

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

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1 Abstract

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2 Introduction

Resistance training (RT) is an effective method to induce skeletal muscle hypertrophy [1]. However, the individual responses to the same RT-protocol can vary greatly. Research has shown a wide array of responses when it comes to improvements in skeletal muscle hypertrophy, maximal strength and metabolic markers of health [2,3]. There have been several proposed mechanisms as to why this inter-individual response exists, and includes but is not limited to genetics, epigenetics and environmental factors (sleep, stress and dietary patterns) [4].

Within genetics, the myonuclei are thought to play a major role in the process of skeletal muscle hypertrophy [5]. Skeletal myofibres are multinucleated cells, each of them containing hundreds to thousands of myonuclei. Each one of these myonuclei govern a finite amount of cytoplasm and serve an important function for enhancing both gene transcription and protein synthesis [5]. In the early stages of hypertrophy, myonuclei can expand their domains to accommodate the initial rate of growth, also known as myonuclear expansion [6]. Beyond a certain threshold, it has been hypothesized that quiescent satellite cells (SC) must proliferate and differentiate into new myonuclei to facilitate further skeletal muscle hypertrophy. This concept is also known as myonuclear accretion. However, this concept remains controversial, and the research is equivocal as to whether this threshold exists [7].

There has been extensive research on the roles of myonuclei accretion and expansion for skeletal muscle hypertrophy. Petrella and colleagues in 2008 found that the differences in hypertrophy between high and low responders to RT can be explained by the degree of myonuclear accretion [6]. However, Haun and colleagues found no group differences in pre- to post-measurements in myonuclear density following a 6-week RT-protocol, despite significant differences in measured hypertrophy [8]. There is also other literature challenging the notion that myonuclear accretion is associated with skeletal muscle hypertrophy [9].

On the topic of myonuclear expansion, Petrella and their group has suggested that there exists a maximum ceiling for how much the domains of individual myonuclei can expand to [6]. This consequently led to the suggestion that a fibre size increase of more than ~26-27% must be accompanied by myonuclear accretion [10]. The research here is also diverging, as another group of researchers found significant myonuclear accretion without myonuclear expansion,

despite the type-II fibres of the participants growing with more than 40% from pre- to post-measurements [5].

The question of whether baseline myonuclear density in type-I and type-II myofibres predict the magnitude of the hypertrophic response following RT remains unclear. Previous research has found association between baseline myonuclear *number* in type-I and type-II and the hypertrophic response following a 12-week RT protocol in young, untrained men [9]. Another study found a moderate correlation between satellite cell count and myofibre growth [6]. This brings us to the study performed by Haun's research group from 2019 on young, trained males, with their data suggesting that pre- and W3 values of type-I myonuclear number was a significant predictor of hypertrophy. While the researchers performed baseline testing of the participants myonuclear density of type-I and type-II fibres, it was not used in the final analysis. Since there is divergent research on whether myonuclear accretion occurs in the presence of significant hypertrophy, an unexplored baseline predictor to examine is *myonuclear density*, i.e the amount of myonuclei per cross-sectional area. A logical assumption would be that a increased baseline myonuclear density would reflect a greater transcriptional machinery, thus allowing for a superior hypertrophic response to RT.

The aim of this study was to analyse the data from the experimental studies performed by Haun and colleagues [8,11] and perform an observational study to determine whether baseline myonuclear density in type-I and type-II fibres is predictive of the hypertrophic response following a 6-week resistance-training protocol in young, trained men. We hypothesize a positive association between myonuclear density in both type-I and type-II fibres and hypertrophy in vastus lateralis functional cross-sectional area (fCSA), as greater myonuclear density is reflective of increased transcriptional capacity and protein synthesis.

3 Materials and methods

The data in the study were obtained from Haun and colleagues study in 2018 [11]. 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 [8,11]. 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). Samples were obtained, cut and then stained with dystrophin antibody solution to identify fiber boundaries. 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 used [8].

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 Table of baseline characteristics of participants

Table 1: Participant characteristics at baseline. Data from [8].

Variable	Mean \pm SD
n	31
Age (years)	21.5 \pm 2.1
Height (cm)	179.8 \pm 7.9
Body mass (kg)	82.9 \pm 11.5
Training age (years)	5.4 \pm 2.6
Baseline Type II fCSA (μm^2)	4103 \pm 836
Baseline Type II myonuclei density (nuclei/fiber)	2.63 \pm 1.07
Baseline Type I fCSA (μm^2)	3837 \pm 1004
Baseline Type I myonuclei density (nuclei/fiber)	2.49 \pm 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 R-packages used for statistical analysis was the *lme4* package. For complete information regarding the R-packages used throughout the study, see Del2.qmd file found within the GitHub repository.

Statistical approach

The primary estimand of interest was the **time x myonuclear density** interaction effect, which was determined by fitting the data to a linear-mixed effect model (LMM). We fit two different LMMs: one for type-II myofibres (FAST), and one for type-I myofibres (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 particularly useful, since it is evident from the dataset that baseline fCSA in the vastus lateralis is not equal across the participants. And thirdly, it allows the model to leverage shrinkage. This has the benefits of pulling each individual participants estimate closer towards the group mean, allowing for more accurate predictions by accounting for outliers in the dataset. Time was treated as a numeric factor given that we expect a linear relation between observed hypertrophy and the short intervention period of six-weeks.

Several statistical approaches were considered but ultimately discarded. A simple change score in pre- to post-measurements of fCSA was an alternative, 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 into the model. A repeated measures ANOVA was also considered, as it could allow us to model change score from pre- to post with multiple measurement points. However, since our predictor variable is continuous and not categorical, it would require us to cluster the participants into arbitrary groups based on their baseline myonuclear density values. 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 was consequently chosen, seeing as it satisfies all our requirements: our 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.

Variables for analysis

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 [8]. 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.

The rationale for fCSA as our hypertrophy outcome is because our hypothesis is at the cellular level. Therefore, a logical extension would be to assess changes in hypertrophy at the cellular level. However, we do acknowledge that there exist several limitations to this approach compared to whole-muscle outcomes such as lean mass measured by DEXA or muscle thickness measured by B-mode ultrasound. The reader is referred to section 5 for further discussion regarding interpretation of the outcome variable.

Statistical power

Haun reported that with a sample of $n = 29$ participants, 2 predictors, $\alpha = 0.05$ and $1 - \beta = 0.8$, their minimum detectable effect size was $f^2 = 0.35$. The researchers acknowledged that the study design was underpowered to detect small but significant effects [8]. However, since Haun employed a stepwise linear regression, this power calculation does not directly apply to our LMM.

Since we employed an LMM, we focused on effect estimates, standard errors (SE) and confidence intervals (CIs) rather than relying on statistical significance to aid with interpretation.

Testing our model assumptions

Our LMM assumes that our residuals are normally distributed around zero, and that they do not differ across the fitted values. Figure 1 visualises the residual vs. fitted values for both of our models, respectively. The residuals appear randomly scattered around zero, suggesting homoscedasticity for both of our models. Figure 2 indicated that our residuals were approximately normally distributed. We see some slight deviations at the tail-end of the distributions, but this is to be expected with extreme values and a small sample such as ours ($n = 30$).

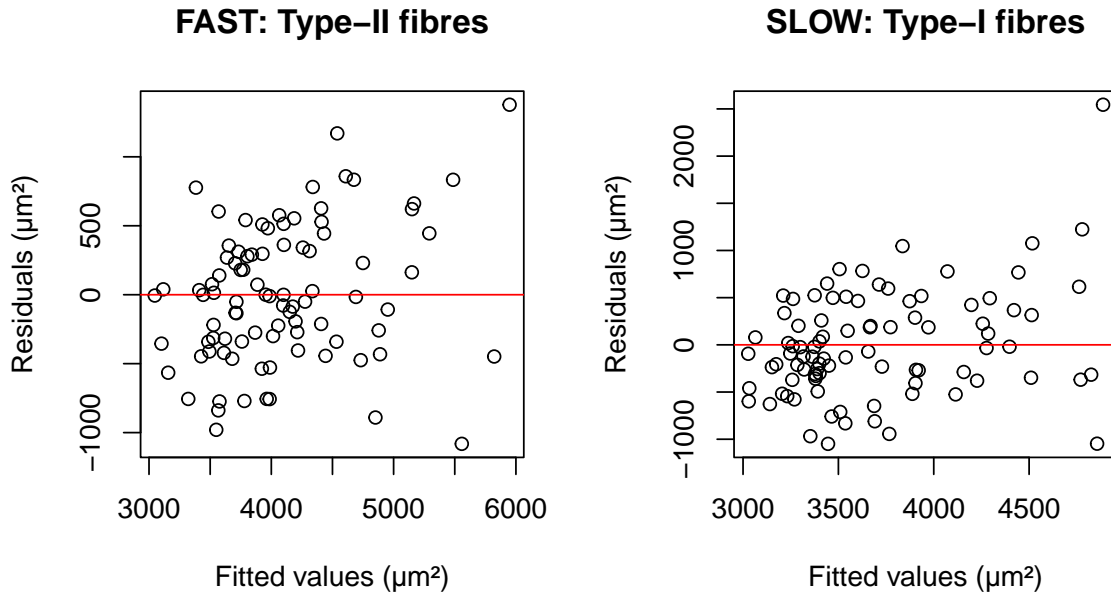


Figure 1: Residuals versus fitted values for FAST (Type-II fibres, left) and SLOW (Type-I fibres, right).

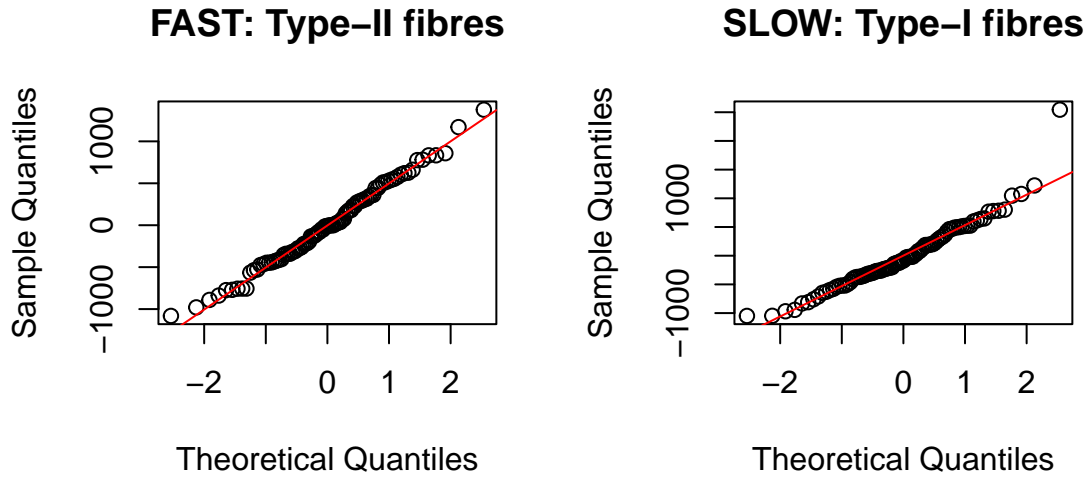


Figure 2: Q-Q plots for FAST (Type-II fibres, left) and SLOW (Type-I fibres, right)

4 Results

Descriptive statistics

Table 2: Model parameters for FAST (type-II) and SLOW (type-I)

	FAST	SLOW
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 \times myonuclear density	53.6 ± 23.7 [7.1 to 100.0]	-11 ± 29.1 [-67.9 to 45.9]

Values shown as estimate (μm^2) \pm SE [95% CI].

Interaction effect between time and baseline myonuclear density

The interaction effect time \times baseline myonuclear density is our primary estimand for both models (see Table 2). All values in this section are reported as estimate SE \pm , [95% CI] unless stated otherwise. In FAST, the interaction estimate between time \times myonuclear density in type-II fibres was $53.6 (\pm 23.7, [7.1 \text{ to } 100.0])$. For each additional nucleus per type-II fibre at baseline, there was a weekly increased area of $53.6 \mu\text{m}^2$ per type type-II fibre. 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 indicates that there is a positive effect of having higher myonuclear density for hypertrophy outcomes in type-II fibres.

In SLOW, the interaction estimate between time x myonuclear density in type-I fibres was -11 (± 29.1 , [-67.9 to 45.9]). For each increase in nucleus per type-I fibre at baseline, we observed a weekly decrease of 11 μm^2 in type-I fibres. 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 fibres.

Secondary findings

Intercepts, main effects of time and baseline myonuclear density are reported in Table 2. On average, fCSA increased by 38.5 μm^2 per week in type-II fibres and 6.2 μm^2 per week in type-I fibres at mean baseline myonuclear density. However, both confidence intervals include zero, precluding us from drawing strong inferences.

The random intercept standard deviation was $\pm 559.2 \mu\text{m}^2$ (type-II) and $\pm 540.1 \mu\text{m}^2$ (type-I), indicating substantial between-participant variability in baseline fCSA.

Figure 3 shows us the predicted trajectories of fCSA in both type-I and type-II fibres, at different baseline values of myonuclear density. In type-II fibres, ..

In type-I fibres, ..

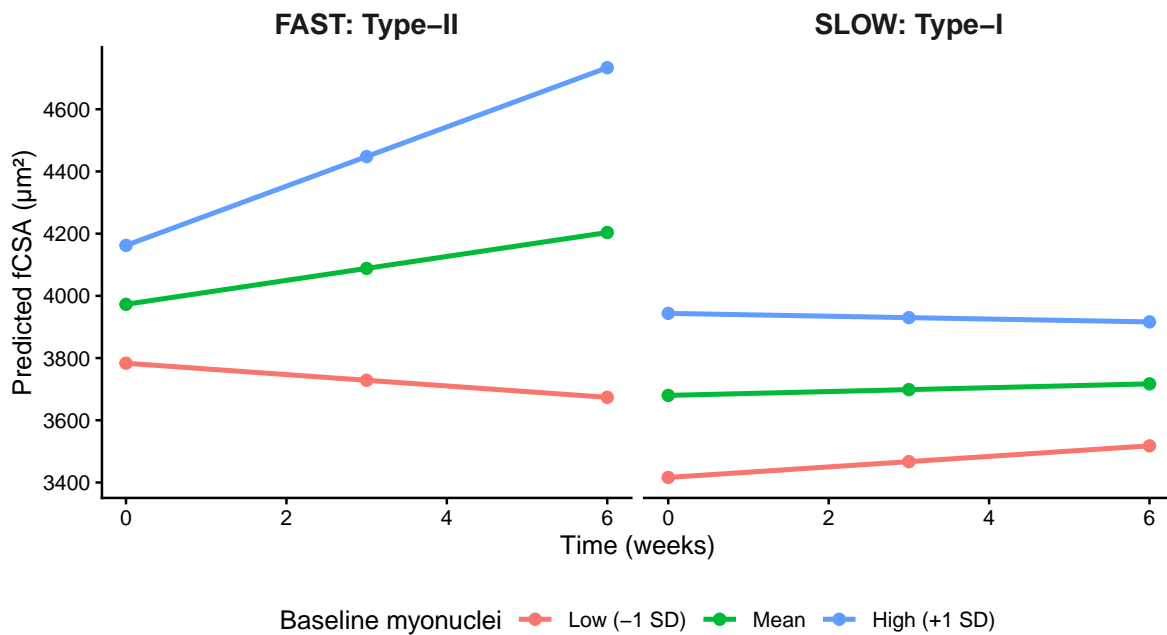


Figure 3: Line plots showing predictions at population level for changes in fCSA at different baseline myonuclear densities.

5 Discussion

5.1 Limitations

Outcome variable of fCSA - Allows us to draw mechanistic inferences, but not at a whole-muscle level. Also limited by the amount of fibres that have actually been examined.

Limited ecological validity. The population of the study is highly homogenous, and represents sampling bias.

Inability to establish causality with an observational design.

Philosophy of science - Frequentist framework. We did not report p-values, even though we employed a frequentist framework. This is because we do not regard p-values as particularly useful when employing an LMM.

6 Conclusion

Bibliography

- [1] Roberts MD, McCarthy JJ, Hornberger TA, Phillips SM, Mackey AL, Nader GA, et al. Mechanisms of mechanical overload-induced skeletal muscle hypertrophy: Current understanding and future directions. *Physiological Reviews* 2023;103:2679–757. <https://doi.org/10.1152/physrev.00039.2022>.
- [2] Erskine RM, Jones DA, Williams AG, Stewart CE, Degens H. Inter-individual variability in the adaptation of human muscle specific tension to progressive resistance training. *European Journal of Applied Physiology* 2010;110:1117–25. <https://doi.org/10.1007/s00421-010-1601-9>.
- [3] Ahtiainen JP, Sallinen J, Häkkinen K, Sillanpää E. Inter-individual variation in response to resistance training in cardiometabolic health indicators. *Scandinavian Journal of Medicine & Science in Sports* 2020;30:1040–53. <https://doi.org/10.1111/sms.13650>.
- [4] Ross R, Goodpaster BH, Koch LG, Sarzynski MA, Kohrt WM, Johannsen NM, et al. Precision exercise medicine: Understanding exercise response variability. *British Journal of Sports Medicine* 2019;53:1141–53. <https://doi.org/10.1136/bjsports-2018-100328>.
- [5] Snijders T, Smeets JSJ, van Kranenburg J, Kies AK, van Loon LJC, Verdijk LB. Changes in myonuclear domain size do not precede muscle hypertrophy during prolonged resistance-type exercise training. *Acta Physiologica* 2016;216:231–9. <https://doi.org/10.1111/apha.12609>.
- [6] Petrella JK, Kim J, Mayhew DL, Cross JM, Bamman MM. Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: A cluster analysis. *Journal of Applied Physiology* 2008;104:1736–42. <https://doi.org/10.1152/japplphysiol.01215.2007>.
- [7] Murach KA, Englund DA, Dupont-Versteegden EE, McCarthy JJ, Peterson CA. Myonuclear Domain Flexibility Challenges Rigid Assumptions on Satellite Cell Contribution to Skeletal Muscle Fiber Hypertrophy. *Frontiers in Physiology* 2018;9. <https://doi.org/10.3389/fphys.2018.00635>.
- [8] Haun CT, Vann CG, Mobley CB, Osburn SC, Mumford PW, Roberson PA, et al. Pre-training Skeletal Muscle Fiber Size and Predominant Fiber Type Best Predict Hypertrophic Responses to 6 Weeks of Resistance Training in Previously Trained Young Men. *Frontiers in Physiology* 2019;10. <https://doi.org/10.3389/fphys.2019.00297>.
- [9] Mobley CB, Haun CT, Roberson PA, Mumford PW, Kephart WC, Romero MA, et al. Biomarkers associated with low, moderate, and high vastus lateralis muscle hypertrophy following 12 weeks of resistance training. *PLOS ONE* 2018;13:e0195203. <https://doi.org/10.1371/journal.pone.0195203>.
- [10] Petrella JK, Kim J, Cross JM, Kosek DJ, Bamman MM. Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *American Journal of Physiology-Endocrinology and Metabolism* 2006;291:E937–46. <https://doi.org/10.1152/ajpendo.00190.2006>.

- [11] Haun CT, Vann CG, Mobley CB, Roberson PA, Osburn SC, Holmes HM, et al. Effects of Graded Whey Supplementation During Extreme-Volume Resistance Training. *Frontiers in Nutrition* 2018;5. <https://doi.org/10.3389/fnut.2018.00084>.