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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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# 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 of 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 guidlines 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, influences and challenges

Recommendations of systematic physical exercise for the purpose of improving 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 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 physiological and medical understanding of that time [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 individuals 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 was also evaluated in the context 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 primarily concerned physical fitness in terms of cardio-respiratory fitness [45]. 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 coincides with 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 contemporary 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]. Scientific research into contemporary exercise prescription can thus be understood as a part of the era of *personalized medicine*.

A challenge facing this program 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.

[58]; A key aspect of successful exercise diagnostics would be to take advantage of the relationship between exercise variables (i.e. modality, intensity, volume etc.) and exercise response for a given individual. By adapting an individuals training program based on some prior knowledge about the individual, it is possible that the response could be positively affected. Observations supporting such notion exists as 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) [59]. Even changing training variables within a specific modality have been shown to convert non-responders to responders as endurance training volume was increased [60].

Although apparent reversal of non-response to exercise training has been observed by manipulating training variables, decisive indications for such manipulations are still lacking.

## 2.2 Adaptations to resistance training

### 2.2.1 Muscle hypertrophy and strength

Systematic resistance training typically increases muscle mass and strength, adaptations through which many beneficial effects on health (increased amino acid storage physical independence) and athletic performance are conveyed. Muscle growth is a well characterized response to resistance training. On the whole muscle level, healthy untrained individuals can be expected to increases their muscle mass by $\sim$ 5-20% when training is conducted over two weeks to 6 months [13, 61, 52]. Over this time span, muscle growth is approximately linear with time [62, 63, 64] and has been detected as early as 3-4 weeks after training initiation, without apparent muscle edema [62, 63].

Relative muscle growth can be expected to be more pronounced in upper- compared to lower-body muscles when loading patterns are similar [61, 65]. This possibly relates to greater every day activity levels of lower-body muscles requiring a larger stimuli for adaptation [66]. Small, but detectable differences in muscle growth is typically seen between sexes for training induced muscle growth in the upper-body [52] but not for lower-body muscles [67]. Furthermore can hypertrophic responses be expected to be reduced with increasing age [65, 68] but increased with sufficient addition of dietary protein [69]. Additionally, training variables such as intensity, volume, frequency (reviewed below) together with other training aids, e.g. manipulation of blood flow through pressure cuffs [70] can effectively modulate resistance training induced hypertrophy. Together, this underlines that both non-modifiable (e.g. sexual dimorphism and age) and modifiable factors (e.g. training variables and protein supplementation) affects training induced muscle hypertrophy.

Whole muscles growth in response to short term resistance training occurs primarily through growth of individual muscle fibers (muscle cells). This can be assumed as training-induced splitting of existing fibers and/or myogenesis, formation of new muscle cells are likely slow processes and any increase in the number of fibers by such mechanisms would only represent a small addition to the whole muscle mass [71, 72, 73, 66]. Growth of muscle fibers transfers to greater muscle strength trough an expansion of the fibers contractile elements. The muscle

cell is to a large degree occupied by myofibrils (about 80% of the cell volume [74]), which in turn contain sarcomeres, arranged in series. Upon neural activation and subsequent $Ca^{2+}$ release, the sarcomere shortens through interaction between actin and myosin resulting in force generation [75]. With resistance training, the number of parallel myofibrils increases with the growth of individual fibers [76] leading to a greater force generating capacity of the whole muscle [74].

Measures of whole muscle size corresponds well with strength, particularly when a measure of muscle size reflects the muscles cross-sectional area [77, 78]. However, in response to resistance training, increases in strength are typically greater in magnitude than muscle growth [79, 80, 64, 81]. When relationships between resistance-training induced change in muscle size and strength in previously untrained individuals are analyzed, only a small portion of the variation in muscle strength can be accounted for by changes in muscle size ($\sim$ 2.5-28%) [78, 13, 82] depending on type of measurements and statistical models used [82] This underlines that muscle hypertrophy is one contributing factor to muscle strength gains, but not the only one.

Different experimental models have shown that strength can increase without concomitant muscle hypertrophy. Getting acquainted to the actual strength test through repeated training of maximal performance produces similar gains in strength without concomitant hypertrophy [83]. If resistance training is performed unilaterally and the contralateral limb act as a control, strength gains are typically also seen here [84, 85]. Additionally, systematic imagery training, without muscle activation produces greater strength gains than control and low-intensity training conditions [86]. Together these observations indirectly points to the central nervous system and motor learning as important factors for strength gains. In addition to effects that mainly can be attributed to motor learning, resistance training leads to changed behavior of motor units, estimated from surface electromyograms [87]. Such changes could be attributed to morphological and functional changes of motoroneurons [88].

### Connective tissue

In addition to changes in the contracting apparatus of single muscle fibers and neural mechanism regulating their activity, resistance training modulates connective tissue, tendon and bone.

### 2.2.2 Muscle fiber-type transitions

Skeletal muscle tissue is heterogeneous in terms

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 [89, 90], and readily switch from IIX to IIA phenotypes in response to mechanical loading [91, 92, 93], suggesting that these fibres display greater plasticity in response to resistance training.

### 2.2.3 Mitochondrial function

Increased mitochondrial respiration after 12 weeks of RT in young men [94]

Fiber type distributions do not predict muscle mitochondrial density in endurance trained individuals [95]

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

## 2.3 Effects of exercise prescription on muscle mass and strength

Precise exercise-training[1] 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, [96, 97] training frequency [98], and intensity [99]. 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 [100, 99]. For development of strength, factors such as intensity and within session organization of exercises is of importance [101, 102], however, when other factors are held constant, increased training volume generally leads to increased strength [101,103, 22], similarly to effects of training volume on muscle growth [23,24].

[1] Exercise 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.

### 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 [104]. 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) [105]. 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]. Reveiwing 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 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 [106,107] 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 [18,106]. Indeed, individual studies do not generally agree on dose-dependent effects of training volume on muscle mass and strength gains [108, 109, 110, 111, 112, 113, 40, 114, 115, 116, 117, 118], including studies performed within participants, where different training volumes are allocated to either extremity [119, 120]. For example, differences in strength are between volume conditions are found in older individuals [108, 109, 40] but not confirmed in another study [112]]. Studies shows that more volume does not lead to increased muscle gains in young individuals [116, 114, 110] a conclusion challenged by others [118, 111].

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 [[101]; [103]; [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, 106], 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 were 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 whitin-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 [120]. 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 [119]. However, the analyses were performed without taking the correlation between individuals into account due to the mixed design [119]. 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 [121, 122, 123, 124, 125, 126] after being blunted during exercise [121]. Mixed muscle protein synthesis and breakdown are highly correlated [127] [123] 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 [127] [123]. When resistance exercise is performed in the fasted state, post exercise net protein balance can be expected to be negative [127] [123], in contrast when performed in the fed state where net balances are positive [128] [129]. 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

In recent years, this view has been supplemented by evidence suggesting that chronic RT leads to increased resting synthetic rate of muscle protein [126, 130, 131], which has been postulated to be associated with increased translational capacity, i.e. accumulation of ribosomes [131, 132]. This notion is supported by exercise-induced increases in total RNA, a proxy marker of ribosome abundance, which is closely connected to protein synthesis [133, 134] and muscle hypertrophy [135, 89, 136]. Conversely, inhibition of ribosomal RNA (rRNA) transcription and inhibition of its up-stream transcription factors act to diminish muscle cell growth [89,133,137].

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 [138, 139, 137]. 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 [139]. Specifically, activation of the of the upstream binding factor (UBF) through phosphorylation is needed to initiate transcription [140, 141]. 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 [142, 143]. Interestingly the availability of UBF *per se* has been shown to be a determinant of rRNA transcription [144] through control of rDNA gene activity [145].

[146] [147]

[148]

### 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) [149]. 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 [150].

[151]

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 [152] and activation of its associated downstream target S6 kinase 1 (S6K1) correlates with increases in muscle protein synthesis and subsequent muscle growth [19, 153]. 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.

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, 154, 89, 153].

### 2.4.2 Ribosomal biogenesis

Conversely, inhibition of ribosomal RNA (rRNA) transcription and inhibition of its up-stream transcription factors act to diminish muscle cell growth [89,133,137].

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 [138, 139, 137]. 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 [139]. Specifically, activation of the of the upstream binding factor (UBF) through phosphorylation is needed to initiate transcription [140, 141].

[141] [155]
[156]
[125]
[157]

### 2.4.3 Transcriptional regulation of muscle mass

[158]
[159]

Ribosomal biogensis as a determinant of RT-induced hypertrophy

Resistance exercise is a potent stimuli for rRNA transcription as a single session leads to increases in pre-rRNA [160,161] and repeated bouts lead to accumulation of mature rRNA thus also total RNA and presumably functional ribosomes [160, 162, 136, 89,135, 163, 131]. 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 [162]. Using voluntary contractions, peak values were reported after nine sessions, followed by a slight decrease to after 18 sessions [163], 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). [136]. 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. [136]. 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.

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 [135]. In accordance with this, training-induced increases in rRNA are larger in muscle hypertrophy high-responders than in low-responders [89,

164]. Secondly, elderly participants typically show blunted ribosome biogenesis, coinciding with attenuated hypertrophic responses [160, 163]. 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.

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## 2.5 Effects of exercise volume on molecular determinants of muscle growth

[165]

[166]

### 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, 167, 69]. 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 [117, 115, 119], meta-analyses conclude in favor of moderate volume protocols [22–24,168]. 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.

## 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 [169], with the opposite happening during inactivity [170]. Despite this generalized view, muscle responsiveness and plasticity vary, both in response to different resistance-training protocols [136] 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 [171, 89, 172 ], which implies interactions with environmental factors, genetics, epigenetics, and composites of the intra physiological milieu [69, 173]. 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 [174, 175, 176] and chronic resistance training [177, 178, 175, 179, 174, 154], as well as described associations between transcriptome characteristics and degrees of muscle growth [154, 180], and function [181, 182]. 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$\alpha$ [183]. 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 [136, 184] 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 Concea *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 [185]. 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 [186, 187].

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 [185, 188]. Of these steps, data normalization is particularly decisive [187], as it aims to transform naïve transcript counts into biologically meaningful results. This essentially means expressing them as *per*-cell abundances [189]. 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 [189]. In cell models that exhibit high degrees of plasticity, gene expression events result in increased amounts of total RNA and mRNA transcripts per cell [190], specifically violating the assumption that most genes are not differentially expressed [189, 186]. 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 [136], 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 [136,89], 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 [123], with subsequent repeated bouts leading to accumulation of muscle protein over time [191,192].

# 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) | 64.3 (6.2) |
| | | Male | 23.6 (4.1) | 183 (6) | 75.8 (10.7) | 20.4 (6.0) | 79.3 (5.0) |
| | Excluded | Female | 22.9 (1.6) | 166 (8) | 64.6 (9.7) | 28.8 (8.7) | 68.6 (9.1) |
| | | Male | 24.3 (1.5) | 189 (5) | 88.2 (22.4) | 24.3 (15.3) | 76.8 (12.7) |
| Study II | Training | Female | 23.4 (2.9) | 168 (8) | 64.0 (9.2) | 30.8 (7.1) | 65.5 (6.8) |
| | | Male | 25.7 (5.8) | 177 (3) | 77.5 (8.0) | 25.3 (3.9) | 71.3 (2.4) |
| | Control | Female | 24.1 (3.5) | 166 (4) | 63.8 (0.6) | 30.5 (6.4) | 66.3 (5.2) |
| | | Male | 25.5 (5.5) | 182 (5) | 76.5 (7.7) | 18.2 (5.1) | 78.7 (4.2) |

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*[193] 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.

## 4.3 Muscle strength assessments

Isokinetic and isometric unilateral knee-extension strength was assessed in a dynamometer (Cybex 6000, Cybex International, Medway USA). Participants were seated and secured in the dynamometer with the knee joint aligned with the rotation axis of the dynamometer. Maximal isokinetic torque was assessed at three angular speeds (60°, 120° and 240° $\times$ sec$^{-1}$). Prior to testing, participants were familiarized with the test protocol by performing three submaximal efforts at each angular speed. Participants were given two attempts at 60° $\times$ sec$^{-1}$ and three attempts at 120 and 240° $\times$ sec$^{-1}$ performed in immediate succession. The highest value was used for statistical analyses. After isokinetic testing, maximal voluntary contraction torque (MVC) was assessed at a knee angle of 30° (full extension = 90°). Participants were instructed to push with maximal force against the lever for 5 sec. Participants were given two attempts, with 30 sec rest in-between. The highest value was used for downstream analyses.

Maximal strength was assessed as one repetition-maximum (1RM) in unilateral leg-press and knee-extension. The test session for each exercise started with a specific warm-up consisting of ten, six and three repetitions at 50, 75 and 85% of the anticipated maximum. Thereafter, 1RM was found by increasing the resistance progressively until the weight could not be lifted through the full range of motion. For each exercise, the highest load successfully attempted was defined as 1RM. Each participant was given four to six attempts.

At baseline, 1RM, isokinetic and isometric strength assessments were performed twice, separated by at least four days. The maximum value achieved for each of the tests was used in subsequent analysis. Strength tests were separated by at least 48 hours from preceeding training sessions. A combined measure of muscle strength was calculated as the average of all tests (1RM, isometric and isokinetic), wherein each test modality was given equal weight. A subset of the participants (n=18) performed strength assessment during the course of the study (at week two, five and nine). For the remaining participants, ordinary training sessions were prioritised when participants missed training or testing due to illness or scheduling difficulties.

Muscle strength was assessed as maximal voluntary isokinetic (90° sec-1) and isometric (60° angle, fully extended leg 0°) knee extension torque. After a brief warm-up (5-min cycling, RPE 12-14), participants were seated and secured in

the individually adjusted dynamometer. Participants were instructed to gradually increase their effort during three warm-up repetitions (50, 60 and, 70% of subjective maximal effort). After a 30-sec restitution period participants were instructed to perform three repetitions with maximal effort in the concentric phase. Sixty seconds after the isokinetic test the lever automatically moved to a 60° angle and participants were instructed to push against the lever enough to see feedback from the visual feedback system. After an additional 15-sec restitution period, participants were instructed to push against the lever with maximal effort. Within the same assessment session, participants remained seated in the dynamometer for measurement perfomed on both legs. The first measurement was alternated between legs every other session. For statistical treatment of the data, all successful attempts were used. The last strength assessment at baseline was performed at least seven days prior to the first biopsy sampling. At least one of the baseline strength tests was performed on separate day with two sessions allowed to be perform on the same day with a short rest between assessments. Post training assessments were performed 48 hours and eight days after the last session.

## 4.4 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, m. vastus lateralis muscle thickness was measured by B-mode ultrasonography (SmartUs EXT-1M, Telemed, Vilnius, Lithuania) using a 39 mm 12 MHz, linear array probe. At least three images were captured for each leg per time-point. Between each image acquisition the probe was relocated to the same position where on the skin and subsequently marked on a soft transparent plastic

sheet superimposed on the thigh.

Muscle thickness of m. vastus lateralis and m. rectus femoris were measured using Transverse images were obtained $\sim$ 60 % distally from the trochanter major towards the femoral lateral epicondyle. Landmarks such as moles and scars were also marked on the plastic sheet for relocation of the scanned areas during post-training measurements. During analysis, pre and post images from the same participant were analyzed consecutively using the Fiji software,66 and by two independent researchers. The average muscle thickness of the three images captured per muscle was used for further analyses.

For determination of body composition participants were scanned using DXA (Lunar Prodigy densitometer, GE Healthcare, Madison, WI, USA) with the standard scanning mode (13-25 cm). Participants were lying supine within the scanning bed reference lines, with a strap secured around the ankles to ensure a standardized body position in each scan. The scans were conducted with participants in a fasted state between 07.00-10.00 AM, with empty bladder and wearing only under-wear. Prior to each scan, a phantom scan was run to prevent baseline drifting from affecting analyses. The same technician was used at each time point. Analyses was performed using GE enCORE version 17.0 software (GE Healthcare). Region of interest was customized for covering upper thigh, marked with a sqaure from pubic symphysis to lateral part of tuberculum major, and distal to art. genu.

Muscle thickness (MT) was measured using a B-mode ultra sound unit (SmartUS EXT-1M, Telemed, Vilnius, Lithuania). Participants lay supine in a relaxed position for 20 min before assessments, with their feet strapped in a standardized position. A mark was set on the line 60% of the distance between Spinia Iliac Anterior Superior and the lateral femur condyle. MT of *m.vastus lateralis* was measured applying a water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission Gel; Parker Laboratories Inc., Fairfield, NJ, USA), and a 39 mm 12 MHz ultrasound probe was placed perpendicular to the site of interest without pressing the skin. When the quality of the image was satisfactory, evident as distinct upper and lower muscle fascia, three images were captured, where the probe was relocated to the same position between each image. Position of the probe was marked on the skin and subsequently marked on a transparent paper to ensure similar probe placement for both the right and left *m.vastus lateralis* at subsequent assessments. Analyses were done in ImageJ Fiji [194] with images cropped and coded to ensure blinding of the assessor.

## 4.5 Muscle tissue sampling and preparations for downstream analyses

Muscle samples were obtained under local anesthesia (Study I, Xylocaine, $10\,\mathrm{mg\,ml^{-1}}$ with adrenalin $5\,\mu\mathrm{g\,ml^{-1}}$, AstraZeneca, Oslo, Norway; Study II, Lidocaine Mylan, $10\,\mathrm{mg\,ml^{-1}}$, 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 [195], 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 [195] 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 $\sim$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 [196], 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 [195,197]. Any reported differences in fiber type distributions between sampling techniques have been suggested relating to differences in sampling depth [197,198].

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 dupliacte samples [199]. In Study I, one or several pieces of muscle (total weight $\sim$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...

### Total RNA extraction

Total-RNA was extracted from pre-weighed, frozen muscle samples with a protocol modified from [200] using Trizol reagent (Life Technologies). Muscle tissue was homogenized in 300 ul 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 ul). The upper phase (400 ul in Study I; 450 ul in Study II) was transferred to a fresh tube and RNA was precipitated using isopropanol (500 ul). 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).

### Protein extraction immunoblotting

Aliquots of muscle-tissue (approximately 25 mg wet weight) were homogenised using a plastic pestle in ice-cold lysis buffer (2 mM HEPES pH 7.4, 1 mM EDTA, 5 mM EGTA, 10 mM $MgCl_2$, 1% Triton X-100) spiked with protease and phosphatase inhibitors (Halt, Thermo Fischer Scientific, Life Technologies AS, Oslo Norway), incubated at 4° for 1 hr and centrifuged for 10 min at 10 000 g and 4°C, after which the supernatants were collected. Total protein concentrations were determined on a 1:10 dilution (Pierce Detergent Compatible Bradford Assay Reagent, Thermo Fischer Scientific). The remaining supernatant was diluted to 1.5 $\mu g \times \mu l^{-1}$ total protein in lysis buffer and 4X Laemmli sample buffer (Bio-Rad Laboratories AB, Oslo Norway) containing 2-Mercaptoethanol. Samples were heated to 95°C for 5 min and stored at -20°C until further processing. During analyses, protein samples (20 $\mu g$ of total protein) were separated at 300 V for 30 min using 4-20% gels (Criterion TGX, Bio-Rad), followed by wet transfer to PVDF membranes (0.2 $\mu m$ Immun-Blot, Bio-Rad) at 300 mA for 3 h. Gel electrophoresis and protein transfer were performed at 4°C. Membranes were then stained using a reversible total protein stain (Pierce Reversible Protein Stain, ThermoFischer Scientific) to ensure appropriate protein transfer. Primary antibodies were purchased from Cell

Signaling Technology (Leiden, The Netherlands): mTOR (mTOR$^{Ser2448}$: #5536; pan: #4517), S6 kinase 1 (p85 S6K1$^{Thr412}$: #9206; p70 S6K1$^{Thr389}$: #9234; pan: #2708), ribosomal protein S6 (rpS6$^{Ser235/236}$: #4858; pan: #2317). Membranes were blocked for 2 h in tris-buffered saline (TBS, 20 mM Tris, 150 mM NaCl) containing 3% bovine serum albumin and 0.1% Tween-20, followed by over-night incubation with primary antibodies targeting either the phosphorylated or non-phosphorylated epitope diluted in blocking buffer, followed by 2 h incubation with secondary horseradish peroxidase-conjugated antibodies diluted in TBS containing 0.1% Tween-20 and 5% skimmed milk. Membranes were washed in TBS containing 0.1% Tween-20 for 6 × 5 min after incubation with primary antibody, and for 8 × 5 min after incubation with secondary antibodies. For rpS6 and mTOR antibodies, following chemiluminescent detection (SuperSignal™ West Femto Maximum Sensitivity Substrate, ThermoFischer Scientific), membranes were incubated with hydrogen peroxide (15 min, 37°C) to inactivate the horseradish peroxidase (HRP), as described by Sennepin *et al.* [201], followed by over-night incubation with primary or secondary antibodies as described above. If the phosphorylated epitope was targeted during the first incubation, antibodies for the non-phosphorylated epitope were used in the second and vice versa. HRP inactivation did not affect the phosphospecific to non-phosphorylated signal ratios. Importantly, as this technique did not involve removing the first primary antibody, antibodies from different hosts (mouse or rabbit) were used for phosphorylated and non-phosphorylated epitopes respectively. As the antibody targeting p70 S6K1$^{Thr389}$ had the same host as the pan-antibody, total-protein was used to normalise chemiluminescent signals. All incubation and washing steps were performed at 4°C using an automated membrane processor (BlotCycler, Precision Biosystems, Mansfield, MA, USA), except for p70 S6K1 experiments, which were performed by hand at room temperature with incubations at 4°C. For mTOR and rpS6, total-protein and chemiluminescence quantification was calculated as the mean value of two separate experiments. S6K1 was quantified once for each phospho-specific antibody. Total-protein content was quantified using ImageJ [202], and was defined as the mean grey value of the whole well with between-well values subtracted as background. Chemiluminescence signals were quantified using Image Studio Lite (LI-COR Biotechnology, Lincoln, Nebraska USA).

Protein was extracted from Trizol preparations according to manufacturers instructions and [203] with modifications. The remaining aqueous phase was removed and DNA was precipitated by the addition of 300 ul of absolute ethanol followed by gentle centrifugation (2000 g, 5 min at room temperature). An aliquot

of the phenol-ethanol phase, corresponding to ~1.75 mg of tissue, was transferred to to a fresh tube. After addition of at least two volumes of isopropanol and incubation (10 min at room temperature), samples were centrifuged (7500 g, 10 min 4°C) and a pellet formed. The pellet was washed three times in 95% ethanol with each wash separated by centrifugation (5000g, 5 min at room temperature). After the last wash all liquid was removed and 45 ul of Kopec buffer [203] was added (5% SDS, 10 mM Tris, 140 mM NaCl and 20 mM EDTA, pH 8; containing protease and phosphatase inhibitors). Pellets were incubated at 50°C for three hours after which the majority of samples were dissolved. Any undissolved material was sedimented by centrifugation (10000 g, 10 min at room temperature). Protein concentrations were measured (Pierce Detergent Compatible Bradford Assay, ThermoFisher Scientific). Sample were normalized in 4X Laemmli buffer (Bio-Rad Norway AS, Oslo, Norway), boiled (95°C, 5 min) and stored at -20°C before later use.

## 4.6 RNA analysis

Complementary DNA (cDNA) was synthesized in technical duplicates from 500 ng of total RNA using random hexemer and anchored Oligo-dT primers (Thermo Fisher Scientific) together with Superscript IV (Thermo Fisher Scientific) according to manufacturer's instruction. qPCR reactions were performed with diluted cDNA (2 ul, 1:25 dilution), a SYBR-green based commercial master mix (PowerUp™ SYBR™ Green Master Mix, Thermo Fisher) and, target-specific primers (500 nM) in 10ul reaction volumes using a real-time detection system (QuantStudio 5 Real-Time PCR System, Thermo Fisher Scientific). Fast cycling was used (1 sec denaturing, 30 sec annealing) after UNG (2 min, 50°C) and polymerase (2 min, 95°C) activation. Melt curves were collected from all reactions to confirm single product amplification. Primers were further evaluated by agarose gel electrophoresis which confirmed primer sizes and non-template control experiments confirming no amplification without template. Primer sequences and their respective average performances are shown in Table 2.

Approximately 25 $mg$ of wet muscle-tissue was homogenised in a total volume of 1 ml of TRIzol reagent (Invitrogen, Life technologies AS, Oslo, Norway) using 0.5 mm RNase-free Zirconium Oxide beads and a bead homogeniser (Bullet Blender, Next Advanced, Averill Park, NY, USA) according to the manufacturer's instructions. In order to enable analysis of target gene-expression per-unit tissue weight, an exogenous RNA control ($\lambda$ polyA External Standard Kit, Takara Bio Inc, Shiga, Japan) was added at a fixed amount (0.04 $ng \times ml^{-1}$ of Trizol

reagent) per extraction prior to homogenisation, as previously described [204, 205]. Following phase-separation, 400 $\mu l$ of the upper phase was transferred to a fresh tube and RNA was precipitated using isopropanol. The resulting RNA pellet was washed three times with 70% EtOH and finally eluted in TE buffer. RNA quantity and purity was evaluated using a spectrophotometer, all samples had a 260/280 $nm$ ratio $> 1.95$. RNA was stored at -80°C until further processing. In the analysis of total RNA content per-unit tissue weight, one sample was excluded prior to analysis due to negative deviation from the expected value based on the relationship between sample weight and RNA content suggesting sample loss in washing steps. RNA integrity was assessed by capillary electrophoresis (Experion Automated Electrophoresis Station using RNA StdSens Assay, Bio-Rad) with average integrity scores (RQI) Five-hundred nanograms of RNA were reverse transcribed using anchored Oligo-dT, random hexamer primers (Thermo Scientific) and SuperScript IV Reverse Transcriptase (Invitrogen) according to manufacturer's instructions. All samples were reverse transcribed in duplicates and diluted 1:50 prior to real-time polymerase chain reaction (qPCR). qPCR reactions were run on a fast-cycling real-time detection system (Applied Biosystems 7500 fast Real-Time PCR Systems, Life technologies AS), with a total volume of 10 $\mu l$, containing 2 $\mu l$ of cDNA, specific primers (0.5 $\mu M$ final concentration) and a commercial master mix (2X SYBR Select Master Mix, Applied Biosystems, Life technologies AS). qPCR reactions consisted of 40 cycles (three seconds 95°C denaturing and 30 seconds 60°C annealing). Melt-curve analyses were performed for all reactions to verify single-product amplification. Gene-specific primers were designed for all targets using Primer-BLAST [206] and Primer3Plus [207] and ordered from Thermo Scientific, except for the external RNA control, for which primers were supplied with the kit. Raw fluorescence data was exported from the platform specific software and amplification curves were modelled with a best-fit sigmoidal model using the qpcR-package [208] written for R [**R-base?**]. Threshold cycles (Ct) were estimated from the models by the second-derivate maximum method with technical duplicates modelled independently. Amplification efficiencies were estimated for every reaction [as described by 209,implemented in 208]. For every primer pair, mean amplification efficiencies ($E$) were utilised to transform data to the linear scale using $E^{-Ct}$. Primer sequences and primer characteristics (i.e. average primer efficiencies and Ct values) are presented in Table 2. Gene expression data was log-transformed prior to statistical analysis. As Ct-values, but not efficiencies are related to RNA integrity [210], RQI scores were used in the statistical treatment of qPCR data to control for potential degradation effects on a by target basis (see

below).

### 4.6.1 Hormonal measurements

Hormone analyses were performed on blood samples collected at five time points: alongside muscle biopsies (Figure 1, four sampling events) and 10 minutes after completion of the fifth training session. Samples were drawn from the antecubital vein into serum-separating tubes and kept at room temperature for 30 min before centrifugation (1500 g, 10 min). Serum was immediately aliquoted and stored at -80°C until further processing. Serum concentrations of total testosterone, cortisol, growth hormone and insulin-like growth-factor 1 (IGF-1) were measured on an Immulite 1000 analyzer, using kits from the Immulite Immunoassay System menu (Siemens Medical Solutions Diagnostics, NY, USA), performed according to manufacturer's protocols. Serum Vitamin D (S-25-OH-D) levels were measured in samples collected before and after the intervention using a electrochemiluminescence immunoassay (Roche Cobas Vitamin D total assay, Roche Diagnostics GmbH., Mannheim, Germany) using automated instrumentation (Roche Cobas 6000's module e601, Roche Diagnostics GmbH., Mannheim, Germany).

## 4.7 Statistics and data analysis

TO DO:

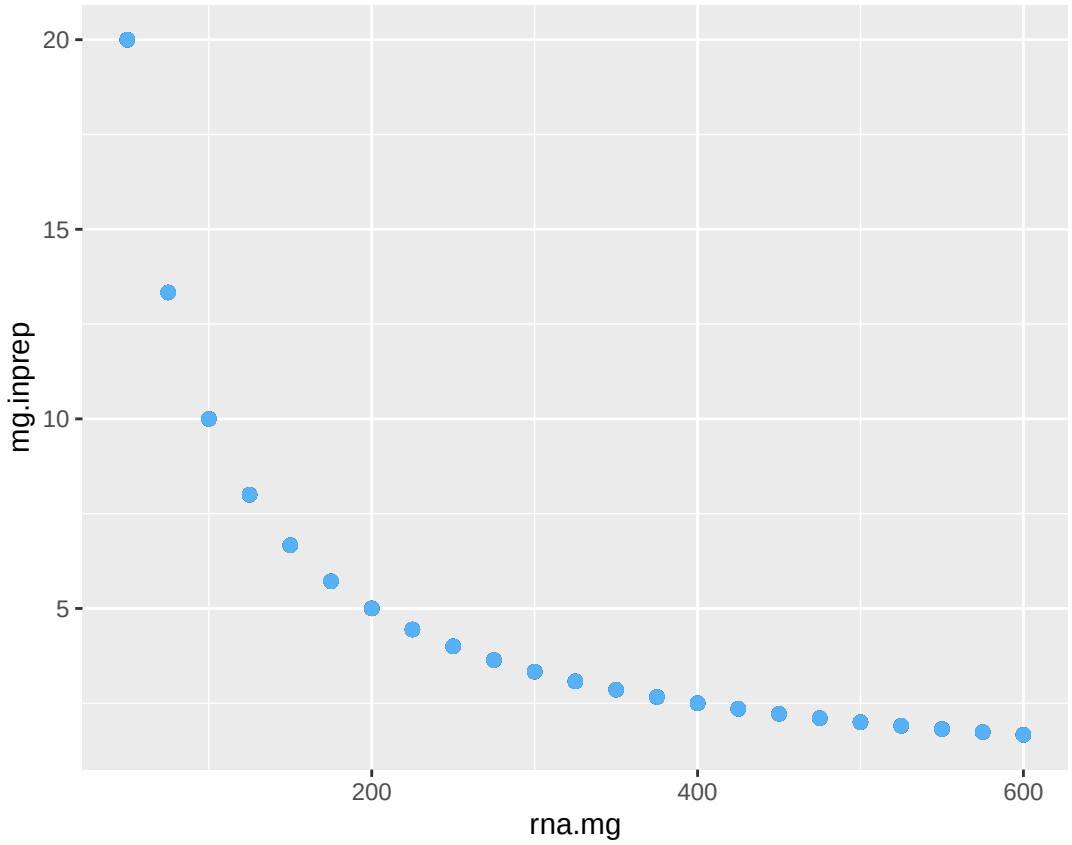
- For methods discussion, compare product length, efficiencies and ct values in relation to RQI-values. See Fleige 2006 for reference.

### 4.7.1 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.

```
# 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
```



## 4.8 Meta-analysis of within-session training volume

### 4.8.1 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.

### 4.8.2 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 [211].

# 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 [212, 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 [103, 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) | |
| | | MOD | 0.07 (0.05) | |
| | Male | LOW | 0.05 (0.05) | 0.11 [0.04-0.26]a |
| | | MOD | 0.07 (0.05) | |
| CSA %-change session | Female | LOW | 0.11 (0.13) | |
| | | MOD | 0.18 (0.15) | 0.08 (0.22)b |
| | Male | LOW | 0.14 (0.12) | |
| | | MOD | 0.19 (0.13) | 0.14 (0.14)b |
| 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) | |
| | | MOD | 1.00 (0.49) | 0.67 (0.35)b |
| | Male | LOW | 0.72 (0.48) | |
| | | MOD | 0.87 (0.46) | 0.47 (0.22)b |

[a] Estimates from Wernbom et al. [212]
[b] Estimates from Ahtiainen et al. (ref:ahtiainen-citation

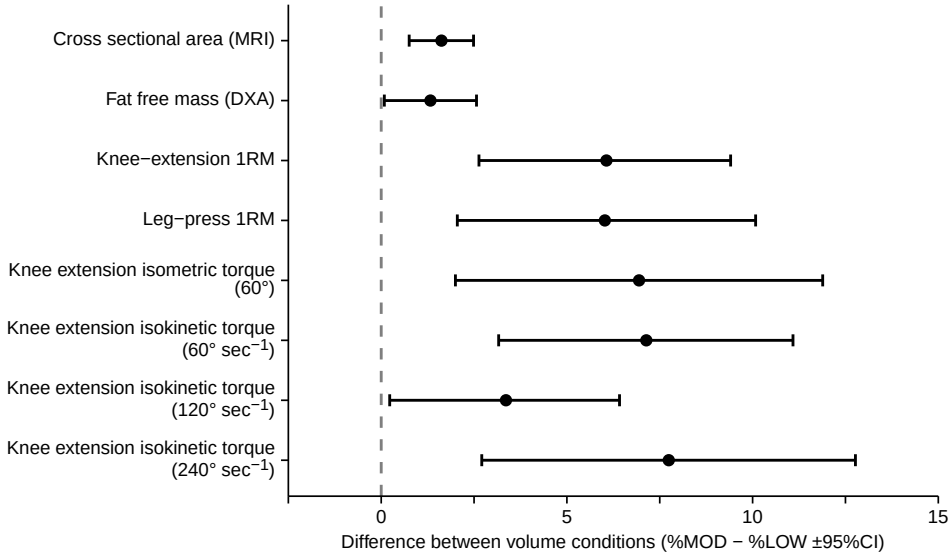



**Figure 5.1:** Differences in training induced relative changes in muscle mass and strength measures. Estimates are derived from ANCOVA models controling for baseline values and sex.

## 5.2 Acute effects of diffrent training volume on determinants of muscle protein synthesis

# 6. General 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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