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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 resis-
tance training of different volumes
with special reference to skeletal
muscle phenotypes

Daniel Hammarström

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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 Feb;598(3):543-565. doi: 10.1113/JP278455.
- 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. *BMC Bioinformatics*. 2020 Nov 30;21(1):548. doi: 10.1186/s12859-020-03866-y
- III. **Hammarström D**, Øfsteng S, Jacobsen N, Flobergseter K, Rønnestad B, Ellefsen S Ribosome accumulation during early phase resistance training. *Manuscript*

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1. Introduction

Skeletal muscle health is essential for physical independence. In a lifespan perspective, measures of muscle mass and/or strength are inversely associated with mortality (1, 2, 3, 4, 5, 6) and disability (7). Besides adverse associations between low muscle mass and strength and clinical conditions, muscle weakness also accounts for increased health care costs in patient populations (8, 9). The intercept between muscle mass, muscle function and health status is interrelated with variables such as age and primary illness or injury (10). 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 counteract the effects of muscle loss due to illness, age or inactivity.

Although a large degree of the observed variations in lean mass and strength are attributed to genetic components (11, 12), environmental factors also contribute, leaving a window of opportunity to increase muscle mass and functional capacity. Among factors affecting muscle mass and functioning are nutrition and pharmacological agents. However, physical activity and specifically systematic 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 mass and strength (13, 14) and is considered safe when performed in a well organized manner (14, 15).

Resistance training can be modulated indefinitely through combined variations of training variables such as frequency, intensity and volume (16, 17). Well designed training prescriptions should incorporate information about the current state and goals of the trainee to maximize the potential outcome of the training program (16, 18, 17). Training volume has received particular attention in the scientific community for many reasons. Evidence suggests that exercise volume affects selected molecular determinants of muscle hypertrophy in a dose-dependent manner (19, 20, 21). Such effects are believed to facilitate long-term training effects as training programs with higher volume generally result in higher gains in muscle mass

and strength with little evidence of differences between age groups or participants with different training backgrounds (22, 23, 24).

A consequence of a more extensive training program is the increased time required to complete such a program. As time constraints has been reported as a limiting factor for engaging in physical activity (25) some merit can be given to arguments against guidelines suggesting higher volume in resistance training prescription (26, 18). From an individual perspective, training prescription that balances time-requirement with efficacy presumably increases the likelihood of participation in physical activity (25). From a more general perspective, increased knowledge about mechanisms governing responses to physical training could improve training prescription also for individuals and populations that experience attenuated benefit of resistance training (27). The overreaching goal of the present thesis is to contribute to understanding individualized training loads. To this end, training volume was used to study the effects of variable training stimulus in within-participant models of exercise-training.

2. Background

2.1 Resistance-exercise prescription, a brief historical note and current challenges

Recommendations of systematic physical exercise with the purpose to improve health or physical performance has long been part of human culture, evident from records dating back to ancient Chinese, Indian and Greek civilizations (28). Today's exercise-training prescription still bear traces of ideas from these eras, further developed during the renaissance and formalized in systems like German Turnen and Ling gymnastics during the nineteenth century (29). German Turnen as a system of physical activities was established in a time when Germany developed from aristocracy to a unified nation. Turnen not only served as a system of preparing men to fight for the developing nation but also as a way of establishing a national identity. Ling gymnastics, developed by Per Henrik Ling, shared common origins with German Turnen and also served as a system of military preparation. However, Ling also established systems for medical, pedagogical and aesthetic gymnastics. Especially Lings medical gymnastics was important for the development of modern exercise prescription as it was scientifically oriented, based on the then current physiological and medical understanding (29). The medical gymnastics of the nineteenth century is referenced in twentieth century texts on therapeutic exercise prescription (30).

With the introduction of “heavy resistance exercises” for the development of muscle strength and mass after injury, DeLorme outlined a system on which modern resistance-exercise prescription is based (31). DeLorme published his system short after the Second World War (31) during which he, as a newly graduated physician, had been working with war injury rehabilitation (32). Inspired by practitioners of weight training (32), DeLorme specifically emphasized high-resistance, low-repetition exercise where progression was achieved with increased resistance (31) as opposed to previous recommendations of endurance-like exercise where progression was achieved through increased number of repetitions (30). DeLorme originally

used the term “heavy resistance exercises” in contrasts to low-resistance exercises (31), but as this could be perceived as exercises performed only with heavy weights, the system was renamed *progressive resistance exercise* to better reflect the method (33). Indeed, central to the system was the concept of repetition maximum (31). Repetition maximum refers to the external resistance that can be overcome with a given number of repetitions. By adjusting external resistance to each individual's progression over the course of a training program, exercises are both individualized and progress is monitored (31). DeLorme originally prescribed sessions of up to 100 repetitions performed in sets of 10 repetitions (31) but later revised this recommendation to three sets of 10 repetitions performed with increasing intensities (33).

Scientific inquiries into prescription of resistance training from the first part of the twentieth century concerned its therapeutic use (e.g. 31, 34) but also came to be introduced as a means of improving strength and physical performance in healthy populations (e.g. 35, 36, 37). Scientific contributions soon moved from questions regarding the effectiveness of resistance training *per se* to comparing outcomes from different modes of resistance training (38, 39, 40, 41, 42, 43). A vocabulary for progressive resistance exercise-training developed through these investigations and parallel practice, the introduction of repetition maximum by DeLorme being one example. These concepts established as modern definitions of exercise variables enabling precise prescription of training loads for a variety of populations and training goals (17).

Although this development started after the Second World War, resistance training was not part of general exercise guidelines until much later. The American College of Sports Medicine (ACSM) position statement on exercise for healthy individuals from 1978 (45) primarily concerned physical fitness in terms of cardio-respiratory fitness. Since the updated 1990 ACSM statement, resistance training is recommended to be included as part of a sensible, general training program (46). The introduction of resistance-training as part of the ACSM recommendation also marks the start of specific recommendations on resistance training being part of other consensus statements (47, Ch. 2). Consequently, informed by epidemiological data, the most recent general guidelines for physical activity do include resistance training (48).

It could be argued that the above account reflects the fact that common understandings of *why* and *how to* exercise are influenced by societal norms and historic events such as the search of national identity in the nineteenth century or war injuries in the twentieth century (29,32). In attempting to outline contem-

porary influences on exercise prescription, one could argue that the development of techniques to collect large amount of biological data is one such influence. The continuously decreasing cost of a sequenced human genome (49) can serve as an example of this development. Such molecular techniques has enabled efforts to describe mechanisms by which exercise training induce favorable adaptations. The newly established Molecular Transducers of Physical Activity Consortium is an example of a large scale effort, explicitly initiated to develop personalized exercise recommendations and identify molecular targets through which effects of exercised may be mimicked (50). Advances in bio-medical technologies are enablers of this enterprise and the quest to *individualize* exercise based on molecular diagnostics can be seen as a motivation for modern exercise science (50, 51). Contemporary exercise prescription can thus be understood as a part of the era of *personalized medicine*.

A challenge facing this enterprise is to accurately describe etiologies of response heterogeneity associated with physical training. A wide variation of individual responses are commonly observed after standardized resistance-training programs where measures of muscle strength-changes varies from -32 to +250% and muscle size-changes varies from negative to (-11%) to impressively large (+59%) (52, 13). By relating such variations to individuals genome (DNA) (53), and messenger RNA (mRNA) profiles (54, 55) we are beginning to gain knowledge about the genetic influence on training responses. In such studies, a common strategy has been to dichotomize responses into “responders” and “non-responders” to exercise training. From a public health perspective this is probably fruitful when *non-response* is defined as the absence of meaningful health-related adaptations, or even adverse effects in response to a given training regime (51, 56). The existence of non-responders would have large implications regarding exercise prescription on the population scale (57), and for any given individual as it, if properly diagnosed, would guide clinical decision-making.

Although “molecular exercise prescription” as a mean of eliminating adverse responses to a given training regime shows potential for future applications (56, 27), the rise of data intensive techniques has also highlighted its complexity. In simplistic terms, genetic information is transferred from DNA to RNA through transcription and from RNA to protein through translation. This flow of information, known as the central dogma of molecular biology (58) indicates a deterministic role of DNA in controlling the characteristics of an organism. It is however apparent that the transcription of specific genes are under tight control of several mechanisms including a group of non-genetic modifications to the DNA organization of each cell,

known as epigenetic modifications (59, 60). Such control determines gene expression in response to progressive resistance training with modifications persisting after a period of de-training (61, 62). Regulation of gene expression is thus related to modifiable variables such as exercise and diet (59, 60, 63, 64) linking environmental factors to the complexity of gene expression.

A key aspect of successful exercise diagnostics would be to map the relationship between exercise variables (i.e. modality, intensity, volume etc.) and exercise response for a given individual. It seems for example that an individual classified as non-responsive to a specific exercise modality (e.g. endurance training) may be classified as a responder to another (e.g. resistance training) (65). Even changing training variables within a specific modality have been shown to convert non-responders to responders as endurance training volume was increased (66).

By manipulating exercise variables in within-participant models we could potentially further our understanding of whom would benefit from a specific manipulation in addition to exploring possible mechanism behind such variability.

2.2 Adaptations to resistance training

2.2.1 Muscle hypertrophy and strength

A well characterized response to systematic resistance training in humans is muscle growth. In healthy, untrained individuals regardless of age, resistance training can be expected to result in increases of $\sim 5\text{-}20\%$ when training is conducted over two weeks to 6 months (13, 67, 52). Responses can be expected to be more pronounced in upper- compared to the lower-body muscles (67)

(68)

(69)

(70)

(71)

2.2.2 Muscle fiber-type transitions

2.2.3 Mitochondrial function

Increased mitochondrial respiration after 12 weeks of RT in young men (72)

Fiber type distributions do not predict muscle mitochondrial density in endurance trained individuals (73)

PMID:158694 Reduced mitochondrial density per fiber area in response to RT

2.3 Effects of exercise prescription on muscle mass and strength

Precise exercise-training¹ prescription gives information on exercises, their sequential order, intensity and volume, rest periods between efforts or sessions and the frequency at which exercise sessions are to be performed (23). By manipulating these variables, resistance training programs can be tailored to better fit goals and starting points of any individual. The relative importance of exercise-training variables for training outcomes has been examined in numerous studies including (but not limited to) the overall organization of exercise sessions, (74, 75) training frequency (76), and intensity (77). It could be argued that training volume is of particular importance for muscle growth as when this variable is held constant, manipulation of other variables has little or no effect hypertrophy (78, 77). For development of strength, factors such as intensity and within session organization of exercises is of importance (79, 80), however, when other factors are held constant, increased training volume generally leads to increased strength (79,81, 22), similarly to effects of training volume on muscle growth (23,24).

2.3.1 Effects of resistance exercise volume on muscle strength and mass

Exercise volume can be prescribed as the within session number of sets performed per muscle group. This unit is practical as it comparable between individuals and muscle groups (82). Berger conducted an early study concerning effects of resistance exercise volume with the goal to determine what method most efficiently produced strength gains (in healthy young males) (83). Berger compared one, two and three sets performed with two, six or ten repetition maximum (RM) in the bench press, three times per week, over twelve weeks. As the combined effect of three sets per session was superior regardless of the number of repetitions performed Berger concluded in favor of three sets. This conclusion was later challenged on the basis of data interpretation (26, 18). Reviwing the study by Berger and others, Carpinelli and Otto arrived to the conclusion that there was “insufficient evidence to support the prevalent belief that a greater volume of exercise (through multiple

¹Exercise is herein defined as an acute bout of physical activity designed to affect physical characteristics such as strength, speed or endurance. Training is defined as the systematic process of combining multiple exercise-sessions performed in sequence over time. Resistance-exercise is defined as an acute strength-promoting program requiring the neuromuscular system to exert force against resistance. Resistance training is defined as a long-term process of multiple resistance exercise-sessions performed over a defined period of time.

sets) will elicit superior muscular strength or hypertrophy” (26). This stand has since been repeatedly put forward as a criticism of higher volume training programs (84,85) and sparked considerable scientific activity. The main argument against the recommendation of additional volume in strength training programs has been the lack of statistically significant results in single studies (18,84). Indeed, individual studies do not generally agree on dose-dependent effects of training volume on muscle mass and strength gains (86, 87, 88, 89, 90, 91, 40, 92, 93, 94, 95, 96), including studies performed within participants, where different training volumes are allocated to either extremity (97, 98). For example, differences in strength are between volume conditions are found in older individuals (86, 87, 40) but not confirmed in another study (90)]. Studies shows that more volume does not lead to increased muscle gains in young individuals (94, 92, 88) a conclusion challenged by others (96, 89).

As previously noted, combining the above results and additional studies, meta-analyses concluded that training volume dose-dependency exists for the development of muscle mass and strength [(79); (81); (22); (23,24). As a second argument against additional volume in resistance training recommendation has been the cost/benefit relationship of adding training volume without meaningful or substantial additional gains (18, 84), a subsequent question is, whom would benefit from greater volumes and whom would not? Schoenfeld *et al.* combined data from published studies to explore if participant characteristics of the above mentioned studies interacted with training volume in explaining study outcomes. Neither sex, muscle groups nor age interacted with volume prescription indicating that no such factor would be able refine training prescription guidelines (24). As the number of studies used to synthesis the meta-analysis was relatively low ($n = 15$) and the studies were heterogeneous in terms of e.g. outcome measurements, it may have lacked in power to detect any meaningful interactions. Additionally, included studies may not have been reporting relevant characteristics for such analysis.

Collectively, the available evidence suggest that there is overlap between training outcomes in studies where different volume has been utilized. The overlap cannot, with available data, be explained by general population characteristics such as age or sex. Studying the effect of different training volumes within participants could potentially help to define determinants of training outcomes in response to different volume conditions. Two within-participant studies have investigated the effects of training volume on strength and hypertrophy outcomes. Sooneste *et al.* compared strength outcomes in response to three- and one-set elbow flexor training for 12 weeks in young males using a within-participant protocol (arms allocated

to either volume condition). The results showed general benefit of three- over one-set training for muscle hypertrophy and tended to do so also for strength gains (98). No attempts were made to relate baseline characteristics to the magnitude of differences between volume conditions, presumably due to the small sample size ($n = 8$). Mitchell *et al.* compared muscle hypertrophy and strength gains in response to three- and one-set of knee-extension exercise performed three times per week for ten weeks. The study contained an additional training condition (low intensity, 30% of 1RM performed with three sets) with participants legs assigned to either of the three conditions in a random fashion. No significant differences were reported between volume conditions for muscle mass or strength gains (97). However, the analyses were performed without taking the correlation between individuals into account due to the mixed design (97). No attempts were made to relate any measured characteristic to differences in responses.

2.4 Molecular determinants of training-induced muscle hypertrophy

Muscle mass fluctuates as a consequence of the balance between muscle protein synthesis and breakdown. When a net-positive balance is achieved, muscle protein accumulates and muscle mass increases. This is a scenario observed in response increased mechanical stress. Following a single bout of resistance exercise, muscle protein synthesis increases over resting levels up to 48-h post-exercise (99, 100, 101, 102, 103, 104) after being blunted during exercise (99). Mixed muscle protein synthesis and breakdown are highly correlated (105) (101) indicating that these processes are mechanistically coupled. And similarly to muscle protein synthesis, acute resistance exercise also stimulates protein breakdown but to a lesser extent than protein synthesis leading to an increase in the net protein balance from baseline (105) (101). When resistance exercise is performed in the fasted state, post exercise net protein balance can be expected to be negative (105) (101), in contrast when performed in the fed state where net balances are positive (106) (107). This indicates an additive effect of

Measurement of protein synthesis (and breakdown) rates in response to a single resistance exercise session has been given a lot of attention

as

(108) (109)

(110)

2.4.1 mTOR

The discovery of an immunosuppressant organic compound called rapamycin in the 1960's led to the characterization of a rapamycin sensitive protein involved in cell growth. The protein was later named mechanistic target for rapamycin (mTOR) (111). This protein has since been shown play a key role in skeletal muscle hypertrophy in relation to mechanical loading. Bodine *et al.* made a comprehensive characterization of mTOR-mediated skeletal muscle hypertrophy using rodent models in 2001, showing that mTOR activation was essential for load-induced hypertrophy. Additionally, using transfection techniques, they showed that constitutively activated Akt signaling led to hypertrophy in an mTOR-dependent manner, confirmed with concurrent administration of rapamycin (112).

(113)

2.4.2 Ribosomal biogenesis

(114) (115)

(116)

(103)

2.4.3 Transcriptional regulation of muscle mass

(117)

(118)

.

.

2.5 Effects of exercise volume on molecular determinants of muscle growth

(119)

(120)

2.5.1 From Training response

In humans, the biological adaptation to resistance training varies with exercise-training variables such as volume, intensity, rest between repetitions and sets, selection and order of exercises, repetition velocity and frequency of training

sessions (16). In addition, genetic and epigenetic disposition and environmental factors play a role in variations in adaptations (57, 62, 121). As time constraints often hinder participation in exercise training-programs (25), numerous studies have searched for the minimal required exercise dose to promote beneficial adaptations. Within-session volume has received particular attention, and although a handful of studies have shown that low-volume training provides similar gains in strength and muscular mass as moderate-volume training (95, 93, 97), meta-analyses conclude in favor of moderate volume protocols (22–24,122). This apparent discrepancy of specific studies to demonstrate benefits of increased training volume is likely due to a combination of small sample sizes and substantial variation in training responses between individuals and experimental groups. In theory, within-participant designs should alleviate these limitations.

Individual response patterns to resistance training, including muscle strength and mass, correlate closely with muscle cell characteristics, measured in both rested-state and acute training-phase conditions (54, 123, 124, 125). In this context, molecular signatures conveyed by the mechanistic target of rapamycin complex 1 (mTORC1) has been in particular focus. Inhibition of mTORC1 impairs protein synthesis in humans (126) and activation of its associated downstream target S6 kinase 1 (S6K1) correlates with increases in muscle protein synthesis and subsequent muscle growth (19, 125). In line with this, surplus exercise volume leads to greater phosphorylation of S6K1 (19, 20, 21) and is accompanied by increases in myofibrillar protein synthesis (19), fitting the notion that increased training volume provides more pronounced adaptations through repeated episodes of increased protein synthesis.

Recent observations in humans are challenging this view by indicating that translational capacity is a limiting factor for training-induced muscle hypertrophy. First, increased abundances of ribosomal RNA (rRNA) in response to resistance training, measured as total RNA per-weight-unit muscle tissue, correlate with muscle hypertrophy (127). In accordance with this, training-induced increases in rRNA are larger in muscle hypertrophy high-responders than in low-responders (124, 128). Secondly, elderly participants typically show blunted ribosome biogenesis, coinciding with attenuated hypertrophic responses (129, 130). Collectively, these observations suggest that muscle growth depends at least in part on increased translational capacity, making it a prime candidate for explaining the diverse response patterns seen in resistance training with different volume in different individuals. To date, no study has investigated the association between training volume, ribosome biogenesis and regulation, and gross training adaptations.

Muscle fibre composition is another potential determinant of muscular responses to resistance training. Type II fibres have greater growth potential compared to type I fibres (124, 131), and readily switch from IIX to IIA phenotypes in response to mechanical loading (132, 133, 134), suggesting that these fibres display greater plasticity in response to resistance training.

2.6 From RNA-seq

Skeletal muscle is a highly adaptable tissue that responds to environmental stress by altering growth rates and differentiation processes. During resistance training, signaling cascades that stimulate muscle plasticity are triggered. Upon repeated exposures, this facilitates growth and a phenotypic shift in a metabolically active direction (135), with the opposite happening during inactivity (136). Despite this generalized view, muscle responsiveness and plasticity vary, both in response to different resistance-training protocols (137) and, perhaps more importantly, between individuals (52, > 13). Selected individuals show a near-complete absence of muscle growth after prolonged resistance training, which markedly reduces the beneficial outcomes of such interventions for muscle function and overall health (13, 52). Currently, little is known about the etiology of this variation. However, it is usually associated with phenotypic traits of skeletal muscle (138, 124, 139), which implies interactions with environmental factors, genetics, epigenetics, and composites of the intra physiological milieu (121, 140). This multifaceted origin makes the training-response-spectrum difficult to study directly, with each of the underlying factors offering limited explanatory value alone (57). Instead, a more indirect approach is necessary, whereby the combined effects of the factors are targeted by studying global patterns of mRNA, protein expression, and skeletal muscle biology.

Previous studies have investigated transcriptome responses to acute resistance exercise (141, 142, 143) and chronic resistance training (144, 145, 142, 146, 141, 123), as well as described associations between transcriptome characteristics and degrees of muscle growth (123, 147), and function (148, 149). Whereas these studies have merited interesting findings, they lack clear coherences in terms of differential expression events, even for classical exercise-inducible genes such as PGC1 α (150). This lack of clear coherence is potentially due to a combination of issues such as differences in study design and methods for synthesis and analysis of transcriptome data. First, biologically founded variability can be attributed to differences in exercise protocols (e.g., differences in exercise-volume or intensity).

This makes it difficult to discern a general transcriptome exercise response, as training variables are not standardized between studies. Biological heterogeneity is also caused by differences between research participants, affecting signal-to-noise ratios and making it difficult to discern the effects of single independent factors such as training variables. Design stage decisions such as the use of within-participant designs (137, 151) are likely to reduce this variation and to provide transcriptome data with increased biological meaningfulness. Second, technical variability can be attributed to decisions made during the bioinformatical treatment of data. As described by Conceia *et al.*, there is no optimal pipeline for sequencing technology as new tools keep evolving and emerging, different tools should be explored to an optimum pipeline for the specific type of data (152). To exploit the potential of any study design, there is a need for identifying an appropriate pipeline for transcriptome analyses to ensure a biologically valid interpretation of data. This entails identifying potential violations of common assumptions caused by the experimental model at hand, relating to, for example, data normalization (153, 154).

For transcriptome data to provide adequate biological information about a given experimental set-up, numerous bioinformatic steps need to be adopted in a customized manner (152, **Arora2020?**). Of these steps, data normalization is particularly decisive (154), as it aims to transform naïve transcript counts into biologically meaningful results. This essentially means expressing them as *per-cell* abundances (155). For most experimental models, this is equivalent to providing transcript-to-total RNA ratios, given the fulfillment of the assumption that total RNA levels remain stable between conditions on a per-unit-cell or per-unit-tissue basis (155). In cell models that exhibit high degrees of plasticity, gene expression events result in increased amounts of total RNA and mRNA transcripts per cell (156), specifically violating the assumption that most genes are not differentially expressed (155, 153). We are not aware of any study that has addressed the need to account for such perspectives during transcriptome analyses of skeletal muscle subjected to mechanical stress, such as resistance training. Indeed, this assumption can be expected to be violated, as total RNA content increases markedly on a per-unit-weight basis (137), with potential global changes also occurring for the mRNA pool, though this remains unknown. The extent to which total RNA, and therefore ribosomal RNA, increases, coincides with the increase in muscle mass (137,124), underlining its importance for cellular growth but also its inevitable presence as a potential confounding factor in RNA sequencing experiments.

2.7 From tr10

Skeletal muscle is a critical target for interventions that promotes health across the lifespan (10), with resistance training (RT) being the advocated remedy.

Prolonged RT leads to changes in the balance between muscle protein breakdown and synthesis, with one bout of resistance exercise acutely increasing protein synthesis for up to 48 hrs after exercise (101), with subsequent repeated bouts leading to accumulation of muscle protein over time (157,158). In recent years, this view has been supplemented by evidence suggesting that chronic RT leads to increased resting synthetic rate of muscle protein (104, 159, 160), which has been postulated to be associated with increased translational capacity, i.e. accumulation of ribosomes (160, 161). This notion is supported by exercise-induced increases in total RNA, a proxy marker of ribosome abundance, which is closely connected to protein synthesis (162, 163) and muscle hypertrophy (127, 124, 137). Conversely, inhibition of ribosomal RNA (rRNA) transcription and inhibition of its up-stream transcription factors act to diminish muscle cell growth (124,162,164).

Biosynthesis of novel ribosomes is a complex, highly coordinated and energy demanding process that involves synthesis of both ribosomal proteins and the four mature rRNA transcripts (165, 166, 164). Ribosomal accumulation is believed to be determined by the rates of pre-rRNA transcription by RNA polymerase I (Pol I), which in turn is regulated by coordinated assembly of a complex of transcription factors at the rDNA promoter (166). Specifically, activation of the upstream binding factor (UBF) through phosphorylation is needed to initiate transcription (167, 114). Such activation is at least partly controlled by the mechanosensitive mTOR pathway, with its inhibition being associated with blocked UBF phosphorylation and subsequent rRNA transcription (168, 169). Interestingly the availability of UBF *per se* has been shown to be a determinant of rRNA transcription (170) through control of rDNA gene activity (171).

Resistance exercise is a potent stimuli for rRNA transcription as a single session leads to increases in pre-rRNA (129,172) and repeated bouts lead to accumulation of mature rRNA thus also total RNA and presumably functional ribosomes (129, 173, 137, 124,127, 130, 160). However, the true time course of ribosomal transcription and accumulation in response to RT remains largely unstudied, with a mere few studies having investigated exercise-induced changes in rRNA over multiple time-points, all of which are either limited to a selected few time-points or a limited time frame. For example, two consecutive bouts of electrically evoked muscle contractions were associated with increased levels of total RNA, with peak values

being observed 72 hrs after the second bout (173). Using voluntary contractions, peak values were reported after nine sessions, followed by a slight decrease to after 18 sessions (130), resembling data from our lab where five sessions of RT led to marked increase in total RNA levels (per-unit muscle tissue), whereby a numerical lowering occurred to after the last training session of the 12 wk interventions (31 sessions). (137). Interestingly, during the initial phase of RT, total RNA accumulation seems to be volume-dependent, as three sets per exercise in leg exercises led to augmented total RNA and rRNA levels compared to one set per exercise, coinciding with the differences in muscle hypertrophy seen after 12 weeks of RT. (137). These data suggest that ribosome accumulation reaches a plateau in the early phase of RT and that increases are sensitive to training volume in constant volume protocols.

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 in muscle molecular characteristics and determine a time course profile of markers of ribosomal biogenesis in response to resistance training. 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 remodeling in response to resistance training
- To determine a time course of markers related to ribosome biogenesis in the early phase of resistance training.

4. Methods

4.1 Study participants, protocols and training interventions

Study I was designed to examine effects of low- and moderate-volume on responses to acute exercise and long-term training within participants. Forty-one healthy individuals were recruited and 34 of these completed at least 85% of the prescribed sessions and were thus included in subsequent data analyses. Reasons for not completing the trial included injury not related to the study ($n = 1$), pain or discomfort during exercises ($n = 5$) and non-adherence to the study protocol. There were no differences in characteristics between participants included in or excluded from data analysis in Study I.

Study II was designed to study the effects of resistance training *per se*, and effects of variable volume on selected markers related to ribosome biogenesis. Participants were therefore recruited to a training group ($n = 11$) and a non-training control group ($n = 8$). Eligible for participation in both studies were young (Study I age 18-40; Study II 18-35), non-smoking men and women. Exclusion criteria included a training history of more than one weekly session during the last 12 (Study I) or six (Study II) months leading up to the study. Participants were also screened for intolerance to local anesthetic, current or previous injuries affecting their ability to perform resistance training, self-reported symptoms or history of disease, intake of medication or supplements with known effects on adaptations to training. Participant characteristics for both studies are shown in Table 4.1.

4.2 Resistance training interventions

Each training session started with a light standardized warm-up (5 min ergometer cycling and 10 repetitions each of push-ups, sit-ups, back-extensions and squats). Before each exercise in the main program, one set of 10 repetitions were performed in the specific exercise with approximately 50% of 1RM.

Table 4.1: Participant characteristics

	Sex	Age (years)	Stature (cm)	Mass (kg)	Fat mass (%)	Lean mass (%)
Study I	Included	Female	22.0 (1.3)	168 (7)	64.4 (10.4)	34.1 (5.6)
		Male	23.6 (4.1)	183 (6)	75.8 (10.7)	20.4 (6.0)
	Excluded	Female	22.9 (1.6)	166 (8)	64.6 (9.7)	28.8 (8.7)
		Male	24.3 (1.5)	189 (5)	88.2 (22.4)	24.3 (15.3)
Study II	Training	Female	23.4 (2.9)	168 (8)	64.0 (9.2)	30.8 (7.1)
		Male	25.7 (5.8)	177 (3)	77.5 (8.0)	25.3 (3.9)
	Control	Female	24.1 (3.5)	166 (4)	63.8 (0.6)	30.5 (6.4)
		Male	25.5 (5.5)	182 (5)	76.5 (7.7)	18.2 (5.1)

Data are means and (SD)

Studies were fully or partially performed as within-participant studies as each participant had their legs assigned to different training conditions (not including the control group in Study II). Allocation was performed after enrollment where each participant had their legs randomized to either low- or moderate volume (Study I), or variable or constant volume (Study II).

In Study I, the low-volume protocol consisted of a single set of each exercise and the moderate-volume consisted of three sets per exercise. Three unilateral leg exercises were used (leg press, leg curl and knee extension). The moderate volume-leg commenced all sessions and the low volume-leg performed a single set of each exercise in the rest between second and third set of the moderate volume training protocol.

In Study II, only unilateral knee-extension was performed in an effort to concentrate the stimulus to the quadriceps muscles. The constant-volume leg performed six sets of 10RM throughout the study and variable leg performed six sets in session one to four, three sets in session five to eight and nine sets in session nine to twelve with same intensity (10RM).

4.2.1 Ethical considerations

Both studies were approved by the local ethics committee Lillehammer University College/Inland Norway University of Applied Sciences and the Norwegian Centre for Research Data. In accordance with the *Declaration of Helsinki*(174) the studies were pre-registered in publicly accessible databases (Study I, ClinicalTrials.gov Identifier: NCT02179307; Study II, <https://osf.io/wa96y>). Participants were informed of the study design, potential risks and sources of discomfort prior to

giving their informed consent.

Measures of muscle mass

In Study I muscle mass was measured by magnetic resonance imaging (MRI) and dual energy X-ray absorptiometry (DXA) prior to and after the intervention. Both MRI and DXA measurements were completed during the same visit to the laboratory. Participants were instructed to refrain from strenuous physical activity during the last 48 h leading up to the measurements. The post-training measurements were completed at least 48 h after the last strength testing session. Participants were asked to refrain from food consumption during 2 h leading up to the measurements.

MRI images were obtained from the mid-thigh and analyzed by the same investigator blinded for time (pre- and post-training) and condition (low- and moderate-volume). Multiple images were used to estimate the cross-sectional area of the extensor muscles at the same distance from the knee-joint.

See figure

In Study II

4.3 Muscle tissue sampling and preparations for downstream analyses

Muscle samples were obtained under local anesthesia (Study I, Xylocaine, 10 mg ml⁻¹ with adrenalin 5 µg ml⁻¹, AstraZeneca, Oslo, Norway; Study II, Lidocaine Mylan, 10 mg ml⁻¹, Mylan Ireland Ltd, Ireland) with a fine needle (12-14 gauge; Universal-plus, Medax, Italy) operated with a spring-loaded instrument (Bard Magnum, Bard Norway AS, Norway). Sampling was performed as previously described (175), with modifications. Anesthesia was injected in the subcutaneous tissue with care taken not to inject anesthesia into the muscle itself. Following pilot experiments we decided not to use an insertion cannula as described in (175) as the biopsy needle itself could be used to puncture the skin and muscle fascia. This also resulted in less discomfort. Several passes through the same skin puncture was made to obtain sufficient material for downstream analyses. A smaller needle (14 vs. 12 gauge) was used to further minimize discomfort in Study II where more biopsies were sampled over a shorter time span, with exception from when material was used for immunohistochemistry. The first biopsy was sampled at one third of the distance between the patella to the *anterior superior iliac spinae* with subsequent biopsies sampled ~2 cm proximal to previous samples.

In Study II, samples obtained more than one week apart were sampled with closer proximity and distally from previous samples but never at previous sampling sites.

The micro biopsy technique produces smaller samples compared to other biopsy techniques (176), and thus requires several passes to produce sufficient material for multiple downstream experiments. However, reports confirms that the micro biopsy technique is comparable to the traditionally used Bergström technique in several measures of muscle characteristics at the same time as being well tolerated (175,177). Any reported differences in fiber type distributions between sampling techniques have been suggested relating to differences in sampling depth (177,178).

For determination of fiber type distributions, a threshold of 200-300 fibers has been suggested as a suitable sample size per specimen as more fibers does not reduce the variation between duplicate samples (179). In Study I, one or several pieces of muscle (total weight ~ 15 mg) were chosen per sampling for analysis of fiber type distributions (described in detail below). The total number of fibers were counted from these specimens (Figure ref fig). Using an average of fibers from the first sampling time point the between leg coefficient of variation was determined to 14% for Type I fibers and 11.3 for type II fibers. The between leg variation in Type I fibers is similar to what has been previously reported. . .

4.4 Gene expression analysis

4.4.1 Total RNA extraction

Total-RNA was extracted from pre-weighed, frozen muscle samples with a protocol modified from (180) using Trizol reagent (Life Technologies). Muscle tissue was homogenized in 300 μ l Trizol with mechanical disruption achieved by Zirconium Oxide Beads (0.5 mm, Next Advance, Inc., New York, USA) and a bead mill (Bullet blender, Next Advance). External, non-mammalian RNA (Lambda PolyA External Standard Kit, Takara Bio Europe, Saint-Germain-en-Laye, France) was added with the initial volume of Trizol to enable per-weight normalization in subsequent analyses. After homogenization, additional Trizol was added to a total volume of 1 ml. Phase separation was achieved by centrifugation after addition of chloroform (200 μ l). The upper phase (400 μ l in Study I; 450 μ l in Study II) was transferred to a fresh tube and RNA was precipitated using isopropanol (500 μ l). After incubation (10 min, room-temperature) and centrifugation (12000 g, 10 min at 4°C) the resulting RNA pellet was washed three times in chilled 75% ethanol.

As previously mentioned, to minimize discomfort with a larger number of

biopsies sampled over a short time, a smaller needle was used for most biopsies in Study II. This generally led to a less tissue used for RNA extractions (Figure XX).

4.5 Determination of protein abundance

4.6 Statistics and data analysis

TO DO:

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

4.6.1 Normalization

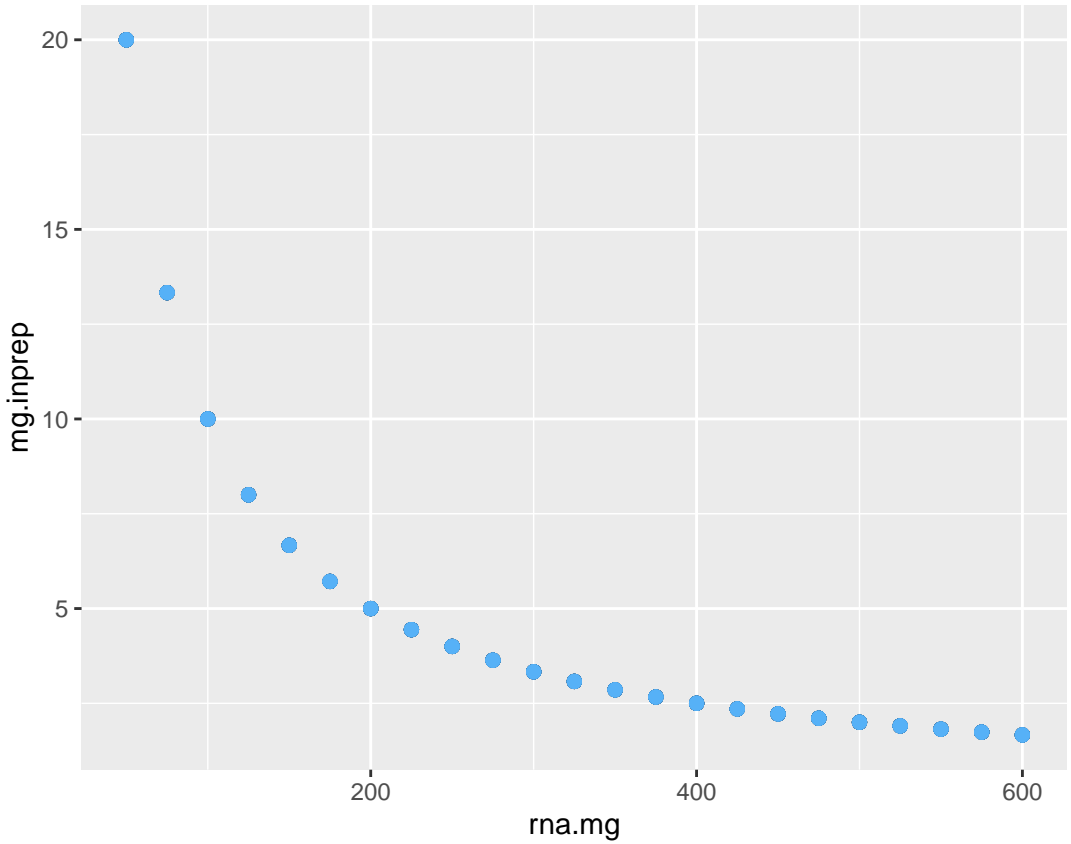
- An external reference gene was added at a constant amount in Trizol preps
- A normalization factor was used to express relative target gene abundance per-weight tissue.
- In qPCR the linearised expression (effectively $2^{-\Delta C_t}$) was used to express the fraction of external reference per total 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 $mw \cdot counts$.

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

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

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# ... with 290 more rows
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4.7 Meta-analysis of within-session training volume

4.7.1 Literature search, inclusion criteria and coding of studies

A first set of studies were coded based previously published meta-analyses (23,24). For more recent studies, PubMed, Google Scholar and SportDiscuss searches were made with search terms being “training volume,” “resistance training,” “strength training,” “set,” “muscle strength,” “muscle hypertrophy” used in different combinations. Studies examining the effect of within-session training volume on muscle strength and mass, with all other training variables kept constant between study groups were considered for inclusion in the meta-analysis. Studies were further assessed for inclusion based on criteria being; (i) participants described as healthy without medications affecting muscle metabolism, (ii) interventions lasting at least 6

weeks and (iii) RT performed without additional stimuli (e.g. blood flow restriction) at intensities above 65% of 1RM or 20RM.

All available outcome measures of muscle mass and strength gains in response to RT were extracted from each study with exception of outcomes reported both as summaries and individual measures (e.g. muscle thickness reported as individual muscles and summarized for the whole muscle group). In such cases the summary was used as outcome. Weekly training volume was calculated as product of weekly sessions, number of sets and exercises for each muscle group assessed for muscle hypertrophy or strength gains. An intervention average of weekly sessions was used when the number of sessions per week differed over the course of the intervention. An exercise was assumed to influence an outcome when it targeted prime movers also assessed for strength or muscle hypertrophy measures. Participant characteristics were coded based on sex (male, female or mixed when a study failed to discern between male and females), age (young, middle-aged, old or mixed), body-mass index (BMI, calculated from average body mass and height when BMI values were not available), training status (trained, > 1 session per week during the last 6 months leading up to the intervention; untrained < 1 session). Study groups were considered independent also in studies utilizing within-participant models.

4.7.2 Calculations of effect sizes and statistical analysis

Group-wise effect sizes were calculated for each outcome measure based on the within-group change score pre- to post-training divided by the pre-training standard deviation (SD). Pre-training SD's were calculated as a pooled SD within outcome and study. Variances of the effect size were calculated using an average effect size across all outcomes within muscle strength or mass, and correlations specific to each measurement type (isokinetic-, isometric- or repetition maximum strength tests; muscle thickness, magnetic resonance imaging, dual energy X-ray absorptiometry) estimated from previous studies.

A correction factor () was applied to both effect sizes and their variances.

Mixed-effects meta-regression models were used to model the effect of weekly number of sets on RT-induced muscle mass and strength gains. Models were fitted in a Bayesian framework using the brms-package (181).

5. Results and Discussion

5.1 Effects of different training volume on changes in muscle size and function

In Study I, the average increases (Table 5.1) in muscle strength and mass in each volume condition corresponded to what could be expected based on previous studies (182, 13).

Average within participant differences in responses between LOW and MOD were consistent across measures of muscle hypertrophy and strength gains (Figure 5.1). These differences were in agreement to what could be expected based on published meta-analyses (81, 23, 22, 24). Taken together, these observations confirmed the efficacy of training programs in general and a dose-response with regard to within-session exercise volume.

In Study II, training efficacy was assessed by comparing outcomes to a non-training control group. The training group displayed increases compared to the control group for both strength muscle thickness measures.

Table 5.1: Training induced changes in muscle CSA and average strength in Study I

	Sex	Volume condition	Mean (SD)	Reference
CSA %-change	Female	LOW	3.05 (3.61)	
		MOD	5.02 (4.04)	
	Male	LOW	3.83 (3.50)	
		MOD	5.10 (3.71)	
CSA %-change day	Female	LOW	0.04 (0.05)	0.11 [0.04-0.26]a
		MOD	0.07 (0.05)	
	Male	LOW	0.05 (0.05)	
		MOD	0.07 (0.05)	
CSA %-change session	Female	LOW	0.11 (0.13)	0.08 (0.22)b
		MOD	0.18 (0.15)	
	Male	LOW	0.14 (0.12)	
		MOD	0.19 (0.13)	
Average strength %-change	Female	LOW	21.0 (9.8)	
		MOD	27.8 (14.4)	
	Male	LOW	19.2 (12.4)	
		MOD	23.1 (12.0)	
Average strength %-session	Female	LOW	0.77 (0.36)	0.67 (0.35)b
		MOD	1.00 (0.49)	
	Male	LOW	0.72 (0.48)	
		MOD	0.87 (0.46)	

^a Estimates from Wernbom et al. (182)^b Estimates from Ahtiainen et al. (ref:ahtiainen-citation)

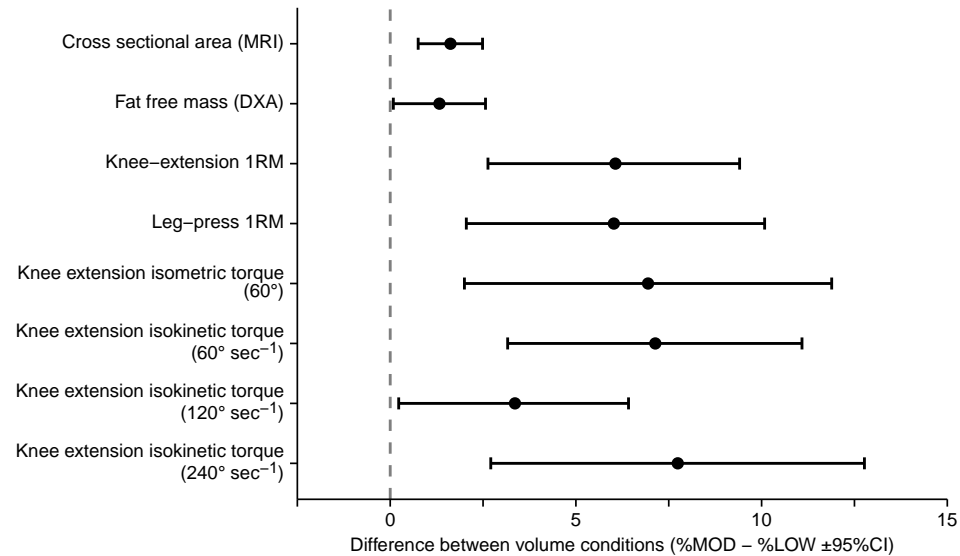


Figure 5.1: Differences in training induced relative changes in muscle mass and strength measures. Estimates are derived from ANCOVA models controlling for baseline values and sex.

5.2 Acute effects of different training volume on determinants of muscle protein synthesis

6. General Discussion

Conclusion

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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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