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DETERMINANTS OF INTRA-INDIVIDUAL VARIATION IN ADAPTABILITY TO RESISTANCE TRAINING OF DIFFERENT VOLUMES WITH SPECIAL REFERENCE TO SKELETAL MUSCLE PHENOTYPES

Determinants of intra-individual variation in adaptability to resistance training of different volumes with special reference to skeletal muscle phenotypes

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Abstract

The preface pretty much says it all.

Second paragraph of abstract starts here.

List of scientific papers

I. **Hammarström D**, Øfsteng S, Koll L, Hanestadhaugen M, Hollan I, Apró W, Blomstrand E, Rønnestad B, Ellefsen S Benefits of higher resistance-training volume are related to ribosome biogenesis. The *Journal of physiology.* 2020;598(3):543-65.

II. Khan Y, **Hammarström D**, Rønnestad B, Ellefsen S, Ahmad R Increased biological relevance of transcriptome analyses in human skeletal muscle using a model-specific pipeline. *Submitted.*

III. **Hammarström D**, Øfsteng S, Koll L, Jacobsen N, Flobergseter K, Rønnestad B, Ellefsen S Ribosome accumulation during early phase resistance training. *Manuscript*

IV. **Hammarström D**, Ellefsen S. generefer: A R package for unbiased selection of reference genes for qPCR in repeated measures designs. *Manuscript*

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1. Introduction

Proper skeletal muscle functioning is essential in everyday life by enabling movement and thus independent interaction with the environment. In a lifespan perspective, measures of muscle mass and/or strength are inversely associated with mortality (Li et al., 2018; Fukasawa et al., 2017; Miyake, Kanazawa, Tanaka, & Sugimoto, 2019; Ruiz et al., 2008; Szulc, Munoz, Marchand, Chapurlat, & Delmas, 2010; Abramowitz et al., 2018) and disability (Janssen, Heymsfield, & Ross, 2002). Besides adverse individual consequences of low muscle mass and strength and associated clinical conditions, muscle weakness accounts for increased health care costs in patient populations (Sousa et al., 2016; Pinedo-Villanueva et al., 2019). Causal links between muscle mass and functioning on the one hand and adverse health outcomes on the other hand are diverse and related to e.g., age and primary illness or injury (Wolfe, 2006). This highlights that interventions designed to increase muscle mass and strength are likely to prevent adverse health outcomes across the lifespan. A higher level of muscle mass and functional capacity would e.g., counteract the effects of muscle loss due to illness (cachexia), age (sarcopenia) or inactivity (athrophy).

Although a large degree of the observed variations in lean mass and strength are attributed to genetic components (Arden & Spector, 1997; Roth, 2012), environmental factors also contribute leaving a window of opportunity increase muscle mass and functional capacity. Among factors possibly affecting muscle mass and functioning are nutrition and pharmacological agents. However, physical activity and specifically resistance training of sufficient volume, intensity and frequency provides a stimulus that promote morphological and functional changes to the human neuromuscular system without adverse side-effects. Irrespective of age, resistance training generally leads to increased muscle muss and strength (Ahtiainen et al., 2016; Grgic et al., 2020) and is considered safe (Grgic et al., 2020).

Exercise training can be modulated indefinitely by combining different variations of training variables (Ratamess et al., 2009)

and in addition, adaptation to exercise training is a phenomenon characterized by great inter-individual variability. The purpose of the present project is therefore

to explore potential determinants of variation in adaptability to resistance-exercise modulated by selected exercise-training variables.

2. Background

2.1 Exercise training variables affecting training outcomes

2.2 Exercise volume

2.2.1 Meta-analysis of exercise volume

2.3 Molecular determinants of training-induced muscle hypertrophy

2.3.1 Protein synthesis

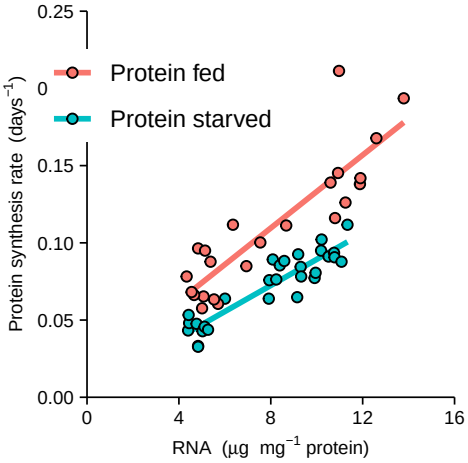


Figure 2.1: Data from Millward et al. 1973. Group A were fed a diet containing protein, group B were starved or fed a diet not containing protein.

2.3.2 The mammalian target of rapamycin (mTOR) and translational efficiency

The mammalian target of rapamycin (mTOR) is a large serine-threonine protein kinase wich in complex with oyher regulatory proteins forms a signaling hub responsible for responses to environmental cues such as nutrients and mechanical stress.

mTOR has several phosphorylation sites

Phosphorylation of Ser2448 is mediated by S6K1 to reduce mTOR activity in a negative feedback loop .

Ser2448 is phosphorylated by S6K1, changes in nutrient avaliability modifies S6K1 and Ser2448, Ser2448 phosphorylation is abolished when S6K1 is depleted

When the C-terminal is deleted, mTOR gets constitutively active

2.3.3 Ribsome biogenesis

Transcription of ribsomal RNA (rRNA)

2.4 Transcriptional activity related to muscle hypertrophy

2.4.1 Methods for studying transcriptional regulation

3. Aims

The primary aim of this thesis was to relate the adaptive response to resistance training with low- and moderate-volume to skeletal-muscle characteristics in previously untrained individuals. The key question was whether manipulation of exercise-volume will have diverse effects in different individuals related to muscular intrinsic characteristics. A further aim was to characterize exercise-volume dependence and time course profiles of molecular mechanism thought to control resistance training-induced muscle growth. Based on these aims, the objectives of the present thesis were;

- to relate skeletal muscle and systemic characteristics to benefit of moderate- compared to low-volume resistance training;
- To determine volume-dependence in molecular networks related to muscle growth and remodelling in response to mechanical stress
- To determine a time course of markers related to ribosome biogenesis in the early phase of resistance training.

4. Methods

4.1 Participants

TO DO:

- For methods discussion, compare product length, efficiencies and ct values in relation to RQI-values. See Fleige 2006 for reference.

4.2 Gene expression analysis

4.2.1 Normalization

- An external reference gene was added at a constant amount in Trizol preps
- A normalization factor was used to express relative target gene abundance per-weight tissue.
- In qPCR the linearised expression (effectivety ^cq) was used to express the fraction of external reference per total RNA.
- In RNA-seq the external reference gene was sequenced and counts were used to express external RNA as a fraction of total RNA.
- In both cases the normalization factor was calculated as mw * counts.

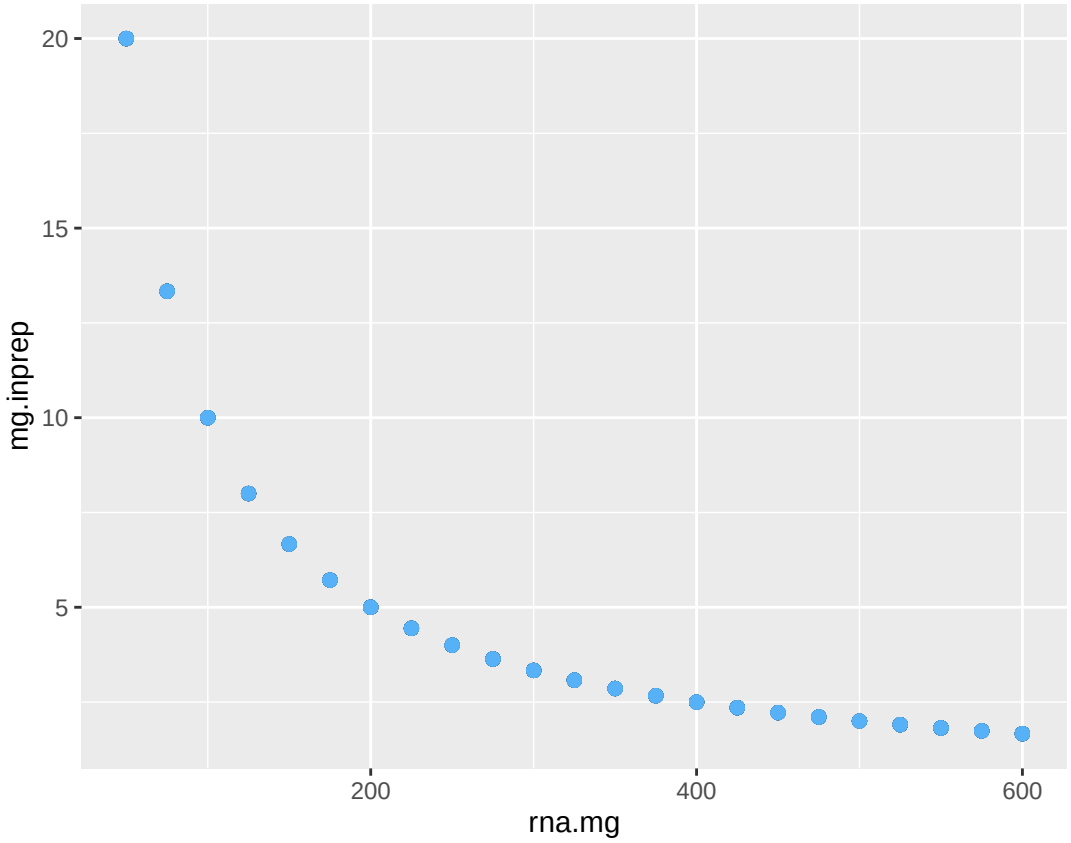
A simulation to see that this is equivalent to tissue used in prep when no measurement errors exists.

```
library(tidyverse)

expand_grid(mg = seq(from = 5, to = 100, by = 5),
            rna.mg = seq(from = 250, to = 600, by = 25),
            ext = 0.04) %>%
  mutate(tot.rna = mg * rna.mg,
         ext.frac = ext / (ext + tot.rna),
         mg.inprep = 1000 / ((ext + tot.rna) / mg),
         nf = ext.frac * mg)
```

```
# A tibble: 300 x 7
      mg rna.mg   ext tot.rna  ext.frac mg.inprep        nf
   <dbl>  <dbl> <dbl>   <dbl>     <dbl>     <dbl>     <dbl>
 1     5    250  0.04    1250 0.0000320      4.00 0.000160
 2     5    275  0.04    1375 0.0000291      3.64 0.000145
 3     5    300  0.04    1500 0.0000267      3.33 0.000133
 4     5    325  0.04    1625 0.0000246      3.08 0.000123
 5     5    350  0.04    1750 0.0000229      2.86 0.000114
 6     5    375  0.04    1875 0.0000213      2.67 0.000107
 7     5    400  0.04    2000 0.0000200      2.50 0.000100
 8     5    425  0.04    2125 0.0000188      2.35 0.0000941
 9     5    450  0.04    2250 0.0000178      2.22 0.0000889
10     5    475  0.04    2375 0.0000168      2.11 0.0000842
# ... with 290 more rows
```

```
expand_grid(mg = seq(from = 5, to = 100, by = 5),
            rna.mg = seq(from = 50, to = 600, by = 25),
            ext = 0.04) %>%
  mutate(tot.rna = mg * rna.mg,
         ext.frac = ext / (ext + tot.rna),
         mg.inprep = 1000 / ((ext + tot.rna) / mg),
         nf = ext.frac * mg) %>%
         ggplot(aes(rna.mg, mg.inprep,  color = mg)) + geom_point(size = 2)
```



4.3 Training protocols

A full body protocol was used in study I including

5. Results

6. Discussion

Conclusion

If we don't want Conclusion to have a chapter number next to it, we can add the `{-}` attribute.

More info

And here's some other random info: the first paragraph after a chapter title or section head *shouldn't be* indented, because indents are to tell the reader that you're starting a new paragraph. Since that's obvious after a chapter or section title, proper typesetting doesn't add an indent there.

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