 Conclusion

Baseline myonuclear density in type-II fibres was positively associated with hypertrophy in fCSA following high-volume RT. The null findings for type-I fibres could be explained by a greater dependence on myonuclear accretion to support hypertrophy, but also by the limited sample size and heterogeneity of participants responses. Future research should aim to assess whether this relationship holds with different populations, different RT-protocols and larger sample sizes.

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