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Sjur Fortun Øfsteng

Exploring the influence of resistance training volume, intensity, and diet on skeletal muscle adaptations

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To Signe & Lykke

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Abstract

Background and Aims. Progressive resistance training programs continue to stand out as the cornerstone for enhancing both maximal strength and muscle mass in individuals. The varied muscle adaptations observed in individuals undergoing resistance training highlight the nuanced impact of training variables and the influence of biological predisposition shaping these adaptations. As a result, the degree to which individuals strength gains and muscle growth respond to training variables, such as number of sets (volume), varies. In some environments, particularly the military, maximal strength and power are increasingly recognized as important components of military performance. However, given the exposure of military personnel to various stressors in their daily routines, including field exercises that may compromise muscular adaptations, it is important to implement optimal training strategies aiming to enhance and sustain muscle strength while mitigating unfavorable changes in body composition.

Study I aimed to explore the effects of low and moderate resistance-training volume on muscle strength and hypertrophy and its association with individual biological characteristics using untrained individuals (Paper I). In Study II, the aim was to explore the temporal patterns of markers associated with ribosomal biogenesis in response to resistance training and to examining how variations in training volume may induce differences in these markers (Paper II). In Study III, we aimed to compare various resistance-intensty strategies incorporated as a part of the soldiers regular training regimen on muscle strength, hypertrophy, and performance in moderately trained soldiers (Paper III). Furthermore, as a part of soldiers regular training regime, we investigated in Study IV whether protein supplementation could mitigate losses of muscle mass and muscular performance during a strenuous military field exercise undergoing severe energy deficit and whether the soldiers could fully recover within a week following the exercise (Paper IV).

Methods. In Study I, 34 individuals (avg. age: 22) underwent 12 weeks of contralateral low (1-SET) and moderate (3-SET) volume resistance training in three lower body exercises. Muscle cross-sectional area (CSA) and maximal strength were measured at baseline and 12 weeks, along with muscle biopsy sampling (m. vastus lateralis), which was also sampled pre- and post-fifth training session in Week 2. In Study II, eleven individuals (avg. age: 24) performed 12 sessions of unilateral knee extension, performing either a variation in training volume or constant volume. Muscle biopsies were taken bilaterally before and 48 hours after the first (baseline), fourth, fifth, eighth, ninth, and twelfth sessions and after eight days of de-training. A non-training control group (n=11) had biopsies at baseline, 48 hours, and 3-5 weeks later. Muscle strength, lean mass, and muscle thickness were measured at baseline, after 12 training sessions, and following the de-training period. In Study III, 27 cadets (avg. age: 20) performed a prolonged 22-week whole-body resistance-training program (7 exercises), performing either 10 repetitions maximum (RM) or 30RM. Muscle strength and mass (DXA) and performance were assessed at baseline and week 22 for the upper and lower limbs, in addition to a mid-intervention assessment at week 10. Biopsy sampling (m.vastus lateralis) was also conducted at the three time points. In Study IV, 38 cadets (ave. age: 21) were recruited and randomly allocated to ingest 1 or 2 g kg^{-1} d^{-1} protein in an isocaloric manner ($\sim$ 15 kcal kg^{-1} d^{-1}) resulting in a severe energy deficit during the 10-day field exercise. Outcome measures of hormone levels,

muscle strength, and performance were assessed before and following the 10-day exercise as well as after seven days of recovery. Changes in muscle mass (DXA) were assessed at baseline and directly after the 10-day exercise.

Results. In study I, 12 weeks of 3-SET training led, in general, to greater strength and muscle mass gains compared to the 1-SET training. These gains were accompanied by increased activation of muscle biomarkers associated with the mTORC1 signaling pathway and a higher abundance of markers linked to ribosome biogenesis. For 13 and 16 of the 34 participants, a distinct advantage was seen for 3-SET over 1-SET for hypertrophy and strength gains, respectively. This benefit was linked to enhanced accumulation of total RNA at Week 2 in the 3-SET leg. In Study II, the three-week short training period, incorporating twelve resistance training sessions, resulted in muscle growth and strength gains within the training group compared to the non-training control group. The initial four sessions led to concomitant increases in total RNA and ribosomal RNA (indicating ribosome biogenesis) before plateauing at $\sim$ 50% above baseline for the remainder of the training period and decreased after eight days of detraining. Upstream binding factor protein levels were associated with increases in total RNA levels, and the increase in total RNA per session predicted muscle hypertrophy. In Study III, implementing a 22-week systematic resistance training program into the regular military training regime of cadets, 10RM training led to greater increases in lower and upper-limb muscle strength, jump height, and upper-limb lean mass compared to 30RM. The two training groups led to similar improvements in sprinting performance and lower-limb muscle mass. Study IV, military cadets undergoing severe energy deficit during the 10-day military exercise, ingesting a higher protein intake (2 g kg^{-1} d^{-1}) did not attenuate losses in body mass, muscle strength, and power compared to lower protein intake (1 g kg^{-1} d^{-1}). Surprisingly, no change was seen for lean body mass. In response to the severe energy deficit during the exercise, both groups experienced alterations in the anabolic/catabolic interplay, leaning towards a cellular catabolic milieu. Following seven days of recovery with ad libitum feeding, most variables returned towards pre-exercise levels, except for counter-movement jump.

Summary. The thesis demonstrates a resistance training volume-dose relationship with muscle growth and strength. It further emphasizes the determinant role of ribosomal biogenesis in resistance training-associated adaptation. The initial accumulation of ribosomal biogenesis serves as a predictive factor for long-term training adaptations, with the benefit of 3-SET training compared to 1-SET training. Moreover, RNA abundance reaches peak values within eight resistance training sessions with a relatively high training volume, and ribosomal biogenesis is sensitive to training cessation.

The thesis further underscores the impact of long-term systematic resistance training in a military training environment. Twenty-two weeks with 10RM training stand out as the preferred resistance training modality in moderately trained cadets, achieving in general superior gains in muscle strength, growth, and performance compared to 30RM training. Furthermore, the ingestion of either a higher protein intake of 2 g kg^{-1} d^{-1} or a lower protein intake of 1 g kg^{-1} d^{-1} in the cadets diets during the demanding field exercise with severe energy deficit did not result in differences between supplementation groups in muscle performance or in body mass composition.

List of papers

The thesis is based on the following research papers:

I. Hammarström, D., **Øfsteng, S.**, Koll, L., Hanestadhaugen, M., Hollan, I., Apro, W., Whist, J. E., Blomstrand, E., Rønnestad, B. R., & Ellefsen, S. (2019). Benefits of higher resistance-training volume are related to ribosome biogenesis. *The journal of Physiology*, 1-23.

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Chapter 1

Introduction

In the ever-evolving world of fitness and exercise science, resistance training[1] repeatedly stands out as a powerful tool for enhancing muscle strength, size, and overall health. There is compelling evidence that a higher level of strength and muscle mass can reduce the risk of all causes of mortality (2). Conversely, physical inactivity (3) and periods of catabolic stress (4,5) are accompanied by loss of functional capacity, bone, and skeletal tissue. Exercise[2] alone or in conjunction with nutritional interventions are components that can improve and ameliorating these adverse physiological changes (7).

Exploring the responses of acute and chronic exercise and understanding the molecular mechanisms involved has facilitated the connection of various molecular pathways associated with skeletal muscle adaptations to diverse training variables and metabolic disorders. Delineating the mechanism by which exercise training alters human physiology will lead to identifying molecules, pathways, and, ultimately, new treatments that confer the benefits of exercise. However, individuals exhibit different responsiveness to resistance training regarding muscle mass and strength gains (8,9). This variability poses a significant challenge in formulating standardized training recommendations that can be universally applied. While resistance-training-induced gains in muscle strength and muscle hypertrophy are affected by gender, age, and training background, including genetic and epigenetic underpinnings of muscle growth capabilities, also training duration and resistance training variables are influencing the observed variability in individuals response to training (10–12). Thus, the complexity of how people react to resistance training underscores the need for personalized approaches to optimize outcomes. As we strive to design effective resistance-training programs, it becomes essential to consider each individuals distinct characteristics and responses, involving shifting away from a universal training approach towards more individualized training strategies. This personalized approach aims to ensure that the exercise program is tailored to the persons unique needs, promoting optimal improvement or maintenance of healthy muscle function throughout their life.

The effectiveness of resistance training can be optimized by strategically adjusting various training variables, including but not limited to frequency, intensity, and volume (13). Furthermore, biological predispositions, such as genetic variation, epigenetic

[1] Resistance training can be defined as repeated exercises requiring the neuromuscular system to exert force against resistance, to increase or maintain a muscle force (1)

[2] Exercise defined as activity that is planned, structured, repetitive, and purposive in the sense that the improvement or maintenance of one or more components of physical fitness is the objective (6)

(12,14), and more modifiable components related to behavior-environmental factors (10), all together affect an individuals trainability. When considering an individuals physical activity level and the varying responsiveness of different muscle groups (such as upper versus lower body) to strength and muscle mass adaptations, it becomes clear that multiple factors influence how effectively someone can respond to training (15). These factors collectively impact an individuals trainability and, consequently, shape the outcomes of their training efforts.

The interplay between genes and the environment in shaping the phenotypes is becoming increasingly relevant in the context of determining training responses (16,17). This information becomes particularly valuable when the underlying biological mechanisms are well understood, as it can enable the use of genetic/biological information to inform and tailor training methodologies (18). This raises the possibility that certain inherent characteristics may serve as moderators, influencing long-term responses to training (19). Exploring these factors could provide valuable insights into predicting and optimizing resistance training outcomes throughout an individuals training progression.

Within the plethora of training variables, the intensity and volume of resistance training have traditionally been considered the most crucial adjustable factors for optimizing strength and muscle mass (20). For example, the modification of training volume influences specific molecular markers that determine muscle hypertrophy in a dose-dependent manner (21–23). These effects are thought to contribute to long-term training outcomes, as programs with higher volume generally lead to more significant gains in muscle mass and strength (24–26). Furthermore, a dose-response concept between higher resistance intensity load[3] and stimulation of muscle protein synthesis has generally been suggested and necessary to maximally facilitate muscle (fiber) hypertrophy (27). This is based on the notion that progressive overloading, utilizing high load, is necessary for maximal fiber recruitment (13,20), consequently activating high threshold fiber type II, a muscle phenotype believed to be more primed for hypertrophic adaptations (19,28,29). Such a concept may be true for maximal strength but does not necessarily be true for muscle mass adaptations (30).

While various assessment methods (i.e., DXA and MRI) provide valuable insights into exercises impact on body composition and muscle morphology, the weak correlation between pre- and post-training change scores across methods poses challenges in interpreting training effects within and across studies (31). Coupled with the variability in strength and muscle adaptability associated with individual training characteristics (13,32), distinct muscle groups (15), and aging (9,33), a comprehensive understanding of the impact of various measurement methods and physiological determinants is crucial for evaluating and developing nuanced, effective resistance training programs.

Resistance training serves as a stimulus that disturbs muscle cells' homeostasis, thereby prompting muscle strength and mass responses. This disruption, however, is not solely attributed to resistance training alone; various other factors can also influence it. For instance, endurance training (34), nutritional considerations (35), and caloric restriction (36) can all contribute to cellular disturbance and subsequent trigger muscular signaling, mediating specific adaptations. Consequently, stress periods with reduced energy intake or caloric restriction, sleep deprivation (factors commonly experienced during military field exercises), and even prolonged physical inactivity create a catabolic environment leading to adverse changes in body

[3]Load is the amount of weight lifted

composition and strength, ultimately reducing muscular performance and decreased metabolic responses (4). In such conditions, nutritional supplementation (i.e., protein) can be a relevant strategy to mitigate negative physiological consequences (37) and sustain, i.e., military readiness (38).

Chapter 2

Background

2.1 The benefit of resistance training for health, sport, and occupation

The prime function of skeletal muscle is to maintain the integrity of our skeleton and generate force and power, enabling the completion of everyday tasks that underpin independence (39). In recent times, there has been a growing focus on the issue of age-related muscle loss (40) and its related health concerns, including bone loss (osteopenia), metabolic decline, fat accumulation, diabetes, and overall increased risk of mortality (40,41). With the rising prevalence of sarcopenia in a population that is becoming more sedentary and older, coupled with mounting evidence demonstrating the effectiveness of resistance exercise in promoting muscle development across all age groups (42), it highlights the need for a health initiative encouraging participation in resistance training.

Adults across all age groups who engage in 2-3 resistance training sessions weekly for 2-3 months demonstrate a simultaneous increase in muscle mass and reduction in body fat (42). An increased muscle mass necessitates more energy at rest for tissue maintenance, thereby raising resting metabolic rate (increased protein turnover) and augmented energy expenditure (43). Since muscle tissue is the primary site for glucose and triglyceride disposal, greater muscle mass in response to resistance training may reduce the risk of glucose intolerance and associated health issues, such as diabetes, obesity, or cardiovascular diseases (44). Notably, indications propose that resistance training regimens integrating elevated volume and intensity protocols might yield greater efficacy in enhancing insulin resistance and glucose tolerance than outcomes associated with lower-volume and lower-intensity exercise protocols (44). Therefore, selecting an appropriate training protocol becomes crucial in attaining the optimal training outcomes aligned with a specific goal. Moreover, resistance training is often associated with enhancing muscular power and the rate of force development, factors frequently acknowledged for contributing to success in a wide range of sports (45). Indeed, in endurance-oriented sports, the advantage of resistance training has been shown to improve factors such as movement economy and delay the onset of fatigue, ultimately improving endurance performance (46). Beyond sports, occupations like the military prioritize strength and muscle hypertrophy to augment military readiness and prevent skeletal muscle injuries (47). As such, maximizing strength gains and muscle hypertrophy is highly desirable across diverse groups of individuals, serving both health and competitive objectives.

2.2 Influencing factors for muscle strength gains and hypertrophy

2.2.1 Resistance training variables and exercise environment

Optimal results from resistance training depend on the manipulation and combination of several variables involved in its prescription. Indeed, adjusting acute training variables (i.e., intensity, volume, frequency, rest period) can enforce differences in mechanical and metabolic stress in the skeletal muscle (13,48,49). During muscle contraction, the mechanical and metabolic stress signals translate into molecular processes that drive physiological responses and subsequent muscular adaptations. These signaling cues activate or repress specific signaling pathways that govern exercise-induced gene expression and protein synthesis/degradation, ultimately determining subsequent muscle growth (50,51). Therefore, the mechanical and metabolic stress induced by manipulating acute training variables are essential stimuli promoting muscle hypertrophy and increasing strength.

One important stimulus for exercise-induced muscle growth is training volume[1]. Indeed, when the volume is held constant, manipulation of other variables (e.g., frequency, set rest, load) seems to have little or no effect on hypertrophy (54,55). Recent advancements in molecular biology and muscle physiology have highlighted the complex relationship between training volume and the molecular markers that drive muscle adaptation and growth. Current training guidelines for hypertrophy and strength recommend the performance of 1-3 sets per exercise for novice individuals, with a higher volume of 3-6 sets per exercise advised for advanced lifters (13,53). These guidelines, as mentioned earlier, are based on the perceived presence of a dose-response relationship between volume and muscle growth, with higher volume eliciting greater hypertrophic and strength gains (24,25,56). However, the relationship between volume conditions and strength and muscle mass development is inconsistent in various studies (57). There are, for example, observed differences in strength development in older individuals (58,59), but they are not apparent in another study (60). Moreover, an increased training volume does not consistently result in improved muscle mass gains in younger individuals (61,62), a finding that others have refuted (15,63). Additionally, are there indications of different responses to training volume between upper and lower body. While greater muscle growth and strength appear in the lower body with increased training volume, this volume dependence is not as evident in the upper body (15,64).

The exercise intensity[2], significantly influences both functional and biological adaptations in skeletal muscle (13). Current guidelines, applicable to both untrained and resistance-experienced individuals, advocate for a high resistance intensity (>65% 1 repetition maximum; 1RM) with a moderate repetition range (6-12 repetitions) as the optimal strategy for building muscle strength and hypertrophy (13,20). However, in recent years, the high-intensity paradigm of resistance training (>65% 1RM (13)) has been challenged (30,65,66). Alternative approaches such as low-intensity training (30-50% 1RM) to failure (67) have been associated with similar muscular responses,

[1]Volume defined as total work: sets × repetitions × load or as a number of sets performed per exercise (20,52,53).

[2]In training studies resistance training intensity is usually defined as a function of 1 repetition maximum (1RM) in the exercise performed (i.e., 90% of 1RM), or a specific target repetition goal (i.e., 10 RM)(20). In the present thesis resistance training intensity is defined as target repetition, nRM, where higher intensities correspond to a lower nRM, and conversely, lower intensities correspond to higher nRM.

including both maximal strength (67,68) and muscle hypertrophy (68–72). Moreover, has utilizing low loads combined with blood flow restriction demonstrated comparable effectiveness in eliciting both strength and hypertrophic responses compared to the traditional high-load paradigm (73,74). Even fiber-type specific adaptations across resistance load (75,76) and with restricted blood-flow (74) are to some extent observed but remain inconclusive (77).

Recognizing the importance of diverse resistance training variables is crucial in various environments, as different training protocols can optimize outcomes for distinct groups of individuals. This adaptability proves especially vital in military contexts, where strategically employing specific training variables becomes important in enhancing the physical readiness and performance of military personnel faced with unique and demanding scenarios (78). In the military environment, resistance training tends to improve physical capabilities such as strength, speed, power, and agility, accompanied by increased lean body mass (79–82), all of which are imperative for a soldiers military performance (78,83). However, the military setting does not consistently observe the benefits of resistance training. (84,85). This lack of consensus may be related to a simultaneous focus on aerobic training, as well as the nature of military-training regimes, which typically include exhausting field operations, leading to a complex range of concurrent physiological stressors that may compromise specific adaptations and hence trainability (82,84,86). While this complexity emphasizes the importance of incorporating resistance training into the annual training routines of soldiers, allowing maintenance of physical capacity throughout the year (80,86), it also emphasizes the need for identifying efficient resistance training strategies that can be performed during deployments.

Consequently, exercise coaches and clinicians should take into account the diverse range of responses in exercise performance parameters during training interventions. Additionally, when designing training programs, it is crucial to consider various exercise variables, as they may yield distinct physiological outcomes, and there is likely a need for increased training intensity and volume as a consequence of increased training experience.

2.2.2 Biological determinants for muscle adaptations

Responses to exercise training varies among individuals, as some individuals respond well to training, while others respond poorly, even when accounting for sex and age (9,87). This large between-subject variability observed in response to resistance training is partly determined by heritability, accounting for $\sim$ 50% of variability in strength-related outcomes and even greater for muscle mass ($\sim$ 80%) (88). Indeed, over 45% of variance in fiber type proportion is explained by genetic variation (i.e., Alpha actinin 3) (89), giving the genetic component a determinant role in individuals muscle strength capacity. Genetic factors apparently have a powerful influence on how people respond to resistance training. Thereby exploring the relationship between resistance-training variables and inter-individual responses using transcriptome and proteom profiles might augment the understanding of underlying mechanisms determinant for trainability (12,14,90).

2.2.2.1 The mTOR pathway a central signaling hub

Responsiveness to resistance training, specifically in terms of muscle strength and growth, is closely associated with cellular signaling pathways regulated by the mechanistic target of rapamycin complex 1 (mTORC1) (91–93). In particular, the mTORC1 is capable of sensing diverse signals from growth factors, nutrients, mechanical stimuli, and energy status of the cell, leading to a multitude of responses, including enhanced transcription, mRNA translation, and subsequent regulation of cellular growth (94–96). These upstream signaling cues follow intra-cellular signaling pathways activating mTORC1 directly or independently (through Akt/protein kinase B, extracellular-signal-regulated kinase 1/2, ERK1/2 and ribosomal S6 kinase, RSK1) by phosphorylation and inactivating the negative regulator tuberous sclerosis 1/2 (TSC1/2) (95). The best-characterized downstream function of mTORC1 is the control of mRNA translation activity through eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), in conjunction with activation of ribosomal S6 kinase (S6K) with its cytosolic (p70S6K) and nuclear (p85S6K) complexes. Phosphorylation of 4E-BP1 detaches it from eIF-4E and allows the small ribosome to bind to the mRNA and subsequently initiate translation (50). The activation of p70S6K1, with its substrate ribosomal protein S6 kinase 1, further leads to translation and elongation through targets such as rpS6 and eucaryotic translation initiation factor 4B (eIF4B) (50,94,97). In response to acute resistance exercise, a correlation between phosphorylation of p70S6K and fractional protein synthesis has been demonstrated (21), presumably crucial for the hypertrophic process. Indeed, resistance training is known to cause a substantial increase in fractional synthesis rate within 3 hours after exercise that is maintained up to 48 hours after training in humans (98,99), and persist up to 72 hours (100) combined with amino acids (101).

Relating the aforementioned signal transduction pathways with resistance training variables reveals a dose-response effect dependent on volume. Higher inter-session volume lead to elevated protein synthesis one hour after leg-extension exercise and sustained S6K1 phosphorylation for up to four hours (33). Furthermore, a single session of either one or three sets observed elevated myofibrillar protein synthesis, especially with three sets, at five and 29 hours, wherein volume-dependent regulation of S6K1 occurred only at 29 hours, and there was no clear volume dependency observed in rpS6 (21). Terzis et al. (22) observed a volume-dependent upregulation of p70S6K1 and rpS6 following 1, 3, and 6 sets. Notably, there was no distinct difference between 1 and 3 sets, thereby challenging the notion of volume-dose dependency, particularly at lower volumes. This aligns with Mitchell et al. (76), who reported equal p70S6K1 phosphorylation between 1 and 3 sets. Increasing training volume to ten sets, Ahtiainen et al. (23) demonstrated a higher level of phosphorylation in p70S6K1 and rpS6, observed 30 minutes after the cessation of exercise, compared to five sets. These signaling events indicate that the activation of translational machinery during exercise is dependent on volume, and a higher volume may, in fact, result in increased muscle protein synthesis (21,33).

However, as individuals get accustomed to the training stress, overall protein synthesis is attenuated (102). A response likely resulting from the diminishing novelty in exercise stimulus with training, indicating a reduced anabolic effect of training over time (103). This emphasizes the uncertainty of using a singular measurement of muscle protein synthesis in an untrained individual to predict an individuals hypertrophic potential after a period of resistance training. This notion finds support in training studies, where a lack of correlation is observed between the acute initial measurement

of protein synthesis and subsequent muscle hypertrophy (104,105). This also highlights that muscle growth through the accumulation of new contractile proteins in response to chronic resistance training is not only determined by the efficiency of existing ribosomes but might also be a consequence of enhanced translational capacity.

2.2.2.2 Ribosome biogenesis

The ribosomes constitute the universal translational apparatus responsible for decoding genetic information into proteins. While ribosomes are linked to cellular growth and proliferation, their number fluctuates depending on the cells physiological state (106). This is because the synthesis of the ribosomes is an energy-consuming biosynthetic process involving multiple factors under tight regulatory control to avoid unnecessary energy expenditure (106). The functional ribosome is composed of ribosomal ribonucleic acids (rRNA) and ribosomal proteins. The synthesis of ribosomal RNAs is initiated in the nucleous of the eukaryotic cell, where RNA polymerase 1 (Pol I) carries out the transcription of pre-rRNA (47S pre-rRNA) from repeats of the ribosomal deoxyribonucleic acid (rDNA) gene. Transcription produces the pre-rRNA as a single long precursor that includes specific rRNAs for the small (18S) and large (5.8S, 28S) ribosomal subunits, separated by internal and external transcribed spacers. Several modification steps involving factors such as ribosomal proteins and exo-/endonucleases enzymes then process the primary 47S pre-RNA into mature 18S, 5.8S, and 28S (107). The first step begins with cleavage off and is followed by a rapid degradation of the 5‘ external transcribed spacer of the pre-RNA (cleaved off to 18S rRNA = 40S small ribosomal subunit) (108). This rapid clearance and modification step thus makes it possible to estimate the rate of Pol I-mediated transcription. Consequently, if the transcriptional activities of Pol I increase relative to the total RNA synthesis within a growing cell, it indicates increased synthesis of ribosomes. The last RNA component, 5S rRNA, complementing the large ribosomal subunit (60S), is, however, transcribed independently by RNA polymerase III. Together with the transcription of ribosomal proteins by polymerase II, all three polymerases are involved in the synthesis of ribosomes. The preribosomal subunits are then actively exported to the cytoplasm, where they bind to the mRNA to form functional 80S ribosome ready to decode the genetic information by translating mRNA into proteins (106,108).

Initiating rRNA transcription requires the presence of various regulatory elements. Within the transcribed segments (rDNA repeat), the regulatory region upstream of the transcription initiation site encompasses the promoter element region and the upstream control region. The initiation of rDNA transcription involves the initial binding of transcription factors to these regions, specifically the upstream binding factor (UBF) and the selectivity factor 1 (SL1) complex, which includes the TATA-binding protein. These factors, along with the recruitment of the transcription initiation factor TIF-1A by SL1, collectively facilitate the recruitment of RNA Pol I, culminating in the formation of the preinitiation complex and the initiation of Pol I-driven rDNA transcription (108). This activation is partly modulated by mTORC1, with increased mTOR activity increasing the UBF availability (109). UBF is, however, considered a master regulator of rDNA transcription, by its direct role with the promotor region of rDNA during cell proliferation, in addition to its role in recruiting and stabilizing SL1 (107). This highlights UBF's integral function in modulating the regulation of rDNA transcription. However, multiple signaling pathways, including Ras-Raf-Erk, Rb-p53, and Myc, are reported to regulate rRNA transcription. (110). ERK signaling has been shown to induce immediate up-regulation of rDNA transcription via TIF-1A

and UBF modification in response to growth factors (106,111). c-Myc also emerges as an essential factor for rDNA transcription (110,112) by directly binding to rDNA loci. Here, it activates the transcription of rRNA both through remodeling chromatin structure (i.e., opening the chromatin available for transcription), facilitating rRNA transcription, and interacting with Pol I cofactors to the rDNA promoter region (113). Conversely, suppressor protein p53 may again suppress c-Myc activation inhibiting rRNA transcription (106).

Human resistance training studies support ribosomal biogenesis role in achieving increased translational capacity and facilitating muscle hypertrophy. For example, following eight and twelve-week resistance training interventions, heightened levels of total RNA were associated with muscle growth in untrained individuals (114–116). Moreover, participants identified as modest and extreme responders to muscle growth exhibit a substantial increase in ribosomal RNA and total RNA after a short four-week (93) and a prolonged 12-week training period (116). In contrast, such enhancements are not prominent in low responders. However, the time course of ribosomal transcription and accumulation in response to human resistance training remains more elusive. Although in a 6-week training study, total RNA peaked after nine sessions (3 weeks), followed by a slight decline at 18 sessions (117). This implies a relatively rapid initial accumulation of ribosomes that peaks in the early 3-week phase of resistance training, followed by a gradual decline in conjunction with increases in muscle mass (117).

An increase in the number of myonuclei has been observed in conjunction with high versus low responders in resistance-training-induced muscle growth (93,118). In studies, the addition of myonuclei through the fusion of satellite cells into muscle fibers has been suggested as necessary to support the expanding cytoplasmic area, maintaining the myonuclear domain in the growing muscle fiber (119,120). The increase in muscle cell transcriptional capacity through myonuclear addition may thus provide more rDNA templates available to facilitate ribosome biogenesis (93), thereby enhancing protein synthesis in high responders to resistance training (118,121). However, muscle growth is also accompanied by the absence of myonuclear accretion. Up to a certain hypertrophic threshold (118,119), the myonuclear domain size is suggested to be flexible in increasing the transcriptional efficiency (yielding a reserve capacity) of resident myonuclei (122,123). Although the temporal pattern of satellite cell activation in a growing muscle fibers is debated (124), the initial muscle growth in response to resistance training may not to be limited by satellite cell activation. However, myonuclear accretion through satellite fusion might be necessary to facilitate and support continued long-term muscle growth (125).

When considered collectively, the process of ribosome biogenesis is a multistep process regulated by various signaling pathways. The coordination of these pathways ensures a finely tuned control mechanism for synthesizing ribosomes, essential for cellular functions and responses to environmental stimuli. Moreover, the association between total RNA and muscle growth suggests that translational capacity might be a determining factor driving skeletal muscle growth.

2.2.3 Resistance training efficacy

For healthy individuals engaging in consistent resistance training, muscle growth evolves essentially linear with time over six months (126), and muscle mass can be expected to increase 5-20% within this time frame in individuals unfamiliar to resistance training (9,20). While these muscle adaptations have shown to be most active during

the early phases of resistance training (3-12 weeks), at which point continued increases are considerably slower in conjunction with the increasing training experience (127). Hence, more resistance-trained individuals exhibit varied responses compared to untrained/novices in resistance training (32). A 'ceiling effect' poses a challenge for trained lifters in increasing muscle mass and strength, prompting the need for more rigorous resistance training protocols to stimulate further muscular gains. As individuals age, a higher training stimulus also becomes necessary to maintain training efficacy, potentially linked to the development of muscular anabolic resistance (128). Consequently, the hypertrophic response is expected to be reduced with aging (27,129,130). However, engaging in exercise and ensuring adequate nutrition, particularly protein intake, may facilitate muscular adaptations in the aging population (131). Training effectiveness may also vary between sexes, with the differences in muscle hypertrophy being small yet discernible (132–134). Although females have demonstrated greater relative strength gains than males (11), especially for the upper body (134). After whole-body resistance training, there are further indications of different responsiveness and development between the upper and lower bodies (133). When loading patterns are similar, one can anticipate that the growth of upper-body muscles will be more evident than that of lower-body muscles (20). This discrepancy possibly relates to the greater habitual activation of lower-body muscles during daily living activities, and they respond less to a given overload stimuli, thereby requiring a more significant stimulus for adaptations (135).

For individuals that are new to resistance training, the relative increase in strength often surpasses the relative increase in muscle size within the initial 4-6 weeks, wherein minimal hypertrophy is typically observed (11,136). During this initial period of resistance training, muscle hypertrophy may explain only 2-28% of the variability in strength improvement (9,137). While shorter studies suggest that strength primarily depends on neurological adaptations (e.g., motor unit firing rates) to resistance training before hypertrophy contributes more substantially in the longer term (138), this time course is more complex (139). This suggests that factors beyond muscle size, including adaptations in muscle connective tissue (100), architectural changes (38), and morphological alterations (140), are also results of systematic training. On an individual level, all these variables can exhibit variability in their contributions to muscle strength relative to muscle size, operating independently of muscle size itself. Hence, the necessity of muscle growth for strength improvements is debated (141), and on a group level, the correlation of these variables can be a result of measurement error/random biological variability (142), rather than physiological adaptations per se. However, it is crucial to note that various measurement techniques come with different measurement errors. Thus, detecting small changes in muscle mass in response to exercise may be limited by the measurement variability itself. This could impact the identification of individuals as responders to resistance training protocols and the relationship between muscle growth and strength. Hence, observing the variability across individuals and studies may result from differences in how strength and muscle size are measured. Subsequently may the varying agreement among different surrogates of hypertrophy (31), hinder the establishment of consensus on the efficacy of different training study outcomes and, consequently, overall trainability. The contribution of muscle hypertrophy to strength gains may depend on the strength task assessed, e.g., isometric, dynamic, or explosive strength. This means that the improved strength gains may be due to skill acquisition (i.e., technique) connected to performing training exercises that more greatly resemble test procedures (76,143). This suggests the importance of incorporating multiple measures for both strength

and hypertrophy when evaluating the impact of any resistance training intervention on muscle strength performance (143), and gains in muscle mass (31). This approach becomes particularly valuable when values from different assessment methods align linearly with the predictor variables (strength and muscle mass). Such alignment can help capture a substantial portion of the variance and enhance the interpretation of the efficiency of various training protocols.

The training efficacy and outcomes of training protocols are directly influenced by the methods employed, the characteristics of training participants, and the assessments used. Therefore, interpreting the outcomes of training studies can be challenging due to the substantial variation in responses observed among individuals, experimental groups, and across studies. While a high degree of variability is common among participants in training studies (11,71), it may negatively affect a study's statistical analysis and data interpretations. In theory, within-participant designs should alleviate some of these limitations. It stands out as a powerful model to explore muscle growth and strength determinants. It efficiently diminishes inter-individual differences through statistical modeling, thereby reducing variability and enhancing the interpretation of biological changes, such as those observed at the translational and transcriptional levels. This assumption is based on the premise that muscle morphology is reasonably well-matched within participants legs at baseline, suggesting that their subsequent responsiveness to stimuli should be relatively similar. Illustrating the potential of the unilateral exercise model, Lindholm et al. (144) found that the variation in resting gene expression was almost five times more pronounced between individuals than within the legs of a single individual. This emphasizes how a unilateral exercise approach can minimize variability in gene expression. At the same time, it's important to acknowledge the potential concern of a transfer effect between limbs, as it may pose a risk to the treatment effect and subsequent interpretation of training protocols. Nevertheless, the suggested transfer effect appears to have minimal impact on a molecular basis, specifically at the protein and mRNA levels (145). However, it may exert a more noticeable influence on cross-education for strength effects (146), likely attributed to the impact on the neuromuscular system (147). The between-subject parallel design may be more feasible when assessing the long-term effectiveness of resistance-training protocols. This approach is more applicable for studying treatment effects across diverse contexts and environments, enhancing generalizability and ecological validity. Taken together, multiple factors need consideration when interpreting the efficacy of resistance training across studies. A comprehensive understanding of the implications of these factors is crucial for accurately assessing the magnitude of training efficacy.

2.3 Preserving muscle mass and strength during periods of catabolic stress

Skeletal muscle hypertrophy is contingent upon maintaining a net positive muscle protein balance, wherein protein synthesis exceeds breakdown. Anabolic processes facilitate protein synthesis, promoting subsequent muscle growth, while catabolism represents counteracting forces acting on skeletal muscle involving protein breakdown, leading to atrophy (148). The detrimental catabolic effects induced by conditions such as illness, injury, physical inactivity, and disuse significantly impact skeletal muscle mass, function, and metabolic regulation (149). The mechanisms driving accelerated catabolism during periods of inactivity can be complex, as evidenced by variations in muscle mass losses between older and younger individuals with similar phenotypes

after a brief period of inactivity (128). While aging individuals seem to undergo disruptions in the regulation of muscle protein metabolism during inactive periods (128,150), it underscores the importance of comprehending the dynamics of anabolism and catabolism to preserve skeletal muscle health across diverse populations and clinical contexts.

Certain athletes often pursue weight loss to enhance performance in an athletic population. Recommended weight-loss strategies include a moderate energy deficit of 500-1000 kcal day^{-1}, achieved by adjusting energy intake, increasing energy expenditure, or combining both (151). However, it is important to note that weight loss resulting from energy restriction may lead to a loss of muscle mass, potentially compromising athletic performance (152). In certain environments, controlling for an adequate energy balance is challenging to maintain body weight, muscle mass, and functional capacity. For example, the military environment is often faced with external stress factors such as prolonged physical activity, negative energy and fluid balance, sleep deprivation, and sustained readiness (86). Intensive military training, comprising demanding field exercises, is integral to military education. These activities involve high activity levels, energy expenditure, and inadequate sleep, resulting in significant energy deficits—reportedly reaching 5000-6000 kcal day^{-1} over a three to seven-day period (153–155). Such periods of severe energy deficits lead to substantial losses in body mass, body fat, and muscle mass (36,155–158). This is, in turn, associated with impaired physical performance, especially strength and power performance, measured as maximal dynamic strength and vertical jump (36,155,156,159,160). Indeed, this is 5-6 times higher than the daily energy deficit recommended to sustain a healthy weight-loss control (152), manage the preservation of muscle mass (151), and attenuate further negative consequences (i.e., reduced muscular performance). For military personnel, it is thus essential to identify strategies to avoid or minimize the loss of muscle mass and performance during periods of energy deficit and psychological and physiological stress.

Dietary intervention with increased protein intake stands out as an interesting approach for maintaining muscle mass (37,161,162), ensuring amino acids availability and sustained anabolic stimuli for muscle protein metabolism (161). Indeed, intake of protein amounting 2-3 times the prevailing recommendation (0.8 g protein kg^{-1} day^{-1}, RDA) leads to the preservation of muscle mass and strength in diet-controlled weight-loss programs (162,163). Although the benefits of excessive protein intake may be affected by aspects such as the degree of energy deficit (164), soldiers initial fat mass levels before entering field exercises (158), consensus remains elusive due to the heterogeneity of studies in design, including variations in time, energy deficit, and supplementation protocols. Furthermore, our understanding of the immediate physiological recovery following military exercises is limited. However, it appears that a period of 2-6 weeks is generally effective in restoring crucial factors for soldier readiness, including physical performance levels and endocrine variables (155,158). However, it remains unclear whether increased protein intake during the exercise positively affects these variables within this relatively short recovery period (165).

Chapter 3

Research aims

This thesis aimed to examine the effects of manipulating resistance-training variables on muscle strength and muscle growth, as well as on physical performance in untrained and moderately trained individuals. Furthermore, we aimed to associate adaptive responses of resistance training volume to molecular muscle characteristics and whether this relationship was related to individuals benefiting from higher training volume. We also aimed to explore the time course profile of ribosomal biogenesis markers in response to resistance training and, lastly, investigate the effect of different protein intakes on soldiers physical capabilities following an energy-restricted field exercise.

The main objectives of this thesis were:

1. to examine the impact of different resistance-training volumes and intensities on muscle strength and muscle mass adaptations (Study I and III), interlimb responses, and muscular performance (Study III),
2. to determine the volume-dependent relationship between resistance training and molecular biomarkers associated with muscle growth, and to investigate how these biomarkers relate to the benefits of 3-SET compared to 1-SET resistance training in individuals (Study I),
3. to determine the time course of markers associated with ribosome biogenesis during the early stages of resistance training and subsequent short-term non-training period (Study II),
4. to examine the potential of increased protein intake in mitigating the loss of muscle mass and preserving muscular performance during a period of severe energy restriction in the context of a demanding military field exercise (Study IV).

Chapter 4

Methods

4.1 Study overview

Study I utilized a within-participant design to investigate the effects of a single set per exercise (1-SET) and three sets per exercise (3-SET) in responses to an acute session and 12 weeks of resistance training. Muscle strength was assessed twice at baseline and further measured at weeks 3, 5, and 9, and after the training intervention (Overview of study design see Figure 4.1). Muscle biopsies were sampled from both legs (*m. vastus lateralis*) before the training intervention at baseline, before and 1 h after the fifth training session (at week 2, acute sample), and after the intervention (Figure 4.1). Body composition measurements using magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DXA) were conducted at baseline and after the intervention.

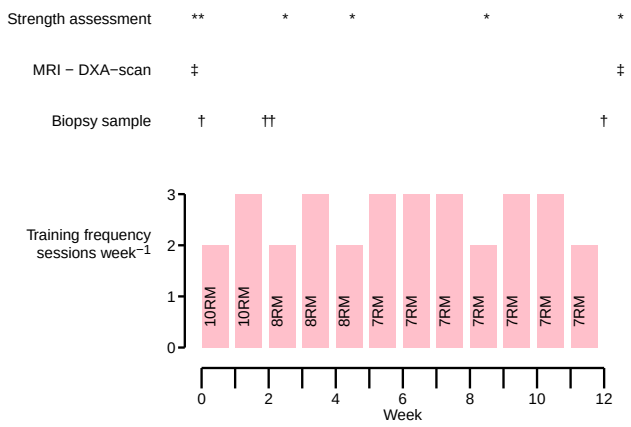

FIGURE 4.1: Overview Study I

Study II was designed to investigate the impact of resistance training per se and the influence of varying inter-session volume compared to a constant volume on selected markers associated with ribosome biogenesis. Hence participants were recruited to an experimental group and a non-training control group. Muscle biopsies were sampled bilaterally in the experimental group before and 48 h after the first session, as well as 48 h after the fourth, fifth, eighth, ninth, and twelfth session, and after

eight days of de-training (Overview of study design see Figure 4.2). The control group had muscle biopsies at baseline, after 48 h, and in one leg after the control period (Figure 4.2). Baseline muscle strength (unilateral isokinetic and isometric knee-extension torque) was measured during three initial visits to the laboratory, with the last baseline measurement performed at least seven days before the first biopsy sampling. Follow-up measures of muscle strength in the experimental group were performed three and nine days after the last training session. Muscle thickness (*m. vastus lateralis*) was measured bilaterally before the study and two and eight days after the last training session in the experimental group. DXA-scans were conducted at similar time points. The control group performed the same initial assessments as the experimental group. Follow-up strength assessments were performed 24 h after the last biopsy.

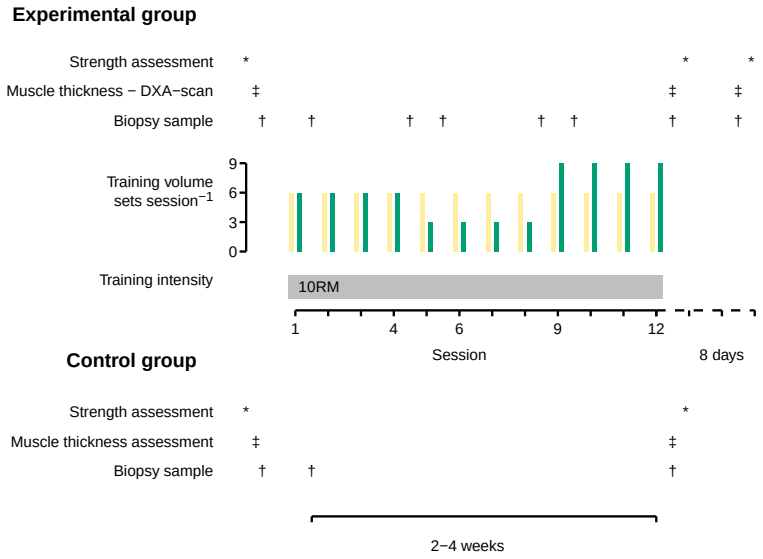

FIGURE 4.2: Overview Study II

Study III used a between-participant, parallel design to examine the long-term effects of either 10RM or 30RM resistance training. Participants reported to the laboratory one week before baseline testing, whereby the participants had a familiarization session containing the entire battery of physical tests. After familiarization, participants assessed physical performance and body composition and sampled a muscle biopsy at week 0 (baseline), mid-intervention at week 10, and after the intervention at week 22 (overview of study design see Figure 4.3). At each time point, testing was organized into three test blocks, conducted on three separate days. Test day 1 started with a blood sample and whole-body DXA-scan followed by counter-movement jump, maximal isometric half squat (MIHS), one repetition maximum in biceps curl, bench press, and leg press, and a muscle endurance performance test. Test-day 2 consisted of an agility test, and on Test-day 3, a muscle biopsy (*m. vastus lateralis*) was performed 24-48 hours after the completion of test-day 2.

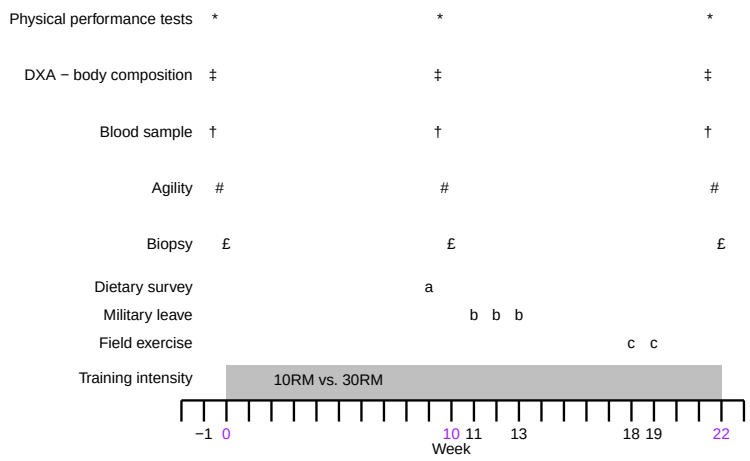

FIGURE 4.3: Overview Study III

Study IV was designed to explore the effects of low ($1\ g \times kg^{-1} \times d^{-1}$) and high ($2\ g \times kg^{-1} \times d^{-1}$) protein intake on body composition and physical performance during a ten days military field exercise with a diet restricted to ($\sim$ $15kcal \times kg^{-1} \times d^{-1}$, equivalent to a $\sim$ %60 reduction in energy intake). Pre-exercise (baseline) testing was conducted 2 days before the field exercise, whereas post-exercise testing was performed immediately after the exercise (Overview of study design see Figure 4.4). A final post-recovery test was conducted after seven days of recovery. All tests were conducted within one test day, starting with a DXA-scanning and blood sample followed by physical performance tests (counter-movement jump, 1RM leg press, bench press, and a Wingate 30-second sprint power test). One week before the start of the field exercise, participants conducted a 24 h recall of habitual diet.

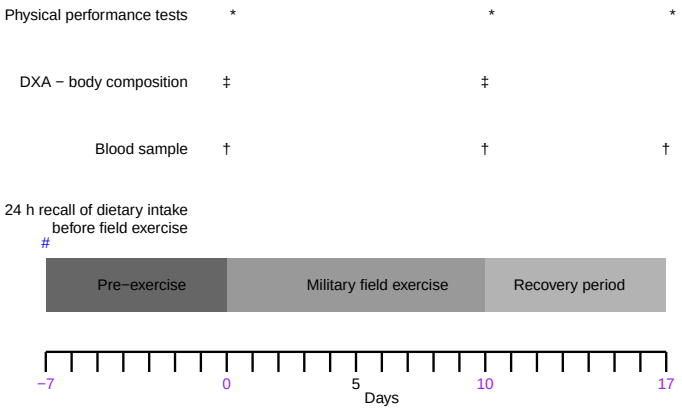

FIGURE 4.4: Overview Study IV

4.2 Participants

Study I and II participants were recruited primarily through advertising and word of mouth at Inland University of Applied Sciences. As for Studies III and IV, cadets were recruited at the military cyber academy through information meetings and individual conversations with the cadets. Participants eligible for inclusion in the studies were given information about the study design and potential risks and sources of discomfort associated with the study before signing an informed consent document. Table 4.1 shows the study participant characteristics. All studies included healthy young men and women (aged 18-40). Eligible participants were non-smokers with a training history of less than one resistance training session per week during the last 12 (study I) or six (study II, III) months leading up to the study. In Study III, individuals engaged in more than two additional sessions per week, in addition to the regular academy training program (which included two sessions with either strength or endurance focus), were excluded to ensure the inclusion of moderately resistance-trained personnel. Further exclusion criteria for these interventions were:

- injuries or disease affecting their ability to perform resistance training or the military field exercise
- any use of dietary supplements or medication with known effects on adaptations to training
- adverse reactions to local anesthetics

Thirty-four out of forty-one included participants (age 22, males n=16, females n = 18) in Study I completed the resistance training protocol. Reasons for not completing the intervention included injury not related to the study (n = 1), discomfort during exercises (n = 5), and non-adherence to the study protocol (n = 1). For Study II, twenty-two volunteered for the study (age 24, males n=9, females n=10), wherein three participants did not complete the intervention due to scheduling difficulties. Twenty-seven participants volunteered for study III (age 20, males n=22, females n=5), wherein three participants did not complete the study for reasons unrelated to the study. And thirty-eight participants were recruited and completed the field exercise in study IV (age 21, males n=31, females n=7). Participants in Study I and II were familiar with physical activity but had no current systematic resistance training. Although ten participants in Study I reported performing resistance-type exercises at enrollment, but this was limited to no more than once a week. For Study III and IV, the participants were soldiers (cadets) regularly doing an academy training program consisting of circuit- and calisthenics exercises, interval training, and combat-like exercises, which was conducted in addition to the resistance-training intervention.

4.2.1 Ethical approvals

The studies were approved by the local ethics committee (Study I, no. 2013-11-22:2; Study II, no. 2017-10-23), the Norwegian center for research data (Study I, 36930/3/LB; Study II, 55300/3/LAR; Study III and IV, reference no. 43901/3/MHM), and Study I and II were pre-registered (Study I: ClinicalTrials.gov Identifier: NCT02179307; Study II, DOI 10.17605/OSF.IO/WA96Y). The four studies were conducted according to the *Declearation of Helsinki*.

Table 4.1: Participant characteristics

	Sex	n	Age (years)	Stature (cm)	Mass (kg)	Fat mass (%)	Lean mass (%)
Study I							
Included	Female	18	22.0 (1.3)	168 (7)	64.4 (10.4)	34.1 (5.6)	64.3 (6.2)
Included	Male	16	23.6 (4.1)	183 (6)	75.8 (10.7)	20.4 (6.0)	79.3 (5.0)
Excluded	Female	4	22.9 (1.6)	166 (8)	64.6 (9.7)	28.8 (8.7)	68.6 (9.1)
Excluded	Male	3	24.3 (1.5)	189 (5)	88.2 (22.4)	24.3 (15.3)	76.8 (12.7)
Study II							
Training	Female	6	23.4 (2.9)	168 (8)	64.0 (9.2)	30.8 (7.1)	65.5 (6.8)
Training	Male	5	25.7 (5.8)	177 (3)	77.5 (8.0)	25.3 (3.9)	71.3 (2.4)
Control	Female	4	24.1 (3.5)	166 (4)	63.8 (0.6)	30.5 (6.4)	66.3 (5.2)
Control	Male	4	25.5 (5.5)	182 (5)	76.5 (7.7)	18.2 (5.1)	78.7 (4.2)
Study III							
10RM	Female	2	20.6 (1.1)	171 (1)	62.0 (2.8)	29.1 (9.6)	75.7 (3.2)
10RM	Male	12	20.8 (0.7)	183 (5)	71.9 (11.7)	19.7 (4.9)	80.8 (5.0)
30RM	Female	3	21.4 (0.4)	174 (6)	65.0 (12.5)	34.4 (6.1)	70.9 (5.0)
30RM	Male	10	21.0 (1.0)	186 (11)	75.3 (11.1)	20.5 (6.2)	80.2 (5.7)
Study IV							
High protein	Female	3	21.6 (1.0)	171 (1)	64.4 (4.8)	22.6 (4.9)	73.1 (5.0)
High protein	Male	8	21.6 (0.9)	183 (7)	83.3 (10.8)	19.7 (6.5)	76.0 (6.1)
Low protein	Female	1	21.6 (NA)	168 (NA)	64.8 (NA)	33.8 (NA)	62.2 (NA)
Low protein	Male	11	21.6 (0.9)	185 (10)	76.3 (11.4)	15.6 (6.8)	80.0 (6.5)

Data are means and (SD). Characteristic data in Study IV is for n = 23, which conducted a DXA-scan (total participants n = 38).

4.3 Resistance training and field exercise protocols

In studies I and II, a within-participant design was used as each participants legs had their legs assigned to different training conditions (except for the control group in Study II). In Study I, each participant had their legs randomized to either performing 1-SET or 3-SET or to a variable or constant volume condition in Study II. Studies III and IV used a between-participant design. Participants in Study III were pair-matched based on initial strength performance and then allocated to either a 10RM group or a 30RM group. In Study IV, participants were randomly allocated into low or high-protein supplement groups.

4.3.1 Studies I-III

Each of the three resistance-training interventions (Study I, II, and III) started with a 5-10 min warm-up on a cycle ergometer followed by a warm-up set of 10 repetitions in each specific exercise with a load corresponding to 30-50% 1RM. Participants in studies I, II, and III performed every set to concentric failure, hence an inability to perform another concentric repetition with proper form. Participants were further encouraged to continuously increase their RM load throughout the intervention period to ensure that they achieved failure in the target repetition. Study I used three unilateral exercises (leg press, leg curl, and knee extension), which were performed with a low-volume protocol consisting of a single set of each exercise and a moderate-volume program consisting of three sets per exercise. The 3-SET-volume leg commenced all sessions, and the contralateral leg performed a single set (1-SET) of each exercise in the rest between the second and third sets of the 3-SET volume training protocol. In Study II, unilateral knee extension was performed. The constant-volume leg performed six sets of 10RM throughout the 12 training sessions. The variable-volume leg performed six sets in sessions one to four, three sets in sessions five to eight, and nine sets in sessions nine to twelve with the same relative intensity. In Studies, I and II, participants also performed upper-body exercises (bench press and either seated

pull-down or shoulder press) to fulfill a whole-body training program. These upper-body exercises were performed in two sets following the leg exercise(s). In Study III the training protocol consisted of three sets of seven exercises per session (squat, leg press, leg curl, bench press, standing rowing, seated pull-down, seated biceps curl), performed two days a week the first 10 weeks and increased to three sessions every other week in the last nine weeks.

4.3.2 Study IV

In Study IV, the participants performed a 10-day strenuous military field exercise in a state of energy deficit, followed by 7 days of recovery. The food was pre-packed rations to be ingested for breakfast, lunch and dinner, providing similar amounts of protein intake (of either 1 g $\times$ kg^{-1} $\times$ d^{-1} or 2 g $\times$ kg^{-1} $\times$ d^{-1}) in every meal throughout the day. Rations were distributed at intervals of 2.5 days. Throughout the field exercise, soldiers engaged in physically and cognitively demanding military tasks within a challenging outdoor environment. The recovery phase was performed without restrictions on energy intake or physical activity.

4.4 Assessment of muscular performance

4.4.1 Muscle strength

All four studies had familiarization to muscular performance tests conducted at least one- to two weeks before baseline.

4.4.2 One-repetition maximum (1RM)

For the lower body, 1RM was assessed in leg press (Study I, III, and IV) and leg extension (Study I, II), and for the upper body, maximal strength was assessed in the bench press (Study III and IV) and bilateral biceps curls (Study III). 1RM tests started with a specific warm-up, consisting of two sets with gradually increasing load (40% and 75% of expected 1RM) and decreasing number of repetitions (10 and 6). The 1RM was defined as the maximal resistance that could be moved through the full range of motion with proper form one time.

4.4.3 Isokinetic and isometric maximal torque

Maximal isokinetic and isometric unilateral knee-extension strength were assessed with a dynamometer (Study I: Cybex 6000, Cybex International, Medway, Usa; Study II: Humac Norm, CSMi, Stoughton, MA, ISA). After a brief warm-up (5-min cycling, RPE 12-14), belts were secured across the participants hips, shoulders, and mid-thigh to reduce any movements of the hips, and to minimize assistance from other muscle groups. Participants were instructed to gradually increase their effort during three warm-up repetitions at each angular speed. In study I, maximal isokinetic torque was assessed at three angular velocities (60°, 120°, $240^\circ \cdot sec^{-1}$), and an angular velocity of $90^\circ \cdot sec^{-1}$ was used in Study II. In Study I, participants performed two attempts at $60^\circ \cdot sec^{-1}$ and three attempts at $120^\circ \cdot sec^{-1}$ and $240^\circ \cdot sec^{-1}$. In Study II, three attempts were made at the designated angular velocity. After completing the isokinetic testing, the lever arm was fixed at 30° (full extension = 90°), participants were instructed to apply force as rapidly and hard as possible for 5 seconds, and the maximal isometric torque was recorded. In Study I, two attempts was made to determine maximal isometric torque and a single attempt was made in Study II. In

both studies, a sixty-second restitution was given between each measurement, except for between isometric contractions in Study II, where a 30-second restitution period was used. The first measurement was performed on alternate legs in the follow-up testing sessions.

4.4.4 Maximal isometric half squat (MIHS)

In Study III, MIHS was conducted using a custom built rack bolted to the floor with an attached fixed bar located over the force plate (SG-9, Advanced Mechanical Technologies, Newton, MA, USA, sampling frequency of 1 kHz). MIHS was measured in a half squat position with a knee angle equivalent to $\sim 60-65^{\circ}$. Knee angle and foot position were marked on the rack and force plate, respectively, to ensure similar body position at the two consecutive test time points. Participants were given 3-4 attempts with 2 min rest between attempts. Verbal encouragement was given throughout the test, and the participants were instructed to push as hard and as fast as possible for 5 sec. The three highest force values were averaged and used in data analyses. For MIHS the coefficient of variation between test attempts averaged for all time points were 4% (SD 3.9, Range: 0.1, 14.5) for 10RM and 4.8% (SD 3.7, Range: 0.1, 14.5) for 30RM.

4.4.5 Performance tests

Jump height was measured with **counter-movement jump test** using the same force plate as MIHS (Study III and IV). The participants hands were placed on their hips and feet at shoulder width on the force plate. Participants descended to a squat position and immediately jumped upward as high as possible. Thirty seconds rest was given between each attempt. The three best jumps were averaged, wherein the lowest jump was removed when >3 attempts were performed, and subsequently used in the data analyses.

Muscle endurance performance (Study III) was measured in the leg-extension exercise using a load corresponding to 60% of pre-test body mass. Participants performed as many repetitions as possible to muscular failure with a cadence of repetitions set to 2 seconds in both concentric and eccentric phases (controlled with a metronome). The test was terminated when the cadence was missed two consecutive times. The same absolute load was used at all test time points, and the maximal repetition was used for analyses.

Agility-running time was assessed using a directional court (Study III). The timer started with participants first movement from a 3-point stance start position (timing system: Brower Timing System, Utah, USA, 2013). Participants ran 4.56 m and touched a line with the hand, reversed direction and ran 9.1 m, touched an opposite line with the hand, and ran back through the timing gate that recorded the elapsed time. Participants had two attempts in each direction (right and left sides). Two minutes of rest was given between trials, and attempts were averaged for each direction and used in analyses.

Wingate 30-second sprint was performed on a cycle ergometer (Lode Excalibur Sport, Lode BV, Study IV). Mean power output (Wmean) was defined as the average power output sustained throughout the 30 seconds, and peak power output was defined as the peak power (Wpeak). The seating position was adjusted according to each participants preference for seat height, horizontal distance between the tip of the seat and bottom bracket, and handlebar position. The accustomed positions

were used in the subsequent test time points.

4.5 Assessment of skeletal muscle mass

4.5.1 Dual x-ray absorptiometry (DXA), magnetic resonance imaging (MRI) and ultrasound (US) measurements

Studies I-IV measured muscle mass with DXA. At each time point, participants were instructed to refrain from strenuous physical activity during the last 24-48 hours leading up to the measurements. Participants were further asked to arrive in a fasted state the morning of the scan. Follow-up scans were performed before (Study III) or at least 48 h after the last maximal strength tests (Study I and II). In Study IV, the DXA-scan was performed 1-2 h after completion of the field exercise, with no repeated scan after the recovery period. A single technician, blinded to treatment allocation and time points, customized the region of interest to encompass the upper thigh in Studies I and II or the whole body in Studies III and IV. Additional muscle mass assessments were conducted in study I using MRI and US in Study II. MRI images were obtained from the mid-thigh and analyzed by the same investigator blinded for time and experimental conditions (1-SET/3-SET). Multiple images were used to estimate the *m. quadriceps* cross-sectional area at the same distance from the knee joint. In Study II, *m. vastus lateralis* muscle thickness was measured using B-mode ultrasonography (SmartUS EXT-1M, Telemed, Vilnius, Lithuania) using a 39 mm 12 MHz linear array probe. Between each image acquisition, the probe was relocated to the same position. The probe position was marked on the skin and subsequently marked on a transparent paper to relocate the same position in the follow-up measurements. Three images were captured for each leg per time-point, with values averaged in analyses. Image analyses were done in ImageJ Fiji (166) by a single assessor blinded for study conditions and time points.

4.6 Biologcial samples

4.7 Muscle biopsy sampling and processing

4.7.1 Micro biopsy sampling

In studies I-III, muscle biopsies were collected using a microbiopsy system (Bard Magnum, Bard Norway AS, Norway) with a fine needle (12-14 gauge; Universal-plus, Medax Italy) in accordance with previous procedure (167). Following local anaesthetization (Lidocaine Mylan, 10 $mg\,ml^{-1}$, Mylan Ireland Ltd, Ireland), biopsies were sampled from *m. vastus lateralis*, at approximately one-third of the distance between basis patella to the anterior superior iliac spine. Subsequent biopsies were sampled $\sim$ 2 cm proximal/distal to the previous sample, and two to four passes were made each time to get sufficient material for the subsequent analyses. The wet muscle weight of aliquots was measured at the collection.

4.7.2 Muscle tissue processing and analyses

4.7.2.1 Total RNA extraction

Frozen muscle tissue was homogenized in 300 µl Trizol with external non-mammal RNA (Lambda PolyA External Standard Kit, Takara Bio Europe, Saint_Germain-en-Laye. France) added to enable per-weight normalization in subsequent analyses (168). After homogenization, additional Trizol was added to a total volume of 1 ml. Prior to centrifugation, 200 µl of chloroform was added to achieve phase separation. Subsequently, the upper phase was (400 µl in Study I, 450 µl in Study II) transferred to a fresh tube, and 500 µl of isopropanol was added to precipitate RNA. After a 10-minute incubation at room temperature, samples were centrifuged (12,000 g, $4^{\circ}C$), and the formed RNA pellet was washed three times in chilled 75% ethanol. Subsequent to the final wash, all ethanol was removed, and the pellet was eluted in 0.1X Tris-EDTA buffer. RNA concentration was determined using spectrophotometry.

4.7.2.2 Protein extraction

In Study I, muscle tissue ($\sim$ 25 mg wet weight) was homogenized 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), added with protease and phosphatase inhibitors (Halt, Thermo Fisher Scientific), followed by 1 h ($4^{\circ}C$) of incubation. After centrifugation for 10 min at 10,000 g and $4^{\circ}C$, the resulting supernatant was diluted 1:10 in distilled water, and total protein quantification was quantified using Bradford reagents. The remaining supernatant was diluted to 1.5 µg μl^{-1} total protein in lysis buffer and 4X Lammeli sample buffer, which was heated to $95^{\circ}C$ for 5 min to denature the proteins and stored at -20$^{\circ}C$ until further separation in SDS_PAGE. In Study II, protein was extracted from Trizol preparations in line with Kopec et al., (169) protocol. The remaining aqueous phase was removed, and DNA was precipitated by the addition of 300 µl of absolute ethanol followed by centrifugation (2000 g, 5 min at room temperature). An aliquot of the phenol-ethanol phase, corresponding to $\sim$ 1.75 mg of tissue, was transferred to a fresh tube. After the addition of at least two volumes of isopropanol and incubation (10 min at room temperature), samples were centrifuged (7500 g, 10 min $4^{\circ}C$), and a pellet formed. The pellet was washed three times in 95% ethanol, with each wash separated by centrifugation (5000 g, 5 min at room temperature). After the last wash, all liquid was removed, and 45 µl of Kopec buffer (169) 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 $50^{\circ}C$ for three hours, after which the majority of samples were dissolved. Any undissolved material was sedimented by centrifugation (10,000 g, 10 min at room temperature). Protein concentrations were measured, and samples were normalized as described in Study I.

4.7.2.3 Immunoblotting

Protein samples of 20 µg were separated on 4-20% Tris-Glycin gels (250-300 V for 30-50 min) and transferred to PVDF membranes (300 mA for 3 hours, 0.2 µm Immun-Blot, Bio-Rad), after which the membranes were stained using a reversible total protein stain (Pierce Reversible Protein Stain, ThermoFischer Scientific) to ensure transfer and confirm equal loading of the samples. The membranes were subsequently blocked for two hours in a blocking buffer (tris-buffered saline, TBS, 20 mM Tris, 150 mM NaCl) containing 3% bovine serum albumin or 5% skimmed milk with 0.1%

Table 4.2: Antibodies for immunoblotting

	Target	Host	Manufacturer	Cat-nr
Primary	$mTOR^{Ser2448}$	Rabbit	Cell Signaling Technology	5536
	pan-mTOR	Mouse		4517
	p85 $S6K1^{Thr412}$	Mouse		9206
	p70 $S6K1^{Thr389}$	Rabbit		9234
	pan-S6K1	Rabbit		2708
	$rpS6^{Ser235/236}$	Rabbit		4858
	pan-rpS6	Mouse		2317
	pan-UBF	Mouse	Santa-Cruz Biotechnology	sc-13125
Secondary	Anti-mouse IgG		Cell Signaling Technology	7076
	Anti-rabbit IgG			7074

Tween-20. Following incubation with primary and secondary antibodies, membranes were washed in TBS containing 0.1% Tween-20 for 3-8 cycles of 5-10 minutes each. Chemiluminescent signals from the membranes were detected after a 5-minute exposure to the substrate (SuperSignal^{TM} West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific) for the quantification of relative target protein abundance. Chemiluminescence signals were quantified using Image Studio Lite (LI-COR Biotechnology, Lincoln, Nebraska, USA). Total protein content was defined as the mean grey value of the whole well, with between-well grey values subtracted as background (conducted in ImageJ Fiji, (166)). All samples from the same participant were run on the same gel and in duplicates. Primary antibodies used in study I and II are found in Table 4.2.

4.7.2.4 Immunohistochemistry

Formalin-fixed muscle biopsies were processed for 2.5 h using a Shandon Excelsior ES (Thermo Scientific, USA), paraffin-embedded, and sectioned into 4 µm. Transverse sections were double stained for determination of muscle fiber types using BF-35 (5 Ug x ml-1, Developmental Studies Hybridoma Bank, deposited by Schiaffino, S.) and MyHCSlow (1:4000, catalog M8421L, Sigma-Aldrich Norway AS, Oslo, Norway). The primary staining was identified by BMU UltraView DAB and UltraView RED (Ventana Medical System, Inc. Tucson, USA). Muscle fiber types were counted as either type 1 (red), type 2A (brown), type 2X (unstained), or hybrid fibers type 2A/2X (light-brown). Care was taken not to circle any fibers along the outside of the cross-section, and the outermost fibers in each section were excluded from quantification (less staining, half fibers, etc.).

4.7.2.5 Quantitative polymerase chain reaction (qPCR)

Complementary DNA (cDNA) was synthesized in technical duplicates from 500 ng of RNA using random hexamer and anchored Olgio-dT primers (Thermo Fisher Scientific) together with Superscript IV (Thermo Fisher Scientific) according to the manufacturer's instruction. qPCR reactions were performed with diluted cDNA (2 µl, 1:25 dilution), a SYBR-green based commercial master mix (PowerUp^{TM} SYBR^{TM} Green Master Mix, Thermo Fischer), and target-specific primers (500 nM) in 10 µl reaction volumes using real-time detection system (Applied Biosystems 7500 fast Real-Time PCR Systems, Life Technologies AS or QuantStudio 5 Real-Time PCR System, Thermo Fisher Scientific). Fast cycling over 40 cycles was adopted for each master mix. Melt curves and agarose gel electrophoresis were used to confirm single

product amplification, product sizes, and no amplification in experiments without a template.

Raw fluorescence data was exported from the real-time detection system, and estimates of quantification cycle (Cq) and amplification efficiency were derived for each reaction using the qpcR package (170). Threshold cycles (Ct) were estimated from the models by the second-derivate maximum method with technical duplicates modeled independently (170). Amplification efficiencies were estimated from every amplification curve (170,171), and the mean amplification efficiency (E) per primer was used to transform amplification data to the linear scale (E^{-Ct}). Gene expression data were log-transformed before statistical analysis and modeled as per total RNA, or per tissue weight, using the external RNA and tissue weight as the normalization factor.

4.7.3 Blood sampling

Blood samples were collected at five time points in Study I and three time points in Study III and IV. Fasting blood samples were obtained from an antecubital vein using serum-separating tubes and incubated for 30 min at room temperature before centrifugation (10 min at 1500 g). Serum was aliquoted and immediately transferred to $-80^{\circ}C$ for storage until analyses. Serum concentrations of total Testosterone, Cortisol, Insulin-like growth factor 1 (IGF-1), and growth hormone were measured using an Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics, NY, USA). Free triiodothyronine (T3), free thyroxin (T4), thyroid-stimulating hormone (TSH), and creatine kinase were measured using a Cobas 6000 (Roche Diagnostics/Hitachi SYSTEMS, Roche Diagnostics Norge AS).

4.8 Diet before and during the field exercise

The participants had a balanced diet prior to the military field exercise (Study IV) according to the 24 h recall survey (see Table 4.3) with a pooled group average daily energy intake of 42.5 (SD 14.9) kcal $\times$ kg^{-1} $\times$ d^{-1}. During the military exercise, total pooled group average energy intake was restricted to 15.2 (0.5) kcal $\times$ kg^{-1} $\times$ d^{-1}, equivalent to a 60% reduction in energy intake. To achieve an isocaloric diet between the supplementation groups, the high protein group (2 g $\times$ kg^{-1} $\times$ d^{-1}) relative content of protein constituted a larger proportion and carbohydrate a lower proportion (50 vs. 146 g) of the total energy intake than the low protein group (1 g $\times$ kg^{-1} $\times$ d^{-1}). Supplementary groups were equal in fat distribution (Table 4.3). The participants were instructed to refrain from eating anything else during the field exercise.

The calculation of energy deficit was based on DXA-estimated changes in fat mass and fat-free mass using the equation:

$$\text{Energy deficit(kcal d}^{-1}) = \frac{((\Delta\text{fat mass} \times 38) + (\Delta\text{fat free mass} \times 6)) \times 238.846}{10 \text{ days}}$$

where Δ is the change in fat mass or fat-free mass in kg times the energy densities of fat mass and fat-free mass (38 and 6 $MJ\ d^{-1}$). The factor 238.846 was used to convert megajoule into kilocalories (172).

TABLE 4.3: Energy and macronutrient composition from the 24-hour recall (prior exercise) and for the diet during the 10-day military field exercise.

	Low protein (1 $g\ kg^{-1}d^{-1}$, n=18)		High protein (1 $g\ kg^{-1}d^{-1}$, n=19)	
	24h recall prior exercise	Field exercise diet	24h recall prior exercise	Field exercise diet
Energy kcal d^{-1} (kcal kg^{-1} d^{-1})	3196.4 [1012.0] (41.7 [13.3])	1183.4 [177.6] (15.3 [0.5])	3337.8 [1313.4] (43.4 [16.7])	1174.2 [170.1] (15.1 [0.5])
Protein g (g kg^{-1} d^{-1})	159.2 [61.4] (2.1 [0.9])	79.2 [12.0] (1.0 [0.0])	144.8 [44.1] (1.9 [0.6])	156.5 [22.6] (2.0 [0.1])
Carbohydrate g (g kg^{-1} d^{-1})	370.4 [121.1] (4.9 [1.8])	146.1 [17.8] (1.9 [0.1])	395.8 [179.6] (5.1 [2.2])	50.1 [10.6] (0.6 [0.0])
Fat g (g kg^{-1} d^{-1})	101.4 [49.1] (1.3 [0.6])	27.8 [7.0] (0.4 [0.0])	123.0 [61.3] (1.6 [0.8])	37.8 [4.4] (0.5 [0.0])

P<0.05 Lower protein and carbohydrate proportions in the high protein field exercise diet compared to low protein group

4.9 Statistics

Overall statistical models employed in the studies

To assess the effect of treatment-conditions (volume, intensity, protein supplement) on muscle-strength and- mass (Study I-IV), linear mixed-effects models were specified with relative changes from baseline (Study I-III) as the dependent variable and time and time to treatment-conditions interactions was included as fixed effects. To control for regression to the mean and sex effect in the models, baseline values (Study I-III) together with sex were added as co-variates. To meet the assumption of independence of repeated measure by subject, random intercepts by participants were included in all models (Study I-IV), and when justified by the data a random slope were included for time and exercise-volume at the level of participants. Training-effects on molecular characteristics (Total-RNA and western-blot data and gene abundances) (Study I-II), muscular performance (Study III), energy and macronutrient (Study IV) were also assessed using linear mixed-effects models using the fixed and random structure specified above. In Study II, we employed a segmented regression model to evaluate the impact of volume condition and time course on ribosomal biogenesis in the training group. This model estimated changes over sessions, divided into three segments corresponding to different volume prescriptions (sessions 1-4, 4-8, and 8-12). In cases where robust effects of volume conditions were not observed, group averages were reported. These segmented models were fitted with time and volume condition as fixed effects and legs nested within participants serving as random level effects.

To validate the assumption of equal variance (homoskedasticity) across predicted values in our models, we conducted visual inspections of residuals plotted against predicted values. In cases where deviations from this assumption were observed, data underwent log-transformation, and models were subsequently refitted. Additionally, to eliminate random effects that did not contribute with meaningful information to our models, we performed model simplification by reducing random-effects parameters through likelihood-ratio tests

In Studies I and III, we computed a factor for both strength and muscle mass. This approach was employed to enhance the interpretability of treatment group effects by calculating a weighted average score of several measures of muscle strength or mass. Furthermore, to interpret the magnitude and direction of differences between treatments groups, Study III and IV employed an effect sizes calculated with the following formula;

$$\frac{\bar{x}_1 - \bar{x}_2}{\mathrm{SD}(x_2)}$$

The scale proposed by Rhea (173) for trained subjects was used to interpret the magnitude of the treatment effect. Statistical significance was set to $p<0.05$ (Study

I, III, IV).

To determine added benefit of 3-SET over 1-SET training in average strength and muscle hypertrophy, a variable was constructed based on the differences between volume conditions within-participants legs. An additional benefit of 3-SET training was considered when the volume difference exceeded the smallest worthwhile change, in the direction of 3-SET training, defined as 0.2 times the baseline between-participants standard deviation.

Model for gene data

The gene-abundance data were analysed using gene counts converted from Cq values obtained from the qRT-PCR as suggested by Matz et al., (174). The Poisson-lognormal model was used to fit these count data and the gene-abundance data[1] were expressed per-unit muscle weight using the external reference gene as an normalization factor. The normalization factor was proportional to the amount of muscle used to prepare cDNA as such we were able to model gene abundance per tissue weight (External reference counts × muscle weight (mg) in each Trizol preparation). To control for technical errors during sample preparation, a random effect for each technical duplicate was included in the models, and due to greater sampling variances are associated with low-abundance target genes a variance function per specific gene was included to adjust for heteroscedasticity over the fittet range.

In Study I-II gene abundance data were fitted with time and time to volume condition as interaction term in the fixed effects, and Study II gene abundance data were fitted with the number of sessions a categorical variable in comparison of volume conditions as fixed effects.

Prediction analyses in Study II

In study II, to investigate an association between total RNA and muscle growth in individuals a linear model was used to estimate the increase per session and average total RNA for every leg in the training group. These estimates were then used to estimate the effect of training-induced increase per session and average total RNA abundance on muscle hypertrophy. A mixed effects model was subsequently fitted with differences in muscle thickness pre- to post-training as the dependent variable and estimated percentage per session increases in total RNA, and the mean total RNA as independent variables. Legs nested within participants was used as random effects. The robustness of this model was assessed by leave-one-out analysis on the level of individual data points in a way to assess any influencing observations on the association. All models in Study II were fitted using a Bayesian framework. Inference about effects of interest was drawn based on point estimates and their 95% credible intervals (CI). Credible intervals not containing null effects were interpreted as robust effects. Models were fitted with default priors. CIs were interpreted as containing the true population value with the specified certainty (95%), given the data and priors.

All statistical analyses and figures were performed in R (175) and statistical models were fitted using packages nlme (176), lme4 (177), brms (178), and MCMCglmm (179) written for R.

[1] genes: rRNA47S ETS, rRNA45S ETS, rRNA45S ITS, rRNA5.8S, rRNA28S, rRNA18S, rRNA5S, and Lambda external reference gene

Chapter 5

Results and Discussion

5.1 Resistance training

5.1.1 Principal findings

The initial part of this thesis explored the effects of resistance training volumes and intensities on muscle strength, growth, and performance among untrained participants (Study I) and moderately trained participants (Study III). The 12- and 22-week training interventions (Studies I and III, respectively) generally demonstrated superior muscle strength gains and hypertrophy with greater training volume (3-SET training) and higher training intensities (10RM) compared to 1-SET and 30RM training, respectively. Furthermore, muscular performance, measured by jump height, favored 10RM training. The volume dose-dependent increase in muscle strength and growth observed after 12 weeks of resistance training (Study I) was associated with higher total RNA content at week 2 in the 3-SET leg, indicating the importance of enhanced translational capacity to meet the increased demand for muscle contractile proteins for muscle growth. Study II further explored this early rise in RNA content in response to resistance training. The study revealed a rapid increase and peak accumulation of total RNA in response to eight resistance training sessions with a relatively high training volume. The increase in total RNA per session was associated with muscle growth over the course of 12 resistance training sessions. Upon the discontinuation of stimuli, a decrease in ribosome synthesis was observed. This finding implies that ribosome biogenesis is rapidly regulated in response to stimuli, aligning with the heightened demand for muscle cell expansion. Conversely, given that ribosome biogenesis is an energy-consuming process, ribosome biogenesis is downregulated when not needed. Finally, Study IV demonstrated that a higher protein intake during a 10-day field exercise with severe energy deficit did not mitigate the loss of muscular performance or alterations in body composition compared to a lower protein intake group. Therefore, during a period when cadets undergo a significant energy deficit, the manipulation of macronutrients probably becomes less crucial than the overall emphasis on total energy intake.

5.1.2 Characteristics of included participants

In Study I and II, individuals who had not participated in consistent resistance training for 6 to 12 months before the baseline assessment were recruited as participants. In Study III, moderately trained cadets recruited from a military environment were familiar with resistance training through their academy exercise training. To verify the training status of the participants in Studies I-III, the estimates from baseline DXA scanning were used to calculate the ratio of total lean mass to body mass for

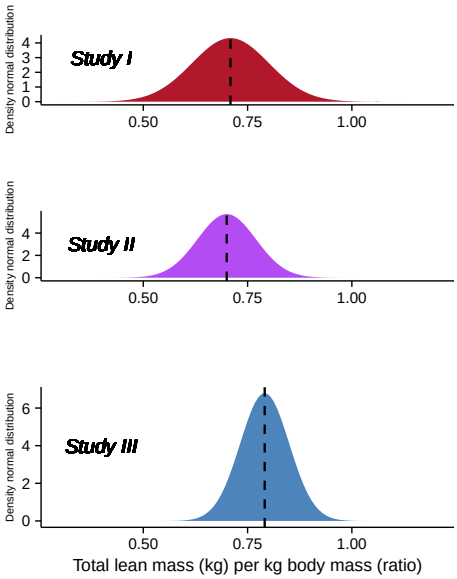

FIGURE 5.1: Baseline distribution for total lean mass (kg) per kg body mass in studies I to III. Vertical line represent mean value.

each participant. The ratio indicates a higher relative muscle mass proportion in the moderately trained cadets (Study III) compared to the expected untrained participants in both Study I (-0.08, 95% CI [-0.12, -0.04]) and II (-0.09, 95% CI [-0.14, -0.05]), as depicted in Figure 5.1). Moreover, the taller distribution curve indicates the cadets were more homogeneous in terms of muscle mass at baseline (lower variance around the mean) compared to the broader and flatter curve in the untrained participants in the two other studies. This is as expected for the moderately trained personnel in Study III, which had a training frequency of (maximal) 2 sessions per week of resistance training before the study start compared to the untrained individuals included in Study I and II.

The training status is further supported by the lower presence of fiber type IIx at baseline (Table 5.1) and their close to complete absence by Week 10, affirming the cadets classification as moderately trained in Study III. While a comparison between the studies in terms of the ratio of maximal muscle strength per kilogram of muscle mass could potentially provide additional support for the participants training status (49), the use of different assessment methods in the studies, such as the unilateral leg press in Study I, the leg extension in Study I-II, and the bilateral leg press in Study III, makes it challenging to make a meaningful comparison across these studies.

5.1.3 General efficiency of the training interventions

Chronic resistance training significantly affects skeletal muscle growth and strength, driving this adaptation depending on training volume, intensity, frequency, and overall organization of exercise sessions (180,181). In order to increase strength and hypertrophy in the aging and younger populations, resistance training is suggested to be performed with a relatively high load (>65% 1RM), with approximately

TABLE 5.1: %-fiber-type distribution

	Fiber-type I	Fiber-type IIa	Fiber-type IIx	Fiber-Type IIax
Study I				
Baseline	44.1 (11.3)	50.6 (10.0)	5.3 (6.7)	5.4 (4.0)
Week 2	46.0 (10.3)	50.3 (8.6)	3.7 (3.8)	6.3 (6.1)
Week 12	45.7 (11.4)	53.0 (10.7)	1.2 (2.6)	1.8 (3.0)
Study III				
Baseline	50.3 (11.2)	46.6 (10.0)	3.1 (4.4)	5.4 (7.7)
Week 10	51.0 (11.5)	49.0 (11.6)	0.1 (0.3)	0.3 (1.0)
Week 22	50.2 (7.9)	49.8 (7.9)	0.0 (0.0)	0.0 (0.0)

data presented as mean (SD)

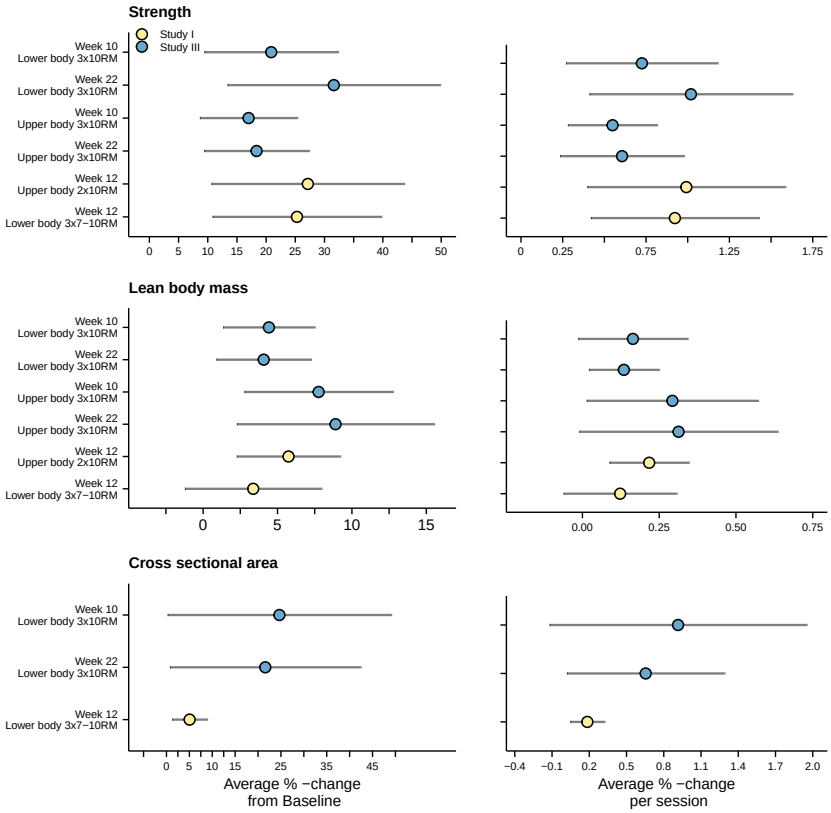

FIGURE 5.2: Training induced changes in strength and muscle size and change per session in Study I (12 week, training volume) and III (22 week, resistance intensity), Cross sectiona area: Study I = muscle CSA, Study III = fiber CSA.

8-12 repetitions, and a minimum of two days a week (13,20,30). Accordingly, the training interventions (Study I and III) were conducted in accordance with the recommended training prescription to augment strength and muscle growth (13,180). Hence, performing 3 sets of either 7-10 RM (Study I) or 10 RM (Study III) for 12 and 10 weeks, respectively, the average increase in muscle strength concurs with previous research findings in young, healthy participants (9,70,71) (Figure 5.2). Increasing the training intensity in accordance with participants progressive resistance-training experience aligns with the expected average increase in strength per session for both untrained individuals (9) and those experienced in resistance training (70,71) (Figure 5.2). Notably, the cadets in Study III demonstrated a percent-strength change per session approximately similar to that of resistance-trained individuals corresponding to 0.82 %-change per session (71). This, along with their lower strength gains per session compared to the untrained participants in Study I, further supports the classification of the cadets as moderately resistance-trained. Opposed to previous studies lasting typically from 8-12 weeks, the cadets engaged in an extended training program lasting 22 weeks (Study III). A continued progression was observed for the lower body strength, while the upper body seemed to level off. Nevertheless, these findings collectively underscore the overall effectiveness of the resistance-training protocols employed in these studies.

The progressive resistance-training protocols promoted mean muscle growth (lean mass, Study I and III) that aligns with anticipated outcomes based on previous research data (9,20,70,71) (Figure 5.2). On the cellular level, the observed increase in fiber CSA up to ten weeks (Study III) exceeded the gains reported in previous studies involving resistance training programs lasting ten (76), twelve (70), and even fourteen weeks (182) (Figure 5.2). Although these studies vary in study design, participants in these studies conducted in general > 30 training sessions over the designated training periods compared to 20 sessions in the present study (III). Given the lower amount of training sessions in Study III and moderately trained cadets vs. untrained participants in the latter studies, it is challenging to rationalize the greater fiber CSA growth observed after 10 weeks in relation to the other interventions. Moreover, the large variability in individual responses to changes in fiber CSA introduces some uncertainty to our data. However, in conjunction with the observed increases in cadets lean body mass at week 10, and similar or greater increases in fiber CSA are detected following 10-11 weeks with resistance training (183,184), the changes in fiber CSA is not uncommon and can thus be justified. These results also highlight that including several measures of muscle hypertrophy is a feasible approach for achieving closer to the true magnitude of hypertrophy (31). Study I exhibited a more modest increase in whole muscle hypertrophy aligning with a typical growth rate of 0.15%/day (20). Interestingly, both training protocols induced superior muscle growth gains in the upper body compared to the lower body. It could be seen in relation to a relatively greater growth potential since the lower body is experiencing greater loading during daily living activities (15) and loading stimuli from the basic military training (Study III), hence some of the growth potential in the leg muscles might already been reached. On the other hand, performance of endurance-oriented training engaging similar muscle groups can have inhibited lower-body muscle adaptations (79). However, the continuity in training-load progression led not to a further increase in muscle mass either as lean mass or CSA in neither upper or lower body (Study III). It thus implies that the training-induced changes in muscle strength observed at the end of the prolonged intervention (Week 22, Study III) may achieve significant contributions from neural

or morphological adaptations other than changes in CSA (137), enhancing specific strength.

5.1.4 The influence of variations in volume and intensity on muscle strength, hypertrophy, and functional performance

In practice, resistance training can be modulated through the variation of multiple training variables, such as exercise intensity used in a training set, volume (total work performed within a session, sets x repetition x load), training frequency, movement velocity, and organization of training sessions throughout the year. Strategic implementation of these variables in a training program determines an individuals likelihood of reaching specified training goals, whether it is for health or performance purposes. Among the training variables, training intensity and volume are considered two of the most deterministic factors that promote adaptations to the neuro-musculoskeletal system.(20,48). Both training intensity and volume can be distributed in several ways (i.e., number of repetitions, % of repetition maximum, number of sets, duration of work) leading to different adaptations (48). Therefore, in the training studies (I-III) training intensity was defined and investigated in terms of the number of repetitions maximum (i.e., 10RM/30RM), and volume was investigated as the number of sets performed per muscle group within a session.

5.1.4.1 Effects of resistance training volume on muscle strength and hypertrophy

Many studies have sought to determine the minimum exercise dose needed to induce beneficial adaptations, as time limitations often restrict participants in exercise training programs (185). There has been considerable debate over the optimal number of sets per exercise to improve muscle strength. Some authors report that multiple sets are necessary to optimize strength gains, particularly in subjects with greater resistance training experience (56,186). Some argue that a single set per exercise is sufficient, and additional sets may not lead to further gains (187). Furthermore, certain studies have not found a significant difference in muscle strength and hypertrophy between single sets (1-SET) and multiple sets (3-SET)(76,188). There are also indications that training volume affects muscle strength and growth differently between the upper and lower body limbs (15).

In Study I, twelve weeks of resistance training led, in general, to greater strength gains with average 5.7 (3.2, 8.1) percent-point difference ($\pm$ 95%CI) in favor of 3-SET compared to 1-SET condition. This difference between volume conditions gradually evolved over the first nine weeks of the study, with no further increase to week 12. The advantage of greater volume was consistent for all measures of strength, including dynamic 1RM as well as isokinetic and isometric strength measures (Figure 5.3). These adaptations, therefore, align with prior meta-analyses, which have indicated that increased training volume leads to favorable outcomes in both single-and multi-joint exercises (24,56). The favor of 3-SET was also evident for muscle growth. There was a clear volume-dose relationship with an average 1.5 (0.4, 2.5) percent-point difference in favor of the 3-SET condition (Study I Figure 5.3), indicated by lean mass and whole muscle CSA that align with prior meta-analyses, which have indicated that increased training volume leads to more favorable muscle growth (25,26). Therefore, although challenged by some researchers (141), the contractile apparatus significantly contributes to muscle strength, as also indicated by our data wherein

increases in muscle mass correlated with increases in muscle strength (averaged over volume conditions (r=0.41 [0.08, 0.66], p=0.016).

Transition in fiber type from Type IIx to Type IIa was also associated with training volume. Specifically, the 3-SET protocol led to a 1.5 percentage point greater reduction in Type IIx fiber expression from baseline to post-intervention when compared to the 1-SET condition. This effect was likely attributed to the more substantial decreases in mRNA expression of the myosin heavy chain IIx gene (MYH1) observed throughout the intervention (189). Interestingly, there was a disconnection between MYH1 mRNA and IIx protein adaptations in the 3-SET compared to the 1-SET leg after two weeks of training, wherein 1-SET led to more pronounced decreases in IIx at the protein level, accompanied by lower abundances of IIx/IIa hybrid fibers. However, this initial change in fiber type IIx levels in the 1-SET leg was not observed at the mRNA level, with MYHC1 being more suppressed in the 3-SET condition. This may indicate an increased need for tissue repair in the 3-SET condition at this early time point (190), resulting in a delayed fiber-type switch. The findings from Study I collectively suggest that the volume of sets significantly impacts the morphological and phenotypic characteristics of skeletal muscle. Moreover, the initial phase of fiber type transition can argue for introducing untrained individuals to lower volume, augmenting fiber type transition towards type IIa, before advancing training volume for further adaptations.

In Study II, the 12 training sessions, with a varying training volume via manipulating the number of sets leading to differences in load × repetitions, did not result in significant differences in maximal strength between the different volume conditions over the short study period (volume profile see Figure 4.2). In general, the resistance training protocol showed a progressive increase in training load at 10RM from the first to second (30%, 95% credible interval (95%CI):[21,41]) and third training block (47%, CI[35, 61]), with each block consisting of four training sessions. The resistance training progression led to increased muscle strength from baseline to after session 12, compared to the non-training control group, measured as isokinetic torque (∼ 9.3%-point difference, Figure 5.4)B). This remained greater in the training group than in the control group following the 8-day de-training period (∼ 6.7%-point difference). Moreover, strength measured with isometric torque displayed a similar pattern following the resistance-training period (7.1%-point difference) but with larger uncertainty as indicated by the wider 95% credible intervals, compared to the control group after the de-training period (∼ 1.9% [-5.2, 9.5]%-point difference, Figure 5.4)B).

The period of fluctuating training volume did not lead to differences between conditions in muscle size, but 12 sessions of resistance training with the relatively high training volume on the legs led to a clear increase in muscle thickness compared to the non-training control group with a ∼ 3.6%-point difference (Figure 5.4)A). This agrees with previous studies, finding early increases in muscle size in response to resistance training (136,191,192). Following the 8 days de-training period, muscle thickness remained greater in the training group compared to the control group (∼ 3.5%-point difference). There was, however, no evident change in leg lean mass estimated by the DXA scan following the short training period. This discrepancy in muscle size changes is likely attributed to the sensitivity of measurement techniques. Specifically, muscle thickness in the *m.vastus lateralis* is directly measured at the largest CSA at the mid-thigh, which is assumed to have the most prominent adaptation in response to resistance training (193). Conversely, DXA-derived estimates of the whole thigh region, encompassing various muscles, are influenced by components such as

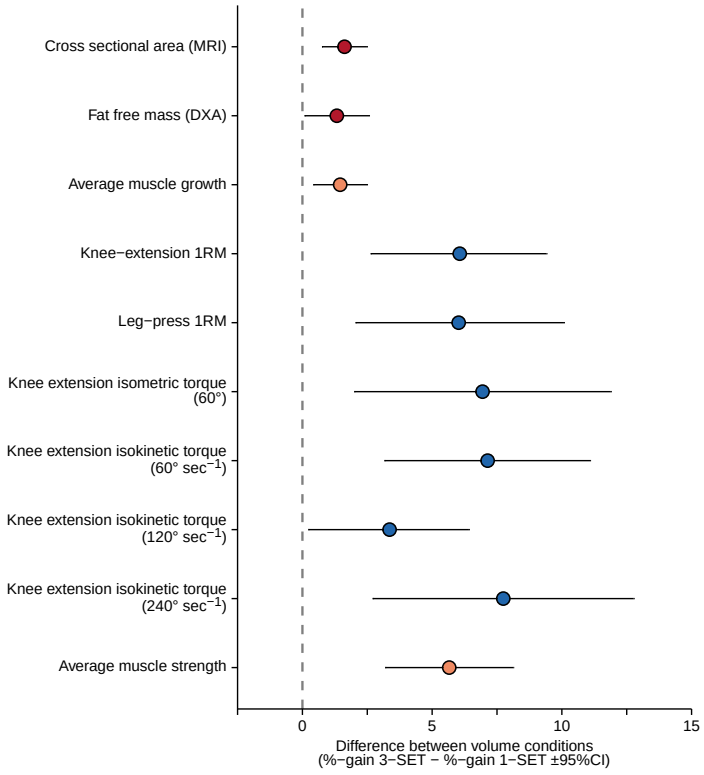

FIGURE 5.3: Differences in training-induced relative changes in muscle-growth (red circless) and strength (blue circles) with a weighted average of all strength and muscle growth measures (orange circles) in Study I.

muscle tissue hydration levels, shifts in body fluids, changes in connective tissue and lean mass between muscle groups, all of which can affect the lean mass coefficient and thus changes in muscle mass (194). Consequently, DXA-derived estimates have been demonstrated to have a larger uncertainty in measurements, which may reduce the ability to detect small changes in muscle mass in response to resistance training (31,195). Nevertheless, these observations emphasize that, during a short-term intervention period of a few weeks, modifying training variables (3-9 sets) will not make a difference in either strength gains or muscle growth when total training volume is equated across conditions at the end of the training period (55,196). It also highlights the difference between measurement techniques and sensitivity in detecting early resistance-training-induced changes in muscle growth.

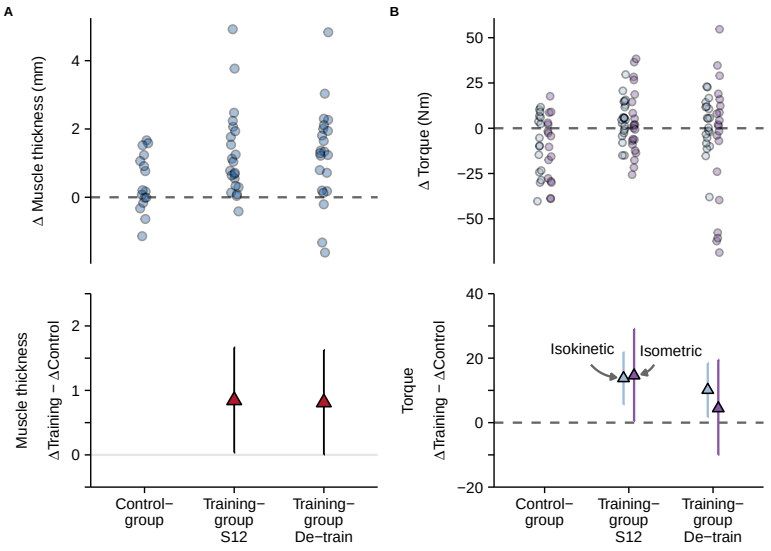

FIGURE 5.4: Study II: Training-induced changes, averaged over volume conditions, in muscle mass (A) and strength (B) compared to non-training controls following 12 resistance training sessions (S12) and eight days of detraining (De-train). Isokinetic: 90° sec^{-1}, Isometric angle: 60°. Points in the upper panels: visualizing changes in participants legs.

5.1.4.2 Effects of resistance training intensity on muscle strength and hypertrophy

In the military environment, adhering to a consistent training program over time might be challenging, making it difficult for soldiers to maintain or improve physical capacity throughout the year. Hence, it is of interest to investigate the effectiveness of different resistance training strategies implemented in soldiers daily routine where the aim is to optimize physiological preparedness and performance.

In addition to training volume, training intensity is regarded as a significant factor influencing strength development (197). Training intensity recommendations are typically referenced to the strength-endurance continuum (180), where the number of repetitions performed at a given load magnitude leads to specific adaptations (20). While heavier intensity is typically recommended for augmenting maximal strength (>85%

1RM) (13), lower intensity of 45-50% of 1RM (and less) have shown to increase dynamic muscular strength in previously untrained individuals (67,68). Furthermore, a study has demonstrated that even resistance-trained individuals can increase strength by lifting lower intensities that allow for a maximum of 15-25 repetitions (71). However, comparisons of high and low-intensity protocols have generally targeted lower-body limbs in predominantly untrained participants, limiting to few studies, with unclear response patterns for shorter training periods (<13 weeks) conducted in lab-controlled environments. Indeed, since strength and power are important physical capabilities for muscular performance in military personnel, we were interested in adding resistance training to cadets regular military training program. Moreover, as previous resistance training studies conducted in military environments are restricted to short time frames <15 weeks (81), and the benefits of resistance training are not consistently seen in the military setting (84,198), we conducted the study over 22 weeks in moderately trained cadets.

In response to 22 weeks of resistance training with either 10RM or 30RM, the cadets utilizing 10RM experienced greater muscle strength gains in both the upper and lower body. This was evident by a larger increase in specific 1RM muscle strength tests (Figure 5.5)A), as well as larger increases in weighted muscle strength, calculated from the weighted average of several measures of strength (Figure 5.5)C). These benefits largely corroborate with conclusions from previous studies (55,71,72,75,76,199), in line with recent meta-analyses (30,66,200), although it contrasts conclusions from other studies (68,70,201,202). Some of the observed advantages of 10RM training could be attributed to skill acquisition linked to performing training exercises that more closely resemble the test procedures (143,203,204), likely related to the two forms of maximal strength measure represent separate neuromuscular domains (204). Indeed, in one meta-analysis, high-intensity training was found to lead to superior performance in RM-tests but not in maximal isometric tests (30), which was also seen in the lower limbs in the present study. This underlines the need for multiple strength measures to ensure proper estimation of the effects of any resistance training intervention on muscle strength performance (143). Furthermore, conducting more frequent testing during a training intervention may diminish the risk of specificity, as shown in Morton et al (70), revealing comparable strength gains between low and high-intensity groups over a 12-week period, where both groups underwent five tests occasions during the intervention. Nevertheless, 10RM training stands out as the preferred intensity modality for moderately trained cadets, offering adaptation benefits while at the same time involving lower training volume and shorter training sessions, all of which are advantageous in a demanding military context with time restrictions (86). However, it must be emphasized that 30RM training also yielded significant improvements. Given these positive outcomes, 30RM training can provide valuable benefits for sustaining operational readiness. This is particularly relevant in situations where access to heavy exercise equipment or formal training facilities is limited, as often experienced during deployments or field exercises.

In contrast to the muscle strength results, 10RM did not offer universal benefits for increments in muscle mass compared to 30RM. Whereas 10RM led to more pronounced muscle mass accretion in the upper limb, measured as arm lean mass, it offered no such benefits in lower limbs, measured as either lean leg mass or muscle fiber CSA assessed in *m. vastus lateralis* or a combination thereof (Figure 5.5)A-B). The differential muscle accretion responses to the two groups between upper and lower limbs may have several explanations. It could be related to a pre-intervention difference in

training status between the limbs. Indeed, upper body limbs are likely to be understimulated in resistance training-naive individuals, as opposed to lower body limbs, which have been utilized during academy-related training (i.e., circuit training, running, marching) and everyday mobility. Furthermore, the differences between upper and lower limbs may have directly resulted from the concurrent aerobically-oriented training conducted alongside the study protocol. This may have compromised resistance training-related adaptations specific to the aerobically trained muscles, i.e. the lower body limbs (79). Thus, upper- and lower-body limbs may represent two slightly different experimental models, and as such, it seems reasonable to assume that they may show differential responses. It is also possible that the difference in response to resistance training between upper and lower limbs may be related to inherent differences in muscle biology. For example, upper body muscle fibers (*m. trapezius*) have previously been observed to express higher densities of androgen receptors compared to lower body muscle fibers (*m. vastus lateralis*) (205). Although, in the few studies that have included upper-and lower body measures, they have not found any difference in muscle growth between extremities across intensity groups in resistance-trained (71) and resistance inexperienced individuals (68). In a previous study, differences in muscle growth between upper and lower body limbs were associated with training volume. Performing 3 sets increased muscle mass (and strength) more than 1 set in the lower body, but no such difference was seen for the upper body muscles following 11 weeks of resistance training in untrained individuals (15). This can be interpreted as the upper body muscles are more untrained and any stimuli in the first weeks of resistance training can augment training adaptation, while a higher training volume is necessary for the muscles in the legs to induce training adaptations. Relating this volume perspective to our study, 30RM with its overall greater training volume (repetitions $\times$ load) compared to 10RM training, muscle growth developed less compared to lower volume but higher intensity (10RM) in the upper body muscles, while there was no group difference in the leg muscles. Although speculatively, manipulating training volume through volume sets rather than volume load could thus have implications for how muscle adapts between body limbs.

We further explored whether the initial stages of muscle strength/mass development would exhibit similarly between training group intensities, anticipating that distinctions in strength and muscle gains might become more evident as the intervention progresses. As experienced lifters advance in their training, it becomes evident that they require progressively heavier loads, with recommendations pointing towards utilizing at least 80% of their 1RM to stimulate further neural adaptations and enhance strength during resistance training (13,206). While this notion was supported by muscle strength development in leg exercises (particularly leg press), other outcome measures such as upper limb strength, upper limb lean mass, and jump height assessed with counter-movement jump showed a different scenario. Indeed, for these measures, the enhanced increase seen in response to 10RM training occurred predominantly during the initial phase of the intervention (Week 0 to Week 10). This course of development is challenging to explain based on the present data but may be related to the physiological demands of the field exercise (conducted weeks 18-19) compromising muscular adaptations. A finding aligning with a resistance-training intervention in military conscripts, albeit over a shorter period of 12 weeks. This study revealed that the most significant improvements in strength occurred during the initial six weeks, followed by a plateau when the training protocol was interrupted by field training and lower training volume (82). However, as lower limb muscle strength increased

without a concomitant increase in muscle mass during the last part of the intervention, it may indicate an increased specific strength in the 10RM group, augmenting muscle strength performance.

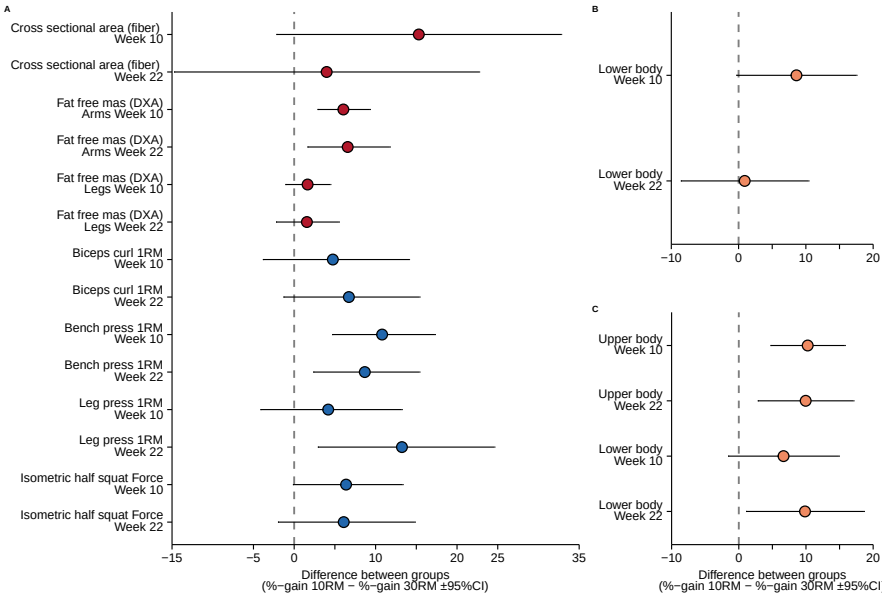

FIGURE 5.5: (A) Differences in training-induced relative changes in muscle-mass (red points) and strength (blue points) from baseline to Week 10 and Week 22 in Study III. (B) The weighted factor (orange points) for lower body fiber cross sectional area and lean body mass and the weighted factor for the strength measures in upper and lower body (C).

5.1.4.3 The effect of resistance intensity on functional performance

Given the importance of strength and power in modern warfare and the limited available evidence for measures of muscular performance in previous resistance-intensity studies (207,208), Study III evaluated certain fitness domains to enhance the transferability of strength and muscle mass to physical performance within the military context (159,209). Since higher-intensity resistance training may promote greater neural adaptations (210) and, both neural adaptations and strength likely mediate improvements in dynamic-muscular performance (211,212), there was not surprising that counter-movement jump height improved more in the 10RM group compared to the 30RM (Figure 5.6). This likely points in the direction of intrinsic factors, such as neural drive and other morphological factors, are enhanced in advance when utilizing higher intensities (10RM) and not at the same extent occur with lower-intensity training (30RM) (211,213) especially when considering that lower-body maximal strength was similar between the groups at week 10. Further, it is likely to impact sprinting and change of direction performance (214). Agility performance slightly favored 10RM resistance training, although the difference was not statistically significant. The relatively narrow confidence intervals, however, provide support for the direction of the observed effect. Muscle endurance, assessed by the number of repetitions performed with a load equivalent to 60% of baseline

body mass (215), did not show differential development between intensity groups or loading-specific adaptations aligned with the strength-endurance loading spectrum (216, 217). Rather, it supports muscular adaptations, and performance can be obtained across a wide spectrum of loading zones (218). Even though the effect of the training groups on muscular endurance was in the direction of using 10RM, the width of the confidence intervals indicates the uncertainty of the true mean effect.

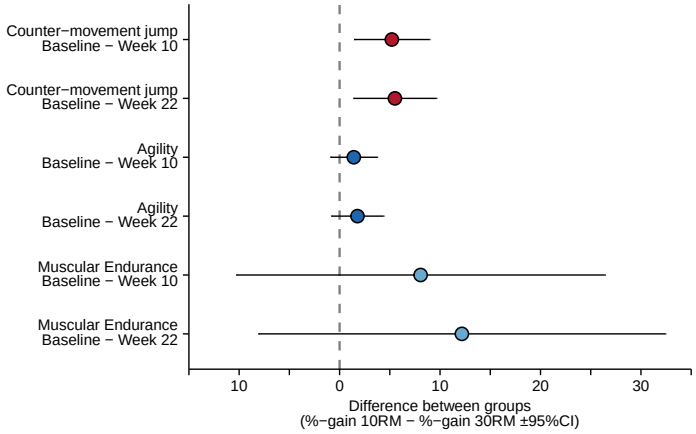

FIGURE 5.6: Differeces in training-induced changes in muscular performance from baseline to Week 10 and Week 22 in Study III.

5.1.5 Resistance-training volume impact on biomarkers

5.1.5.1 Effect of volume on signaling transduction pathway through mTORC1

In conjunction with the greater effect of 3-SET compared to 1-SET training in strength and muscle growth (Study I), 3-SET training corresponded to more pronounced responses in muscle biological markers associated with muscle hypertrophy. This heightened response in biological markers is often linked to signaling through mTORC1. A frequently employed indicator of mTORC1 signaling involves the phosphorylation of S6K1, which subsequently leads to the phosphorylation of rpS6 (22,94). This signaling pathway is suggested to be crucial for inducing protein synthesis and subsequent muscle growth in response to resistance training (219). In the present study, an increase in the phosphorylation of S6K1, specifically in the isoforms p70 and p85, both of which are indicative of heightened mTORC1 activity, were evident at week 2 when measuring acute activation before to after the fifth training session (as shown in Figure 5.7). This heightened phosphorylation of S6K1 coincided with elevated levels of phosphorylated mTOR and rpS6 (upstream and downstream of S6K1). These findings align with previous research that demonstrated an exercise-volume dependency of mTORC1-related signaling in the acute phase post-resistance session (21–23,33). While resistance training typically triggers this signaling cascade, it is important to acknowledge that additional acute-phase signaling pathways may operate in parallel with mTORC1, potentially facilitating downstream targets. This suggests that mTOR phosphorylation is not the sole factor driving p70S6k phosphorylation. For example, studies have shown a

disconnect between mTOR and p70S6k (22,220), hinting at alternative pathways for mTOR-independent p70S6k activation, potentially involving phospholipase D and phosphatidic acid (220). Compensatory signaling pathways like MAPK-ERK1/2 might further influence translation initiation through direct regulating rpS6 phosphorylation (221). However, it is worth noting that previous research has not observed changes in ERK1/2 phosphorylation in response to resistance exercise volume (22,23). Taken together, Study I clearly demonstrates a volume-dependent regulation along the mTORC1 axis. Nevertheless, it is imperative to acknowledge that simultaneous signaling pathways may operate concurrently, influencing shared targets responsible for eliciting muscle hypertrophy.

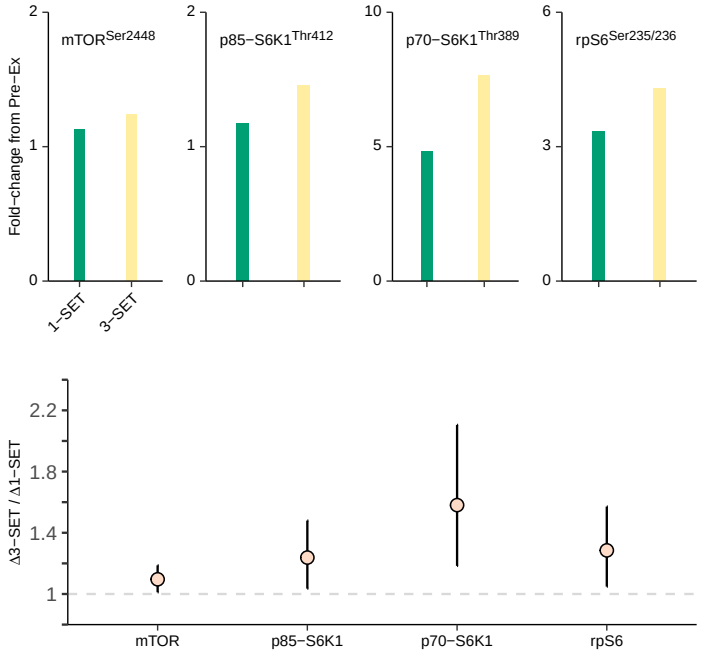

FIGURE 5.7: Acute changes in the phosphorylation of mTor, p85-S6K1, p70-S6K1, and rpS6 in response to Session 5 in Week 2 under different volume conditions and differences between volume conditions (lower panel). Data are estimates with 95% confidence intervals.

It is well-established that resistance training leads to increased mTORC1-related signaling, subsequently facilitating protein synthesis. Furthermore, there are observed volume-dependent increases in mTORC1 signaling coinciding with higher rates of protein synthesis (21,33). Consequently, a higher exercise volume could serve as a substantial stimulus for increased protein synthesis during the acute post-exercise phase. This, in turn, has the potential to promote long-term muscle growth. A connection previously demonstrated between acute signaling from an initial resistance training session with muscle growth measured after 14-16 weeks in untrained individuals (91,92). However, it is important to note that this correlation is not consistently observed in the literature (76). Even in the present study (Study I), there was inconsistencies in this relationship. Specifically, when evaluating the acute activation of S6k1 following the fifth training session, there were no significant associations with

resistance-training-induced changes in muscle mass. In fact, changes in S6k1 (p70-p85) and muscle mass, as determined through MRI, showed insignificant and weak associations (with r values ranging from r = -0.124 - 0.056).

5.1.5.2 Ribosomal biogenesis

Another crucial cellular function under the influence of mTORC1 is the control of ribosome biogenesis, which supports the protein synthetic demands of the hypertrophying cell (109,112,222–224). Accordingly, mTORC1s works as a signaling hub, conveying cell signaling to synthesize new contractile proteins. However, it also regulates the availability of rDNA transcription factors, promoting transcription and, consequently, the accumulation of ribosomal RNA. The accumulation of ribosomal RNA increases the RNA content, with approximately $\sim$ 80% of the total RNA pool composed of ribosomal RNA. Therefore, an increase in total RNA levels can reflect an increase in ribosome abundance when expressed per unit tissue weight (107,109,223). Several studies have demonstrated resistance-training-induced increases in total RNA (93,114,115,117). In agreement with these studies, 3-SET training resulted in 8.8% [1.5, 16.6] greater total RNA abundance per weight-unit of muscle tissue at Week 2 compared to 1-SET condition (Figure 5.8). This difference was also evident at week 12, albeit less extensive (5.9% [-1.9, 13,3]), but still elevated in both conditions compared to baseline. This response complies with the changes in rRNA subspecies, wherein 3-SET training led to a greater abundance of rRNA transcripts at week 2 (18S; 19%, 28S; 15.3%, 5.8S; 14.7%). Collectively, these alterations highlight the notion that training volume is an essential stimulus in eliciting ribosomal content, and it can be regulated in a dose-dependent way.

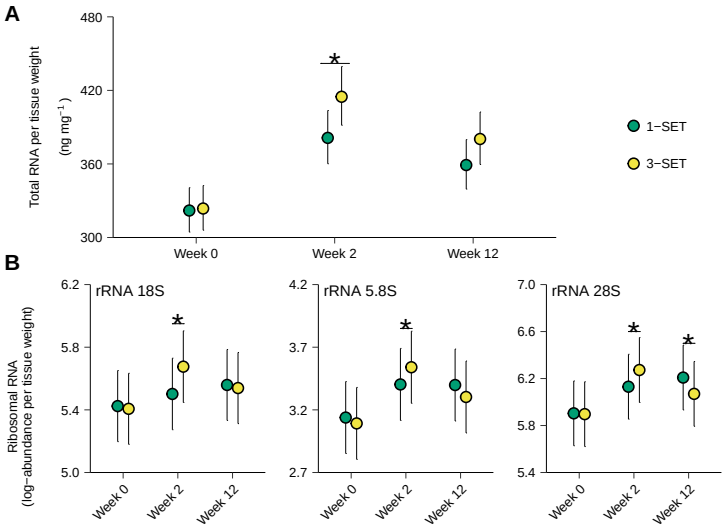

FIGURE 5.8: Total RNA (A) and ribosomal RNA (B) measured at weeks 0, 2, and 12 in Study I. Values are estimated means 95% confidence interval. * is difference between volume conditions.

The resistance-training-induced increase in total RNA indicates enhanced translational capacity, wherein greater rRNA production is associated with larger hypertrophic responses (93). This association between measures of ribosome accumulation,

indicated by the total RNA, and muscle hypertrophy has been observed in several studies (93,114–116). As these events unfold consecutively, with heightened translational capacity preceding observable muscle hypertrophy, an early elevation in total RNA levels may serve as an early indicator of training-induced adaptations in muscle hypertrophy (191,225,226). Indeed, in Study I, exploring a connection between total RNA levels and muscle growth unveiled a significant association. In this regression model, the dependent variable was set to changes in CSA with RNA abundance (RNA per muscle weight ng mg^{-1}) as the independent variable, measured at weeks 0, 2, and 12. The analysis demonstrated a linear relationship at week 2, with total RNA levels coinciding with changes in muscle size (CSA). Specifically, a unit increase in total RNA led to a 0.02% [0.01, 0.03] rise in muscle cross-sectional area. However, this linear relationship at week 2 disappeared at week 12 (0.01% [-0.00, 0.02]), which can indicate a dilution of the RNA pool as a consequence of the progressive increase in muscle mass (107). Hence, these data emphasize the crucial role of heightened translational capacity in the early phases of resistance training, playing a determining role in the adaptation of muscle mass in response to resistance training.

5.1.5.3 Time course profile of early stages of ribosomal biogenesis and related transcription factors

Analysis in Study I underscores the importance of early exercise-induced alterations in translational capacity by demonstrating a robust correlation between RNA abundance and muscle hypertrophy at Week 2, possibly priming the muscle for subsequent growth. However, the time course of total RNA/rRNA changes in response to resistance training has so far remained speculative, with no study investigating responses with multiple sampling time points. Study II was designed to address this gap by examining the early-phase accumulation of total RNA in response to resistance training to determine whether this initial increase is sensitive to training volume and a short period of non-resistance training. To explore RNA accumulation and its likely importance for subsequent muscle hypertrophy, we assessed the effect of resistance training per se by comparing muscular responses in a training group with a non-training negative control group. Additionally, to assess the sensitivity of the muscular translational system to resistance training, different volume protocols were allocated to each leg of the participants (Study design see Figure 4.2).

After twelve resistance training sessions, there was a substantial increase in total RNA per unit muscle weight, demonstrating a robust increase compared to the non-training control group (Figure 5.9)A, Post-training). However, these elevated total RNA levels declined after a de-training period, showing a reduction of 19% [-29, -8] compared to the peak levels but still remained higher than the baseline, although it was no longer robustly different from the control group (Figure 5.9)A, Post-De-training). Concomitant with the rise in total RNA levels, the initial training session led to increases in rRNA transcription, specifically 45/47S pre-rRNA, when compared to non-training controls (Session 1, Figure 5.10). This increase persisted after twelve training sessions, accompanied by higher levels of mature rRNA (28S and 18S, Figure 5.10). After de-training, pre-rRNA levels returned closer to those observed in the control group, but mature rRNA transcripts remained elevated in the training group compared to controls, indicating a decrease in de novo rRNA transcription (Figure 5.10), Post + de-training). In combination with the findings in Study I, the current findings support the notion that resistance training alters total RNA content (93,114,115,117,225–227), likely driven by de novo transcription of rRNA. Furthermore, the normalization of pre-rRNA levels during the eight-day break from

resistance training indicates a strong link between ribosome formation and the stimuli provided by resistance training, which makes sense given the significant cellular resources required for synthesizing ribosomes (106,107).

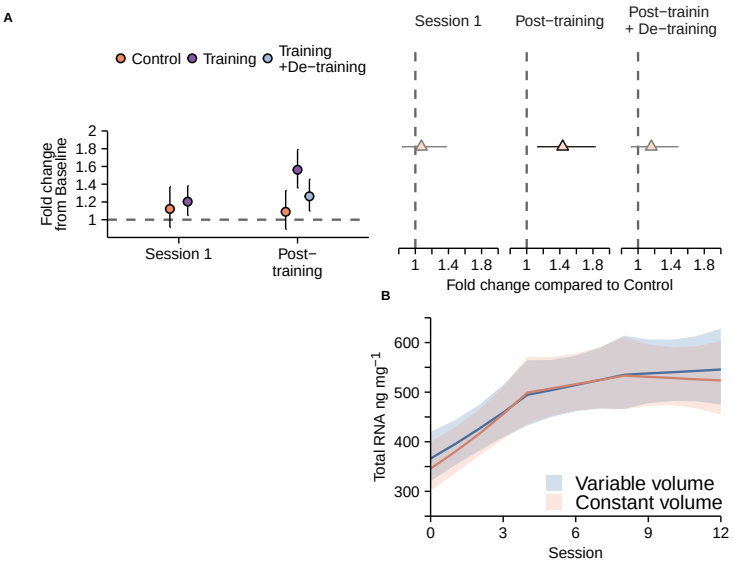

FIGURE 5.9: Resistance-training induced increases in total RNA per tissue weight measured after Session 1 and 12 (Post-training) and after eight days of de-training (A). Time course of total RNA in variable- and constant-volume training conditions (B). Values in the training group are averaged over volume conditions. Errorbars are 95% CrI.

The resistance training resulted in a noticeable session-to-session increase in total RNA abundance, with increases in total RNA predominantly occurring during the first part of the training period (as shown in Figure 5.9)B). Indeed, when averaged over volume conditions, total RNA increased 8.6% [5.6, 11.8] per session during the four first training sessions, after which the changes gradually leveled out from sessions four to eight (1.8% [-1.0, 4.7] increase per session) and eight to twelve (0.0% [-3.1, 3.2] increase per session). This increase corresponded to relative increases from baseline to 48 hours after sessions four, eight, and twelve, respectively, of 39.0% [24.1, 56.1], 49.4% [34.0, 66.9], and 49.5% [32.5, 68.2], with the peak increase from baseline to the eighth session being defined as an accumulation phase (Figure 5.9)B). This aligns with a previous study that reported peak values within four to nine sessions in young individuals (3 weeks) (117), further corroborating the findings in Study I. This implies that a rapid increase in ribosome capacity may prepare muscle fibers for subsequent growth, responding to increasing demand for proteins (114,117). After initial accumulation, the apparent leveling off and plateau may indicate that ribosome production is only marginally influenced by continued training. Instead, the continued training seems to maintain the elevated RNA level, as evidenced by the sustained elevation of pre-rRNA transcripts at session 12, signifying syntheses of novel rRNA (Figure 5.10). This suggests that the continuous transcription of rRNA plays a crucial role in maintaining a relatively constant ribosomal density, aligning with the demands of hypertrophying cells (107).

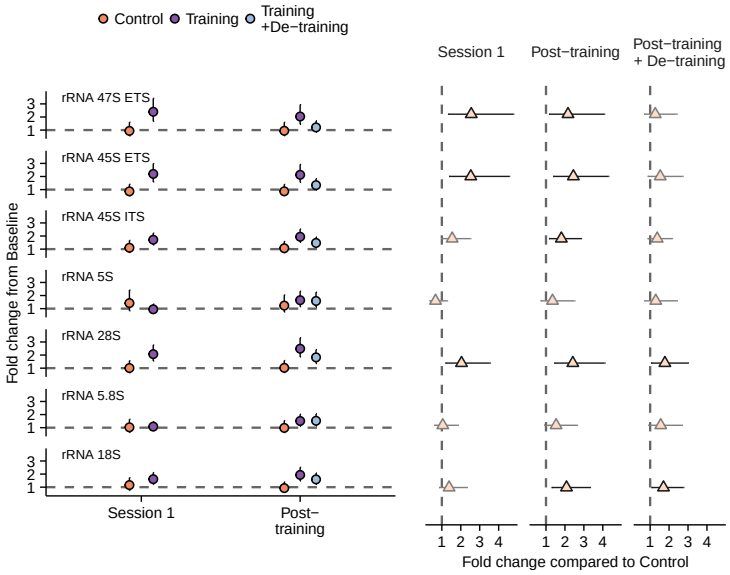

FIGURE 5.10: Compared to the control group, resistance training increased ribosomal RNA per tissue weight measured after Session 1 and 12 (Post-trining) and after eight days of de-training. Values in the training group are averaged over volume conditions. Point estimates are displayed with 95% CrI.

We then explored whether ribosome biogenesis could reflect the degree of training stimuli. Since synthesizing ribosomes is an energy-consuming process involving multiple factors under tight regulatory control, it could be closely connected to changes in training volume. Therefore, during the 12 sessions, one leg had varying training volume while keeping it constant in the other within participants in the training group. Initially, both legs received the same training volume for the first four sessions (six sets). Subsequently, the leg with variable volume was reduced to three sets for the next four sessions, followed by an increase to nine sets for the last four sessions. Both volume conditions showed similar increases in total RNA (Figure 5.9)B) and most rRNA species throughout the training sessions. This suggests that the transcriptional apparatus is not sensitive to a relatively short period of fluctuation in training volume, at least when performing four training sessions per volume adjustment, and hence does not affect subsequent long-term hypertrophy adaptations.

The observed training-induced increases in rRNA and total RNA coincided with increases in ribosomal protein S6 (rpS6) and upstream binding factor (UBF) protein levels. For both rpS6 and UBF, protein levels increased linearly throughout the training intervention, with rpS6 showing estimated increments per session corresponding to 4.2% [1.2, 7.3] during block 1 (session 1-4), 2.6% [-0.3, 5.5] during block 2 (session 5-8) and 4.6% [1.2, 8.1] during block 3 (session 9-12), and UBF showing increments corresponding to 7.3% [2.1, 13.0], 4.5% [-0.5, 9.8] and 6.1% [0.3, 12.1]. This general pattern was confirmed when comparing the training group to the control group, where UBF and rpS6 protein levels were higher in the training group compared to the non-training control group after Session 12. This increase was followed by a partial reversal of rpS6 but maintained UBF levels after the de-training period (Figure 5.11).

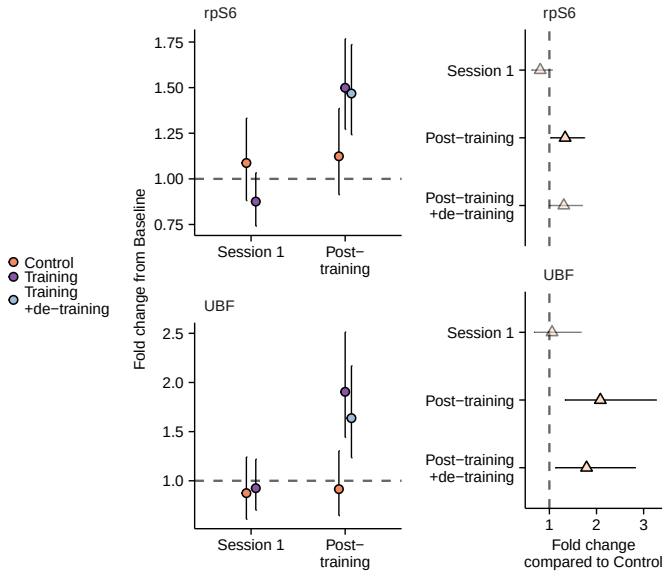

FIGURE 5.11: Ribosomal protein S6 (rpS6) upstream binding factor (UBF) protein measured 48 h after session 1 and 12. Right panel, group comparison between training and control. Values in the training group are averaged over volume conditions. Errorbars are 95% CrI.

The potential link between total RNA and the levels of rpS6 and UBF proteins was examined in order to delve deeper into this potential coordination. UBF levels positively predicted RNA levels in the training group throughout the intervention. Specifically, for each unit (SD-units) increase in UBF, there was a corresponding 5% 95%CI[0.2, 10.2] increase in total RNA levels. The relationship between UBF levels and total RNA thus indicates that the UBF abundance does contribute in facilitating rRNA transcription and, consequently, the regulation of ribosome biogenesis (109,228). However, such a relationship did not appear between RNA levels and rpS6. Even though resistance training led to a general increase in both rRNA and rpS6, which is in agreement with a previous report in young men (127), the disconnect observed in the regression analysis (2% 95%CI[-3, 7]) suggests that the regulation of rpS6 expression and ribosomal RNA transcription follows different temporal patterns in response to resistance training. This can be related to differing half-life of ribosomal proteins (ribosomal proteins mRNA have a shorter half-life than other mRNA transcripts and degraded if not assembled in ribosomes) and ribosomal proteins being identified with other functions without interfering with the ribosomes (228). Free ribosomal proteins may, therefore, interact with other cellular proteins and perform extra-ribosomal functions (i.e., DNA repair), affecting protein expression independent of ribosomal biogenesis (229,230).

After eight days of de-training, total RNA and rRNA levels per weight unit muscle tissue returned toward baseline levels, though without concomitant reversal of muscle thickness, which remained at elevated levels. This was likely caused by attenuated rRNA transcription. This notion was supported by the reversal of pre-rRNA abundances and possibly by lowered UBF protein levels decreasing the total RNA levels with $\sim$ 20% from the last training session (Figure 5.10). A decrease in total RNA levels, comparable in magnitude to the reduction observed after 10-14 days of unilateral limb immobilization in resistance-inexperienced men (231,232). This suggests that resistance training-induced increases in ribosomal content are easily lost, driven by a rapid reduction in ribosome biogenesis and possible increased ribosome degradation (232). The de-training effect on total RNA and rRNA levels highlights that ribosomal biogenesis is a demand-driven cellular activity, and unnecessary biogenesis imposes an energetic penalty on the cell (228,232).

5.1.6 The accumulation rate of total RNA predicts muscle resistance-training-induced hypertrophy

To further explore the connection between total RNA and muscle growth, changes in total RNA abundance over the training period were estimated from each participants leg. These estimates were then used to model muscle growth, measured as increases in *m. vastus lateralis* thickness. Rates of RNA accumulation throughout the entire intervention emerged as a determinant of changes in muscle thickness, corresponding to every percent increase in total RNA per session led to an 0.28 mm increase in *m.vastus lateralis* thickness. Thus, individuals with higher rates of RNA accumulation over the training course period showed larger accretion of muscle mass (Figure 5.12)A). Conversely, individual variation in fixed total RNA (content at session 6) was negatively associated with muscle growth. In fact, higher levels of total RNA content were instead associated with a tendency towards lowered muscle growth (Figure 5.12)B). This analysis gives implications for the importance of making new ribosomes in contrast to having a lot of ribosomes. It may also imply that newly synthesized ribosomes would be more beneficial for muscle growth in response to new stimuli, as novel ribosomes are potentially specialized for growth (233,234).

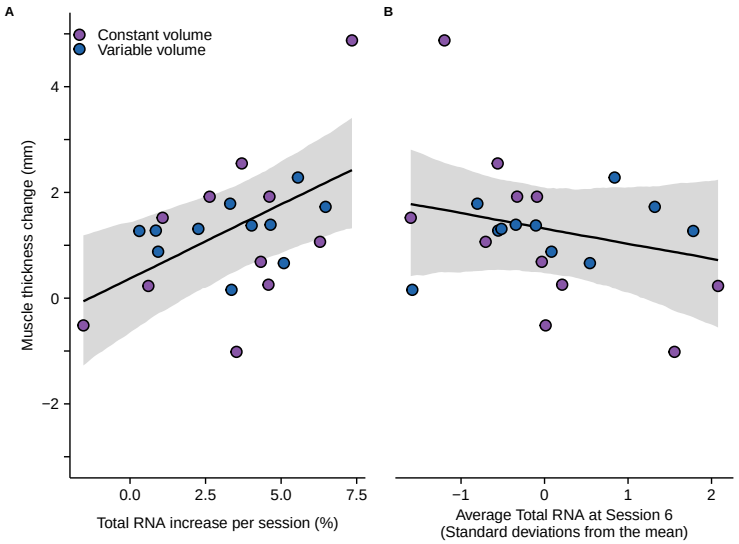

FIGURE 5.12: Total RNA increase per session correlates with muscle thickness in response to the twelve resistance training sessions (A) and average total RNA abundance at Session 6 (B).

The data from the longitudinal 12-week Study I supports the idea that novel ribosomes specialize in the translational process of contractile proteins for muscle growth. Following the 12-week training intervention, thirteen and sixteen participants showed clear benefits of 3-SET training for increases in CSA (SWC 2.7%) and strength (SWC 4.5%), as indicated by the blue points in Figure 5.13. This benefit was accompanied by higher total RNA levels in the 3-SET leg compared to the 1-SET leg after 2 weeks of training (17.6% [5.8, 30.7] and 9.5% [-1.7, 22], respectively).

Further analyses revealed that participants who benefit from 3-SET training on CSA, strength, or both showed higher levels of total RNA in the 3-SET leg than in the 1-SET leg measured as a between-leg ratio (Figure 5.14). Eleven participants showed no benefits of 3-SET training on either CSA or strength, meaning training volume conditions were equally effective in developing strength and muscle growth in these individuals (below the SWC threshold for strength and CSA). This was further reflected by the lower levels of total RNA in the 3-SET leg than in the 1-SET leg, indicated by the lower ratio (0.96 [0.92,1.00], n=11, lower left quadrant in Figure 5.14). In contrast the benefit of 3-SET for both CSA and strength displayed the highest 3-SET-to 1-SET leg ratio for total RNA (1.34 [1.01,1.68], n=6, upper right quadrant), followed by benefit on CSA only (1.13 [1.03,1.22], n=7, lower right quadrant), and strength only (1.12 [0.98,1.27], n=10, upper left quadrant). These findings suggest that increasing ribosomal content in the early phases of resistance training in response to the higher mechanical and metabolic stress accompanying higher training volume may be crucial in facilitating subsequent muscle growth and strength. This likely occurs through an increased capacity for protein synthesis and aligns with the overall impression conveyed by the data set, wherein 3-SET training resulted in larger increases in total RNA and mature rRNA subspecies (rRNA 18S, 28S, and 5.8S, see Figure 5.8) and subsequent muscle hypertrophy and strength gains. Therefore, our data supports the overall consensus of ribosomal biogenesis being a determinant factor for muscle

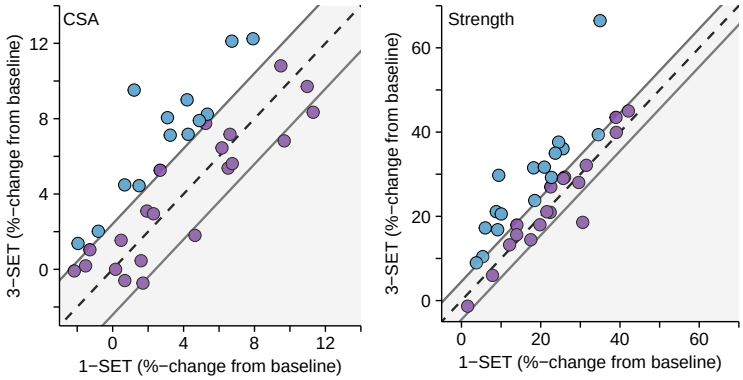

FIGURE 5.13: Relationship between training conditions leg for each participant in study I. The dashed identidy line indicate x = y, and the distance from the dashed identity line to solid lines indicates the smallest worthwhile change (SWC). CSA = *m.vastus lateralis* muscle cross sectional area, Strength = weighted average of all strength measures, blue circles = benefit of 3-SET, purple circles = no benefit of 3-SET

growth in response to resistance training (93,114–116).

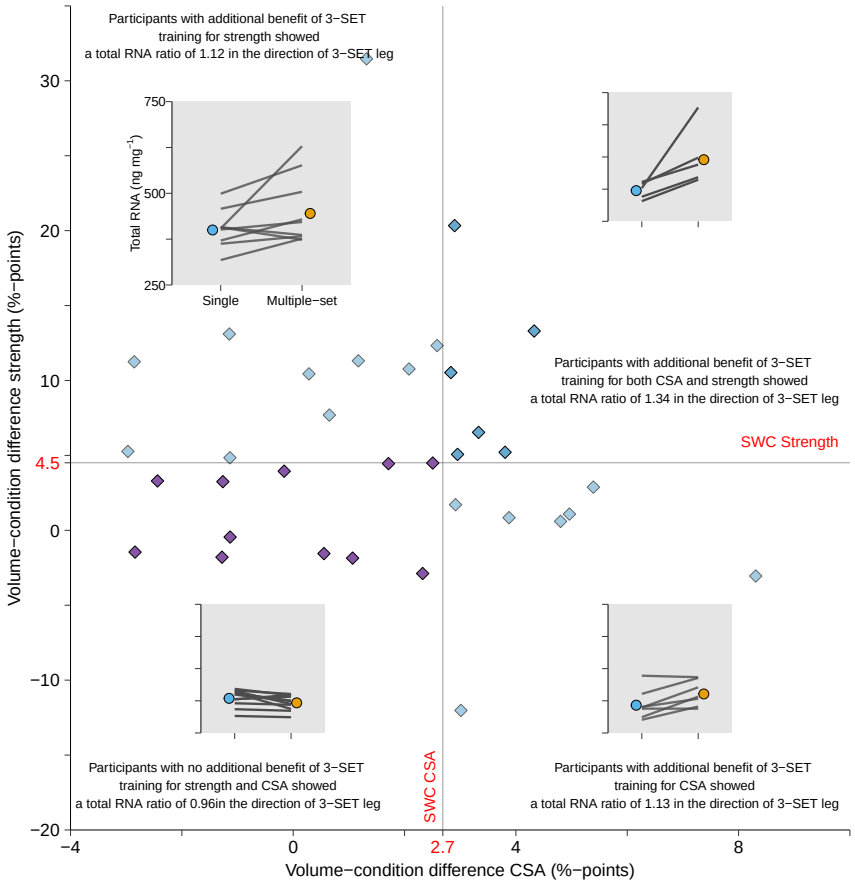

FIGURE 5.14: Additional benefits of 3-SET training on CSA, strength or both, and its association with total RNA levels at Week 2, measured as ratios between 3-SET and 1-SET leg.

5.2 Military field exercise

Field exercises often involve high activity levels and energy expenditure combined with low energy intake and reduced sleep (154,156). Prolonged negative protein balance and concomitant muscle loss may compromise physical performance and increase injury risk and lost duty time, diminishing warfighters readiness (36). Designing training programs aimed at sustaining optimal levels of muscle mass would effectively serve to support soldier performance across a wide range of military-relevant tasks (235), that is frequently linked to strength and power production (83). Therefore, preserving muscle mass becomes imperative, especially during prolonged periods in the field, to sustain muscular performance and overall military readiness. Increased protein intake has been shown to effectively mitigate fat-free mass loss and maintain muscle strength in diet-controlled weight-loss programs (151,162,163). Within this framework, Study IV compared the effects of high and low quantities of proteins under isocaloric conditions on muscle mass and performance in cadets experiencing energy restriction during a field exercise. It is noteworthy that previous research included supplementary protein in addition to the regular diet, resulting in higher total energy intake among the protein-consuming participants compared to the control subjects (236,237). Consequently, these studies did not exclusively examine the direct impact of protein supplementation on muscle mass and performance, as energy availability emerges as a potent modulator of these interconnected variables.

5.2.1 Muscular strength and performance

In the present study, the ingestion of higher protein intake combined with low intake of carbohydrates (in an isocaloric fashion) led to similar decreases in physical performance as the combination of lower protein intake and low carbohydrate intake, measured as counter-movement jump height, maximal strength, and cycling sprint power following the 10-day military exercise (Figure 5.15). The decline in physical performance observed over the 10-day military exercise aligns with findings from other studies that have examined the impact of near-continuous physical activity, sleep deprivation, and underfeeding on muscle strength and power (36,155–159,236). In these studies, the extent of the impaired performance co-varies with the severity of the intervention, including its length (36,156,157,159) and its degrees of energy deficiency (236,237), as well as with differences in the timing of post-exercise testing, varying from 2 hours to 24 hours (157,159,236,237), but independent of the amount of body mass losses. Data from the present study are in the outer-most part of the specter, despite a relatively low level of physically exhausting activities during the intervention and a relatively short duration compared to other studies (36,155,156,236).

The current investigation revealed notably larger reductions in both body mass and muscular performance compared to studies of equivalent duration (159,237). This discrepancy is likely due to a more substantial negative energy balance. According to a meta-regression analysis, participants can tolerate a negative energy balance of approximately -1166 kcal $\cdot$ d^{-1} or a cumulative total of -8162 kcal without experiencing more than a negligible (0%) to minor (-2%) impact on performance (4). Hence, in the present study, a plausible inference is that the marked decline in muscular performance can be attributed to the significant energy deficit, estimated to -4320 kcal $\cdot$ d^{-1} (representing an aggregate energy deficit of about -43203 kcal, $\sim -77\%$), calculated from changes in fat mass (-4.9 $\pm$ 1.4 kg) and fat free mas (0.5 $\pm$ 2.2 kg, FFM), resulting in more pronounced loss of fat mass compared to findings in numerous other

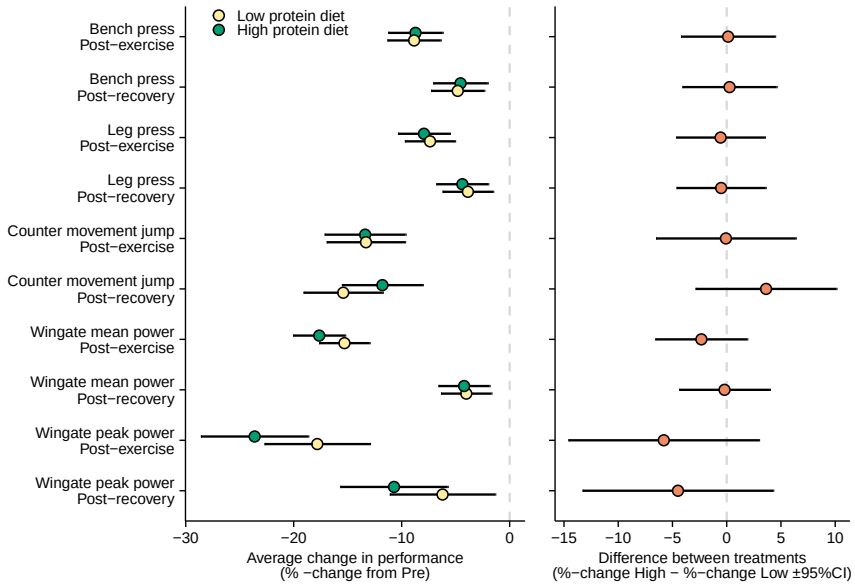

FIGURE 5.15: Changes in performance parameteres following 10-days of military exercise (Post-exercise) and 7 days of recovery (Post-recovery) in High (2 g kg^{-1} d^{-1}) and Low (1 g kg^{-1} d^{-1}) protein diet groups. Values are estimated means with 95%CI.

studies (156,157,159,236–239). This also agrees with a meta-analysis that consolidated data from nine military field exercise studies. This analysis identified a decline in lower-body power and strength as a cumulative consequence of the combination of daily energy deficit and exercise duration (4). The authors concluded that the total energy deficit of military exercises/operations should not exceed -5000 to -19.000 kcal to limit negative effects on physical performance (4). When the energy deficit exceeds 40.000 to 60.000 kcal, moderate to large declines can be expected in physical performance, corroborating well with data from the present study. Moreover, the high protein group (2 g kg^{-1} d^{-1}) experienced severe carbohydrate deficit in addition to the general energy deficit (habitual 5.1 g kg^{-1} d^{-1} vs diet intervention 0.6 g kg^{-1} d^{-1}), a level which is way beyond the recommended intake between 4-8 g kg^{-1} d^{-1} for active warfighters (240). This may have impaired any positive effects of higher protein intake by further increasing the need for gluconeogenesis in order to sustain energy homeostasis (241). Taken together, relative levels of energy deficit seem to be more decisive for whole-body protein loss (239,241) and performance (242), than macronutrient composition in a state of severe energy deficit.

5.2.2 Body composition

A decrease in military training performance is frequently linked to body mass loss, particularly the loss of muscle mass. However, a severe negative energy balance can impact physical performance even before reaching a 10% body mass loss—a threshold considered detrimental to preserving strength and endurance performance in soldiers (243). In fact, periods of low-calorie diets can cause significant muscle atrophy, particularly in type II muscle fibers (244), leading to a loss of strength and power (158,245).

This suggests that the duration of studies and the extent of negative energy balance are crucial factors contributing to the decline in physical performance beyond just body mass (4,243). This idea is consistent with our study, which was relatively short but had a severe negative energy balance, resulting in an average body mass reduction of about 5-6% (Figure 5.16)A). Despite this modest body mass loss, there was a significant decrease in strength and power performance following the field exercise in both intervention groups (Figure 5.15).

Surprisingly, despite a decline in muscle performance in response to the military exercise, neither the high-protein group (2 g kg^{-1} d^{-1}) nor the low-protein (1 g kg^{-1} d^{-1}) group showed any significant changes in fat-free mass. This suggests that the intervention did not affect the amount of muscle mass, which contradicts findings from several studies (36,155,156,159,236,246,247), some of which involved similar (246,247) or less severe energy deficits and shorter exercise duration (155,159,246,247). Some doubts are raised about the fat-free mass measurement process, especially the timing of the post-exercise DXA scanning conducted immediately after the exercise. This timing may have been problematic because physical activity toward the end of the exercise can lead to changes in body fluids and hydration status, potentially impacting the accuracy of fat-free mass estimation (194,248). Additionally, DXA-based fat-free mass measurements are sensitive to changes in carbohydrate stores in skeletal muscle, which can result in tissue dehydration (194). Cadets likely experienced this scenario during the post-exercise scan, which might have influenced the fat-free mass data. However, this could have led to an underestimation of fat-free mass levels, opposing the potential overestimation caused by the timing of DXA scanning (249). Furthermore, the pronounced increase in CK levels, indicating muscle damage, is associated with increases in muscle swelling due to increased fluid transfer into the muscle tissue (250). This may mask any reduction in muscle protein content and hence obscure the estimation of fat-free mass data at post-exercise (194). Therefore, interpreting fat-free mass estimates should be done with caution. Interestingly, in a recent field exercise with a severe energy deficit ($\sim$ 6000 kcal per day) during a 5.5-day field exercise, female soldiers were able to attenuate muscle mass loss, which was also observed in the female cadets in the present study (Figure 5.16)B). This preservation of muscle mass during an energy deficit may be related to females ability to metabolize more fat and fewer carbohydrates and amino acids compared to men (158). Although this explanation does not fully clarify why most male cadets in the present study were able to preserve their muscle mass. However, a recent field exercise study of similar duration and $\sim$energy deficit observed only minor losses in muscle mass (160). Together with our data, encompassing fat mass at a certain level (at least within the normal range) before a demanding field exercise, cadets may be better able to preserve muscle mass. A notion supported in male conscripts wherein individuals with initial higher fat mass levels led to attenuated losses in muscle mass following a demanding exercise with even higher daily energy expenditure and lower energy intake than in our study (158). Although such a correlation was not observed in the present data set (Figure 5.16)C), it may be more evident when the initial fat mass levels are lower (<10%) as in Vikmoen et al., (158). This underscores the importance for soldiers to maintain fat mass levels within the normal range, at the very least, before engaging in demanding field exercises, in order to better protect against muscle mass loss.

5.2.3 Hormonal regulation

Overall, 1 and 2 g kg^{-1} d^{-1} protein supplementation led to similar declines in blood concentrations of anabolic and pro-metabolic hormones (e.g. testosterone and Insulin

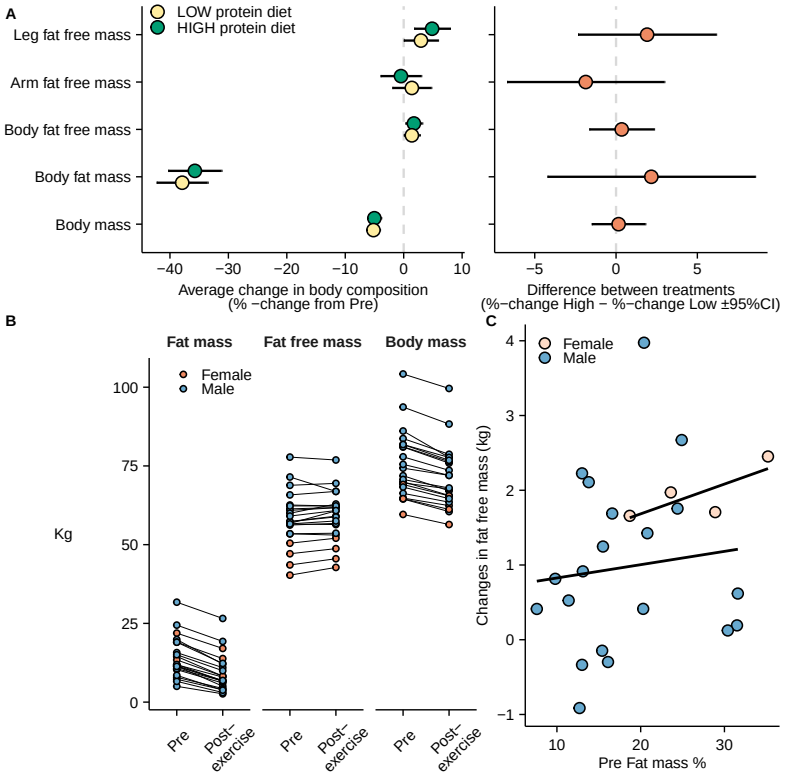

FIGURE 5.16: Changes in body composition from DXA-derived estimates following 10 days of military exercise in High (2 g kg^{-1} d^{-1}) and Low (1 g kg^{-1} d^{-1}) protein diet groups (A). Points are estimated means with 95%CI. Individual changes in body composition from Pre to Post-exercise (B). Correlation plot between pre fat mass (%) and changes in fat free mass (C).

like-growth factor-1; IGF-1) and markers of muscle damage (Creatine kinase), with only Triiodothyroine (T3) and Cortisol (COR) showing differential responses between groups (Figure 5.17). The relatively marked changes in blood variables align with previous research investigating the physiological effects of rigorous military exercises (36,155,157,158,236,251). The observed changes in endocrine variables in response to the military exercise, such as decreased levels of androgen hormones (TESTO, freeTESTO, IGF-1) and pro-metabolic hormones (T3 and T4), alongside elevated COR levels, indicate the development of a catabolic physiological milieu, resembling observations made in previous studies (36,155,157,158,160,236,238,251). This may halt cellular growth and proliferation while allocating available energy resources toward basal metabolic demands (241,251). Accordingly, during the military exercise, the cadets were in a maladaptive state, with a reduced ability to repair muscle tissue and sustain adequate tissue functions, which could explain the observed impairment in physical performance. Furthermore, the substantial increase in Creatine kinase levels post-exercise, indicating damage to the contractile apparatus in muscle fibers, may suggest that muscle damage is a potent contributor to the reduced muscle performance seen (155,158), as there was no significant change in muscle mass during the intervention together with a recent study showing that muscle fiber size does not change in response to a field exercise (160). For most of the endocrine variables, there was no beneficial effect of higher protein ingestion, strengthening the notion that the severe energy deficiency was more decisive for responses to the exercise than amino acid and carbohydrate availability, as carbohydrate has a protein-sparing effect and vice versa (252).

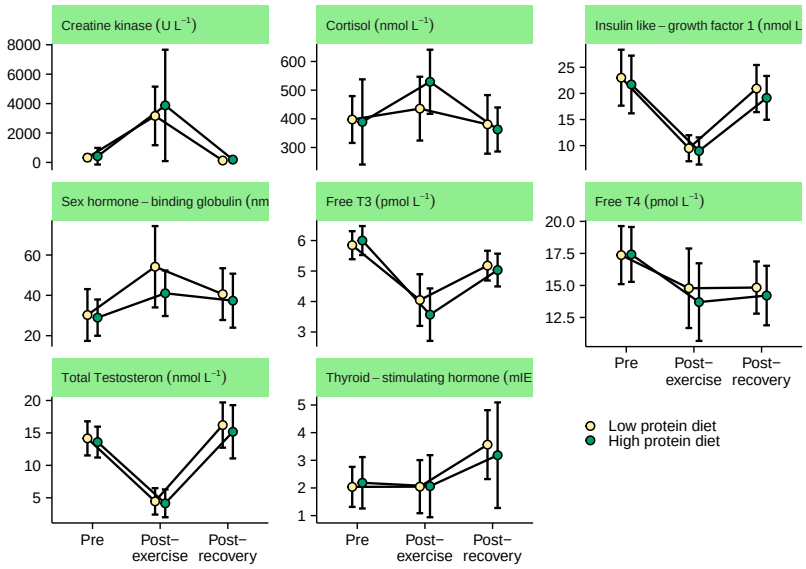

FIGURE 5.17: Absolute changes in endocrine biomarkers before (Pre), after 10-days military exercise (Post exercise) and following seven days of recovery (Post recovery) in High (2 g kg^{-1} d^{-1}) and Low (1 g kg^{-1} d^{-1}) protein diet groups.

5.2.4 Recovery of the variables following the field exercise

The recovery duration for soldiers is a vital aspect that influences their operational readiness during training and combat missions (86). Despite this recognition, the scientific literature is notably lacking in comprehensive insights into the temporal aspects of recovery processes after military field training (155,156,158). This sparse information is somewhat surprising, given the paramount importance of recovery in assessing soldiers health and performance readiness. The seven-day recovery period in the present study proved effective in restoring body mass and most performance and endocrine variables toward pre-exercise levels. Notably, increased protein intake during the field exercise did not influence the recovery of any of these variables. The effectiveness of this recovery period can likely be attributed to the restoration of energy intake and rest, mirroring observations made in previous investigations of military exercises (155,156,160,251,253,254). The restoration of hormone levels towards or even surpassing resting physiological levels may signify a need for cellular growth and repair, a recurrent pattern frequently observed following demanding military field exercises (158,251,254,255).

However, it is worth noting that jump height, as assessed through the counter-movement jump, did not recover in the same pattern as the other performance variables and remained at reduced post-exercise levels (Figure 5.15). This aligns with previous findings where counter-movement jump performance remained compromised even after 10 to 14 days of recovery from intense military exercises (155,158,160,255). The prolonged recovery of counter-movement jump may be due to impaired functions of muscle spindles, possibly linked to elevated Creatine kinase concentrations and/or muscle fiber damage (245,256). Such impairments may affect the stretch reflex, which plays a significant role in counter-movement jump performance, leading to delayed maximal shortening velocity and power (257). This suggests that the ability to generate force at high contraction velocities recovers more slowly compared to the capacity to generate force during low-velocity contractions or isometric actions.

5.3 Methodological considerations

Including a control group in Study I and III would have strengthened our interpretation of the effects of resistance training per se. This would have been especially valuable in confirming beneficial effects overall treatment effects in Study III as compared to conventional military training, which is characterized by light loads and high velocities (81,82,258).

The training groups in Study III were not volume-matched, despite evidence of a volume-dose response relationship with muscle growth and strength (26,259). This decision was made because equating training volume to, for example, the 10RM group could have led to insufficient stimuli for muscular adaptations in the 30RM group (55). To ensure maximal muscular stimuli for each training group, reaching true failure in each training set within the designated target repetitions, the training load was progressively adjusted to induce maximal responses. Nevertheless, the significantly larger volume load lifted in the 30RM group did not translate into superior muscular adaptations compared to the 10RM group, contrasting with the expected effects of increased training volume.

In Study I and II we allocated volume conditions within participants using unilateral exercises. These studies effectively minimize differences between study conditions

otherwise attributed to inter-individual differences. As such, inferences drawn from these studies can be regarded as robust and less prone to differences between study participants with regards to i.e. behavioral and genetic characteristics. In contrast, a between-participants design is more likely to mask meaningful effects, such as relationship between training volume and associated adaptations, due to larger variation in training responses between conditions caused by inter-individual differences. Indeed, this effect was evident in Study I. We observed a wide range of responses to resistance training, with variations in muscle growth and strength changes across participants as shown in (Figure 5.18)A). However, when looking at individual participants, the differences in response to different training volumes were consistently smaller than differences between participants. An observation which was supported by a strong correlation between the responses to 3-set and 1-set conditions in terms of increased average strength gain ($r = 0.80$, [0.55, 0.87]) and muscle hypertrophy ($r = 0.75$, [0.55, 0.87]). This suggests that, despite the overall variability, the impact of training on individuals remained consistent across different conditions. Consequently, examining effects within participants enhances the power to detect small yet important differences in outcomes, like changes in muscle signaling (biomarkers), muscle strength or mass. Furthermore, even with randomization balanced for known co-variates, small between-participants designs, suffers from the risk imbalance of un-measured co-variates and as a consequence, allocated treatment groups may by chance exhibit systematic imbalance in outcomes. A larger sample size and longer intervention period would presumably display a similar correlation between volume conditions in Study II, assuming participants are drawn from the same population (Figure 5.18)B).

However, the practical relevance to the broader population is somewhat limited with the within-participant design, resulting from a lower ecological validity. Conversely, integrating the training intervention into cadets weekly training routine enhances its relevance and applicability for sustaining the training program within their operational environment. Additionally, the utilization of a parallel between-participant design enhances the generalizability of the findings to a broader population, as each participant is performing only one condition (treatment), engaging in a whole-body training program reflective of typical real-life experiences.

In evaluating the benefit of training volume (3-SET training), we must acknowledge that the rationale of using smallest worthwhile change with a cut off threshold set to between-participants baseline standard deviation greater than 20% is not based on empirical evidence but justified by using an objective factor other than just assume any meaningful benefit is above/under zero line (null hypothesis). By using this threshold, we may have been naive in assuming any meaning full change is beyond such threshold, considering the threshold does not account for typical errors associated with our tests. Consequently, we might have been overly optimistic in believing that the observed score closely reflects an individuals true score. However, repeated familiarization with the test battery and standardization efforts could, to some extent, mitigate the uncertainty associated with an individuals true score.

The immunohistochemistry analysis shows average fibers per sample of $\sim$ 500 and $\sim$ 235 from *m.vastus lateralis* in Study I and Study III, respectively. Accordingly, there should be enough fibers for a representative analysis for changes in fiber type proportion and size (260,261). However, we must acknowledge that half the number of fibers in Study III is causing a greater variance and uncertainty in the analysis, which leads to more imprecise estimates and interpretation of the muscle fiber type

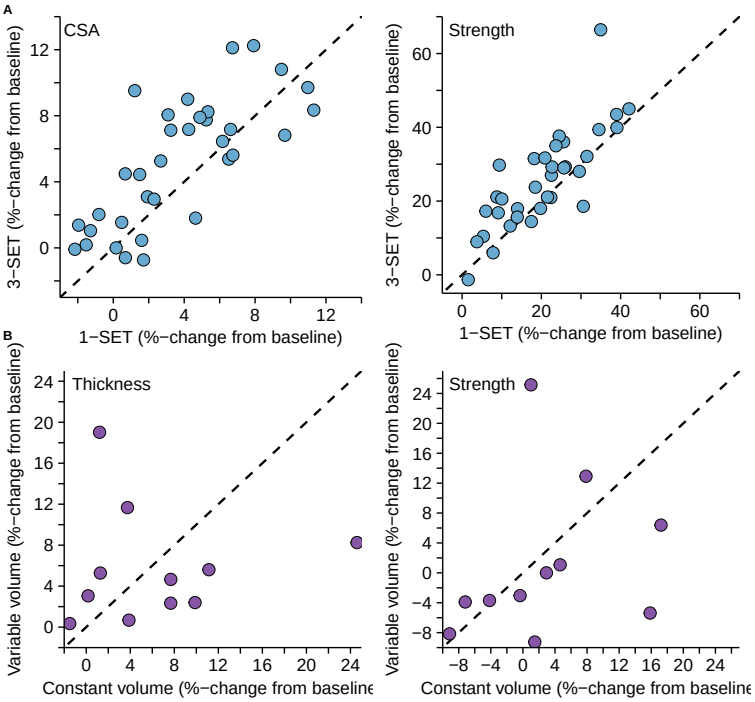

FIGURE 5.18: Relationship between training conditions leg for each participant in study I (A) and Study II (B). The dashed identidy line indacate x = y. CSA = *m.vastus lateralis* muscle cross sectional area, Thickness = muscle thickness *m.vastus lateralis*, Strength = weighted average of all strength measures.

proportion and size. Furthermore, biological variation may affect subsequent analysis and interpretation due to, i.e., differences in sampling depth and acute changes in signaling events in response to multiple sampling at the same site. At the same time, this variation is difficult to account for (260). Study II randomly sampled biopsies 1 cm proximal/distal to the first sampling point to reduce possible changes in response to the previous biopsy (i.e., adaptations to tissue healing, regeneration).

As previously noted, conducting DXA measurements immediately after the field exercise might have compromised the accuracy of the fat-free mass data (Study IV). Although a later follow-up scan could have provided validation for our data interpretation, limited access to the apparatus prevented this option. Nevertheless, it's important to emphasize that these uncertainties should not have had an impact on the group comparison analyses.

In evaluating cadets dietary intake and steady-state energy requirements we used a dietary recall (24 h), which was fast and easy to administer on the cadets telephone. Such self-report energy intake can lead to underestimation of the true energy requirements, , as caused by underreporting (262). However, the energy data provided by the cadets was similar to the body energy requirement calculated from cadets body composition measured by the DXA-scanning at baseline showing no difference in total kcal (-21 kcal, $p = 0.69$). Thus, the validity of the energy intake provided by the recall should be acceptable.

Chapter 6

Conclusion

- The results from Study I highlight the importance of training volume wherein higher training volume through 3-SET training led to greater increases in muscle strength compared to 1-SET training, performed as within-session number of sets. This also aligned with a more pronounced muscle hypertrophy observed in the 3-SET training compared to the 1-SET training.
- In Study III, 10RM led to more pronounced improvements in upper limb muscle strength and mass than 30RM in moderately trained cadets following 22 weeks. Furthermore, 10RM training led to superior gains in lower limb muscle strength and jump performance compared to 30RM. However, there was no difference in other lower limb characteristics, such as muscle endurance performance, agility, and measures of muscle mass.
- When characterizing biomarkers associated with muscle hypertrophy, 3-SET training exhibited higher stimulation of mTORC1-related signaling (measured acutely at week 2) along with greater total RNA and ribosomal RNA levels at this time point compared to 1-SET training. The difference between volume conditions in total RNA levels at week 2 predicts the benefit of 3-SET training over 1-SET training after twelve weeks of resistance training.
- Study II showed that total RNA peaks after eight training sessions with a relatively high training volume. The fluctuation in training volume across 12 resistance sessions does not impact the accumulation of total RNA or ribosomal RNA. However, an interruption in the resistance training stimulus for eight days resulted in decreased pre-RNA and total RNA content.
- Lastly, after 10 days of military exercise in a severe energy-deficient state, there was no difference in the preservation of physical performance or alterations in body composition parameters when comparing higher protein intake (2 g kg^{-1} d^{-1}) to lower protein intake (1 g kg^{-1} d^{-1}) in an isocaloric setting.

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Chapter 7

Norsk sammendrag

Bakgrunn. Systematisk styrketrening fører til økning i muskelstyrke og vekst hos personer. Imidlertid observeres det variasjoner i personers respons på styrketrening og tilpasninger i muskulaturen. Disse variasjonene kan knyttes til individets biologiske predisposisjon, og dermed spille en rolle i hvordan enkeltpersoner responderer med hensyn til for.eks treningsvolum og påfølgende endringer i muskelstyrke og vekst.

Maksimal muskelstyrke er en viktig faktor for soldaters fysiske yteevne. Dermed er implementering av styrketrening i soldatenes daglige rutiner avgjørende for å optimalisere soldaters fysiske prestasjonsevne. Soldatens eksponering for ulike stressfaktorer i hverdagen, som feltøvelser, kan imidlertid hemme tilpasninger i muskulaturen. Derfor er utvikling av treningsstrategier som ikke bare forbedrer og opprettholder muskelstyrken, men også reduserer ugunstige endringer i kroppssammensetningen viktig.

Studie I undersøkte effekten av lav- og moderat styrketreningsvolum på utviklingen av maksimal styrke og muskelvekst. Videre ble individuelle muskeltilpasninger relatert til treningsvolum og knyttet til biologiske markører for muskelvekst. Studie II undersøkte utviklingen av biologiske markører for ribosomal biogenese aktivitet over en tre-ukers styrketreningsperiode. Videre ble disse markørene evaluert i forhold til forskjellig treningsvolum og en kort periode uten styrketreningsstimuli. Studie III sammenlignet effekten av to ulike styrketreningsbelastninger på utviklingen av maksimal styrke, muskelvekst og prestasjon implementert som en del av soldatenes rutinemessige treningshverdag. Til slutt, i Studie IV, ble det undersøkt om økt proteininntak kunne redusere tap av muskelmasse og fysisk prestasjon under et relativt stort energiunderskudd i forbindelse med en feltøvelse. Samtidig ble restitusjonen av disse parameterne evaluert etter en uke med hvile etter øvelsen.

Metode. Studie I inkluderte 34 utrente personer (gj.snitt alder: 22), som gjennomførte 12 uker med lav (1-SET) og moderat (3-SET) treningsvolum som en kontralateral protokoll i tre beinøvelser. Målinger av muskelens tverrsnitts areal og maksimale styrke ble utført før og etter de 12 ukene med styrketrening. Muskelbiopsi (*m.vastus lateralis*) ble utført på samme tidspunkt samt før og etter den femte økten i uke 2. Nitten utrente personer (gj.snitt alder: 24) deltok i Studie II og ble delt inn i en treningsgruppe (n=11) og en kontrollgruppe (n=8) som ikke deltok i styrketreningen. Treningsgruppen gjennomførte 12 økter med kneekstensjon, der det ene beinet gjennomførte varierende treningsvolum (6-3-9 sett, med hver blokk bestående av 4 økter), mens det andre beinet gjennomførte øktene med konstant treningsvolum (6 sett). Muskelbiopsier ble utført bilateralt før og 48 timer etter treningsøkt 1, 4, 5, 8, 9, 12, og etter en uke uten treningsstimuli. Kontrollgruppen gjennomførte biopsier ved inklusjon, etter 48 timer, og etter 3-5 uker. Muskelstyrke, muskelmasse, samt muskeltykkelse ble målt ved inklusjon, etter 12 økter, og etter 8 dager uten styrketrening. I

Studie III gjennomførte 27 moderat trente kadetter (gj.snitt alder: 20) et helkropps styrketreningsprogram med enten 10 repetisjoner maksimum (RM) eller 30RM over en 22 uker lang periode. Maksimal styrke og muskelprestasjon ble målt før og etter 22 uker, både for over- og underkroppen, med ytterligere en måling etter 10 uker fra studiens oppstart. Muskelmassemålinger og muskelbiopsier ble gjennomført ved alle tre tidspunktene. I Studie IV ble 38 kadetter rekruttert til å innta 1 eller 2 g kg^{-1} d^{-1} protein i en isokalorisk diett tilsvarende $\sim$ 15 kcal kg^{-1} d^{-1} under en 10 dager lang militærøvelse. Før og etter øvelsen ble muskelsyrke, prestasjon og hormonnivå målt, samt etter syv dager med restitusjon. Videre ble endringer i muskelmasse målt før øvelsen samt rett etter øvelsen.

Resultater. I Studie I resulterte 12 uker med styrketrening til større økning i muskelstyrke og masse i benet som gjennomførte 3-SET sammenlignet med 1-SET. Denne økningen samsvarte videre med en økning i muskulære signalmarkører assosiert med mTORC1-proteinkomplekset, samt markører knyttet til aktiviteten av ribosomal biogenese. I tillegg ble den positive effekten av å trene med høyere treningsvolum, i henhold til større økning i muskelstyrke og masse, assosiert med en større akkumulering av total RNA i 3-SET beinet målt ved uke 2. I Studie II resulterte tre uker med totalt 12 styrketreningsøkter i økt muskelmasse og styrke i treningsgruppen sammenlignet med kontrollgruppen. Videre førte treningsøktene til en økning i ribosomal biogenese aktivitet. Markører for ribosomal biogenese viste en umiddelbar økning i total RNA i løpet av de første 4 øktene, med maksimal synteseaktivitet etter 8 økter ($\sim$ 50% over baseline). Denne økningen i total RNA var videre assosiert med muskelvekst over treningsperioden. Åtte dager uten styrketrening resulterte i reduksjon i total RNA og ribosomal RNA nivåene, men uten videre reduksjon i muskelmassen. I Studie III førte 22 uker med 10RM styrketrening, integrert som en del av kadettenes militære treningsregime, til en større økning i styrke i både over- og underkroppen, hopp høyde samt en større økning i muskelmassen i overkroppen sammenlignet med 30RM gruppen. Begge treningsgruppene viste lik økning i hurtighet og muskelmasse i underkroppen i løpet av perioden. I Studie IV var det ingen forskjell mellom høyt (2 g kg^{-1} d^{-1}) og lavt (1 g kg^{-1} d^{-1}) proteininntak når det gjaldt endringer i kroppsmasse, muskelstyrke eller prestasjon etter 10 dager med feltøvelse med et betydelig energiunderskudd. Muskelmassen viste imidlertid ingen endring i noen av gruppene etter feltøvelsen. Etter syv dager med hvile (ad libitum), retunerte de fleste variablene mot verdiene målt før øvelsen, bortsett fra hopphøyden målt som svikt hopp som var på samme nivå som etter øvelsen.

Oppsummert. Studiene (I-II) indikerer at responsen på styrketrening knyttet til muskeltilpasninger, som muskelvekst og styrke, følger et volum-dose-responsforhold. Fordelen av et høyere treningsvolum i muskeltilpasninger etter 12 uker ble videre knyttet til større akkumulering av ribosomer i 3-SET-beinet etter de fem første øktene. Videre faciliterer et relativt høyt treningsvolum maksimal RNA-synteseaktivitet etter kun få treningsøkter (8 økter). Samtidig viser resultatene at synteseaktiviteten er sensitiv overfor cellens krav til ekspandering, da nydanning av ribosomer avtar når treningsstimuliet opphører. Videre viser studie III at 22 uker med systematisk styrketrening fremmer økning i muskelstyrke, muskelvekst og prestasjon hos moderat trente soldater. Imidlertid viste gruppen som trente med 10RM generelt større endringer i muskeltilpasninger sammenlignet med gruppen som trente med 30RM. Studie IV viste at et høyere proteininntak under den ti dager lange feltøvelsen ikke resulterte i bedre forutsetninger for å hindre tap av muskelstyrke og prestasjon sammenlignet med lavere proteininntak, når soldatene samtidig opplevde et betydelig energiunderskudd. Dermed kan studiene tyde på at tung treningsbelastning foretrekkes for å

optimalisere utviklingen av soldatens fysiske prestasjonsevne, og det kan være mer hensiktsmessig å sikre tilstrekkelig energitilførsel enn å manipulere energisammensetningen under feltøvelser for å minimere tap av muskelprestasjon.

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Paper I

The Journal of Physiology

Benefits of higher resistance-training volume are related to ribosome biogenesis

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Key points

- For individuals showing suboptimal adaptations to resistance training, manipulation of training volume is a potential measure to facilitate responses. This remains unexplored.
- Here, 34 untrained individuals performed contralateral resistance training with moderate and low volume for 12 weeks. Moderate volume led to larger increases in muscle cross-sectional area, strength and type II fibre-type transitions.
- These changes coincided with greater activation of signalling pathways controlling muscle growth and greater induction of ribosome synthesis.
- Out of 34 participants, thirteen displayed clear benefit of MOD on muscle hypertrophy and sixteen showed clear benefit of MOD on muscle strength gains. This coincided with greater total RNA accumulation in the early phase of the training period, suggesting that ribosomal biogenesis regulates the dose–response relationship between training volume and muscle hypertrophy.
- These results demonstrate that there is a dose-dependent relationship between training volume and outcomes. On the individual level, benefits of higher training volume were associated with increased ribosomal biogenesis.

Abstract Resistance-exercise volume is a determinant of training outcomes. However not all individuals respond in a dose-dependent fashion. In this study, 34 healthy individuals (males $n = 16$, 23.6 (4.1) years; females $n = 18$, 22.0 (1.3) years) performed moderate- (3 sets per

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exercise, MOD) and low-volume (1 set, LOW) resistance training in a contralateral fashion for 12 weeks (2–3 sessions per week). Muscle cross-sectional area (CSA) and strength were assessed at Weeks 0 and 12, along with biopsy sampling (m. vastus lateralis). Muscle biopsies were also sampled before and 1 h after the fifth session (Week 2). MOD resulted in larger increases in muscle CSA (5.2 (3.8)% *versus* 3.7 (3.7)%, $P < 0.001$) and strength (3.4–7.7% difference, all $P < 0.05$. This coincided with greater reductions in type IIX fibres from Week 0 to Week 12 (MOD, −4.6 percentage points; LOW −3.2 percentage points), greater phosphorylation of S6-kinase 1 (p85 S6K1^{Thr412}, 19%; p70 S6K1^{Thr389}, 58%) and ribosomal protein S6$^{Ser235/236}$ (37%), greater rested-state total RNA (8.8%) and greater exercise-induced c-Myc mRNA expression (25%; Week 2, all $P < 0.05$). Thirteen and sixteen participants, respectively, displayed clear benefits in response to MOD on muscle hypertrophy and strength. Benefits were associated with greater accumulation of total RNA at Week 2 in the MOD leg, with every 1% difference increasing the odds of MOD benefit by 7.0% ($P = 0.005$) and 9.8% ($P = 0.002$). In conclusion, MOD led to greater functional and biological adaptations than LOW. Associations between dose-dependent total RNA accumulation and increases in muscle mass and strength point to ribosome biogenesis as a determinant of dose-dependent training responses.

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Introduction

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 (Ratamess *et al.* 2009). In addition, genetic and epigenetic disposition and environmental factors play a role in variations in adaptations (Timmons, 2011; Morton *et al.* 2018; Seaborne *et al.* 2018). As time constraints often hinder participation in exercise training programmes (Choi *et al.* 2017), 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 gains in strength and muscular mass similar to moderate-volume training (Ostrowski *et al.* 1997; Cannon & Marino, 2010; Mitchell *et al.* 2012), meta-analyses conclude in favour of moderate-volume protocols (Rhea *et al.* 2003; Krieger, 2009, 2010; Schoenfeld *et al.* 2016). 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 (Terzis *et al.* 2008; Raue *et al.* 2012; Thalacker-Mercer *et al.* 2013; Stec *et al.* 2016). 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 (Drummond *et al.* 2009) and activation of its associated downstream target S6 kinase 1 (S6K1) correlates with increases in muscle protein synthesis and subsequent muscle growth (Terzis *et al.* 2008; Burd *et al.* 2010). In line with this, surplus exercise volume leads to greater phosphorylation of S6K1 (Burd *et al.* 2010; Terzis *et al.* 2010; Ahtiainen *et al.* 2015) and is accompanied by increases in myofibrillar protein synthesis (Burd *et al.* 2010), 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 rRNA in response to resistance training, measured as total RNA per weight-unit of muscle tissue, correlate with muscle hypertrophy (Figueiredo *et al.* 2015). In accordance with this, training-induced increases in rRNA are larger in muscle hypertrophy high-responders than in low-responders (Stec *et al.* 2016; Mobley *et al.* 2018). Secondly, elderly participants typically show blunted ribosome biogenesis, coinciding with attenuated hypertrophic responses (Stec *et al.* 2015; Brook *et al.* 2016). 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 (Jespersen *et al.* 2011; Stec *et al.* 2016), and readily switch from IIX to IIA phenotypes in response to mechanical loading (Widrick *et al.* 2002; Ellefsen *et al.* 2014*b*; Andersen & Gruschy-Knudsen, 2018), suggesting that these fibres display greater plasticity in response to resistance training.

The purpose of the present study was to evaluate the effects of single- and multiple-set training protocols on strength, muscle hypertrophy and fibre-type composition using a within-participant design. We also aimed to compare the effects of the two volume conditions on phosphorylation of proteins relating to the mTORC1 pathway, as well as abundances of total RNA, ribosomal RNA and selected mRNA.

Methods

Ethical approval

All participants were informed about the potential risks and discomforts associated with the study and gave their informed consent prior to study enrolment. The study design was pre-registered (ClinicalTrials.gov Identifier: NCT02179307), approved by the local ethics committee at Lillehammer University College, Department of Sport Science (no. 2013-11-22:2) and all procedures were performed in accordance with the *Declaration of Helsinki*.

Participants and study overview

Forty-one male and female participants were recruited to the present study with eligibility criteria being non-smoking and age between 18 and 40 years. Exclusion criteria were intolerance to local anaesthetic, training history of more than one weekly resistance-exercise session during the last 12 months leading up to the intervention, impaired muscle strength due to previous or current injury, and intake of prescribed medication that could affect adaptations to training. During data analyses, seven participants were excluded due to not completing at least 85% of the scheduled training sessions with reasons being: discomfort or pain in the lower extremities during exercise ($n = 5$), injury not related to the study ($n = 1$), failure to adhere to the study protocol ($n = 1$). At baseline, there were no differences in maximal voluntary contraction (MVC) normalised to body mass or anthropometrics between included and excluded participants (see Table 1). Among the included group, one participant chose to refrain from biopsy and blood sampling at Week 2. Additionally, blood was not collected from three of the participants at different time-points due to sampling difficulties. All included participants reported previous experience with sporting activities (e.g. team-sports, cross-country skiing and gymnastics). Twenty participants reported that they were engaged in physical training at the time of enrolment (median number of sessions per week, 2; range, 0.5–4), 10 of whom performed sporadic resistance-type training, though none more than once per week.

The intervention consisted of 12 weeks of full-body resistance training (all participants commenced the trial during September–November). Leg exercises were performed unilaterally to allow within-participant differentiation of training volume. Accordingly, for each participant, the two legs were randomly assigned to perform resistance exercises consisting of one set (single-set condition) and three sets (multiple-set condition); i.e. each participant performed both protocols. Muscle strength was assessed at baseline, during (Weeks 3, 5 and 9) and after the training intervention. Body composition was measured before and after the training intervention. Muscle biopsies were sampled from both legs (vastus lateralis) at four time-points during the intervention: at baseline (Week 0, rested state), before and 1 h after the fifth training session (Week 2 pre-exercise, rested; Week 2 post-exercise, acute-phase biopsy) and after completion of the intervention (Week 12, rested state). For an overview of the study protocol, see Fig. 1. Starting at Week 6, participants performed a dietary registration in which they weighed and logged their dietary intake for four to five consecutive days, including one weekend day (Table 1).

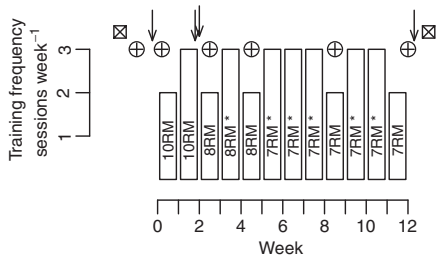


Figure 1. Study overview
Bars represent weekly training frequency with training intensity expressed as repetition maximum (RM). * indicates that one session per week was performed at 90% of prescribed RM intensities. ↓ indicates muscle biopsy: before (Week 0, $n = 34$) and after the 12 week intervention (Week 12, $n = 34$), as well as before and after (1 h) the fifth exercise session (Week 2 Pre-Ex and Post-Ex, $n = 33$). The plus inside a circle symbol indicates a strength test: before the intervention (Week 0, $n = 34$), during 3, 5 and 9 weeks of training ($n = 18$), and after finalisation of the intervention (Week 12, $n = 34$). Baseline strength was determined as the highest value obtained during two test sessions performed prior to the intervention. Body composition was measured prior to the intervention (Week 0) and after its finalisation (Week 12, $n = 34$) using full-body DXA and knee-extensor muscle MRI (cross inside a square symbol).

Table 1. Participant characteristics and habitual dietary data

	Female		Male	
	Included	Excluded	Included	Excluded
N	18	4	16	3
Age (years)	22.0 (1.3)	22.9 (1.6)	23.6 (4.1)	24.3 (1.5)
Mass (kg)	64.4 (10.4)	64.6 (9.7)	75.8 (10.7)	88.2 (22.4)
Stature (cm)	168 (7)	166 (8)	183 (6)	189 (5)
Body fat (%)	34.1 (5.6)	28.8 (8.7)	20.4 (6.0)	24.3 (15.3)
MVC (N m kg^{-1})	3.1 (0.5)	3.6 (0.5)	3.7 (0.6)	3.9 (0.7)
	Dietary survey			
kcal day^{-1}	Protein kg^{-1} day^{-1}		Fat kg^{-1} day^{-1}	CHO kg^{-1} day^{-1}
1994 (839)	1.33 (0.40)		1.10 (0.44)	3.36 (1.17)

Data are means and standard deviations (SD). Habitual dietary data from $n = 21$. CHO, carbohydrate.

Resistance-exercise training protocol

Prior to all training sessions, participants performed a standardized warm-up routine consisting of (i) 5 min ergometer cycling (rating of perceived exertion, RPE 12–14), followed by (ii) 10 repetitions each of body weight exercise (push-ups with individually adjusted leverage, sit-ups, back-extensions and squats), and (iii) one set of 10 repetitions at ∼50% of one repetition maximum (1RM) for each resistance exercise. Leg resistance exercises were performed in the following order: unilateral leg press, leg curl and knee extension, performed as either one set (single set) or three sets (multiple set) per exercise. Single sets were performed between the second and third set of the multiple-set protocol. Following leg exercises, participants performed two sets each of bilateral bench-press, pull-down, and either shoulder-press or seated rowing (performed in alternating sessions). Rest periods between sets were 90–180 s. Training intensity was gradually increased throughout the intervention, starting with 10RM for the first 2 weeks, followed by 8RM for 3 weeks and 7RM for 7 weeks (Fig. 1). To better fit the training programme to a participant's daily schedule, some sessions were performed unsupervised. The average number of supervised sessions were 91% (SD = 10%, range: 67–100%) of performed sessions. In order to monitor unsupervised sessions, participants were instructed to keep detailed logs. These were continuously checked by the research team together with participants to ensure progression and adherence to the protocol. From the ninth training session, every week (containing three training sessions) had one session with reduced loads, corresponding to 90% of the previous session with the same target number of repetitions. Training sessions with maximal effort were separated by at least 48 h. Training sessions with submaximal efforts (90%) were separated from other sessions by at least 24 h. To aid immediate recovery, a standardised drink was given after each session containing 0.15 g kg^{-1} protein, 11.2 g kg^{-1} carbohydrates and 0.5 g kg^{-1} fat.

Muscle strength assessments

Isokinetic and isometric unilateral knee-extension strength was assessed in a dynamometer (Cybex 6000, Cybex International, Medway, MA, 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° s^{-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° s^{-1} and three attempts at 120 and 240° s^{-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 s. Participants were given two attempts, with 30 s 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 10, 6 and 3 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

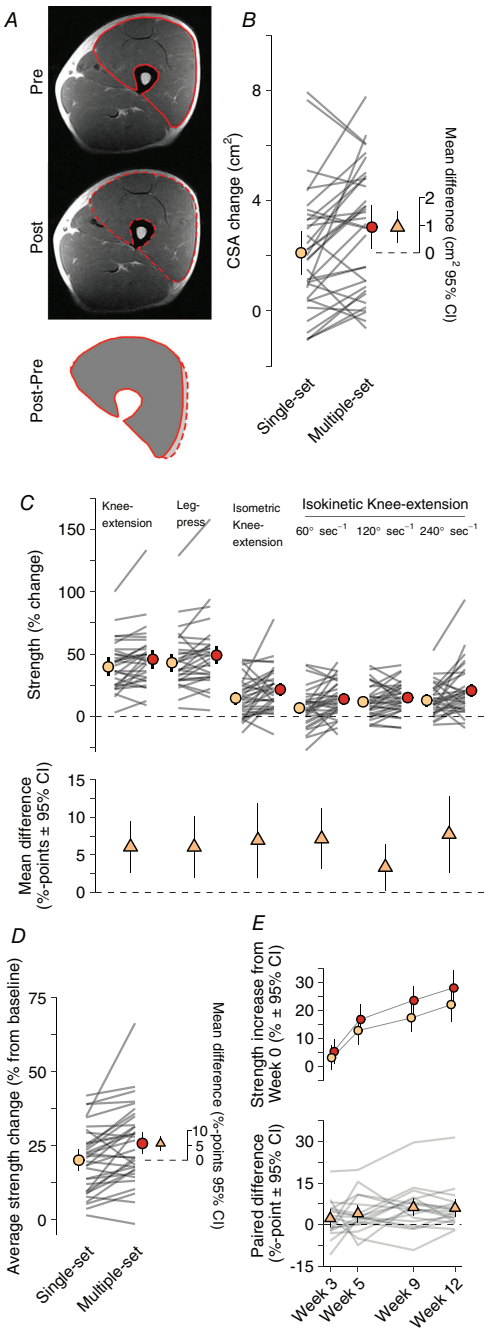


Figure 2. Volume-dependent effects on muscle mass and strength
Training volume-dependent changes in muscle mass and strength after 12 weeks of resistance training, evident as larger increases in knee-extensor muscle CSA (measured using MRI, *A* and *B*) and larger increases in one-repetition maximum knee extension and leg press, isometric isokinetic knee-extension strength in the multiple-set leg (*C*). A weighted average of all strength measures (*D*) was used to study the time course of strength changes ($n = 18$), showing a gradually increasing difference between volume conditions (in favour of multiple-set training) until Week 9, with no further increase to Week 12 (*E*). Summary values (circles) are estimated means ± 95% CI. Triangles signify mean paired differences ± 95% CI. [Colour figure can be viewed at wileyonlinelibrary.com]

4 days. The maximum value achieved for each of the tests was used in subsequent analysis. Strength tests were separated by at least 48 h from preceding 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 Weeks 2, 5 and 9). For the remaining participants, ordinary training sessions were prioritised when participants missed training or testing due to illness or scheduling difficulties.

Muscle cross-sectional area and body composition

Knee-extensor muscle cross-sectional area (CSA; vastus lateralis, medialis, intermedius and rectus femoris) was determined before and after the training intervention using magnetic resonance imaging (MRI) in accordance with the manufacturer's protocol (S-Scan, Esaote Europe B.V., Maastricht, the Netherlands). Images were analysed in a blinded fashion by the same investigator, using OsiriX (v.5.6, Pixmeo Sarl, Bernex, Switzerland). For each participant, CSA was determined at the same distance from the knee joint pre- and post-intervention (mid-thigh), using at least four consecutive images (5 mm thickness, 10 mm separation; see Fig. 2*A* for representative images). Body composition was determined before and after the intervention using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, GE Healthcare, Oslo, Norway), in accordance with standard protocol. Prior to MRI and DXA measurements, participants were asked to stay fasted for 2 h and to refrain from vigorous physical activity for 48 h. Two days separated the last strength test session from body composition measurements.

Hormonal measurements

Hormone analyses were performed on blood samples collected at five time-points: alongside muscle biopsies (Fig. 1, four sampling events) and 10 min 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 analyser, using kits from the Immulite Immunoassay System menu (Siemens Medical Solutions Diagnostics, Malvern, PA, United States), 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 module e601, Roche Diagnostics).

Muscle tissue sampling and processing

Muscle biopsies were obtained bilaterally from m. vastus lateralis under local anaesthesia (Xylocaine, 10 mg ml^{-1} with adrenaline 5 μg ml^{-1}, AstraZeneca AS, Oslo, Norway) using a 12-gauge needle (Universal-plus, Medax, San Possidonio, Italy) operated with a spring-loaded biopsy instrument (Bard Magnum, Bard, Rud, Norway). For each participant, resting samples were collected at the same time of day at all time-points and all sampling was done in the morning after a standardised breakfast. Participants were instructed to standardise meals during the last 24 h leading up to sampling and to refrain from strenuous physical activity during the last 48 h. Biopsy sampling prior to the fifth sessions was performed in the morning 2 days after session four. Post-intervention biopsy sampling was performed 3 and 6 days after the last training bout and strength-testing session, respectively. Samples were obtained within 10 min from both legs at all time-points. The first biopsy was sampled at 1/3 of the distance from the patella to the anterior superior iliac spine; subsequent biopsies were sampled ∼2 cm proximal to the previous sample. The tissue was quickly dissected free of blood and visible connective tissue in ice-cold sterile saline solution (0.9% NaCl). Samples for immunohistochemistry (∼15 mg) were transferred to a 4% formalin solution for fixation for 24–72 h, before further preparation. Samples for protein and RNA analyses (∼60 mg) were blotted dry, snap-frozen in isopentane cooled to −80°C and stored at −80°C until further analyses.

Immunohistochemistry

Formalin-fixed muscle biopsies were processed for 2.5 h using a Shandon Excelsior ES (Thermo Scientific, Oslo, Norway), paraffin-embedded and sectioned into 4 cm transverse sections. For determination of muscle fibre types, sections were double-stained using BF-35 (5 μg ml^{-1}; Developmental Studies Hybridoma Bank, deposited by S. Schiaffino, Venetian Institute of Molecular Medicine (VIMM), Padova, Italy) and MyHCSlow (1:4000, cat. no. M8421L, Sigma-Aldrich Norway AS). The primary staining was visualised using BMU UltraView DAB and UltraView Red (Ventana Medical Systems, Inc., Tucson, AZ, USA). Muscle fibres were counted as either Type I (red), Type IIA (brown), Type IIX (unstained) or hybrid fibres Type IIA/IIX (light brown) (for representative image, see Fig. 3*A*). Fibres identified as hybrid fibres were analysed as 0.5 × Type IIA and 0.5 × Type IIX.

Protein extraction and 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 Fisher Scientific), incubated at 4°C for 1 h 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 Fisher Scientific). The remaining supernatant was diluted to 1.5 μg $μl^{-1}$ 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 μg 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 μm 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, ThermoFisher Scientific) to ensure appropriate protein transfer. Primary antibodies were purchased from Cell Signaling Technology (Leiden, the Netherlands): mTOR (mTOR^{Ser2448}: no. 5536; pan: no. 4517), S6 kinase 1 (p85 S6K1^{Thr412}: no. 9206; p70 S6K1^{Thr389}: no. 9234; pan: no. 2708), ribosomal protein S6 (rpS6$^{Ser235/236}$: no. 4858; pan: no. 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 overnight 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 chemiluminescence detection (SuperSignal West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific), membranes were incubated with hydrogen peroxide (15 min, 37°C) to inactivate the horseradish peroxidase (HRP), as described by Sennepin *et al.* (2009), followed by overnight 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 phospho-specific 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 (Rueden *et al.* 2017), 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, NE, USA).

Total RNA extraction, quantitative real-time reverse transcription polymerase chain reaction

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 (λ polyA External Standard Kit, Takara Bio Inc., Shiga, Japan) was added at a fixed amount (0.04 ng ml^{-1} of Trizol reagent) per extraction prior to homogenisation, as previously described (Ellefsen *et al.* 2008, 2014*a*). Following phase separation, 400 µl 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 nm/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 score (RNA quality indicator; RQI) 8.1 (SD = 2.1). 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 the manufacturers' instructions. All samples were reverse transcribed in duplicate and diluted 1:50 prior to quantitative 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 µl, containing 2 µl of cDNA, specific primers (0.5 µM final concentration) and a commercial master mix (2X SYBR Select Master Mix, Applied Biosystems, Life Technologies AS). qPCR reactions consisted of 40 cycles (3 s 95°C denaturing and 30 s 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 (Ye *et al.* 2012) and Primer3Plus (Untergasser *et al.* 2012) and ordered from Thermo Scientific, except for the external RNA control, for which primers were supplied with the kit. Raw fluorescence data were exported from the platform-specific software and amplification curves were modelled with a best-fit sigmoidal model using the qpcR-package (Ritz & Spiess, 2008) written for R (R Core Team, 2018). 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 Tichopad *et al.* 2003; implemented in Ritz & Spiess, 2008). 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 were log-transformed prior to statistical analysis. As Ct values, but not efficiencies are related to RNA integrity (Fleige & Pfaffl, 2006), RQI scores were used in the statistical treatment of qPCR data to control for potential degradation effects on a by target basis (see below).

Data analysis and statistics

All descriptive data are presented as mean and standard deviation (mean (SD)) unless otherwise stated. *A priori* sample-size calculations indicated that 40 participants was

Table 2. Primer sequences and performance

Gene symbol	Full name	Accession[a]	Primer sequence (forward and reverse)	Ct mean (SD)	*E*
MYH7	Myosin heavy chain 7 (MyHC-1)	NM_000257.3	5′-AGGAGCTCACCTACCAGACG-3′ 5′-TGCAGCTTGTCTACCAGGTC-3′	21.70 (0.77)	1.88
MYH2	Myosin heavy chain 2 (MyHC-2A)	NM_017534.5	5′-CCAGGGTACGGGAGCTG-3′ 5′-TCACTCGCCTCTCATGTTTG-3′	17.65 (0.62)	1.92
MYH1	Myosin heavy chain 1 (MyHC-2X)	NM_005963.3	5′-GGCCAGGGTTCGTGAACTT-3′ 5′-TGCGTAGACCCTTGACAGC-3′	23.33 (1.94)	1.88
c-Myc	v-myc avian myelocytomatosis viral oncogene homologue	NM_002467.4	5′-GGGTAGTGGAAAACCAGCAG-3′ 5′-TCCTCGTCGCAGTAGAAATACG-3′	30.23 (2.03)	1.93
rRNA5.8S	5.8S ribosomal RNA	NR_003285.2	5′-ACTCTTAGCGGTGGATCACTC-3′ 5′-GTGTCGATGATCAATGTGTCCTG-3′	15.64 (0.45)	1.88
rRNA28S	28S ribosomal RNA	NR_003287.2	5′-TGACGCGATGTGATTTCTGC-3′ 5′-TAGATGACGAGGCATTTGGC-3′	12.39 (0.66)	1.78
rRNA18S	18S ribosomal RNA	NR_003286.2	5′-TGCATGGCCGTTCTTAGTTG-3′ 5′-AACGCCACTTGTCCCTCTAAG-3′	13.16 (1.45)	1.81
rRNA45S	45S pre-ribosomal RNA	NR_046235.1	5′-GCCTTCTCTAGCGATCTGAGAG-3′ 5′-CCATAACGGAGGCAGAGACA-3′	25.60 (1.75)	1.76
λ polyA	External Standard Kit	—	Proprietary sequences	23.96 (0.82)	1.98

Average threshold cycles (Ct) and priming efficiencies (*E*) were calculated from all qPCR reactions. [a]NCBI Reference Sequence.

sufficient to detect ∼3 and 5 percentage-point differences in the primary outcomes, muscle cross-sectional area and maximal voluntary strength, respectively, between volume conditions. Sample-size calculations were based on a desired 80% power, assuming differences between volume condition corresponding to effect sizes of 0.47–0.51, as estimated from previous studies (Ronnestad *et al.* 2007; Mitchell *et al.* 2012). To assess the effect of volume conditions (number of sets) on muscle hypertrophy and strength, linear mixed-effects models (LMMs) were specified with relative changes from baseline as the dependent variable and number of sets as the main fixed effect. Baseline values were used as a co-variate together with sex. The interaction between sex and number of sets was explored for all hypertrophy and strength outcomes. Training effects on molecular characteristics (total RNA and western blot data) were also assessed using LMMs specified with time and the time to exercise–volume interaction as fixed effects. Models were specified with random intercepts for participants and when appropriate, random slopes for time and exercise volume at the level of participants. Model simplification was performed through reduction of random-effects parameters based on likelihood-ratio (LHR) tests. Plots of residual and fitted values were visually inspected to assess uniformity of variance over the fitted range. Whenever deviations from these assumptions were identified, data were log-transformed and models were re-fitted.

Generalised linear mixed-effects models (GLMMs) were used to fit muscle fibre distributions and gene family-normalised myosin heavy-chain mRNA data (Ellefsen *et al.* 2014*b*; after transformation to transcript counts as described by Matz *et al.* 2013) using the fixed and random effects structure specified above for molecular characteristics. A binomial variance/link function (logit-link) was used for muscle fibre distributions with the number of counted fibres per sample used as weights to account for sample size. A beta variance/link-function (logit-link) was used to model gene family-normalised myosin heavy-chain mRNA data. This was done in order to account for the non-normal nature of relative fibre-type/myosin-isoform distribution data, where specific fibres/transcripts are analysed as a proportion of the total number of fibres/transcripts in each sample and thus bound between 0 and 1. The beta model was used for gene-family mRNA data as the denominator could be regarded as arbitrary. Gene-abundance data, either expressed as per total RNA or per unit muscle weight using the external reference gene were analysed through the modelling of gene sets as suggested by Matz *et al.* (2013) using mixed linear models with within-model normalisation through the addition of random effects of technical replicates. To allow for gene-specific variances, variance functions were specified per strata (per gene) (Pinheiro & Bates, 2000). RNA integrity scores (RQI) were included in the model on a per target basis to control for RNA degradation.

Tests against the null-hypotheses of no differences between volume conditions and no effect of time were performed on model-parameter estimates resulting from LMMs and GLMMs. LMMs were fitted using the nlme-package (Pinheiro & Bates, 2000), binomial GLMM

models using the lme4-package (Bates *et al.* 2015) and beta GLMMs using the glmmTMB-package (Magnusson *et al.* 2019) written for R.

To explore the determinants of the additional benefit of multiple-sets, dichotomous response variables were constructed from individual differences in single- and multiple-set outcomes in muscle hypertrophy (cross-sectional area, CSA) and average muscle strength. When the difference between volume conditions in training-induced outcomes were larger than the smallest worthwhile change (SWC) in the direction of the multiple-set, variables were coded as additional benefits of multiple-set. The SWC was calculated as between-participants SD $\times$ 0.2. To account for sex differences in CSA and strength measures, standard deviations were estimated from data mean-centred per sex. SWCs were expressed as percentages of the sex-specific mean and the averages thereof were used to classify benefits. For the combined strength variable, a weighted SWC was used in order to avoid underestimation of between-participant variability due to regression toward the mean. The probability of benefits of the multiple-set was related to a wide range of predictors using logistic regression. Prior to model fitting, *a priori* selection of relevant predictor variables was done; these included blood variables, baseline strength and muscle mass, volume-dependent molecular responses to training (i.e. total RNA content and S6K1 phosphorylation expressed as a percentage of single-set readouts) and baseline fibre-type composition. Two participants were excluded from variable selection due to missing data in selected variables. Purposeful selection of variables was done in a step-wise manner following Hosmer *et al.* (2013). First, each possible predictor was fitted into a univariate linear model, controlling for sex, providing estimation of the between-benefit groups difference for the variable of interest. Predictors with $P < 0.20$ from the first step were kept for further considerations. All predictors from the first step were fitted in a preliminary model from where predictors were sequentially removed if they were not significant at the $P < 0.1$ level using Wald-based P values or influenced other predictors. All predictors from the first step were checked for linearity (logit) by creating design variables and plotting each category median against coefficients from a logistic model. Non-linear variables were categorised into biologically meaningful categories (e.g. Vitamin D insufficient/sufficient), dichotomised based on measurement detection limits (testosterone in females) or sex-specific median values (e.g. lean body mass). Thirty-two participants were included in the variable selection as two participants had missing data in some of the pre-selected variables.

Logistic models fitted with small samples have been shown to give biased estimates (Nemes *et al.* 2009); this was recognised and bias-corrected estimates were reported (Kosmidis, 2019) with P values from likelihood-ratio tests comparing sequentially reduced models.

The level of statistical significance was set to $\alpha = 0.05$. All data-analysis was done in R (R Core Team, 2018).

Results

Volume-dependent regulation of muscle strength, muscle mass and fibre type composition

Overall, 12 weeks of resistance training led to a 25% (95% confidence interval (CI): [20, 29], $P < 0.001$) increase in average muscle strength and a 4.4% ([3.2, 5.6], $P < 0.001$) increase in muscle mass (mean values of both volume conditions). Adherence to the protocol was 96 (5)% of the prescribed 31 sessions (range 81–100%), which gives an efficiency for developing muscle strength and mass equivalent to 0.84 (0.42)% and 0.15 (0.12)% per session, being within the expected range of training-induced changes (Ahtiainen *et al.* 2016).

Training had no effect on serum levels of cortisol and testosterone (Table 3). IGF-1 decreased ~5.4% from Week 0 to Week 2, and increased ~3.6% from pre- to post-exercise in Week 2. Growth hormone concentrations increased in response to acute exercise, with patterns differing between sexes (Table 3). Vitamin D levels were different at baseline between males (76.6 (16.4) nmol l^{-1} and females (100.0 (33.4) nmol l^{-1}, P = 0.006) and were similarly reduced from Week 0 to Week 12 in both sexes (63.1 (19.8) and 91.4 (31.7) nmol l^{-1} for males and females, respectively; time effect $P < 0.001$).

The difference in number of sets per exercise between multiple- and single-set conditions resulted in a ratio of performed work (number of repetitions $\times$ external resistance) between legs corresponding to 2.9 (0.3) in knee extension and 3.0 (0.5) in leg press. This was accompanied by higher ratings of perceived exertion in response to multiple sets than single sets (7.09 (1.95) *vs.* 6.22 (1.82), $P < 0.001$). Concomitantly, multiple-set resistance-training led to greater increases in muscle strength over the course of the intervention than single-set training (all variables $P < 0.05$, Fig. 2*C* and *D*). This difference in strength gain gradually increased over the first 9 weeks of the study (Fig. 2*E*). In line with this, multiple-set training led to greater increases in knee extensor CSA (mean percentage-point difference 1.62, [0.75, 2.50], $P < 0.001$, Fig. 2*B*). There was no difference between sexes in relative muscle strength and mass gains, and sex did not interact with responses to different volume conditions. There were strong correlations between responses to multiple-set and single-set conditions with respect to average strength gain ($r = 0.80$, [0.64, 0.90], $P < 0.001$, Fig. 6*B*) and muscle hypertrophy ($r = 0.75$, [0.55, 0.87], $P < 0.001$, Fig. 6*A*). Increases in muscle strength correlated

Table 3. Hormone measurements

			Week 2 (fifth session)							
	Week 0		Pre-exercise		Post-exercise (10 min)		Post-exercise (60 min)		Week 12	
	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*
Cortisol (nmol l^{-1})										
Female	584 (217)	17	586 (166)	18	541 (201)	18	521 (195)	18	580 (177)	17
Male	412 (71)*	16	406 (127)	14	451 (135)	15	384 (105)	15	355 (95)	16
Growth hormone (μg l^{-1})										
Female	1.40 (2.21)	17	1.17 (1.70)	18	7.27 (3.46)‡	18	0.94 (0.76)‡	18	1.83 (3.02)	17
Male	0.08 (0.02)*	6	0.11 (0.07)	6	2.75 (2.49)	15	1.76 (3.82)§	12	0.08 (0.03)	7
IGF-1 (nmol l^{-1})										
Female	19.9 (6.0)	17	18.7 (6.0)†	18	19.3 (6.1)‡	18	18.8 (5.8)	18	19.4 (6.2)	17
Male	21.0 (4.0)	16	19.6 (4.7)	14	20.1 (4.8)	15	19.1 (4.3)	15	19.9 (3.9)	16
Testosterone (nmol l^{-1})										
Female	0.9 (0.2)	5	1.4 (0.4)	2	1.8 (2.5)	8	1.1 (0.1)	3	1.2 (0.2)	5
Male	14.0 (3.4)	16	13.7 (2.5)	14	13.8 (4.2)	15	13.6 (4.6)	14	14.8 (3.9)	16

Differences between resting samples (Week 0, Week 2 pre-exercise and Week 12), between rest and post-acute-exercise in Week 2, and between males and females, were tested in mixed-effects models where * denotes significant main effect of sex; † resting samples different from Week 0; ‡ acute samples different from Week 2 pre-exercise; § change from Week 2 pre-exercise different between men and women, all $P < 0.05$. Missing values in growth hormone and testosterone are measurements below the detection limit (0.05 μg l^{-1} and 0.69 nmol l^{-1} for growth hormone and testosterone, respectively). Due to the small number of detectable testosterone samples in females, statistical tests were carried out in males only.

with increases in mass ($r = 0.41$, [0.08, 0.66], $P = 0.016$) assessed as averaged effects of the two volume conditions.

In muscle tissue, multiple-set training led to more pronounced conversion of Type IIX fibres into Type IIA fibres from Week 0 to Week 12 than single-set training, measured as both cell counts using immunohistochemistry (odds ratio (OR): 0.53, [0.30, 0.92], Fig. 3*B*) and mRNA abundance using gene-family profiling (OR: 0.76, [0.62, 0.91], Fig. 3*B*). Surprisingly, at Week 2, the relationship between training volume and fibre conversion was the opposite, with single-set legs showing greater IIX to IIA transition (OR: 1.60, [1.04, 2.48]). This volume-dependent effect was accompanied by a difference in the abundance of IIX/IIA hybrid fibres at Week 2, with the multiple-set condition showing higher levels (Fig. 3*C*). Notably, from baseline to Week 2, a pronounced decrease was seen in *MYH1* gene expression (coding for the Type IIX myosin heavy chain transcript), and more so in response to multiple-set training than to single-set training. This change was partly reversed in Week 12 (Fig. 3*D*).

Volume-dependent regulation of mTOR signalling and ribosomal biogenesis

Acute exercise led to greater phosphorylation of S6K1 observed in isoforms p85 and p70, both indicative of mTORC1 activity (Fig. 4*A* and *B*, mean percentage difference from single-sets with [95% CI]: phospho-p70 S6K1^{Thr389}, 58.2 [13.1, 121.5]; phospho-p85 S6K1^{Thr412}, 18.7 [0.4, 40.4]). This coincided with greater levels of phosphorylated rpS6$^{Ser235/236}$ and mTOR^{Ser2448} (phospho-rpS6, 37.4 [7.3, 75.9]%, Fig. 4*C*; phospho-mTOR, 9.3 [0.9, 18.4]%, Fig. 4*D*), both targets of S6K1 (Fig. 4*F*). Notably, non-phosphorylated (pan-) levels of S6K1 and rpS6 decreased from before to after the fifth training session with no difference between volume conditions (Fig. 4*E*). As this could potentially affect analyses of phosphorylated proteins, total-protein stains were used to normalise phosphorylated signals of S6K1 and rpS6. Normalising to pan-signals resulted in larger estimated changes pre- to post-exercise but similar estimates of volume-dependent phosphorylation patterns (data not shown).

In line with these data, multiple-set training resulted in 8.8% [1.5, 16.6] greater total RNA abundance per weight-unit of muscle tissue at Week 2 than single-set training. This difference was also evident at Week 12, albeit less extensive (5.9% [−1.0, 13.3], Fig. 5*A*). Accordingly, the multiple-set leg showed greater abundances of mature rRNA transcripts at Week 2 (18S, 19.0% [3.9, 36.4]; 28S, 15.3% [2.7, 29.4]; 5.8S 14.7% [1.8, 29.2], Fig. 5*B*). The abundances of these rRNA subspecies remained elevated at Week 12 with a tendency towards greater levels in the single set condition, an effect most pronounced in 28S (Fig. 5*B*). The rRNA precursor transcript 45S also increased from baseline to Week 2 when measured per weight-unit of muscle tissue with no clear differences

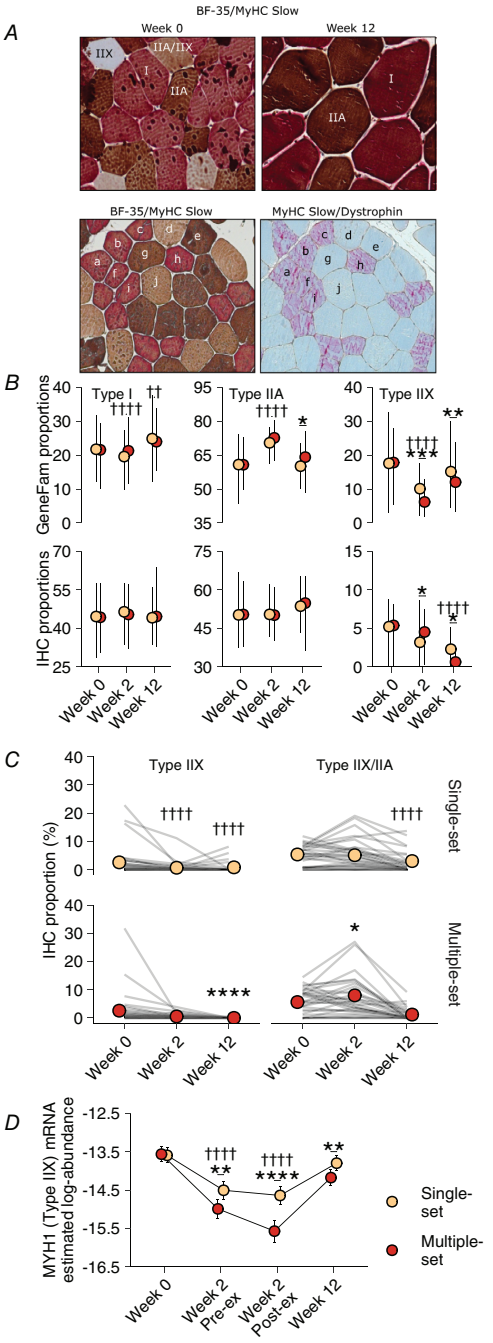


Figure 3. Fibre-type distributions
Muscle cross-sections were stained for myosin-heavy chain isoforms, Type I (MyHC Slow) and all but Type IIX (BF-35). Red staining separated Type I fibres from other fibres (*A*, lower panel). No staining was analysed as Type IIX fibres (*A*, upper panel), while weak brown staining was analysed as Type IIX/IIA hybrids. Volume-dependent changes in muscle fibre-type distribution was evident in m. vastus lateralis after 2 and 12 weeks of multiple- and single-set resistance training, measured as relative cell counts using immunohistochemistry (IHC) and gene family profiling (GeneFam)-normalised myosin heavy-chain mRNA expression (*B*). Volume-dependent effects were identified for proportions of Type IIX fibres and IIX/IIA hybrid fibres (*C*). Volume-dependent effects were also evident at the transcript level, measured as surplus reductions in Type IIX mRNA (MYH1) abundance in the multiple-set leg at all time-points (*D*). Values are mean ± 10th–90th percentile in *B*, and individual values and means in *C*, and estimated means ± 95% CI in *D*. † represents difference from Week 0, †–†††† for $P < 0.05$ to $P < 0.0001$; * represents differences between sets *–**** for $P < 0.05$ to $P < 0.0001$. [Colour figure can be viewed at wileyonlinelibrary.com]

between volume conditions (Fig. 5*C*, upper panel). When measured per unit of total RNA, levels of 45S pre-rRNA showed a clear increase only at Week 12 compared to baseline values (43.1% [4.9, 95.0] in the single-sets condition) with multiple-set remaining near baseline levels (−29.8% [−48.5, −4.2] of single-set, Fig. 5*C* lower panel). Overall, these data suggest that resistance training-induced increases in ribosomal content depend on training volume. Further supporting this view, mRNA expression of the transcription factor c-Myc, which is important for initiating rRNA transcription (van Riggelen *et al.* 2010), increased 1.58 [1.14–2.17]-fold more in response to multiple-set training than to single-set training (Fig. 5*D*, measured before and after the fifth training session).

Determinants of additional benefit of multiple-set training

Thirteen and sixteen participants showed clear benefits of multiple-set over single-set for increases in CSA and strength, respectively, defined as differences in training-induced changes greater than the SWC in favour of multiple-set (SWC CSA, 2.7%; SWC strength, 4.5%, Fig. 6*A* and *B*). In contrast, only three participants showed an additional benefit of single-set training on CSA and one participant showed an additional benefit of single-set training for strength. To identify determinants of multiple-set benefit, we performed logistic regression analyses with purposeful selection of variables. Variables initially selected for modelling are listed in Table 4. After variable selection, total RNA content measured at rest in the multiple-set leg at Week 2 (expressed as percentage of the single-set leg), remained as the only predictor for additional benefits of moderate volume for both CSA and strength (Table 5). Total RNA content was elevated in the multiple-set-trained leg in participants with clear benefits of multiple-set (Fig. 6*A* and *B*). For every percentage-point increase in total RNA in the multiple-set leg (compared

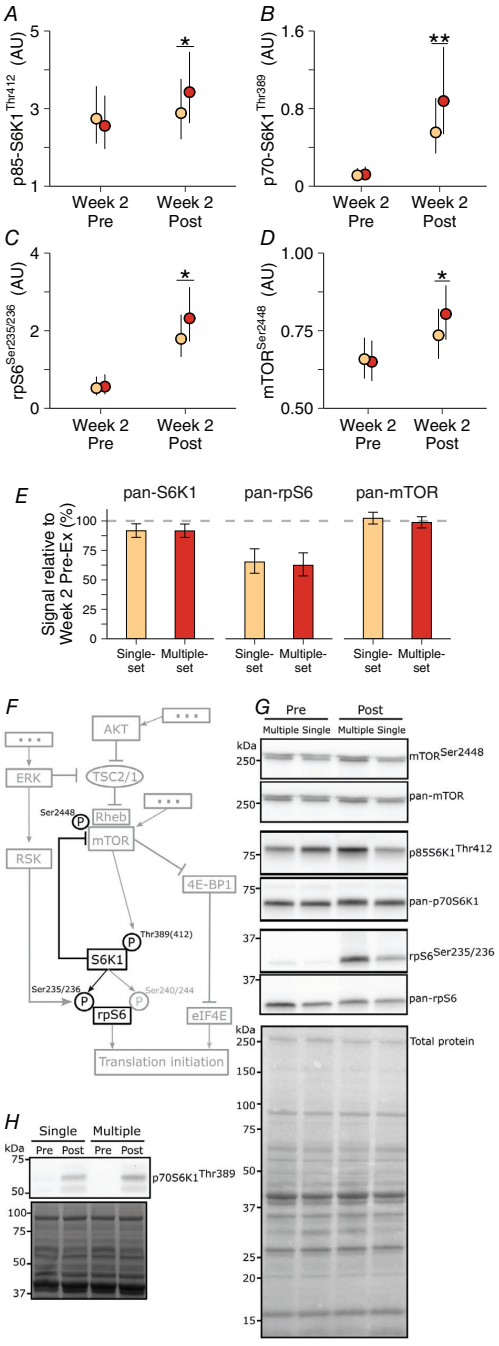


Figure 4. Western blot analysis of the mTOR signalling pathway

to the single-set leg), the odds of multiple-set benefit increased by 1.07 [1.00, 1.15] and 1.1 [1.01, 1.19] for muscle CSA and strength, respectively (CSA-model no. 6 and strength-model no. 4, Table 5). Notably, lean body mass also remained a significant predictor of benefit of moderate training volume on muscle CSA after variable selection: baseline lean body mass proportions lower than the sex-specific median reduced the odds of benefit of multiple-set to 0.21 [0.04, 1.17] (CSA-model no. 6, Table 5). The association between benefit of moderate volume on CSA and total RNA levels at Week 2 was independent of baseline lean body mass.

In all models, sex was included as a calibrating variable to account for potential predictors with sex-dependent regulation (e.g. blood variables). However, excluding sex and apparent sex-dependent variables from the variable selection, did not affect the conclusion (data not shown), nor did it affect the remaining variables when excluded as a final step in variable selection (Table 5).

We performed further analyses to explore the association between benefits to moderate volume and total RNA levels at Week 2. Eleven participants showed no benefits of moderate training volume on either CSA or strength (Fig. 6*C*). These participants also showed lower levels of total RNA in the multiple-set leg than in the single-set leg (multiple- to single-set leg ratio for total RNA of 0.96 [0.92,1.00]). In contrast, all other response patterns (benefit CSA, benefit strength or benefit CSA and strength) showed higher levels of total RNA in the multiple-set leg. These data showed a progressive nature, with benefit of moderate volume for both CSA and strength showing the highest multiple- to single-set leg ratio for total RNA (1.34 [1.01,1.68], $n = 6$), followed by benefit on CSA only (1.13 [1.03,1.22], $n = 7$) and benefit on strength only (1.12 [0.98,1.27], $n = 10$, all $P < 0.05$ compared to no benefit, Fig. 6*C*).

Discussion

In the present study, multiple-set resistance training led to greater increases in muscle strength and mass than single-set training. This is in agreement with

Training volume-dependent phosphorylation of S6K1 (p85, *A*; p70, *B*), rpS6 (*C*) and mTOR (*D*) proteins was evident in m. vastus lateralis after the fifth training session. (*E*) Pan levels of S6K1 and rpS6 but not mTOR were affected by acute exercise. Measured phosphorylation sites are shown in context (*F*) where phosphorylation of S6K1 (Thr389) is indicative of mTOR activity; S6K1 mediates negative feedback to mTOR through phosphorylation of the Ser2448 site. mTOR and MEK/ERK signalling converges on rpS6 as both pathways phosphorylate Ser235/236. Representative blots and total protein stains are shown in *G* and *H*. Values are means ± 95% CI. * represents differences between volume conditions, * and ** for $P < 0.05$ and $P < 0.01$, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

results from meta-analyses concluding in favour of moderate- compared to low-volume training (Krieger, 2009, 2010; Schoenfeld *et al.* 2016). The greater effect of multiple-set training coincided with greater responses in muscle biological traits indicative of hypertrophic response (Andersen & Aagaard, 2000; Terzis *et al.* 2008; Goodman *et al.* 2011; Stec *et al.* 2016; Luo *et al.* 2019), including greater transition from Type IIX to IIA muscle fibres, greater post-exercise phosphorylation of S6K1 and ribosomal protein S6, greater post-exercise expression of c-Myc and greater rested-state levels of total RNA and ribosomal RNA. While most of these variables are already

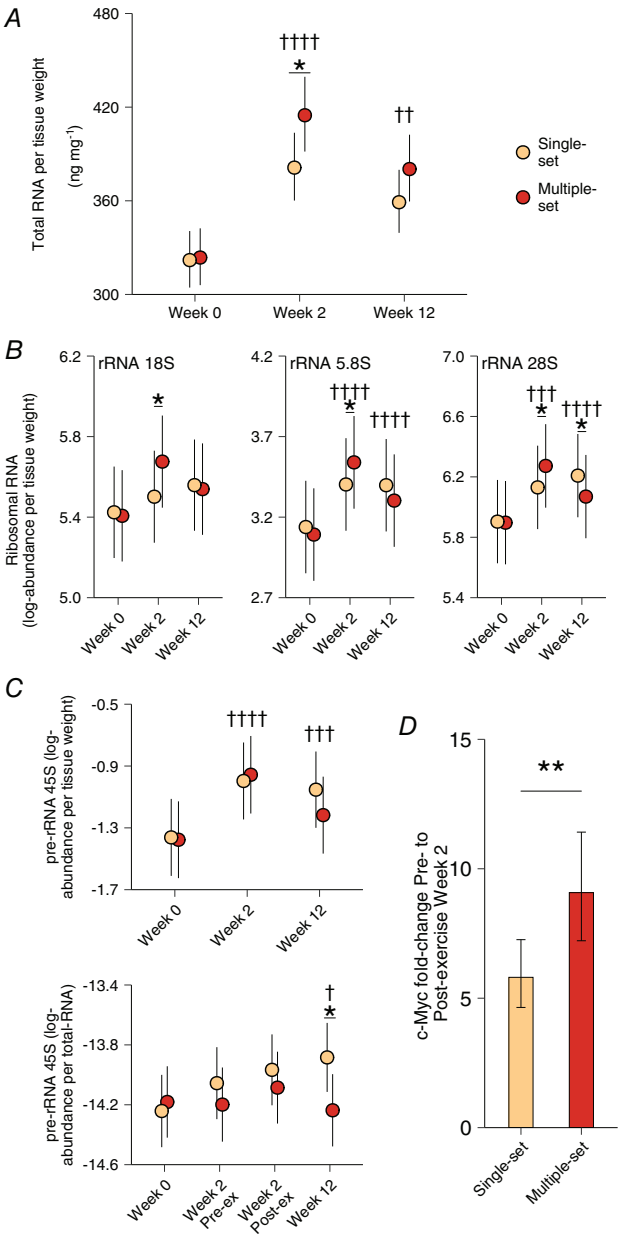
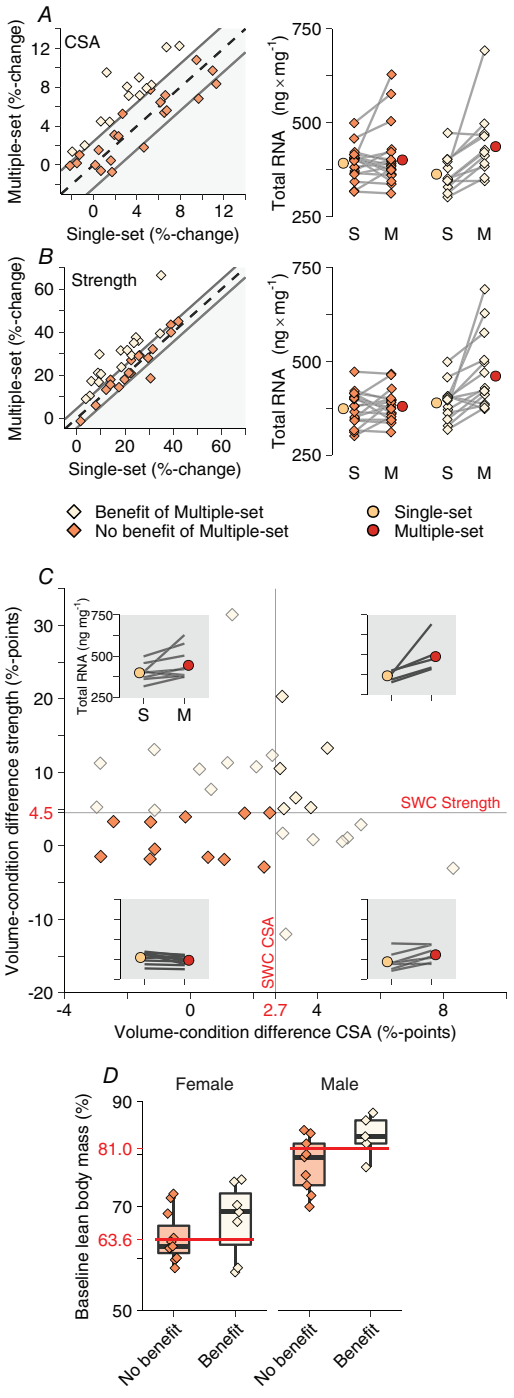


Figure 5. Total RNA and ribosomal RNA
Training volume-dependent changes in total RNA and ribosomal RNA 18S content were apparent in m. vastus lateralis after 2 weeks of resistance training (measured per unit muscle weight, Week 2, *A* and *B*). Other mature ribosomal RNA species exhibited similar expression patterns without reaching statistical significance (*B*). Increases in c-Myc mRNA abundance, measured 1 h after the fifth session, also showed volume dependency (*C*). Ribosomal pre-RNA 45S, expressed relative to total RNA, showed greater relative abundances at Week 12 than at Week 0 in the single-set leg (*D*). Values are estimated means ± 95% CI. * represents difference between volume conditions for $P < 0.05$. †represents difference from Week 0, †–†††† for $P < 0.05$ to $P < 0.0001$. [Colour figure can be viewed at wileyonlinelibrary.com]



assumed to be volume sensitive, such as muscle mass and strength (Krieger, 2009, 2010; Schoenfeld *et al.* 2016) and mTOR signalling (Burd *et al.* 2010; Terzis *et al.* 2010), this is the first study to suggest that the IIX → IIA fibre switch is also volume sensitive. Importantly, this adaptation is a hallmark of resistance training adaptations (Andersen & Aagaard, 2000). This study also suggests that the volume-sensitive increase in ribosomal content is essential for beneficial effects of increases in training volume on muscle growth and strength, as shown by thirteen and sixteen of the participants, respectively. Arguably, the biological resolution of the present data was high due to the use of a within-participant training model, facilitating disclosure of volume-dependent effects. Indeed, previous studies have typically used between-participant models to assess the volume dependency of muscle development (e.g. Starkey *et al.* 1996; Rhea *et al.* 2002; Ronnestad *et al.* 2007). This makes their interpretations prone to the large individual-to-individual variation in exercise adaptability (seen in e.g. Ahtiainen *et al.* 2016), which has been linked to variation in genetic and epigenetic predisposition (Timmons, 2011; Seaborne *et al.* 2018), and may potentially explain the long-standing lack of consensus (Carpinelli & Otto, 1998; Krieger, 2010).

In the present study, a large range of changes was evident for both muscle strength and muscle mass. The observed variation in muscle hypertrophy (SD of average %Δ CSA ∼4%) was comparable to that seen in larger cohorts (Ahtiainen *et al.* 2016). The strong correlation between responses to the two volume conditions (see Fig. 6*A* and *B*) highlights the importance of within-participant analyses: if the response to one training protocol was strong, the response to the other protocol was also strong. Consequently, our contralateral protocol resulted in lower estimates of differences between volume conditions at the population level, expressed as relative gains in muscle mass per weekly set, compared to a previous meta-analysis (∼1.6 *vs.* ∼2.5% estimated from Table 3 in

Figure 6. Analysis of additional benefit of multiple set training on muscle mass and strength
Participants that showed additional benefit of multiple-set on muscle hypertrophy had higher levels of total RNA in m. vastus lateralis of the multiple- compared with the single-set leg after 2 weeks of training (*A*, 17.6% [5.8, 30.7], $P = 0.004$). The same tendency was seen in strength analyses (*B*, 9.5 [−1.7, 22.0], $P = 0.095$). Dashed lines in *A* and *B* are identity lines ($y = x$). The distance from dashed lines to continuous line represents the smallest worthwhile change (SWC). Participants with additional benefits of multiple-set training on CSA, strength, or both, showed greater total RNA levels (*C*), measured as ratios between the multiple-set leg and the single-set leg, than participants with no additional benefit (*C*, lower left quadrant). SWC in strength and CSA analyses constitutes the four-way grouping. Baseline lean body mass was higher in participants displaying benefit to multiple-set training (*D*). Sex-specific median values are denoted with red (in *D*). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 4. Univariate analysis of predictors of additional benefit of multiple-sets on training-induced muscle hypertrophy and strength

			Muscle CSA					Muscle strength				
				Model coefficients[a]					Model coefficients[a]			
Variable	Classification		Mean (SD)[b]	Estimate	SE	*t/z*	*P*	Mean (SD)[b]	Estimate	SE	*t/z*	*P*
Ribosome biogenesis												
Total RNA Week 2 (% of single-sets)	No benefit		3.2 (15)	18	6.2	2.9	0.007	2.2 (11)	16	6.5	2.4	0.021
	Benefit		22 (21)				0.007	20 (24)				
Total RNA Week 12 (% of single-sets)	No benefit		5.7 (15)	5.5	7.1	0.78	0.444	7.7 (20)	2.6	7.3	0.36	0.720
	Benefit		11 (26)				0.444	7.7 (20)				
mTOR signalling												
$S6K1^{Thr389}$ (fold of single-sets)	No benefit		1.40 (0.59)	0.20	0.33	0.61	0.548	1.77 (1.01)	−0.73	0.30	−2.4	0.023
	Benefit		1.62 (1.26)				0.548	1.13 (0.51)				
Endocrine parameters												
Cortisol (mean Weeks 0–2)	No benefit	F	544 (145)	13	48	0.27	0.792	625 (196)	−84	47	−1.81	0.080
		M	417 (54)				0.792	419 (76)				
	Benefit	F	577 (197)				0.792	503 (112)				
		M	402 (100)				0.792	393 (58)				
Testosterone (mean Weeks 0–2)[c]	No benefit	F	0.67 (0.47)	−1.15	0.81	−1.43	0.163	0.42 (0.46)	0.79	0.83	0.95	0.350
		M	15 (3.1)				0.163	14 (3.6)				
	Benefit	F	0.75 (1.62)				0.163	0.93 (1.30)				
		M	12 (2.8)				0.163	15 (1.76)				
Growth hormone (mean post-exercise Week 2)	No benefit	F	4.0 (2.0)	1.03	0.71	1.46	0.156	4.7 (2.3)	−0.037	0.75	−0.050	0.960
		M	1.44 (1.36)				0.156	1.68 (1.42)				
	Benefit	F	4.3 (1.93)				0.156	3.6 (1.52)				
		M	3.4 (2.5)				0.156	3.3 (3.0)				
IGF-1 (mean pre-exercise Weeks 0–2)	No benefit		20 (5.2)	0.38	1.85	0.21	0.838	19 (4.8)	1.10	1.86	0.59	0.560
	Benefit		20 (4.7)				0.838	20 (5.2)				
IGF-1 (mean post-exercise Week 2)	No benefit		19 (5.7)	1.42	1.97	0.72	0.478	19 (4.8)	2.0	1.98	1.02	0.315
	Benefit		20 (4.5)				0.478	20 (5.8)				
Vitamin D (mean Weeks 0 and 12)	No benefit	F	100 (39)	−12	9.5	−1.24	0.226	101 (34)	−10	9.7	−1.08	0.289
		M	74 (18)				0.226	73 (18)				
	Benefit	F	90 (15)				0.226	92 (30)				
		M	60 (14)				0.226	60 (15)				
Baseline characteristics												
Baseline strength (kg^{-1}, AU)	No benefit	F	6.4 (1.10)	0.41	0.35	1.17	0.250	6.8 (1.11)	−0.43	0.35	−1.24	0.226
		M	7.7 (0.76)				0.250	8.1 (0.88)				
	Benefit	F	6.5 (0.96)				0.250	6.2 (0.89)				
		M	8.6 (0.85)				0.250	7.9 (0.98)				

(*Continued*)

Table 4. Continued

			Muscle CSA					Muscle strength				
				Model coefficients[a]					Model coefficients[a]			
Variable	Classification		Mean (SD)[b]	Estimate	SE	*t/z*	*P*	Mean (SD)[b]	Estimate	SE	*t/z*	*P*
Baseline lean mass (%)	No benefit	F	64 (4.8)	4.3	1.96	2.2	0.037	65 (5.9)	−2.2	2.1	−1.06	0.298
		M	78 (5.3)				0.037	82 (4.4)				
	Benefit	F	67 (7.2)				0.037	65 (6.2)				
		M	83 (4.1)				0.037	76 (6.3)				
Muscle fibre types												
Type IIA (% of total MHC)	No benefit		50 (7.3)	0.64	2.7	0.23	0.817	51 (7.5)	−0.69	2.8	−0.25	0.805
	Benefit		51 (8.2)				0.817	50 (7.8)				
Type IIX (% of total MHC)	No benefit		3.3 (2.2)	3.1	1.67	1.84	0.076	4.0 (3.9)	0.74	1.78	0.41	0.681
	Benefit		6.4 (7.0)				0.076	5.0 (5.8)				
Type I (% of total MHC)	No benefit		46 (8.1)	−3.7	3.4	−1.10	0.280	45 (8.8)	−0.053	3.5	−0.015	0.988
	Benefit		43 (11)				0.280	45 (10)				
Pre-study training habits												
Pre-study training habits (*n* sessions >0/0)[c]	No benefit		*n* = 13/8	−0.32	0.71	−0.45	0.654	*n* = 10/8	0.27	0.70	0.38	0.702
	Benefit		*n* = 7/6				0.654	*n* = 10/6				
Pre-study strength training (strength-type training, yes/no)[c]	No benefit		*n* = 6/15	0.12	0.77	0.16	0.874	*n* = 5/13	0.16	0.75	0.21	0.831
	Benefit		*n* = 4/9				0.874	*n* = 5/11				
Training characteristics												
Supervised sessions (100%/<100%)[c]	No benefit		*n* = 9/12	−0.16	0.72	−0.22	0.823	*n* = 9/9	−0.74	0.71	−1.03	0.301
	Benefit		*n* = 5/8				0.823	*n* = 5/11				
Total number of sessions (100%/<100%)[c]	No benefit		*n* = 12/9	−0.42	0.71	−0.59	0.555	*n* = 8/10	0.69	0.70	0.99	0.323
	Benefit		*n* = 6/7				0.555	*n* = 10/6				
Dietary data[d]												
Protein kg^{-1} day^{-1}	No benefit		1.34 (0.46)	−0.015	0.18	−0.083	0.93	1.34 (0.46)	−0.18	0.18	−1.05	0.31
	Benefit		1.32 (0.36)				0.93	1.32 (0.36)				
kcal day^{-1}	No benefit		2169 (1036)	−334	368	−0.91	0.38	2169 (1036)	−227	373	−0.61	0.55
	Benefit		1835 (620)				0.38	1835 (620)				

[a]Model coefficients from univariate analysis using linear regression with benefit groups as the independent variable for continuous data and logistic regression with benefit groups as the dependent variable for dichotomous data. Sex was included in all models to account for sex differences.
[b]Sex-specific mean and SD are reported when significantly different between sexes.
[c]Dichotomous variable, logistic regression model used to determine association.
[d]Dietary data on *n* = 21, not used in variable selection. M, male; MHC, myosin heavy chain; F, female.

Table 5. Multivariate logistic regression on additional benefit of multiple-set training on muscle hypertrophy (CSA) and strength

	Muscle CSA				
Variable	Estimate[a]	SE	*Z* value	*P* value	LRT *P* value
Model 1					
Intercept	−0.61	1.39	−0.44	0.662	
Sex (male)	0.67	0.98	0.68	0.495	
Total RNA Week 2 (% of single-set)	0.054	0.034	1.57	0.115	
Testosterone (mean Weeks 0–2)[b]	−1.02	0.93	−1.09	0.274	
Growth hormone (mean post-exercise Week 2)	0.18	0.23	0.80	0.422	
Baseline lean mass (%)[c]	−1.32	0.90	−1.47	0.142	
Type 2X (% of total MHC)[d]	−0.27	0.95	−0.29	0.775	
Model 2					
Intercept	−0.85	1.16	−0.73	0.463	Model 1 *vs.* 2 $P = 1.000$
Sex (male)	0.75	0.98	0.76	0.446	
Total RNA Week 2 (% of single-set)	0.058	0.034	1.67	0.095	
Testosterone (mean Weeks 0–2)[b]	−1.14	0.91	−1.26	0.209	
Growth hormone (mean post-exercise Week 2)	0.21	0.22	0.95	0.344	
Baseline lean mass (%)[c]	−1.34	0.90	−1.49	0.137	
Model 3					
Intercept	−0.10	0.86	−0.12	0.907	Model 2 *vs.* 3 $P = 0.292$
Sex (male)	0.44	0.91	0.48	0.629	
Total RNA Week 2 (% of single-set)	0.065	0.035	1.86	0.062	
Testosterone (mean Weeks 0–2)[b]	−1.03	0.88	−1.18	0.239	
Baseline lean mass (%)[c]	−1.35	0.89	−1.52	0.128	
Model 4					
Intercept	−0.59	0.76	−0.77	0.439	Model 3 *vs.* 4 $P = 0.197$
Sex (male)	0.44	0.88	0.50	0.617	
Total RNA Week 2 (% of single-set)	0.068	0.035	1.93	0.054	
Baseline lean mass (%)[c]	−1.51	0.88	−1.71	0.087	
Model 5					
Intercept	−1.34	0.66	−2.0	0.043	Model 4 *vs.* 5 $P = 0.043$
Sex (male)	0.51	0.84	0.61	0.545	
Total RNA Week 2 (% of single-set)	0.063	0.031	2.1	0.039	
Model 6					
Intercept	−0.38	0.61	−0.61	0.539	Model 4 *vs.* 6 $P = 0.653$
Total RNA Week 2 (% of single-set)	0.068	0.036	1.91	0.057	
Baseline lean mass (%)[c]	−1.58	0.89	−1.78	0.075	
	Muscle strength				
Variable	Estimate[a]	SE	*Z* value	*P* value	LRT *P* value
Model 1					
Intercept	1.59	1.56	1.02	0.308	
Sex (male)	−0.90	0.98	−0.92	0.356	
Total RNA Week 2 (% of single-set)	0.086	0.043	1.99	0.047	
S6K1^{Thr389} (fold of single-set)	−1.43	0.95	−1.51	0.132	
Cortisol (mean Weeks 0–2)	−0.003	0.004	−0.83	0.407	
Model 2					
Intercept	1.56	1.46	1.07	0.285	Model 1 *vs.* 2 $P = 0.333$
Sex (male)	−0.88	0.96	−0.92	0.359	
Total RNA Week 2 (% of single-set)	0.090	0.043	2.1	0.036	
S6K1^{Thr389} (fold of single-set)	−1.43	0.89	−1.60	0.110	
Model 3					
Intercept	−0.67	0.62	−1.07	0.282	Model 2 *vs.* 3 $P = 0.011$
Sex (male)	−0.36	0.86	−0.42	0.671	
Total RNA Week 2 (% of single-set)	0.076	0.037	2.1	0.037	
Model 4					
Intercept	0.79	1.15	0.69	0.493	Model 2 *vs.* 4 $P = 0.261$
Total RNA Week 2 (% of single-set)	0.093	0.041	2.3	0.022	
S6K1^{Thr389} (fold of single-set)	−1.16	0.78	−1.49	0.136	

[a]Estimates are log-odds ratio. Variables not linear in the logit were transformed to meet assumptions.
[b]Testosterone dichotomised to above and below the detection limit (0.69 nmol l^{-1}) in females and above and below the median in males (13.5 nmol l^{-1}).
[c]Percentage lean body mass dichotomised to the sex-specific median (females, 63.6; males, 81.0).
[d]Percentage Type IIX fibres dichotomised above and below the median (3.7%). LRT, likelihood-ratio test.

Schoenfeld *et al.* 2016). Notably, in the present study, this comparison was prone to systemic contralateral adaptions to training, which would diminish differences between volume conditions. However, this effect is likely negligible as non-trained limbs typically do not show increased protein synthesis, hypertrophy or muscle fibre type transitions (Wilkinson *et al.* 2006; Brook *et al.* 2016). Instead, it is plausible that the overall effect of added training volume, as reported in Schoenfeld *et al.* (2016), is overestimated due to small sample sizes, a known weakness in meta-analyses (Nüesch *et al.* 2010). Comparing our study to the similarly designed study by Mitchell *et al.* (2012) is not straightforward. The present study used two exercises to activate knee extensor muscles instead of one, resulting in a doubled training volume compared to Mitchell *et al.* (2012). It remains unclear if this discrepancy could explain the dissimilar between-conditions effect (∼1.6 *vs.* ∼3.8% percentage-point differences in CSA change). This perspective is clouded by the fact that strong within-participant correlations were not accounted for in Mitchell *et al.* (2012). Arguably, contralateral designs improve comparisons of responses to different training volumes and regimes by accounting for inter-individual differences in training responses. Failing to account for within-participant correlations could lead to biased conclusions.

In our search for determinants that could explain the variation in acquired muscle mass and muscle strength in response to the two volume protocols, potential explanatory factors included baseline characteristics, blood variables, indices of mTOR signalling (S6K1 phosphorylation) and ribosome biogenesis, as well as training characteristics. Following variable selection, the multiple- to single-set ratio of total RNA at Week 2 remained as a significant predictor of additional multiple-set benefit in both muscle CSA and strength. As total RNA is a valid proxy marker of rRNA abundance (Zak *et al.* 1967; Chaillou *et al.* 2014), this suggests that early-phase, volume-dependent ribosomal accumulation is a determinant of dose–response relationships between training volume and muscle hypertrophy. In other words, the ability to induce superior increases in ribosomal content in response to the higher mechanical and metabolic stress of accompanying higher training volume is necessary to induce subsequent superiority in growth and strength increases. This probably acts through an increased capacity for protein synthesis, and fits well with the overall impression conveyed by the data set, wherein multiple-set training resulted in larger increases in total RNA and mature rRNA species (rRNA 18S, 28S and 5.8S).

In untrained participants, early accumulation of ribosomal content seems to be a generic response to training (Brook *et al.* 2016; Stec *et al.* 2016). This accumulation follows a progressive nature during the first 3 weeks of training (Brook *et al.* 2016), whereupon total RNA remains at elevated levels for at least 12 weeks (Figueiredo *et al.* 2015; Mobley *et al.* 2018), presumably preceded by an increased expression of the 45S pre-rRNA. The latter was not evident in the present data, suggesting that the timing of muscle biopsy-sampling was not suited for investigating *de novo* transcription of rRNA measured as increased levels of pre-rRNA relative to total RNA as evident in previous studies (Nader *et al.* 2014; Stec *et al.* 2015; Figueiredo *et al.* 2016). However, when assessed before the fifth session and expressed per unit tissue weight, 45S pre-rRNA followed the same pattern seen in mature rRNA species indicating an accumulative behaviour of rRNA in response to repeated bouts of resistance exercise (Figueiredo & McCarthy, 2019). A limitation in our assessment of 45S pre-rRNA abundances is that we only targeted the 5′ external transcribed spacer. During the processing of pre-rRNA, several sequential splicing events occur (Henras *et al.* 2015). This may have prohibited us from measuring *de novo* synthesis in the appropriate manner, as we would have missed acute accumulation of transcripts downstream of early splicing events. This may also explain differences in expression patterns of pre-rRNA seen in some studies (Figueiredo *et al.* 2015) but not others (Figueiredo *et al.* 2016, 2018; Fyfe *et al.* 2018).

The potential link between ribosomal content in muscle and trainability is not surprising. Several studies have shown that ribosomal biogenesis measured as total RNA per tissue weight is positively associated with training-induced muscle hypertrophy (Figueiredo *et al.* 2015; Stec *et al.* 2016; Mobley *et al.* 2018) in addition to early observations of a relationship between RNA content and rate of protein synthesis (Millward *et al.* 1973). Our data provide further evidence for a relationship between increased translational capacity and long-term protein accretion, potentially mediated by increased basal protein synthesis (Kim *et al.* 2005; Reidy *et al.* 2017). Notably, transcription of precursor rRNA is also induced by stimuli other than training, including protein supplementation (Figueiredo *et al.* 2018), which indeed also affects training responses (Morton *et al.* 2018). The lack of a comprehensive dietary control in the present study poses a limitation, as we cannot exclude dietary aspects from exerting confounding effects. However, the within-participant nature of our design arguably limits its impact on volume-dependent comparisons. Between-participants comparisons could still be affected, though indices of habitual dietary patterns did not differ between response groups (benefit *vs.* no benefit to multiple-set training, Table 4).

Variable selection did not identify other variables that could explain the benefits of moderate training volume, discarding biological traits such as sex and muscle fibre composition. For example, variable selection discarded post-exercise phosphorylation of S6K1, indicative of

mTORC1 activity, as a potential explanatory variable, though increased exercise volume led to more pronounced activation of mTORC1-related signalling. This seems somewhat counterintuitive, as this pathway is a known regulator of translation initiation and elongation, as well as of ribosomal biogenesis (Nader *et al.* 2005; Chauvin *et al.* 2014; von Walden *et al.* 2016; West *et al.* 2016) giving it a role in acute control of protein synthesis and accumulation of rRNA and subsequent moderate-volume beneficence. However, signalling cues that are measurable and provide insight into mTORC1 activity, such as S6K1 phosphorylation, are acute-phase responders to resistance exercise that show phasic and time-dependent regulation. This means that the measured changes in S6K1 phosphorylation status depend on factors such as timing of biopsy sampling, giving it low resolution and making it less suited for explanatory analyses. In addition, mTORC1-related signalling is under regulation from mechanisms other than mere feed-forward AKT-based activation such as negative feedback phosphorylation from downstream targets (e.g. from S6K1, Chiang & Abraham, 2005). There is also likely signal redundancy as input from parallel signalling systems such as the MEK/ERK pathway (Roux *et al.* 2007) and c-Myc induction (von Walden *et al.* 2012; West *et al.* 2016) regulates common targets. Indeed, in the present study we observed the volume dependence of mTOR phosphorylation at Ser2448, which could be a sign of negative feedback from mTORC1-based activation of S6K1 (Figueiredo *et al.* 2017). We also observed volume-dependent regulation of rpS6 phosphorylation at Ser235/236, which is a common target of both S6K1 and the p90 ribosomal S6 kinase, downstream of MEK/ERK (Roux *et al.* 2007) and volume-dependent induction of c-Myc representing a synergist pathway. Given these limitations in using mTORC signalling as a marker of muscle hypertrophy, it is not surprising that previous studies are ambiguous in their associative approach between acute mTORC1-related phosphorylation and hypertrophy in humans. Some studies find a strong correlation (Terzis *et al.* 2008; Mitchell *et al.* 2013) while others do not (Mitchell *et al.* 2012; Phillips *et al.* 2017). To conclude, exercise-induced mTORC1 activity is transitory, along with other parallel acute-phase processes. However, its effects on muscle biology is long-lasting, leading to steady-state adaptations on a longer time scale. Many of these adaptations, including ribosome biogenesis, are easily detectable in rested muscle (Nader *et al.* 2005; von Walden *et al.* 2012, 2016; Chauvin *et al.* 2014). Targeting such rested-state muscle characteristics obviates issues such as biopsy-sampling timing, making them better suited as biomarkers.

We identified baseline percentage of lean body mass as a predictor of additional benefit to multiple-set training on muscle hypertrophy. Although this estimate was associated with considerable uncertainty, the finding is in line with current guidelines advocating higher training volume for individuals with more training experience (and thus likely higher percentage of lean body mass) (Ratamess *et al.* 2009). Contrary to this interpretation, baseline lean body mass was not related to any measure of self-reported training practice. This indicates that within a homogeneous group (in terms of training experience), baseline muscle mass could be more informative for exercise prescription. More data are needed to confirm this as a valid diagnostic tool. Using this line of logic, we initially hypothesised that participants with lower proportions of Type IIX muscle fibres and thus likely more training experience, would benefit more from moderate volume training (and vice versa) than subjects with higher proportions of IIX, as outlined in the pre-study clinical trials registration. Indeed, during variable selection, baseline IIX fibre proportions were selected as one potential explanatory factor behind volume benefits on hypertrophy (Table 4). However, contrary to our hypothesis, higher levels of IIX tended to be associated with the beneficial effects of multiple sets. Although this trait was discarded during variable selection, the tendency towards a positive effect of higher IIX levels could be ascribed to their greater growth potential (Jespersen *et al.* 2011; Stec *et al.* 2016), with these fibres having been in a state of disuse prior to the intervention. This implies a relatively rapid transition of type IIX fibres into IIA fibres, which indeed was present in the data already after 2 weeks of training at both protein and RNA levels. Correlation analyses revealed that this transition was more pronounced in individuals with higher baseline levels of IIX, with an r value >0.95 (data not shown), far exceeding the bias expected from regression-towards-the-mean.

To our knowledge, this is the first study to show that muscle fibre transitions from Type IIX to IIA depend on resistance training volume. Moderate volume resulted in 1.5 percentage points greater reduction in Type IIX fibre expression from baseline to post-intervention compared to low volume, presumably driven by more pronounced reductions in mRNA expression of the myosin heavy chain IIX (*MYH1*) gene (-61% *vs.* -31%). Previous studies have not compared this transition directly between volume protocols. However, Pareja-Blanco *et al.* (2017) observed blunted IIX $\rightarrow$ IIA transitions in response to non-exhaustive high-load resistance training compared to load-matched training to volatile failure. Together with our data, this makes exercise volume and subsequent metabolic stress and dosage of neuromuscular activity plausible candidates for regulation of IIX $\rightarrow$ IIA reprogramming, as opposed to mechanical stimuli. Indeed, in rodents, mechanical load does not affect fibre-type transitions (Eftestol *et al.* 2016), which is instead linked to neural activation. Interestingly, after 2 weeks of training, the volume effect on IIX $\rightarrow$ IIA transitions was opposite to our main finding after

12 weeks, with low-volume resistance training resulting in more pronounced decreases in IIX at the cellular level, accompanied by lower abundances of IIX/IIA hybrid fibres. This seemingly early benefit of single-set training on overall IIX levels was not observed at the mRNA level, with *MYH1* being more heavily suppressed in the moderate volume condition. Instead, at Week 2, there seemed to be a disconnection between *MYH1* mRNA and IIX protein adaptations in the multiple-set leg compared to the single-set leg. Whether this phenomenon was caused by increased need for tissue repair in the moderate-volume condition at this time-point (Kim *et al.* 2005; Damas *et al.* 2016) or other causalities, rather than myofibril-specific adaptations remains unclear. Regardless of causality, these data underline the importance of optimising exercise volume to achieve optimal training progression, such as making use of progressive volume protocols. Although such protocols remain largely unexplored, previously untrained individuals will likely benefit from careful calibration of training volume during early phases of resistance training. Too large or too small a training volume may lead to suboptimal adaptations.

In conclusion, resistance training with higher volume led to augmented increases in muscle CSA, muscle strength and fibre-type transitions, as well as greater responses in molecular hypertrophy signalling and effectors. Beneficial effects of multiple-set over single-set training on muscle hypertrophy coincided with higher total RNA levels at Week 2 in response to moderate- compared to low-volume training, suggesting that volume-dependent early-phase regulation of ribosomal biogenesis contributes to the dose–response relationship between training volume and muscle adaptations.

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Additional information

Competing interests

The authors have no conflicts of interest to disclose.

Author contributions

Data collection was done in the Sport Science Laboratory at Inland University of Applied Sciences and the Hospital for Rheumatic Diseases with molecular analyses partly performed at Åstrandlaboratoriet, The Swedish School of Sport and Health Sciences and Innlandet Hospital Trust. D.H., S.E. and B.R.R. designed the study; D.H., S.Ø., L.K., M.H., S.E. and W.A. performed experiments; D.H. analysed the data; D.H. S.E., S.Ø., L.K., M.H., W.A., J.E.W., I.H., E.B. and B.R.R. interpreted the results; D.H. drafted the manuscript; D.H., S.Ø., L.K., M.H., B.R.R., E.B., W.A., J.E.W., I.H. and S.E. edited and revised the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

resistance-training, ribosome biogenesis, training-volume

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document

Paper II

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EXERCISE PHYSIOLOGY

ACTA PHYSIOLOGICA

Ribosome accumulation during early phase resistance training in humans

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Abstract

Aim: To describe ribosome biogenesis during resistance training, its relation to training volume and muscle growth.

Methods: A training group (n = 11) performed 12 sessions (3-4 sessions per week) of unilateral knee extension with constant and variable volume (6 and 3-9 sets per session respectively) allocated to either leg. Ribosome abundance and biogenesis markers were assessed from vastus lateralis biopsies obtained at baseline, 48 hours after sessions 1, 4, 5, 8, 9 and 12, and after eight days of de-training, and from a control group (n = 8). Muscle thickness was measured before and after the intervention.

Results: Training led to muscle growth (3.9% over baseline values, 95% CrI: [0.2, 7.5] vs. control) with concomitant increases in total RNA, ribosomal RNA, upstream binding factor (UBF) and ribosomal protein S6 with no differences between volume conditions. Total RNA increased rapidly in response to the first four sessions (8.6% [5.6, 11.7] per session), followed by a plateau and peak values after session 8 (49.5% [34.5, 66.5] above baseline). Total RNA abundance was associated with UBF protein levels (5.0% [0.2, 10.2] per unit UBF), and the rate of increase in total RNA levels predicted hypertrophy (0.3 mm [0.1, 0.4] per %-point increase in total RNA per session). After de-training, total RNA decreased (−19.3% [−29.0, −8.1]) without muscle mass changes indicating halted biosynthesis of ribosomes.

Conclusion: Ribosomes accumulate in the initial phase of resistance training with abundances sensitive to training cessation and associated with UBF protein levels. The average accumulation rate predicts muscle training-induced hypertrophy.

KEYWORDS

muscle hypertrophy, resistance training, ribosome biogenesis

See related editorial: Henriksson J. 2022. The accumulation rate of ribosomes predicts muscle training-induced hypertrophy *Acta Physiol* (Oxf). e13817.

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1 | INTRODUCTION

Skeletal muscle is a critical target for interventions that promote health across the lifespan,[1] 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 hours after exercise,[2] and subsequent repeated bouts leading to accumulation of muscle protein over time.[3,4] In recent years, this view has been supplemented by evidence suggesting that chronic RT leads to increased basal muscle protein synthesis rates,[5-7] which has been postulated to be associated with increased translational capacity, that is, accumulation of ribosomes.[7,8] This notion is supported by exercise-induced increases in total RNA, a proxy marker of ribosome abundance, which is closely connected to protein synthesis[9,10] and muscle hypertrophy.[11-13] Conversely, inhibition of ribosomal RNA (rRNA) transcription and inhibition of its up-stream transcription factors act to diminish muscle cell growth.[9,12,14]

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.[14-16] 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.[16] Specifically, activation of the of the upstream binding factor (UBF) through phosphorylation is needed to initiate transcription.[17,18] 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.[19,20] Interestingly, the availability of UBF per se has been shown to be a determinant of rRNA transcription[21] through control of rDNA gene activity.[22]

Resistance exercise is a potent and specific[23] stimuli for rRNA transcription as a single session leads to increases in pre-rRNA.[24,25] Repeated bouts lead to the accumulation of mature rRNA reflected in total RNA and presumably functional ribosomes.[7,11-13,24,26,27] 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 hours after the second bout.[26] Using voluntary contractions, peak values were reported after nine sessions, followed by a slight decrease to after 18 sessions,[27] resembling data from our lab where five sessions of RT led to marked increase in total RNA levels (per-unit muscle tissue), followed by lower levels measured after the last training session of the 12 wk interventions (31 sessions).[13] 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.[13] 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.

Based on these observations we hypothesize that (1) ribosome accumulation occurs during the early phase (3-4 weeks) of RT, within which this accumulation (2) reaches a plateau when RT volume is kept constant, (3) displays fluctuations in response to fluctuating training volume and (4) is partially reversed one week after cessation of RT. In addition to addressing these hypotheses we aimed to relate RNA accumulation to total UBF levels and muscle growth. We utilized a within-participant unilateral training model where one leg was assigned constant volume (CONST, 6 sets per session) and the contra lateral leg variable volume (VAR, 6, 3 and 9 sets in sessions 1-4, 5-8 and 9-12 respectively). Effects of training were assessed by comparison to a non-training control group (CTRL).

2 | RESULTS

All participants allocated to TRAIN successfully completed their prescribed RT on both legs, with the two volume conditions resulting in diverging volume profiles (load × repetitions) over the course of the study (Figure 1B). Exercise intensities (resistance at 10RM) increased similarly in both conditions from the first to the second (30%, 95% credible interval (CI): [21, 41]) and third (47% [35, 61]) training block, with each block consisting of four training sessions. Concomitantly, in TRAIN, isokinetic strength and thickness of *m. vastus lateralis* increased from baseline to after Session 12 compared to CTRL (isokinetic strength ~9.2%-point difference; muscle thickness ~3.6%-point difference, Figure 1C,D), a difference that was sustained to after eight days of de-training (~6.7%-point and ~3.5%-point difference in change in isokinetic strength and muscle thickness, respectively; Figure 1C,D). Isometric strength showed the same general pattern to after Session 12 (~3.5%-point difference), though with considerably larger degrees of uncertainty, as indicated by wider 95% CI normalization compared to CTRL after de-training (~1.9%-point; Figure 1C). No

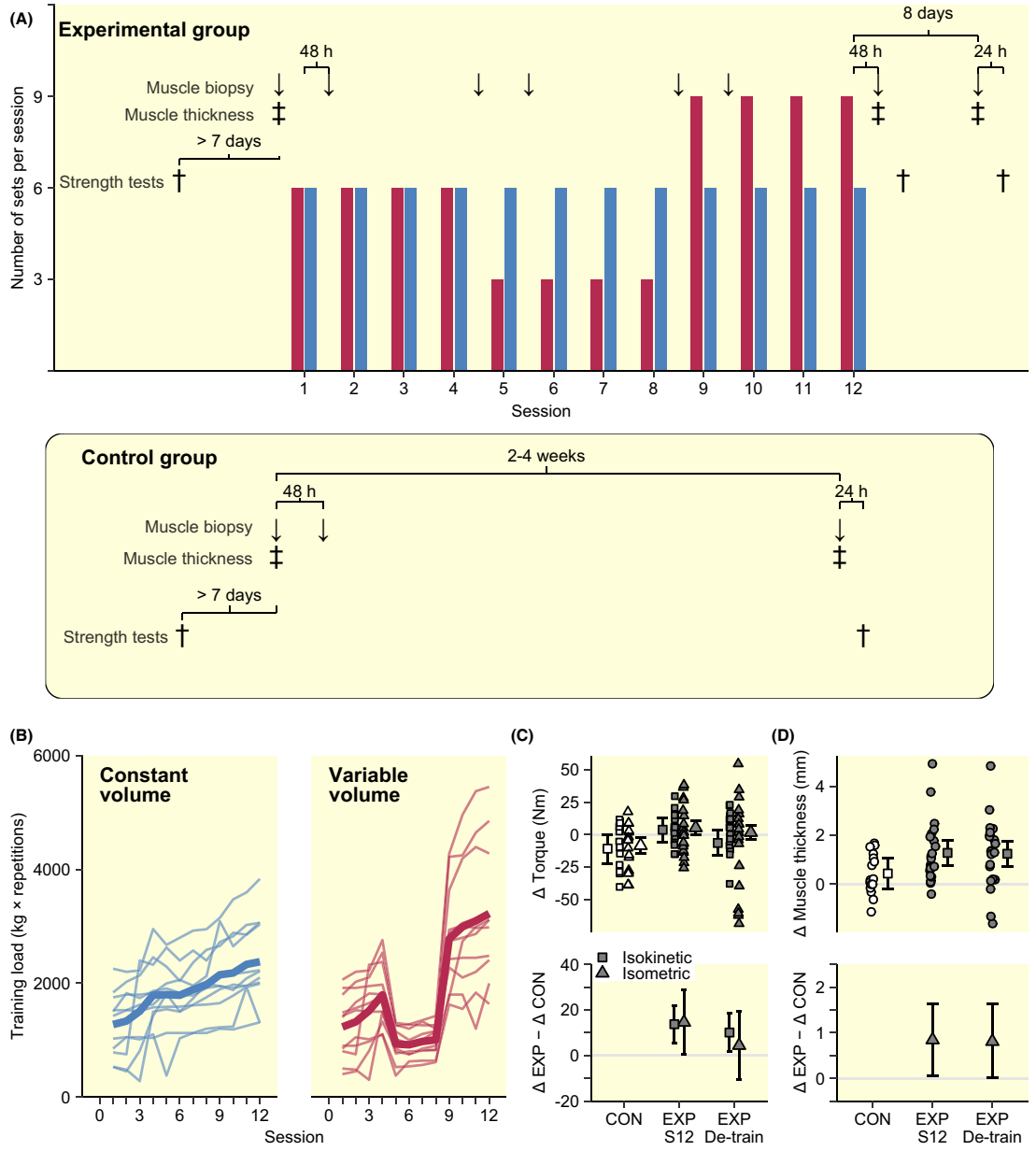

FIGURE 1 (A) Study design showing muscle biopsy sampling, thickness and strength assessments time points together with number of sets per session (CONST blue bars, VAR red bars). Assessments time points in the negative control group is shown in the lower panel. (B) Observed training loads in response to CONST and VAR volume protocols. Training outcomes are shown as within condition changes and in comparison to the control group (muscle strength, C; muscle thickness D). Intervals in C and D indicate 95% CI.

differences were observed between volume conditions for either strength or muscle thickness.

For both ribosomal protein S6 (rpS6) and UBF, protein levels increased linearly throughout the training intervention, with rpS6 showing estimated increments per session corresponding to 4.2% [1.2, 7.3] during block 1 (session 1-4), 2.6% [−0.3, 5.5] during block 2 (session 5-8) and 4.6% [1.2, 8.1] during block 3 (session 9-12), and UBF

showing increments corresponding to 7.3% [2.1, 13.0], 4.5% [−0.5, 9.8] and 6.1% [0.3, 12.1]. This general pattern was confirmed when comparing TRAIN to CTRL where UBF and rpS6 protein levels were higher in TRAIN compared to CTRL after Session 12, and remained elevated after eight days of rest (Figure 2A,B), with no robust differences being observed after the first training session (48 hours). Increases did not differ between volume-conditions but for UBF, there was a tendency towards lower levels in VAR after Session 12 (−19.2% [−41.8, 13.0]). After de-training, UBF-levels tended to decrease in CONST (−22.3% [−43.5, 7.3]) while levels in VAR remained at elevated level compared to after Session 12 (7.6% [−22.6, 47.4]; interaction effect: 33.2% [−15.7, 110.9]). For rpS6, de-training did not affect protein levels, which remained similar between volume conditions. At the mRNA level, UBF showed robust increase from before to 48 hours after the first session in TRAIN compared to CTRL (Figure 2D), while rpS6 showed no robust differences between TRAIN and CTRL at any time point. No differences were observed between volume conditions for either transcripts (Figure 2D,E).

A single session of RT (Session 1) led to robust increases in precursor ribosomal RNA (pre-rRNA 47S ETS and 45S ETS) abundance per unit tissue weight, measured as changes from baseline to 48 hours after exercise within TRAIN (Figure 3B), as well as compared to CTRL (Figure 3C). After Session 1, pre-rRNA 47S ETS and 45S ETS levels remained at similar levels at all measured timepoints in TRAIN (Figure 3D), confirmed in comparison to CTRL after Session 12 (Figure 3C). Other rRNA transcripts showed increases in response to training with slightly different temporal patterns with exception of rRNA 5S which did not change and rRNA 5.8 which tended to follow other mature transcript spliced from pre-rRNA 45S ETS, but without statistical robustness (Figure 3C,D). After eight days of rest, 18S and 28S remained at elevated levels compared to CTRL (Figure 3B). This general pattern of rRNA expression was reflected by total RNA abundance per unit tissue weight, which increased robustly and steadily in TRAIN throughout the initial part of the intervention (Figure 3E,G), leading to robust increase compared to CTRL after Session 12 (Figure 3F), followed by decreased levels after de-training (−19.3%, [−29.0, −8.1]). For both rRNA expression and total RNA levels, the training-associated increases in abundances occurred predominately during the first four sessions, evident as 8.6% [5.6, 11.7] increase per session, followed by sustained levels from sessions four to eight (1.8% [−1.0, 4.7] increase per session) and from sessions eight to twelve 0.0% [−3.0, 3.3], corresponding to 39.3% [24.4, 55.9], 49.5% [34.5, 66.5] and 49.8% [33.0, 68.9] increases from baseline to 48 hours after session 4, 8 and 12 respectively. In TRAIN, the two volume conditions led to similar changes for most variables (Figure 3D,G), with 45S ETS abundance only

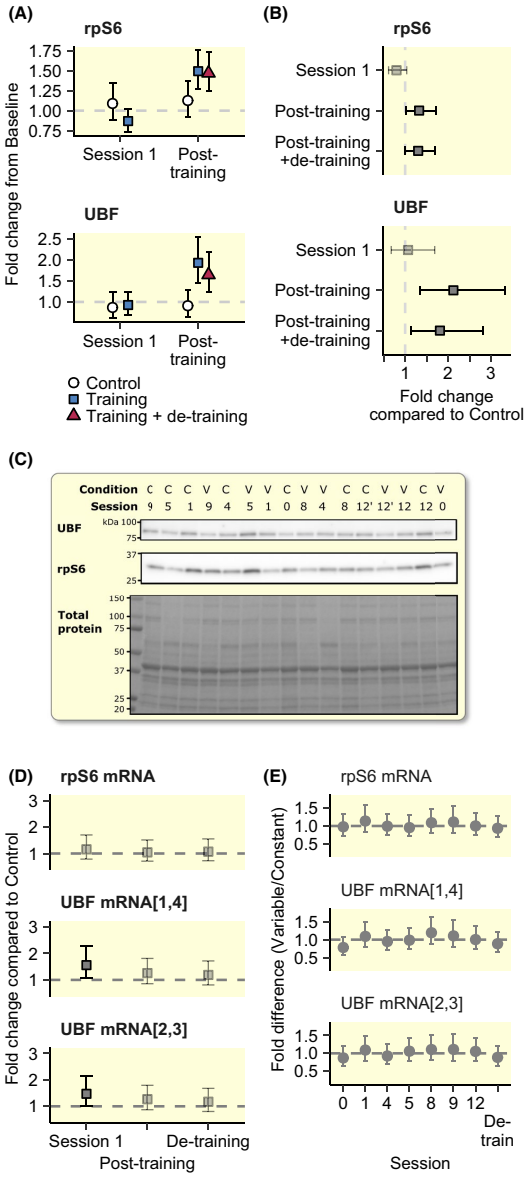

FIGURE 2 Protein (A and B) and mRNA abundances (D and E) of rpS6 and UBF. Non-transparent gray points and error bars represent statistically robust results (a 95% CI not containing 0). C shows western blots and total protein stains from a representative participant. mRNA data are normalized per total RNA. Intervals in A, B, D and E indicate 95% CI

showing differential expression, evident as robustly higher levels in VAR compared to CONST after the 12th session (Figure 3D), coinciding with the increased training volume towards the end of the intervention for this condition.

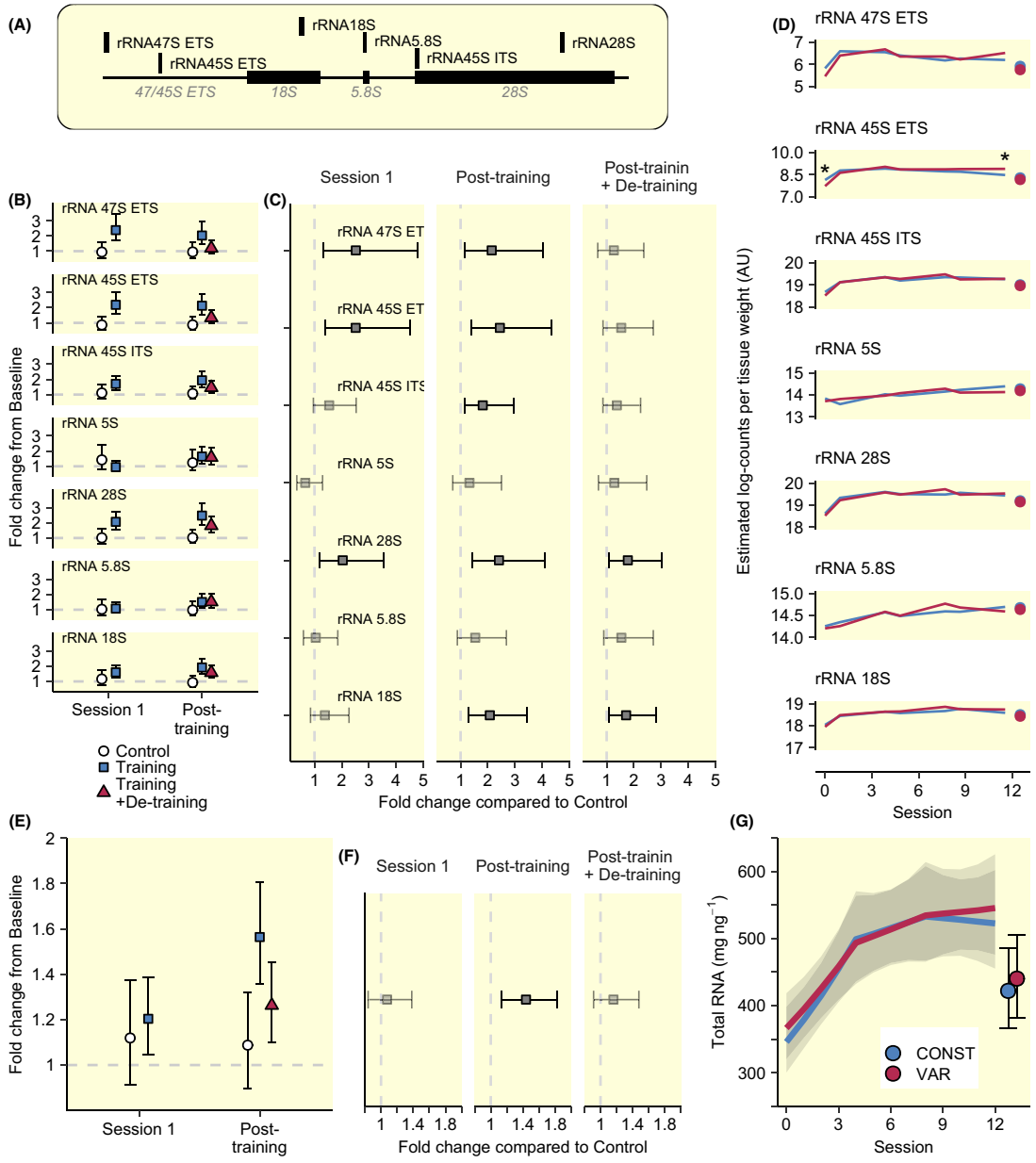

FIGURE 3 Total RNA and ribosomal RNA subspecies in response to resistance training. (A) shows primer locations targeting different ribosomal RNA subspecies in qPCR analyses. Ribosomal RNA species measured by qPCR and compared to control was affected by training (B,C), but did not show clear differences between volume conditions (D). Total RNA increased compared to non-training controls to post-training (12 sessions) and tended to normalize after de-training (E,F). Time-course analysis revealed the greatest increase during the first four sessions (G). Error bars shows 95% CI. Asterisk in D indicates robust differences between volume conditions (a 95% CI of pairwise differences not containing 0). Points in D and G show abundances after de-training for reference

In TRAIN, total RNA levels were robustly predicted by UBF levels (after controlling for time), with 5.0% [0.2, 10.2] increases in total RNA per unit tissue weight coinciding with one unit increase in UBF levels (corresponding to one standard deviation; Table 1). In contrast, no evidence was found for a relationship between total RNA and rpS6 protein levels (Table 1).

In TRAIN, there was a robust positive relationship between rates of increase in total RNA in response to training and muscle growth measured as increases in *m. vastus lateralis* thickness (Table 2, Figure 4A), with changes in total RNA over the course of the training intervention being estimated in each leg using a regression model containing number of sessions as the independent variable. Conversely, there was a tendency towards a negative relationship between average total RNA levels at Session 6 and changes in muscle thickness (Table 2, Figure 4B), with the average total RNA levels estimated as the predicted value at Session 6 (estimated as the intercept-term) from the model used to estimate the rate of total RNA increase per session.

To assess the robustness of the model for predicting muscle growth, individual relationships between sessions and total RNA levels (Figure 4C) were recalculated after the removal of single data points from each participant. The model predicting muscle growth was refitted using new estimates of changes in total RNA abundances and increases thereof per session. Each refitted model resulted in slightly different estimates (displayed as means and 95% CI in Figure 4D). No single data point influenced the results in any meaningful way. Next, we

TABLE 1 Effect of UBF and rpS6 levels, sessions and de-training on RNA-levels

Coefficient	Estimate[a]	SD	Lower 95% CI	Upper 95% CI
Intercept	5.91	0.06	5.79	6.04
UBF protein levels (SD-units)	0.05	0.02	0.00	0.10
Session 1-4[b]	0.09	0.02	0.05	0.12
Session 4-8[c]	−0.08	0.03	−0.13	−0.02
Session 8-12[d]	−0.02	0.03	−0.08	0.04
De-training	−0.23	0.08	−0.38	−0.08
Between participant variation	0.11	0.05	0.03	0.23
Between participant:leg variation	0.04	0.03	0.00	0.11
Residual SD	0.23	0.01	0.20	0.26
Intercept	5.90	0.06	5.78	6.03
rpS6 protein levels (SD-units)	0.02	0.03	−0.03	0.07
Session 1-4[b]	0.09	0.02	0.06	0.12
Session 4-8[c]	−0.08	0.03	−0.13	−0.02
Session 8-12[d]	−0.02	0.03	−0.08	0.04
De-training	−0.26	0.08	−0.41	−0.11
Between participant variation	0.11	0.05	0.03	0.24
Between participant:leg variation	0.04	0.03	0.00	0.11
Residual SD	0.23	0.02	0.20	0.26

[a]The dependent variable is total RNA levels (log), n = 10.
[b]Slope in response to session 1-4.
[c]Change in slope in session 4-8.
[d]Change in slope in session 8-12.

TABLE 2 Total RNA as a predictor of muscle growth

Coefficient	Estimate[a]	SD	Lower 95% CI	Upper 95% CI
Intercept	3.21	2.16	−0.70	7.88
Baseline muscle thickness	−0.15	0.09	−0.35	0.02
Sex (male)	1.30	0.64	0.06	2.64
Mean RNA at session 6 (SD units)	−0.27	0.27	−0.76	0.32
Mean total RNA increase per session (%)	0.28	0.09	0.10	0.44
Between participant variation	0.71	0.39	0.09	1.64
Residual SD	1.00	0.13	0.78	1.30

[a]The dependent variable is Δ Muscle thickness (mm).

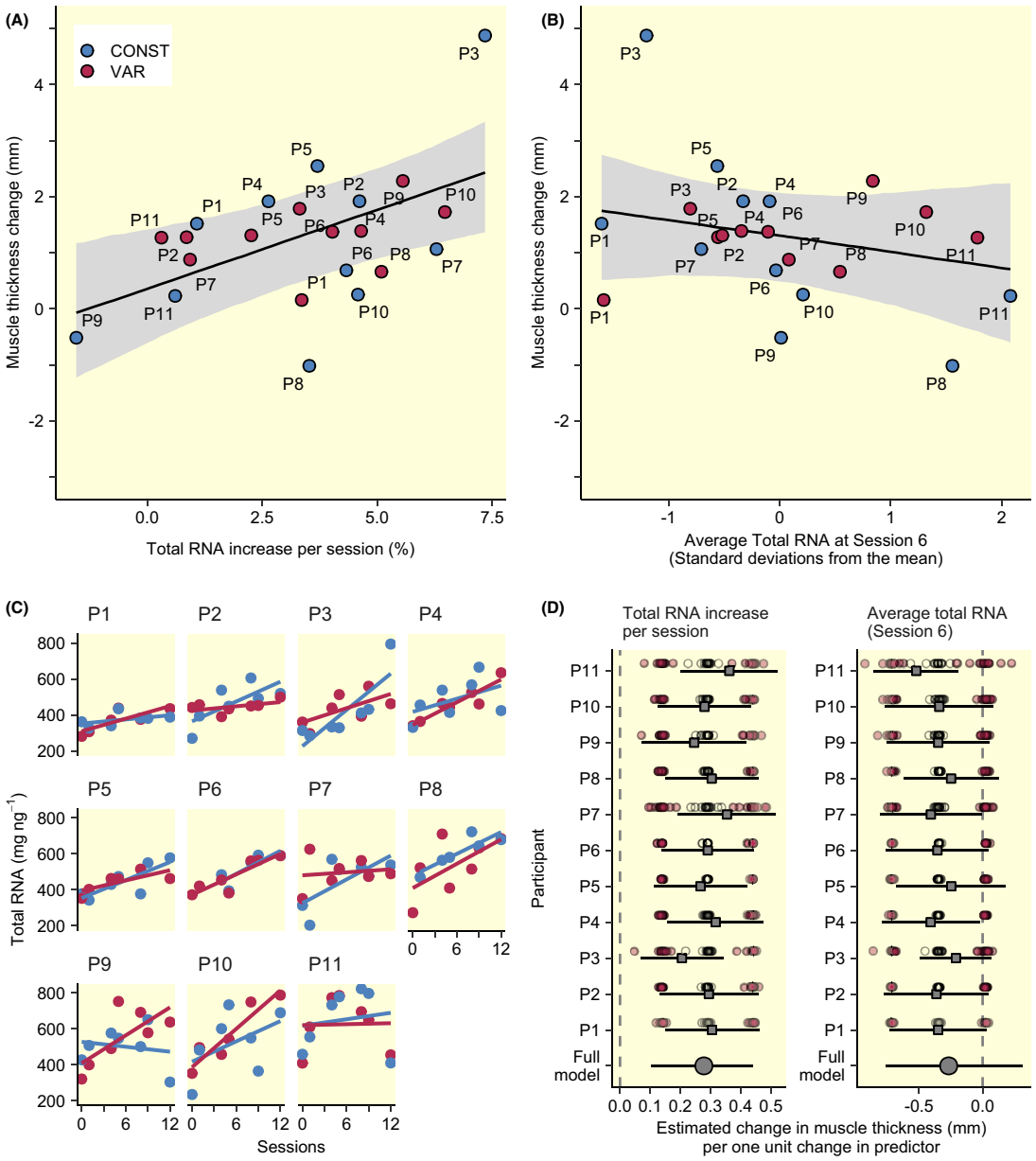

FIGURE 4 Predictions of muscle thickness increase based on total RNA increases (A) and total RNA abundance (B; see Table 2). Model estimates shown as black lines with 95% CI are averaged over values from men and women. Individual plots of estimates total RNA increases over time are shown in C together with results from leave-one-out analysis (D). Leave-one-out analysis shows the effect of removing a single participant (grey point and error bars) and individual values from the total RNA per time estimates where red points represent bounds of the 95% CI and circles represent mean estimates

assessed the robustness by iteratively removing one participant from the data set, similarly this showed that estimates of the effect of total RNA increase on muscle growth was robust but the effect of average total RNA estimates were more variable (eg, Participants 11 and 3 in Figure 4D).

3 | DISCUSSION

Here, we confirm that resistance training leads to increased abundance of markers of ribosome density, measured as total RNA, ribosomal RNA subspecies and rpS6 protein in previously untrained individuals compared to a non-training control group. These markers accumulated progressively during the initial part of the intervention before they levelled out, establishing a plausible time course for changes in ribosomal concentration in response to RT that plateaus after ~8 sessions. This increase in total RNA was interconnected with increases in UBF protein abundance, suggesting UBF levels to play a role in regulation of rRNA transcription regulation in response to RT. Total RNA increases were not affected by weekly fluctuations in training volume. However, eight days of de-training led to lowered levels of total RNA and rRNA content, suggesting that training cessation halts ribosome biogenesis. Finally, individual rates of increases in total RNA abundance predicted the magnitude of muscle growth, confirming the likely link between ribosomal biogenesis and muscle protein accretion[9,10] and muscle hypertrophy.[11-13]

Total RNA seems to be a valid proxy marker of ribosomal density, as most of the RNA is assumed to be ribosomal RNA,[28] which in turn is a valid marker of translational capacity.[10] Several studies have shown that total RNA content is altered by RT,[7,11-13,24,26,27,29,30] as was also the case in the present data set. However, the time course of total RNA/rRNA changes in response to RT has so far remained speculative, with no study investigating responses to prolonged interventions with multiple sampling time points. In the present data, RT led to a clear session-to-session increase in total RNA per unit tissue weight in response to the first four sessions, whereupon the changes gradually levelled out before peaking after the 8th session, with the peak increase from baseline being ~50%, defining an accumulation phase. This corroborates well with previous suggestions of peak values being reached within four to nine sessions in young males and females,[13,27] and may be essential for preparing muscle fibres for subsequent growth.[11,13,27] After the 8th session, no meaningful increase or decrease were observed for total RNA or rRNA content within the training period, suggesting a plateau phase with attenuated net synthesis of novel ribosomes. Within this last part of the intervention, synthesis of novel rRNA still seemed to be elevated *per* weight unit muscle tissue compared to baseline, as suggested by sustained elevation of pre-rRNA transcripts, coinciding with peak values of UBF protein levels. This may indicate that during the plateau phase, the ribosomal concentration is balanced by muscle growth.[31] This balance, measured as a constant ribosomal density in a growing cell, still requires the biosynthesis of ribosomes to match the volumetric expansion of the cell. As such, indirect measures of translational capacity such as the concentration of total RNA may mask the absolute increase in ribosomes that occurs during periods of muscle hypertrophy.

The observed rates of RNA accumulation over the entirety of the intervention were found to be a determinant of changes in muscle thickness (after controlling for average total RNA levels). Individuals with higher rates of accumulation showed larger accretion of muscle mass. This supports the notion that ribosomal biogenesis is an important determinant of RT-induced muscle hypertrophy, with previous studies showing that increases in total RNA are positively correlated with increases in muscle mass,[7,11,32] differs between individuals displaying low versus high levels of muscle hypertrophy in response to RT[12] an contribute to explain RT volume-dependent changes in muscle mass and strength.[13] In addition, suppression of ribosomal biogenesis in in vitro models leads to halted muscle cellular growth in some[9,12,19] but not all studies.[33] Conversely, individual variation in fixed amounts of total RNA was not found to determine muscle mass accretion, and higher levels of total RNA were instead associated with a tendency towards lowered muscle growth. Overall, the rate of increases in ribosomal density hence seems to be a better predictor for individual RT-induced changes in muscle mass than absolute ribosomal density, suggesting that net increases in ribosomal biogenesis may be a core determinant of RT responsiveness. Interestingly, the interaction between rRNA synthesis rate and muscle mass accretion (but not between ribosomal content and muscle mass accretion) may shed light on observed differences in muscular responses to RT between young and old individuals. Whereas aged muscle display higher levels of total RNA at rest[24] they show reduced changes in total RNA levels in response to RT,[27] potentially explaining their alleged poorer overall hypertrophic responses.[27] Whether these cellular characteristics are related to, for example, differences in fibre type distributions[34] remains to be determined. Furthermore, ribosomal accumulation is unlikely to be the only ribosome-derived trait that is important for training responsiveness. Evidence suggests that mechanical loading may lead to changes in ribosome characteristics[35] potentially leading to heterogeneous tissue-specific ribosome populations.[36,37] Our results support a model where specialized, newly synthesized ribosomes contribute to muscle hypertrophy as the increase in ribosomal content but not absolute levels predicted muscle growth. We, however, acknowledge that the present study does not provide substantial insight into this perspective. Together, these results and perspectives emphasizes on the potentially crucial role of RT-induced ribosomal synthesis for adaptations

to training, making ribosomal responses to RT an interesting biomarker in relation to manipulation of training loads for specific populations.

In the present study, training induced increases in rRNA and total RNA coincided with increases in rpS6. Conversely, changes in total RNA levels and rpS6 in response to de-training did not correspond, as rpS6 protein levels remained elevated after the de-training period. The training induced increases in rpS6 seen in the present study are in agreement to those previously reported in young men,[4] but not in elderly men and women, where a decrease was observed in response to training despite increases in total RNA and rRNA.[12] Although increases were seen for both rpS6 and total RNA, rpS6 did not explain variation in total RNA (after controlling for the number of sessions). Together with a disconnect after the de-training period, this suggest that regulation of rpS6 expression and ribosomal RNA transcription display differential temporal responses to RT. Such difference in temporal regulation of ribosomal RNA and proteins has previously been deduced in cell culture experiments. Briefly, inhibition of protein degradation led to accumulation of ribosomal proteins suggesting that excess amounts of ribosomal proteins are synthesized, imported into the nucleus and rapidly degraded if not incorporated into ribosomes.[38] This inherent capacity of cells to provide sufficient access to ribosomal proteins also suggests rRNA transcription (and not synthesis of ribosomal proteins) is not rate-limiting during ribosomal biogenesis.[38] Currently, it remains unknown if RT leads to expansion of the pool of unbound ribosomal proteins in humans. Indeed, in the present study, the disconnect between rpS6 and total RNA after de-training may have been associated with increased numbers of myonuclei, rather than accumulation of rpS6 in existing myonuclei, providing an alternative explanation to the elevated rpS6 levels (which accordingly may have been distributed over a larger number of nuclei). Interestingly, in a recent study, Murach and colleagues showed that newly acquired myonuclei (from satellite cell fusion) contribute to the ribosomal pool in myofibers.[39] Importantly, the present study was not designed to investigate these perspectives, and observations are limited to one single ribosomal protein, with no investigation of RT-induced myonuclear accretion. A parallel mechanism that could further help explain our observations is the possibility of extra-ribosomal functions in selected ribosomal proteins affecting their expression independent of ribosomal biogenesis.[40]

UBF levels robustly explained total RNA levels over the course of the intervention. As these analyses were done while accounting for the number of training sessions, estimates are likely to be unbiased. Unrealistically strong relationships could have been otherwise expected as both the dependent variable (total RNA) and the covariate (UBF levels) varies with the number of sessions. From a mechanistic perspective, UBF is an important transcription factor for rDNA transcription as it, in its active state recruits a secondary transcription factor (SL1) to the rDNA promoter and enables transcription by RNA Pol I.[18] Activation of UBF is controlled by the mechanosensitive mTOR pathway, and rapamycin, a specific mTOR inhibitor, blocks UBF from recruiting SL1 and subsequent rRNA transcription.[19,20] Evidence from human exercise studies confirms training-induced activation of UBF through phosphorylation.[11,41] In addition to exercise-induced activation of UBF, mechanical loading also leads to increased levels of total UBF.[11,41] Increases in UBF was determined to be rapamycin insensitive after synergist ablation in mice[42] pointing to an effect observed in cell models where c-Myc induces UBF mRNA transcription.[43] Interestingly, the availability of UBF has been shown to regulate rRNA transcription[21] through control of rDNA gene activity.[22] Together with our observations, this underlines the importance of UBF as a regulator of RT-induced ribosomal biogenesis. However, the lack of measurements of UBF in the context of active chromatin interaction may have inhibited us from further explaining the role of UBF in response to the de-training period.

After eight days of de-training, total RNA and rRNA levels per weight unit muscle tissue returned toward baseline levels, though without concomitant reversal of muscle thickness, which remained at elevate levels. This was likely caused by attenuated rRNA transcription, a notion that was supported by reversal of pre-rRNA abundances and possibly by lowered UBF protein levels, though this was not confirmed as statistically robust. The magnitude of the detraining-associated decrease in total RNA (~20%) is similar to that seen in response to unloading of untrained human muscle over a similar time frame (7-10 days).[44,45] This suggests that RT-induced increases in ribosomal content is easily lost, likely driven by a combination of reduced synthesis and enhanced degradation (ribophagy). While the relative contribution of these two remains to be determined, inactivity has previously been shown to induce ribophagy in rat muscle (subjected to hindlimb suspension).[45] Future studies should investigate the interrelationship between ribosome biogenesis and ribophagy in response to training and detraining. We suspect that their relative importance for ribosomal homeostasis will vary substantially between different physiological perturbations, as they are regulated by a complex interconnection of stimuli and pathways.[15,16,46] For example, Kim et al showed that cancer cachexia is associated with reduced rDNA transcription (and hence reduced translational capacity),[47] contrasting the inactivity-driven

reduction in translational capacity which to a larger degree seems to involve ribophagy.[45]

The de-training effect on total RNA and rRNA seen in the present study supports the idea that ribosomal biogenesis is a cellular activity on demand, possibly relating to its relative expense[15] also in muscle tissue. Based on this notion, and the fact that RT volume is known to be a potent modulator of molecular mechanisms determining protein synthesis and ribosomal biogenesis including induction of c-Myc expression, mTOR activation,[13,48,49] subsequent total RNA increases[13] and post exercise protein synthesis[49] and subsequent training outcomes,[13,50] we hypothesized that fluctuations in training volume would be reflected in markers of ribosomal biogenesis. When comparing VAR to CONST in the present study we found only one part of the pre-rRNA, 45S ETS, to be differentially expressed and only so after Session 12 in favour of VAR together with a tendency towards rescued UBF levels after de-training in response to increased volume in the VAR but not CONST protocol. These observations do not give support to a clear effect of fluctuations in training volume on total RNA levels or rRNA expression within a relatively short and training-intensive intervention, though it should be noted that the time point with increased 45S ETS expression was preceded by a period of increased training volume, suggesting a potential interaction between time and volume. Indeed, both training protocols utilized in the present study increased muscle strength and induced muscle hypertrophy to a similar degree. From a general perspective, albeit volume is an important determinant of increases in muscle strength and mass,[50,51] differences in organization of training loads is likely of minor importance when training volumes are equated over time.[52] It is important to note that RT in the current study was performed with the same volume in the first four sessions, something that could have been more than enough to maximize rRNA transcription in previously untrained individuals. This is supported by the observation that pre-rRNA increased rapidly initially in both protocols with minimal changes in response to subsequent sessions, regardless of exercise volume. The CONST protocol in the present study corresponded to volumes used in the moderate volume condition in a previous study from our lab (three sets in two exercises activating knee extensor muscles).[13] There, higher levels of total RNA were observed after four sessions in the moderate compared to a low volume protocol.[13] Interestingly, using a progressive volume protocol in well-trained participants, increases in total RNA have been reported throughout six weeks of training.[29] Although this observation was done in well-trained participants performing a high volume protocol without a control condition with constant volume, compared to constant volume protocols,[13,27] progressive volume may thus increase ribosomal abundance to a higher degree and provide a measure to avoid the plateau phase seen in the present study.

In conclusion, RT-induced ribosome accumulation reached peak values in the initial phase of RT (eight sessions) and was interconnected with increases in UBF protein levels. The rate of total RNA accumulation predicted RT-induced muscle hypertrophy. Fluctuations in training volume did not transfer to fluctuations in ribosomal biogenesis, but training cessation led to decreased ribosomal content.

4 | MATERIALS AND METHODS

4.1 | Study overview

Nineteen volunteers were recruited to the study. Eligible participants were non-smokers between 18 and 35 years of age with a training history of less than one RT session per week during the six months leading up to the study. Exclusion criteria were consumption of dietary supplements or medication with known effects on muscle metabolism, injuries causing impaired strength and/or affecting their ability to perform RT, symptoms or history of disease, and known adverse reactions to local anaesthetics. Participants were allocated to either a training group (TRAIN, n = 11) or a non-training control group (CTRL, n = 8; see Table 3 for participant characteristics;

TABLE 3 Participant characteristics

	Experimental group		Control group	
	Female	Male	Female	Male
n	6	5	4	4
Age (years)	23.4 (2.9)	25.7 (5.8)	24.1 (3.5)	25.5 (5.5)
Body mass, (kg)	64.0 (9.2)	77.5 (8.0)	63.7 (0.5)	76.0 (7.0)
Stature (cm)	167.8 (8.1)	177.2 (3.3)	166.0 (3.7)	181.8 (5.0)
Body mass index (kg m^{-2})	22.7 (2.7)	24.7 (2.7)	23.2 (1.1)	23.1 (3.2)
Body fat (%)	30.8 (30.8)	25.1 (25.1)	30.3 (30.3)	17.9 (17.9)

see Figure 1A for overview of the intervention). TRAIN performed a 12-session RT protocol lasting for 3-4 weeks, consisting of 10 repetition maximum (RM) unilateral knee-extension, with the two legs conducting RT with different volume profiles, allowing within-participant comparison of the effects of volume regimes. In TRAIN, one leg conducted RT with constant volume throughout the intervention (CONST, 6 sets per session) and the other leg performed RT with variable volume (VAR, 3 blocks of four sessions with 6, 3 and 9 sets per session respectively; Figure 1A). CTRL did not partake in RT and were instructed to continue their everyday activities. Muscle biopsies were sampled bilaterally in TRAIN before and 48 hours after the first session, as well as 48 hours after the fourth, fifth, eight, ninth and twelfth session, and after eight days of de-training. Muscle biopsies were obtained from CTRL at three occasions; at baseline and 48 hours and 3-5 weeks (average (SD) 3.6 (0.7)) after the first sampling event. TRAIN and CTRL performed strength assessments > seven days prior to the first biopsy sampling (TRAIN; CTRL), 72 hours after the twelfth session (TRAIN) and 24 hours after the last biopsy (TRAIN, following de-training; CTRL). Appendicular lean mass (Dual-energy X-ray absorptiometry, DXA) and muscle thickness of *m. vastus lateralis* were assessed prior to the first biopsy (TRAIN and CTRL) as wells as before the second to last (TRAIN) and last (TRAIN and CTRL) biopsy.

All participants gave their informed written informed consent prior to data collection. The study was conducted according to the Declaration of Helsinki, approved by the local ethics committee (no. 2017-10-23) and the Norwegian center for research data (ref: 51549/3/AH), and pre-registered (DOI: 10.17605/OSF.IO/WA96Y).

4.2 | RT protocol

Prior to all RT sessions, participants performed a standardized warm-up consisting of 5 minutes ergometer cycling (rating of perceived exertion (RPE): 12-14), followed by ten repetitions of push-ups, sit-ups and back-extensions. After warm-up, participants performed unilateral knee-extension with the prescribed number of sets. Each set was prescribed with 10 repetitions maximum (RM). When sets were completed with either fewer (8) or more (12) repetitions, the resistance was adjusted accordingly. Inter-set rest periods were 90 seconds. Throughout the intervention RT sessions were alternatingly initiated by training the right and left leg, changing every other session. The contralateral leg was trained in the rest period between sets of the first leg, still allowing for complete rest between efforts (~60 seconds). The second session of each four-session block (session 2, 6 and 10) was performed at a sub-maximal resistance (~90% of the previous session) with the same number of repetitions (10). Within each session, participants also conducted two sets of three upper-body exercises (bench press, lateral pull-down and shoulder press; 10RM). After completion of each session participants were given a standardized drink to aid recovery (0.15 g kg^{-1} protein, 11.2 g kg^{-1} carbohydrates and 0.5 g kg^{-1} fat).

4.3 | Muscle strength, body composition and muscle thickness assessments

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-minute 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-seconds rest 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-seconds 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 performed 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.

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 and 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 square 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 minutes 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[53] with images cropped and coded to ensure blinding of the assessor.

4.4 | Muscle biopsy sampling

Muscle specimens were sampled bilaterally from *m. vastus laterlis* under local anaesthesia (Lidokain 10 mg mL^{-1}, Mylan, Mylan Ireland Limited, Dublin, Ireland) using a disposable needle (12-14 gauge, Universal plus, Medax, Poggio Rusco, Italy), operated with a spring loaded device (Bard Magnum, Bard Norway, Rud, Norway). Two to four passes were made to get sufficient material. Material from all passes was quickly dissected free from connective and fat tissue and divided into one to two aliquots (depending on amount of available material). Aliquots were weighed and frozen in isopentan chilled to −80°C and stored at −80°C until further processing. Due to difficulties during the sampling procedure, we could not obtain a sample from one participant's leg belonging to TRAIN at baseline.

4.5 | RNA and protein extraction

Frozen muscle tissue was homogenized in 1 mL of Trizol (ThermoFisher Scientific, Oslo, Norway) spiked with 0.04 ng of an external, non-mammalian, RNA spike-in (Lambda PolyA External Standard Kit, Takara Bio Europe, Saint-Germain-en-Laye, France). The addition of the external spike-in allowed for normalization of target RNA to muscle weight, see below. Mechanical disruption of the samples was achieved using Zirconium Oxide Beads (0.5 mm, Next Advance, Inc, New York, USA) and a bead mill (Bullet blender, Next Advance). Chloroform (200 µL) was added prior to centrifugation (12 000 *g*, 15 minutes at 4°C) to achieve phase separation. Four hundred fifty microlitre of the upper aqueous phase was transferred to a fresh tube and 500 µL of isopropanol was added to precipitate the RNA. After a 10 minutes incubation at room temperature, samples were centrifuged (12 000 *g*, 10 minutes at 4°C), after which a pellet formed. The pellet was washed three times in chilled 75% ethanol with centrifugation between each wash (7500 *g*, 5 minutes at 4°C). After the final wash all ethanol was removed and the pellet was eluted in 0.1X Tris-EDTA buffer. RNA concentration and purity was assessed by spectrophotometry. All samples had 260/280 ratios > 1.95.

Protein was extracted from Trizol preparations according to the manufacturer's instructions and[54] with modifications. The remaining aqueous phase was removed and DNA was precipitated by the addition of 300 µL of absolute ethanol followed by gentle centrifugation (2000 *g*, 5 minutes 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 minutes at room temperature), samples were centrifuged (7500 *g*, 10 minutes 4°C) and a pellet formed. The pellet was washed three times in 95% ethanol with each wash separated by centrifugation (5000 *g*, 5 minutes at room temperature). After the last wash all liquid was removed and 45 µL of Kopec buffer[54] 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 (10 000 *g*, 10 minutes at room temperature). Protein concentrations were measured (Pierce Detergent Compatible Bradford Assay, ThermoFisher Scientific). Sample were normalized to a common protein concentration, 4X Laemmli buffer (Bio-Rad Norway AS, Oslo, Norway) was added and samples were boiled (95°C, 5 minutes) and stored at −20°C before later use.

4.6 | Quantitative polymerase chain reaction (qPCR)

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 the manufacturer's instructions.

qPCR reactions were performed with diluted cDNA (2 μL, 1:25 dilution), a SYBR-green based commercial master mix (PowerUp™ SYBR™ Green Master Mix, Thermo Fisher) and, target-specific primers (500 nM) in 10 μL reaction volumes using a real-time detection system (QuantStudio 5 Real-Time PCR System, Thermo Fisher Scientific). Fast cycling was used (1 second denaturing, 30 seconds annealing) after UNG (2 minutes, 50°C) and polymerase (2 minutes, 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 amplicon sizes and non-template control experiments confirming no amplification without template. Primer sequences and their respective average performances are shown in Table 4.

Raw fluorescence data were exported from the QuantStudio software and estimates of quantification cycle (Cq) and amplification efficiency was derived for each reaction using the qpcR package.[55]

4.7 | Immunoblotting

Protein samples (20 μg) were separated on 4%-20% Tris-Glycin gels (Criterion TGX Precast Gels, Bio-Rad) at 250 V for 45 minutes using the recommended running buffer (25 mM Tris, 192 mM Glycin, 0.1% SDS). All samples from the same participant were run on the same gel and all samples were run in at least duplicates. When samples were compared between participants, signals were

TABLE 4 Primer sequences and average performance

Symbol	Transcript name	Sequence	Mean Cq (SD) and efficiency
rRNA47S ETS	45S pre-ribosomal RNA	F: 5′-CTGTCGCTGGAGAGGTTGG-3′ R: 3′-GGACGCGCGAGAGAACAG-5′	27.3 (1.9), E = 1.84
rRNA45S ETS	45S pre-ribosomal RNA	F: 5′-GCCTTCTCTAGCGATCTGAGAG-3′ R: 3′-CCATAACGGAGGCAGAGACA-5′	24.0 (2.2), E = 1.89
rRNA45S ITS	45S pre-ribosomal RNA	F: 5′-TCCGAGACGCGACCTCAG-3′ R: 3′-TCGCCGTTACTGAGGGAATC-5′	12.2 (2.2), E = 2.14
rRNA5.8S	5.8S ribosomal RNA	F: 5′-ACTCTTAGCGGTGGATCACTC-3′ R: 3′-GTGTCGATGATCAATGTGTCCTG-5′	15.7 (1.9), E = 1.96
rRNA28S	28S ribosomal RNA	F: 5′-TGACGCGATGTGATTTCTGC-3′ R: 3′-TAGATGACGAGGCATTTGGC-5′	10.7 (1.8), E = 2.07
rRNA18S	18S ribosomal RNA	F: 5′-TGCATGGCCGTTCTTAGTTG-3′ R: 3′-AACGCCACTTGTCCCTCTAAG-5′	10.3 (2.9), E = 1.98
rRNA5S	5S ribosomal RNA	F: 5′-TACGGCCATACCACCCTGAAC-3′ R: 3′-GGTCTCCCATCCAAGTACTAACC-5′	17.1 (2.2), E = 2.00
RPL32	Ribosomal protein L32	F: 5′-AAGTTCCTGGTCCACAACG-3′ R: 3′-CGGCACAGTAAGATTTGTTGC-5′	22.0 (1.6), E = 1.93
RPS6	Ribosomal protein S6	F: 5′-TTGAAGTGGACGATGAACGC-3′ R: 3′-GGACCACATAACCCTTCCATTC-5′	22.3 (1.7), E = 1.96
UBTF [1,4]	Upstream binding transcription factor	F: 5′-CCGATTCAGGGAGGATCACC-3′ R: 3′-ACCTCCTTCGTAGTGGCATC-5′	28.4 (2.7), E = 1.87
UBTF [2,3]	Upstream binding transcription factor	F: 5′-CGGCCAGATGAGATCATGAGAG-3′ R: 3′-GGGTGGACTTGGTGATACCC-5′	28.0 (1.8), E = 1.88
MYH7	Myosin heavy chain 7 (MHCslow)	F: 5′-AGGAGCTCACCTACCAGACG-3′ R: 3′-TGCAGCTTGTCTACCAGGTC-5′	19.5 (2.3), E = 1.93
MYH2	Myosin heavy chain 2 (M)	F: 5′-CCAGGGTACGGGAGCTG-3′ R: 3′-TCACTCGCCTCTCATGTTTG-5′	18.0 (1.9), E = 1.99
MYH1	Myosin heavy chain 1 (M)	F: 5′-GGCCAGGGTTCGTGAACTT-3′ R: 3′-TGCGTAGACCCTTGACAGC-5′	22.0 (2.5), E = 1.94
Lambda	Lambda external reference	F: 5′-Proprietary-3′ R: 3′-Proprietary-5′	22.2 (2.0), E = 1.98

expressed per a calibrator sample from each participant that in turn was measured on a separate gel with all calibrator samples. Due to technical difficulties, the calibrator sample from one participant was excluded from analysis, reducing the sample size in analyses between participants in TRAIN to n = 10 (Table 1). Separated samples were transferred to PVDF membranes (Immun-Blot, Bio-Rad) using wet transfer (25 mM Tris, 192 mM Glycine, 10% vol/vol methanol) at a constant voltage of 300 mA for 3 hours. Membranes were then stained to confirm transfer and enable total protein quantification using a reversible protein stain (Pierce Reversible Protein Stain, Thermo Fisher Scientific). Primary antibodies were acquired to detect UBF (F-9, sc-13125, Santa-Cruz Biotechnology, Dallas, Texas, USA) and rpS6 (54D2, #2317, Cell Signaling Technology, Danvers, MA, USA). After blocking (Tris-buffered saline blocking buffer, 20 mM Tris, 150 mM NaCl, 5% fat-free milk, 0.1% Tween-20), membranes were incubated overnight with primary antibodies diluted in blocking buffer (UBF, 1:200; S6, 1:1000) followed by incubation with a secondary antibody conjugated to horseradish peroxidase (Anti-mouse IgG, #7076, Cell Signaling Technology, 1:10 000). Membranes were washed 6 × 5 minutes after incubation with primary antibodies and 8 × 5 minutes after incubation with the secondary antibody. All incubation and washing steps were performed at 4°C using an automatic membrane processor (BlotCycler, Precision Biosystems, Mansfield, MA, USA). Chemiluminescent signals from membranes were detected after 5 minutes incubation in substrate (Super Signal West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific) using a documentation system. Total protein content was quantified from whole membrane images and defined as the mean gray value of the whole lane. Between-lane gray values were used as background subtracted from protein values. Total protein quantification was done using ImageJ Fiji.[53] Chemiluminescence signals were quantified using Image Studio Lite (LI-COR Biotechnology, Lincoln, NE, USA).

The average coefficient of variation across replicates were 20.2 and 22.8%, for rpS6 and UBF respectively.

4.8 | Statistics and data analysis

Descriptive data are presented as mean and standard deviation (SD). The effect of training on muscle strength, muscle thickness, UBF/rpS6 protein, total RNA and gene abundances were assessed using mixed effects regression models. Time and group (TRAIN vs. CTRL) were treated as population (fixed) effects and leg nested within participant included as group level (random) effects. These analyses were performed on data with matching time points between TRAIN and CTRL with the exception that all post-training data from TRAIN were included (post-training and de-training). Relative interactions between groups were estimated as Δ TRAIN - Δ CTRL. The effects of different volume conditions and general time-course patterns were assessed using all pairwise observations from the TRAIN group. For protein and total RNA data, segmented regression models were used to estimate changes over sessions in three segments (session 1-4, 4-8 and 8-12; corresponding to blocks of different volume prescription in TRAIN). When no robust effects of volume conditions were detected, group averages are presented. Segmented models were fitted with time and volume condition as population effects and legs nested within participants as group level effects. Muscle strength, muscle thickness, protein and total RNA data was modeled after log transformation.

Gene abundance data were fitted with number of sessions as a categorical variable in comparisons of volume conditions, and Cq values converted to counts as suggested by Matz et al.[56] A Poisson-lognormal model was used to fit these count data, using data from all genes and including group level effects for each technical duplicate, controlling for technical errors during sample preparation. An offset consisting of a normalization factor proportional to the amount of muscle used to prepare cDNA was used to model gene abundance per tissue weight. The external reference gene was used to calculate the normalization factor (External reference counts × muscle weight (mg) in each Trizol preparation). The offset was specified as a predictor with the coefficient fixed to 1.

A linear model was used to estimate the increase per session and average total RNA for every leg in the TRAIN group. These estimates were then used to estimate the effect of training-induced increase per session and average total RNA abundance on muscle hypertrophy. For each leg, session was used as the independent variable centered on Session 6 and log transformed RNA per tissue weight as the dependent variable. Mean-centring of the independent variable was done to obtain an estimate of the average RNA concentration per leg. This also assured that the slope and intercept did not correlate, something that could lead to issues with collinearity in subsequent modelling. A mixed effects model was subsequently fitted with differences in muscle thickness pre- to post-training as the dependent variable and estimated percentage per session increases in total RNA, the mean total RNA scaled as standard deviations from the mean and sex as independent variables. Participants were used as group levels effect. The robustness of this model was assessed by leave-one-out analysis on the level of individual data points in the relationship between total RNA and sessions and on the level of participants (see Results).

All models were fitted using a Bayesian framework using either the brms[57] or MCMCglmm[58] package written for R.[59] Inference about effects of interest was drawn based on point estimates and their 95% credible intervals (CI). Credible intervals not containing null effects were interpreted as robust effects. Models were fitted with default priors. CIs were interpreted as containing the true population value with the specified certainty (95%), given the data and priors. Fitting performance was assessed by confirming convergence of at least four different chains of MCMC samples (graphically assessed and confirmed with $\widehat{R} \approx 1$). Model performance was assessed from comparing simulated data from each model to observed data graphically (posterior predictive checks).

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CONFLICT OF INTEREST

The authors do not have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data and code are available at github.com/dhammarstrom/ribo-accum-paper.

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Paper III

Superiority of high-load vs. low-load resistance training in military cadets

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Brief running head: High-load vs. low-load resistance training

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Abstract

Muscle strength and power are important determinants of soldiers' performance in modern warfare. Here, we compare the efficacy of 22 weeks of whole-body resistance training with high load (HL, 10 repetitions maximum/RM) and low loads (LL, 30RM) for developing maximal muscle strength and power, performance, and muscle mass in moderately trained cadets (20 ± 1 years, f; n=5, m; n=22). Outcome measures were assessed at baseline and at week 22, in addition to a mid-intervention assessment at week 10. Twenty-two weeks of HL led to greater increases in muscle strength (upper limb, Δ 10%, 95% CI [2.8, 17.1], p=0.01;lower limb, Δ 9.9%, CI [1.1, 18.6], p=0.029), jump height (Δ 5.5%, CI [1.4, 9.6], p=0.011), and upper-limb lean mass (Δ 5.2%, CI [1, 9.4], p=0.018) compared to LL. HL and LL led to similar changes in agility, muscle endurance performance, lower-limb muscle mass, and cross-sectional area in *m.vastus lateralis*. For all variables, training-associated changes occurred primarily during the initial ten weeks of the intervention, including the differential responses to HL and LL. In conclusion, while 22 weeks of HL led to greater increases in lower- and upper-limb muscle strength, power, and upper-limb lean mass than LL, the two load conditions led to similar improvements in agility performance and lower-limb muscle mass. Our results thus indicate that both loading regimes elicit multifaceted physiological improvements important for military readiness.

Key words

military environment, resistance-training modalities, prolonged exercise, cadets

INTRODUCTION

Soldiers need to exhibit high aerobic fitness, muscular strength, and mental capabilities to ensure optimal performance during demanding operations (38). Military training programs thus need to target a large range of demands (15,38). Unfortunately, prevailing programs often focus on aerobic endurance training, and thereby largely fail to develop muscle strength and power, which are recognized as increasingly important components of modern military practice and warfare (15,17). At present, the best-practice resistance-training plan in the military setting is challenging to prescribe, as physical endeavors such as field exercises and deployments often complicate day-to-day predictability and consistency (38).

Progressive high-load resistance training is the primary approach for developing muscle mass, maximal strength, and power (36). In the military environment, it tends to improve physical capabilities such as strength, speed, power, and agility, accompanied by increased lean body mass (11,16,20), all of which are all imperative for a soldier's military performance (15). However, the benefits of resistance training are not consistently seen in the military setting (31,42). This lack of consensus may be related to a simultaneous focus on aerobic training, as well as the nature of military-training regimes, which typically include exhausting field operations, leading to a complex range of concurrent physiological stressors that may compromise specific adaptations (25,31). While this complexity emphasizes the importance of incorporating resistance training into the annual training routines of soldiers, allowing maintenance of physical capacity throughout the year (17,20), it also emphasizes the need for identifying efficient resistance training modalities that can be performed during deployments.

In recent years, the high-load paradigm of resistance training (>65% 1 repetition maximum; 1RM (28)) has been challenged (29,32). Alternative approaches such as low-load training (30-50% 1RM) to failure (7) have been associated with similar muscular responses, including both maximal strength (7,40) and muscle hypertrophy (18,24,34,39,40). Still, the scientific standing remains equivocal, with other studies reporting favorable effects of high-load training for developing both maximal strength (4,18,23) and muscle mass (4). These discrepancies may be related to differences in study characteristics, such as the participants' training status and the study's duration, with studies typically being performed over a short

time frame (range 6-13 weeks). In addition, comparisons of high- and low-load protocols have generally targeted the lower-body limbs, and although there is evidence to suggest that differential load conditions affect lower and upper body muscle groups somewhat similarly (34,40), this may be sensitive to the exercise performed (14), and there are studies indicating that lower and upper body muscle groups respond differently to changes in training volume (26,30). Our current insight is hence limited to a few studies, with unclear response patterns, restricted to shorter periods of training (<13 weeks). Intriguingly, low-load training promises to be particularly beneficial for military personnel, as it seems suitable for maintaining and developing physical capabilities and resilience in settings where heavy-loading exercise equipment or training facilities are unavailable.

The purpose of the present study was to compare the efficacy of 22 weeks of whole-body resistance training with high (10 repetitions maximum/RM, HL) and lower loads (30RM, LL) for improving maximal strength and power, performance, and muscle mass in moderately trained cadets undergoing military training. Secondary aims included comparing the efficacies of the two loading conditions between upper and lower limbs.

METHODS

Experimental Approach to the Problem

The study was conducted using a randomized, repeated-measures, between-subject, parallel design. The resistance-training intervention lasted for 22 weeks (Figure 1A; September-March), whereby testing of maximal muscle strength, speed, muscle endurance performance and muscle mass were performed at three time points (Weeks 0, 10 and 22). At each of the time points, testing was organized into three test blocks, conducted at three separate days. Test day 1 included blood sampling and whole-body dual-energy X-ray absorptiometry (DXA-scan) followed by counter movement jump (CMJ), maximal isometric half squat (MIHS), one repetition maximum in three exercises, and a muscle endurance performance test. Test day 2 included an agility test. Test day 3 included muscle biopsy sampling (m. vastus lateralis; >48h after test day 2). One week prior to testing at week 0 (baseline), all participants conducted a familiarization session containing the entire battery of physical tests to reduce possible learning effects. All cadets tested approximately

at the same time of the day (± 1-2h) to control for circadian variations. All tests were supervised by the same trained test personnel. All test sessions were monitored by personnel not otherwise involved in the study, meaning that all test personnel were blinded for training load allocation. Participants were instructed to refrain from performing any additional resistance-type training for the duration of the study. During week nine, participants conducted a 4-day weight-controlled dietary registration.

(Include Figure 1 here)

Subjects

Twenty-seven cadets (20 ± 1 years, 75.5 ± 12.9 kg, 182 ± 9 cm) from the 2nd year of the Norwegian Defense Cyber Academy volunteered for the study. Prior to enrollment, the cadets had conducted two weekly exercise training sessions throughout the last year as part of their Cyber Academy training program, consisting of exercises such as circuit training exercises, calisthenics, and high-intensity interval training. Cadets that had conducted systematic heavy resistance training (>2 sessions/week) during the last six months leading up to the study were not eligible for participation. The participants did not have any existing musculoskeletal injuries, were non-smokers, and did not report any use of anabolic steroids. Participants were pair-matched based on initial strength performance and then randomly allocated to either a heavy-load group (HL, male: 12, female: 2, 10RM) or a low-load group (LL; male: 10, female: 3, 30RM). The study was performed according to the ethical standards established by the Helsinki Declaration of 1975 and was pre-registered in a Norwegian public database (Norwegian Center for Research, project number 43901/3), and approved by the local Ethics Committee at Inland Norway University of Applied Sciences. All participants gave their informed consent before enrollment in the study. Baseline characteristics are shown in Table 1.

(Include Table 1 here)

Resistance training procedures

The resistance training intervention lasted for 22 weeks, and was conducted in two phases, separated by an obligatory three-week military leave. It was initiated by a 10-week period (September to December), followed by a 9-week period from January to March. The training protocol consisted of three sets (2-3 min inter-set rest) of seven exercises per

session, performed two days a week during the first 10 weeks (total 20 sessions) and increased to three days a week every other week during the last nine weeks (total 17 sessions). Every set was performed to concentric failure, i.e. the inability to perform another concentric repetition with proper form. Participants were instructed to continuously increase their RM load throughout the intervention period to ensure that they reached the state of failure towards the end of each series (39). Exercises were performed in the following order: squat, leg press, leg curl, bench press, standing rowing, seated pull-down, seated biceps curl. At the start of each resistance training session, participants performed a ~10 min general warm-up using a cycle ergometer (self-selected intensity). This was followed by specific warm-up during the first lower- (squat) and upper-body (bench press) exercise, consisting of 10 repetitions at ~30-50% of 1RM. During each exercise session, training loads and numbers of repetitions were registered for each exercise. The training sessions were supervised by experienced strength coaches to ensure proper technique and training progression. After each exercise session, participants consumed a standardized drink (30 g chocolate Whey protein powder, Proteinfabrikken, Norway) to ensure adequate protein intake for resistance-trained persons (1.3-1.8 $g \cdot kg^{-1} \cdot d^{-1}$).

Dietary intake and reporting of other training

To assess total energy intake and macronutrient intake, a 4-day (3 weekdays + 1 weekend day) weight-controlled self-reported food diary was conducted at week nine (Figure 1A). Participants were instructed to record all food items and respective portion sizes consumed during the designated time frame using a digital food weighing scale and a dietary journal. Dietary data were analyzed using the Norwegian Directorate of Health's diet tool (available at www.kostholdsplanleggeren.no). Throughout the study, participants recorded all habitual endurance training performed outside the mandatory military training. During the entirety of the 22-week intervention, HL and LL performed a mere 9.3 ± 7.7 and 7.5 ± 7.3 hours (P=0.546), respectively, of additional endurance-oriented training. All participants conducted 1-2 weekly mandatory training sessions organized by the academy. These sessions had alternating training focus with a duration of 60 min. Typically sessions included aerobic training such as long-distance running (low intensity), marching with military gear (moderate intensity), combat conditioning (moderate intensity) and interval training (high intensity) or circuit-based calisthenics, core strength, coordination, and

mobility sessions. All additional sessions were, if possible, conducted on weekdays with no resistance training. If additional exercise was conducted on the same day, at least >4 hours separated the sessions to minimize muscle adaptations interference (1).

Testing days

Hormonal, body composition and physical performance (Day 1)

Blood sample

Fasting blood samples for hormonal analyses were obtained from an antecubital vein using serum-separating tubes while participants rested in a supine position. At all three time points (baseline, 10 week and 22 week), samples were obtained at the same time of the day (between 08.00-10.00 AM). Blood samples were incubated for 30 min at room temperature before they were centrifuged at $1500g$ for 10 min. Serum was aliquoted and immediately transferred to -80° C for storage until analyses. Serum concentrations of total Testosterone (TESTO), Cortisol (COR), Insulin-like growth factor 1 (IGF-1) and growth hormone (GH) were measured using an Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics, NY, USA), using kits from the Immulite Immunoassay System menu (Siemens Medical Solutions Diagnostics, NY, USA), performed according to manufacturer's protocols. Reference intervals were as follows; TESTO (8.0-35.0 $nmol.L^{-1}$), IGF-1 (17-63 $nmol.L^{-1}$), COR (138-690 $nmol.L^{-1}$), GH (< 0,5 µg/l). Coefficient of variation (analytic) for the analyses were TESTO 14%, IGF-1 9%, COR 14%, GH 6%.

Muscle mass and fat mass (DXA)

DXA-derived estimates for lean body mass and fat mass were obtained using the standard scanning mode 13-25 cm (Prodigy Advance PA+302047, Lunar, San Francisco, CA, USA). Participants were positioned supine within the marked lines on the scanning bed and a strap secured around the ankles to ensure standardized body position in each of the two followup scans, in accordance with the manufacturer. At all test time points the soldiers were scanned in a fasted state between 07.00-09.00 AM, wearing limited clothing (boxer-short and sports top) and no jewelry. Before onset of each scanning session, a phantom scanning was conducted to prevent baseline drifting from affecting analyses. The same technician conducted all tests. Analyzes were performed using GE enCORE version 17.0 software (GE Healthcare, Madison, WI, USA).

Physical testing

Physical performance was measured using four functional tests, performed in the following order: CMJ, MIHS, 1RM bilateral biceps curl, leg press and bench press, and muscle endurance performance. The test session started with 10 minutes of general warm-up on a cycle ergometer, with intensities equivalent to 10-12 on the 6-20 Borg Rating of Perceived Exertion Scale. CMJ was performed on a force plate (SG-9, Advanced Mechanical Technologies, Newton, MA, USA, sampling frequency of 1 kHz). Participants were instructed to place their hands on their hips and feet in shoulder width on the platform. Participants descended to a squat position of self-selected depth and immediately jumped upward as high as possible. If the third attempt resulted in the highest jump, an additional jump was performed. Thirty sec rest was given between each attempt. Participants were blinded to the results and the three best jumps were averaged, wherein the lowest jump were removed when >3 attempts were performed, and subsequently used in the data analyses (20). The coefficient of variation between test attempts averaged for all time points were 3.6% (SD 2.2, Range: 0.9, 11.7) for HL and 4.3% (SD 2.4, Range: 0.8, 9.9) for LL.

MIHS was conducted on the same force plate as CMJ using a custom-built rack bolted to the floor with an attached fixed bar located over the force plate. MIHS was measured in a half squat position with a knee angle equivalent to ~60-65°. Knee angle and foot position was marked on the rack and force plate, respectively, to ensure similar body position at the two consecutive test time points. Participants were given 3-4 attempts with 2 min rest between attempts. Verbal encouragement was given throughout test and the participants were instructed to push as hard and as fast as possible for 5 sec. The three highest force values were averaged and used in data analyses. For MIHS the coefficient of variation between test attempts averaged for all time points were 4% (SD 3.9, Range: 0.1, 14.5) for HL and 4.8% (SD 3.7, Range: 0.1, 14.5) for LL.

1RM tests started with a specific warm-up, consisting of two sets with gradually increasing load (40% and 75% of expected 1RM) and decreasing number of repetitions (10 and 6). The first attempt was performed with a load approximately 5% below the expected 1RM. If a lift was successful, the load was increased by approximately 2-5%. Upper limb 1RM tests

were performed using biceps curl and bench press. In the biceps curl participants were sitting on a curl bench, using an olympic-curl barbell starting with the elbows extended. An attempt was considered successful when the curl barbell was lifted to full elbow flexion with their seat kept in contact with the bench and feet touching the floor throughout the lift. Bench press was determined with the participants laying supine with their shoulders and hips kept in contact with the bench throughout the test and with their feet touching the floor. Attempts were considered successful when the barbell in a controlled fashion touched the chest during the eccentric phase and the elbows were fully extended at the end of the concentric phase. For the lower limb, 1RM was determined in the leg press. Participants were seated with knee and hip flexed at approximately 90°-96° and 45°, respectively. Individual knee angle depth was marked on the side rack to ensure similar knee position across test time points. A successful 1RM was defined as the maximal resistance that could be moved through the full range of motion with proper form one time. Participants made 3-4 attempts and were given 2 min rest between each attempt in the biceps curl, bench- and leg press. The best attempt was used in data analyses. For each participant, the same supervisor controlled the lifts, seating adjustments and body positions, and gave vocal encouragement in all strength tests, at all time points.

Muscle endurance performance was determined in the leg-extension exercise using a load corresponding to 60% of pre-test body mass. Participants were instructed to perform as many repetitions as possible to muscular failure with proper form. Cadence of repetitions was set to 2 seconds in both concentric and eccentric phase, which was controlled with a metronome. Participants were positioned with the lever arm two fingers proximal of the medial malleol, and the knee joint was aligned with the lever-arm axis. The test was terminated when the cadence was missed two consecutive times. The same absolute load was used at all test time points (Week 10 and 22). Maximal repetition was used for analyses.

Speed (Day 2)

Agility run time was measured using timing gates from a 3-point stance start position. The electronic timer (Brower Timing System, Utah, USA, 2013) started with participant's first movement. From the starting position, participants were given instruction to run to either

the right or left (determined by coin flip) for 4.56 m and touch a line with the hand, reverse direction and run 9.1 m, touch an opposite line with the hand, and run back through the timing gate that recorded the elapsed time. Participants had two attempts in each direction (right and left sides). Two minutes of rest was given between trials and attempts were averaged for each direction and used in analyses.

Biological tissue sampling (Day 3)

Muscle micro biopsy.

A muscle specimen was obtained from *m. vastus lateralis* under local anesthesia (Xylocain, 10 mg ml-1, AstraZeneca AS, Oslo, Norway) using a fine needle (12g Universal-plus, Medax, San Possidonio, Italy) operated with a spring loaded biopsy instrument (Bard Magnum, Bard Nordic, Helsingør, Denmark). The first biopsy was sampled at one third of the distance from basis patella to anterior superior iliac spine, and subsequent biopsies were sampled two cm proximal/distal to the first incision. The tissue sample was quickly dissected free of connective tissue and blood in ice-cold sterile saline solution (0.9% NaCl), and fixated in formalin (~10 mg) for immunohistochemistry preparations.

Immunohistochemistry. Formalin-fixed muscle biopsies were processed for 2.5 h using a Shandon Excelsior ES (Thermo Scientific, USA), paraffin-embedded and sectioned into 4 µm. Transverse sections were double stained for determination of muscle fiber types BF-35 (5 µg x ml-1, Developmental Studies Hybridoma Bank, deposited by Schiaffino, S.) and MyHCSlow (1:4000, catalog M8421L, Sigma-Aldrich Norway AS, Oslo, Norway). The primary staining was identified by BMU UltraView DAB and UltraView RED (Ventana Medical System, Inc. Tucson, USA). Fiber types were counted as either type 1 (red; mean fiber count = 76.2, range =17-164), type 2A (brown; mean fiber count = 75.7, range = 15-223), type 2X (unstained; mean fiber count = 5.9, range = 1-22) or hybrid fibers type 2A/2X (light-brown; mean fiber count = 8.8, range = 1-30).

Statistical Analyses. Descriptive data are presented as mean and standard deviation (SD). Linear mixed-effects models (2) were used to estimate differences between treatment conditions. Relative changes from baseline for 1RM strength, muscle mass and performance variables were used as dependent variables and groups (HL vs. LL), and time as main fixed effects. Baseline values were used as a co-variate together with sex.

Interaction term between conditions and time points were included as fixed effects, and models were specified with random intercept by participant. Estimated means and pairwise comparisons were computed with the contrasts function from the emmeans package for R (19), allowing interpretation of the direction and width of the 95% confidence interval (CI), indicating the magnitude of treatment effects and the certainty of the true population value (mean) (9). Additionally, Hedge's g effect size was calculated and interpreted as small 0.35-0.80, moderate 0.80-1.50, and large >1.5. Assumptions were checked by visual inspection of residual plots to assess uniformity of variance over the fitted range. Whenever deviations from the assumption were observed, data were log-transformed, and models were refitted. Weights functions was applied to fiber cross sectional area data to account for variances in standardized residuals across the fitted range (homoscedasticity). Weighted combined factors were calculated for muscle mass and muscle strength. For muscle mass, lean mass and cross sectional area were normalized to the highest value of the variables baseline test. Likewise, for muscle strength, upper limb combined factor included maximal strength in biceps curl and bench press and lower limb combined factor included leg press and maximal isometric in half squat. Subsequently, computed factors for each subject were calculated as the mean of the normalized values for each outcome variable. To measure specific strength, we used the DXA derived estimates calculated as the ratio between the weighted combined factor for upper and lower limb muscle strength and mass. All analyses were run in R (41). Significance level was set to $\alpha = 0.05$.

RESULT

Adherence to the protocol, training characteristics and dietary intake

Both HL and LL groups showed high degrees of adherence to the protocol, completing an average of 92% (SD 8) and 96% (SD 7) of the prescribed 37 resistance exercise sessions (range 29-37), respectively, with no difference between groups (P=0.172). HL was associated with a higher relative training load (75.5 %1RM, 95% confidence interval (CI): [71.7, 79.2] vs 50.8 %1RM, [47.2, 54.5], P<0.001) and a lower average training volume (load x repetitions per week) compared to LL (13687 kg, [12324, 15049] vs 25119 kg, [23720, 26518], respectively P<0.001) corroborating with previously observed differences between high-load and low-load training modalities performed to concentric failure (34).

Both training protocols led to marked increases in muscle strength and lean-body mass in upper and lower limbs over the course of the study, evident as 0.46 (SD 0.34) % and 0.76 (SD 0.56) % increases in muscle strength $\times$ training session^{-1}, respectively, and 0.17 (SD 0.17) and 0.10 (SD 0.13) % increases in lean mass $\times$ training session^{-1}, with muscle strength measures representing a pooled average for training modalities and muscle strength exercises. The efficacy was thus in the expected range of responses to resistance training in moderately trained individuals (34).

For both HL and LL, the average training-load per-session increased less for upper than for lower body exercises from baseline to week 10 (35 vs. 66%, respectively, P<0.001, group interaction; p=0.423, 0.262, respectively, Figure 1B), whereupon the elevated load was maintained to Week 22 (P=0.154 and P=0.976, respectively, group interaction; P=0.208, 0.186, Figure 1B). Furthermore, dietary intake of macronutrients (Range p-values: 0.203-0.616) and total energy (kcal, P=0.420) were similar in HL and LL at Week 9 (Table 1). HL and LL had similar blood levels of endocrine variables throughout the intervention (testosterone, IGF-1, GH, cortisol, no effects of time, Table 2).

(Include Table 2 here)

Comparing the effects of HL and LL on muscle strength, muscle mass and muscle fiber characteristics

For upper body limbs, 22 weeks of HL led to larger increases in muscle strength than LL (Figure 2), evident as larger increases in both 1RM in specific resistance exercises (Biceps curl, 9.1 % mean difference, 95% CI [0.1, 18.1], P=0.049; Bench press, 7.8 % mean difference, [1.6, 14.1], P=0.016) and as larger increases in muscle strength measured as a weighted combined muscle strength score (10% mean difference, [2.8, 17.1], P=0.010, ES [95% CI] 1.31 [0.28, 2.31]; Figure 2A). The more pronounced effects of HL were accompanied by greater increases in lean mass of the arms (5.2 % mean difference, [1, 9.4], P=0.018, ES: 0.99 [0.15, 1.81]; Figure 3A).

The benefits of HL in the upper body were manifested during the first phase of the training intervention for both muscle strength (baseline to Week 10: 10.3 % mean difference, [4.4, 16.1], P<0.001) and lean mass (5.3 % mean difference, [1.2, 9.3], P=0.013; Figure 2 and 3), with no differences being seen during the second phase (Week 10 to Week 22: -0.3 % mean difference, [-8, 7.4], P=0.938 and -0.1 % mean difference, [-3.4, 3.3], P=0.956, respectively; Figure 2 and 3). HL and LL led to similar improvements in specific strength (baseline to Week 22; 4 % mean difference, [-3.6, 11.6], P=0.275. Figure 3E), calculated as ratios between the weighted combined upper body muscle strength score and arm lean mass.

(Include Figure 2 and 3 here)

For lower body limbs, 22 weeks of HL led to larger increases in muscle strength measured as 1RM leg press than LL (14.7 % mean difference, 95% CI [3.9, 25.6], P=0.016; Figure 2), while the two training modalities led to similar improvements in MIHS (7.1 % mean difference, [-2.3, 16.5], P=0.130, Figure 2). After combining the two measures into a weighted muscle strength score, HL led to more pronounced increases in muscle strength (9.9 % mean difference, [1.1, 18.6], P=0.029, ES 0.87 [0.05, 1.68]; Figure 2B). The superior effect of HL seemed to accumulate gradually throughout the intervention, as no significant differences were observed between the groups during either of the two intervention phases (baseline to 10 weeks 6.7 % mean difference, [-1.6, 14.9], P=0.123, and 10 weeks to 22 weeks 3.2 % mean difference, [-1.2, 7.5], P=0.158, respectively; Figure

2B). For lower body limbs, HL and LL led to similar increases in markers of muscle mass, measured as both lean leg mass (1.3 % mean difference, 95% CI [-1.6, 4.1], P=0.374, ES 0.41 [-0.38, 1.19], Figure 3B), pooled muscle cross-sectional area (4.0 % mean difference, [-14.7, 22.7], P=0.656, no difference between groups in either fiber type; Figure 3C), and the weighted combined measure of lean leg mass and cross-sectional area (0.9 % mean difference, [-8.6, 10.4], P=0.845, Figure 3D), contrasting observations made for upper body limbs. Consequently, there seemed to be a decoupling of development of muscle strength and muscle mass between load conditions in the lower limb, with HL tending to induce more pronounced improvements in specific strength compared to LL, at least during the second phase of the intervention (9.6 % mean difference, [-0.8, 20.1], P=0.079, Figure 3E). HL and LL led to similar changes in muscle fiber proportions in *m. vastus lateralis*, with both leading to complete eradication of type IIX fibers (Table 3), presumably due to transition from IIX to IIA.

(Include Table 3 here)

Effects of HL and LL on muscle power, agility, and endurance

Twenty-two weeks of resistance training (HL and LL) led to improved performance in CMJ, agility and muscle endurance (time effect: P<0.001, P=0.0268, P=0.004, respectively; Figure 4). For CMJ, HL led to larger improvements than LL (5.5 % mean difference, 95% CI [1.4, 9.6], P=0.011, ES 0.60 [-0.20, 1.38]; Figure 4A), a phenomenon that was manifested during the first phase of the training period (baseline to Week 10, 5.2 % mean difference, [1.4, 9], P=0.012), with no additional advantage being observed during the second phase (Week 10 to Week 22, 0.3 % mean difference, [-3.2, 3.8], P=0.865). For agility and muscle endurance, no differences were observed between training modalities (Figure 4B-C).

(Include Figure 4 here)

DISCUSSION

To the authors' knowledge, this is the first study to compare the effects of prolonged whole-body resistance exercise training (>13 weeks) with high vs. low loads on muscle strength, performance, and biological characteristics in active military cadets. Briefly, for upper body limbs, HL led to greater increases in muscle strength and lean mass than LL. Similarly, for lower body limbs, HL led to more pronounced improvements in muscle strength and jump performance, though this was not the case for other outcome measures such as muscle endurance performance, agility, and measures of muscle mass (LBM and muscle fiber CSA), and muscle fiber composition, for which the two load conditions led to similar changes. Together, these data advocate high-load training as the preferred resistance training modality for young, moderately trained military cadets.

The observed differences between upper and lower body muscles underline the notion that different muscle groups can display differential responses to different training modes (22,26,30). This phenomenon is likely linked to differences in muscle properties, and potentially covaries with characteristics such as genetics, age, health, and training status (37,43). Indeed, these sources of variation may underlie the current lack of consensus for the effects of varying training variables such as load for development of muscle strength and mass (4,23,24,34), together with variation in study protocols (4,21,24,40). Notably, the present study protocol was conducted as part of a military training and education program, with the study population consisting of prospective soldiers. Hence, while the data provide insight into the effects of high- and low-load resistance training modalities that are generalizable to the overall population, they also provide insight into their specific efficacies in a military environment. For this purpose, our findings suggest that two weekly resistance training sessions are sufficient to increase strength and muscle mass in military cadets, in both upper and lower body, irrespective of training load. Resistance training thus seems to be a potent way of maintaining and developing muscle characteristics during military training programs, a perspective that is supported by a recent 15-week study (11). Importantly, the military setting is quite unique, and despite its potential drawbacks such as periods of exhaustive field exercises and military leave, it stands out as an intriguing model for exercise interventions. For example, as it offers remarkable standardization of variables

such as nutrition (25), levels of physical activity, and circadian rhythm across study participants and groups (6), while also involving non-protocol stressors such as field exercises (17). In the present study, this likely reduced the potential negative impact of these confounding factors between the two groups, acting to improve the biological validity of training load-based interpretations, together with study design characteristics such as close supervision of every training session and blinding of test personnel to load conditions.

The superiority of HL for increasing muscle strength in both upper and lower-body limbs was evident by larger increases in specific 1RM muscle strength tests, as well as larger increases in weighted muscle strength, calculated from the weighted average of several measures of strength, as previously described (10). These benefits largely corroborate with conclusions from previous studies (4,18,23,34), including recent meta-analyses (29,32), though it contrasts conclusions from other studies (21,24,40). Of note, in one meta-analysis study, high-load training was found to lead to superior performance in RM-tests but not in maximal isometric tests (32), which was indeed also seen in the lower limbs in the present study. While this implies that some of the observed benefits of high-load training may be due to skill acquisition connected to performing training exercises that more greatly resemble test procedures (3,23), it also underlines the need for multiple strength measures to ensure proper estimation of the effects of any resistance training intervention on muscle strength performance (3). Of note, in the present study, both loading conditions led to increases in muscle strength irrespective of factors such as sex (no interaction; data not shown) and the concurrent performance of endurance-oriented training. Thus, albeit high-load training led to more pronounced strength responses, low-load training also led to marked improvements. Therefore, heavy-load training stands out as the preferred load modality for moderately trained cadets , supported by the moderate effect size of HL compared to LL, offering adaptational benefits while at the same time involving lower training volume and shorter duration of training, all of which are advantageous in a demanding military context with time restrictions (17). Still, low-load training is likely to offer valuable benefits to maintain operational readiness.

In contrast to the muscle strength results, HL did not offer universal benefits for increments in muscle mass compared to LL. Whereas HL led to more pronounced muscle mass

accretion in upper limb, measured as arm lean mass, it offered no such benefits in lower limbs, measured as either lean leg mass, muscle fiber CSA of *m. vastus lateralis* or a combination thereof. In light of the universal benefits of HL for improving muscle strength, the muscle mass discrepancy may suggest a more pronounced decoupling between strength and muscle mass development in the lower limbs (i.e. that strength can be improved without increasing mass) (5), reflected by a less pronounced effect estimate between groups in lower-body muscle mass accretion. This is supported by a tendency for HL to lead to greater improvements in specific strength, potentially mediated by neuromuscular adaptations or factors such as changes in muscle morphology (i.e improved force transmission) (8).

The differential muscle accretion responses to the two load conditions between upper and lower limbs may have several explanations. First, it may be related to a pre-intervention difference in training status between the limbs. Indeed, upper body limbs are likely to be rather unstimulated in resistance training-naive individuals, as opposed to lower body limbs, which are necessary for everyday mobility. Adding to this, the military cadets had more than one year of academy-related training prior to the intervention, during which they conducted at least two training session per week, targeting primarily lower-limb muscles (i.e., circuit training, running, and marching). It is thus reasonable to speculate that tweaking other training variables, such as adding a higher training volume, may have been necessary to induce sufficient training stimuli for muscular adaptations to occur in the more trained lower body limbs (30). Based on this line of arguments and our observations, upper- and lower-body limbs represent two rather different experimental models that are likely to show differential responses to a given training stimuli. This is supported by findings in a previous study, where eight weeks of resistance training with heavy loading (90% 1RM) led to more pronounced muscle growth (and increases in strength) than lighter loading (70% 1RM) in the upper body but not in the lower body of resistance training-experienced men (22). Second, the observed differences between upper and lower limbs in the present study may have been a direct consequence of the concurrent aerobically-oriented training conducted alongside the study protocol. This may have compromised resistance training-related adaptations in the lower body limbs (44). Importantly, however, any such academy-related training was predominately conducted on separate days, ensuring at least 12 h

between aerobic and resistance training sessions. This should have been enough to reduce its negative effects (1). Furthermore, it cannot be ruled out that lower limb muscles are more sensitive to periods of disturbed sleep and energy deficit, such as experiences during the field exercise, potentially activating molecular signaling pathways that are antagonistic to muscle protein synthesis and, therefore, leading to attenuated muscle mass and strength adaptations (12). Having noted this, all cadets were part of the same unit, and hence experienced similar stressors during the training intervention, making them less likely to have had an impact on group comparisons. Third, the difference in response to resistance training between upper and lower limbs may be related to inherent differences in muscle biology. For example, upper body muscle fibers (*m. trapezius*) have previously been shown to express higher densities of androgen receptors compared to lower body muscles fibers (*m. vastus lateralis*) (13). Albeit speculative, such biological differences may lead to differential responses to a given anabolic stimulus, mirroring a differential responsiveness to either mechanical stimulus (high-load training) or metabolic stimulus (low-load training) (27).

Initially, we anticipated any differences in the efficacy of high-load and low-load training protocols for developing muscle strength and mass to emerge towards the end of the intervention. While this is supported by the observed development of specific strength in lower body limbs, though without leading to differential responses over the course of the intervention, all other outcome measures deviated from our expectation. Indeed, for variables such as upper limb strength and upper limb lean mass, the time-course development showed the opposite relationship with time, with benefits of high-load training emerging during the initial phase of the intervention (Week 0 to Week 10). The causative explanation behind these observations is difficult to address thoroughly based on the present data. However, for the differential development of specific strength in the lower limbs, it seems plausible that the benefits of high-load training involved neuromuscular or structural adaptations (8), as previously discussed. As for the upper limbs, the benefits of high-load training involved simultaneous increases in muscle strength and mass, with no observable differences in the development of specific strength between load conditions. This suggests that the enhanced force-generating capacity was associated with greater muscle hypertrophy, with responses being more pronounced in the initial phase. Despite

this, no associations were found between changes in muscle strength and muscle mass on an individual level, reiterating on the debated relationship between changes in muscle strength and muscle hypertrophy (5).

Limitations

In the present study, resistance training protocols were not volume-matched between groups (reps x load x set). As there is a dose-response relationship between resistance-training volume and increases in muscle strength and hypertrophy (33), this may have affected the outcomes. However, the significant larger volume load lifted in LL did not translate into superior muscular adaptations compared to HL, making our observations inversely related to the expected effects of increased training volume. Furthermore, the study did not include a negative control group, i.e, a group of cadets that did not conduct resistance training. While this should not have affected the ecological validity of our between-group comparisons, this cannot be ruled out, as standard military training with light loads and high velocities are known to be beneficial for muscle strength (11,14), and may interact with HL and LL stimuli in different manners.

In conclusion, 22 weeks of high-load resistance training led to more pronounced improvements in upper limbs muscle strength and lean mass compared to low-load resistance training in moderately trained cadets. Similar benefits of high-load training were seen for lower limb muscle strength and jump performance, but not for other lower limb characteristics such as muscle endurance performance, agility, measures of muscle mass (lean mass and muscle fiber cross-sectional area in *m. vastus lateralis*) and muscle fiber composition (*m. vastus lateralis*). Overall, the benefits of high-load training were manifested during the initial ten weeks of training. Although further interventions are needed to establish the full benefits of resistance load in challenging military contexts, our data provide evidence for high-load training as being the preferred resistance training modality for in-training prospective soldiers. Still, low-load training is likely to offer a valuable asset for soldiers during extreme circumstances such deployed operations, when high-load resistance-training may not be feasible.

PRACTICAL APPLICATIONS

Based on the current data, it is apparent that incorporating resistance exercises into the weekly training routines of military personnel, as previously suggested (11,16,20), can yield significant benefits. In addition to augmenting muscle strength, endurance, and mass, these exercises contribute to enhancements in jump height and agility performance. These aspects are particularly pertinent as they align with well-established evaluation protocols that hold relevance for military-specific tasks (35).

Comparing the impact of high-load and low-load training, it becomes evident that high-load training produces more noticeable effects on muscle strength and mass, particularly in the upper body. Nevertheless, it's important to note that both training modalities yield considerable improvements. Notably, the viability of low-load training emerges as an attractive option for soldiers, especially in scenarios where access to heavy exercise equipment or training facilities is restricted, such as during deployment or field exercises. This underscores the potential suitability of low-load calisthenics exercises as a viable substitute. However, given the paramount importance of maximal strength and power in enhancing soldiers' performance in operational settings, it is advisable to capitalize on the advantages of high-load training whenever feasible.

It's worth mentioning that the findings from this study are likely transferrable to other populations of moderately trained individuals, thus broadening the scope of applicability beyond just military personnel.

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Figure Legends

Figure 1. A) Time-line of the study, including overview of measurements performed at baseline and at week 10 and week 22, a three-week military leave, a two-week military field exercise, and a dietary survey (Week 9). Prior to baseline testing, familiarization to all physical test protocols were conducted. DXA, scan for body composition; Blood, blood sampling; CMJ, counter movement jump; MIHS, maximal isometric half squat; 1RM, 1 repetition maximum; END, muscle endurance performance; Agility, running time; Biopsy, muscle sample from *m. vastus lateralis*.

B) Relative increases in training load from week 1 to week 2, week 10 and week 22 for upper limb (exercises combined; seated pull down, standing rowing, bench press, biceps curl) and lower limb exercises (squat, leg press, leg curl) in HL and LL. Data are mean ± 95% CI.

Figure 2. Effects of high-load (HL) and low-load (LL) resistance training on muscle strength in upper-and lower body exercises. A) Muscle strength in Upper body exercises combined (weighted average of 1RM biceps curl and bench press; left panel) and in specific exercises (right panel). B) Muscle strength in lower-body exercises combined (weighted average of 1RM leg press an maximal isometric half squat) and in specific exercises. Values are estimated means ± 95% CI. In both A and B, differences between groups are presented as weighted percent point mean difference (Δ HL - Δ LL; means ± 95% CI; left panels).

Figure 3. Effects of high-load (HL) and low-load (LL) resistance training on markers of muscle mass in upper- and lower body limbs. A, B) Lean body mass in upper- (A) and lower-body limbs (B), shown as group specific estimated means (left panel) and as differences between groups (right panels). C) Cross-sectional area of muscle fiber types I and II in *m. vastus lateralis*, shown as group-specific estimated means (left panel) and as differences between groups (right panel). D) Weighted combined measure of markers of lower-body muscle mass (lean mass and muscle fiber CSA), shown as changes from baseline (left panel) and as differences between groups (right panel). E) Specific strength in upper (weighted muscle strength per lean mass) and lower limbs (weighted muscle strength

per weighted muscle mass), shown as percent changes from baseline to weeks 10 and 22, and as differences between groups (right panel). Group-specific estimated means are shown as mean ± 95% CI. Differences between load conditions (groups) are shown as mean percentage point difference ± 95% CI (ΔHL - ΔLL).

Figure 4. Effects of high-load (HL) and low-load (LL) resistance training in muscle performance measured as counter movement jump (A), agility (B), and muscle endurance performance (C). Data are presented as groups-specific estimated means (estimated means ± 95% CI; left panel) and as differences between groups (mean percent point difference ± 95% CI; Δ HL - Δ LL).

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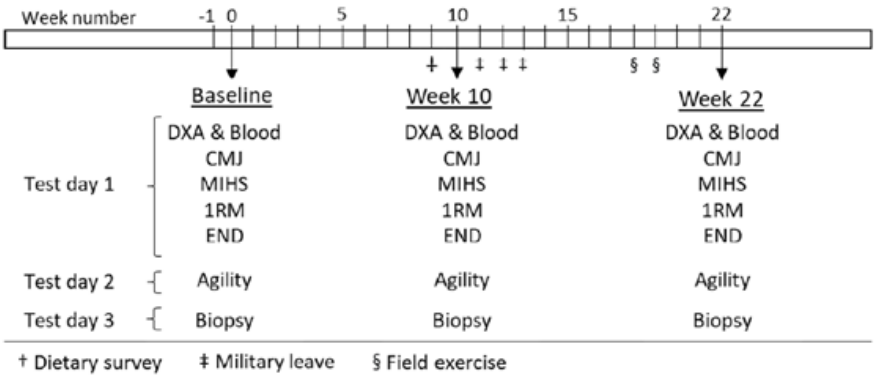
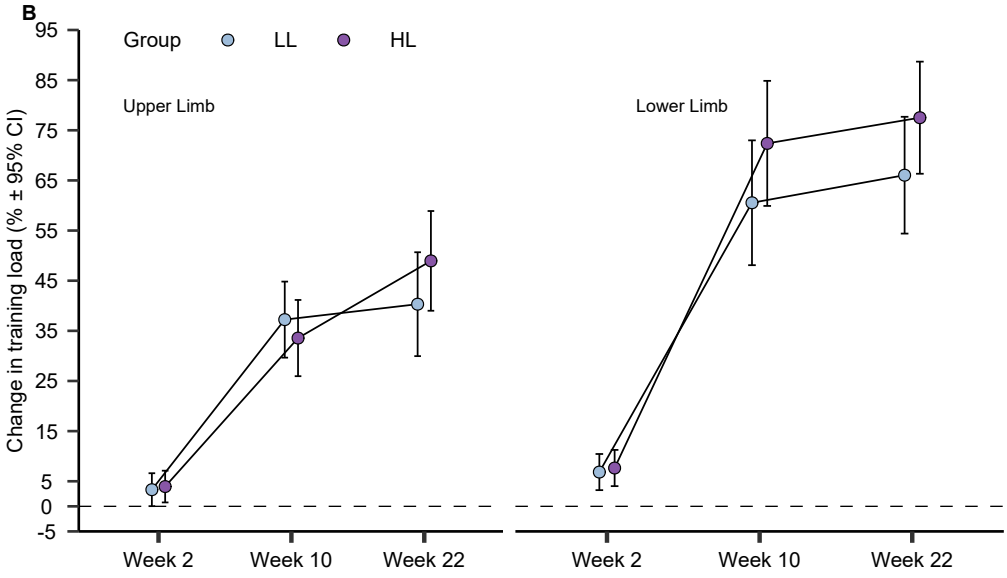
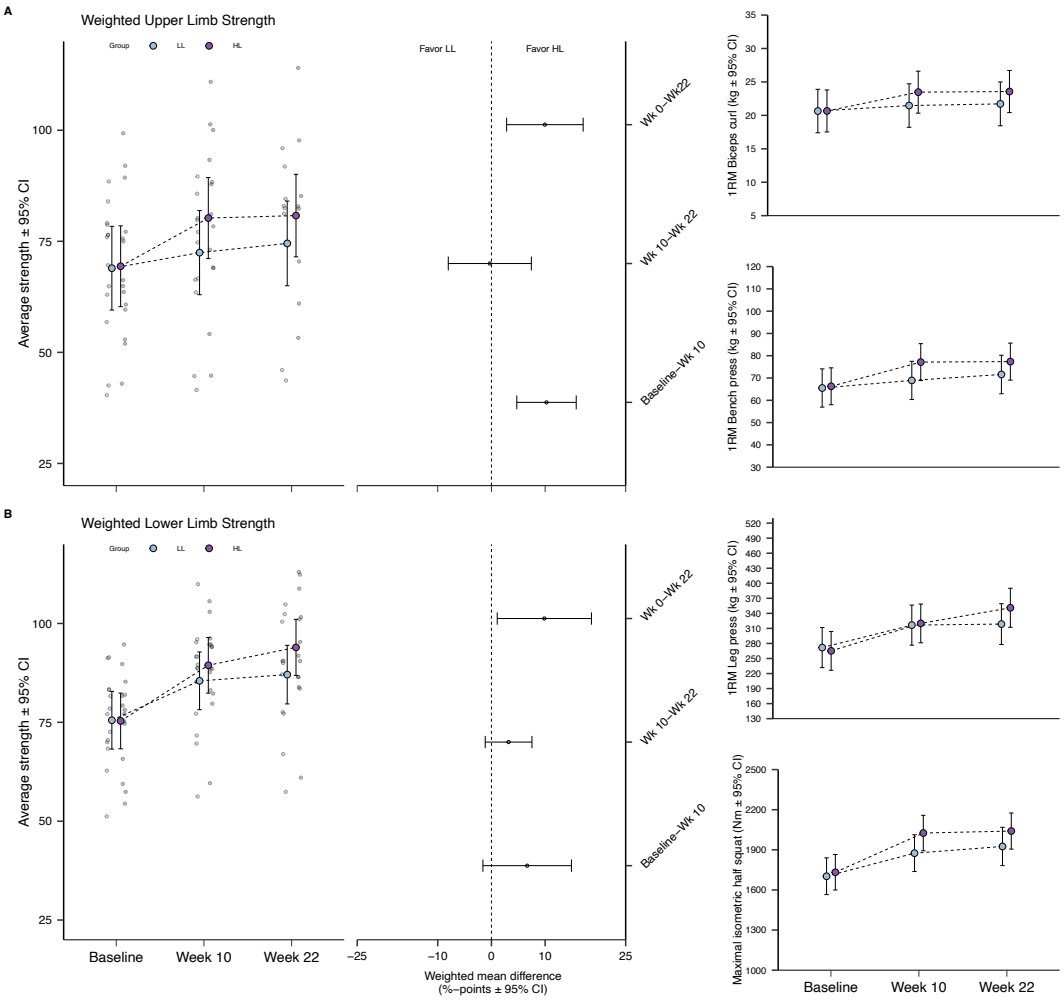
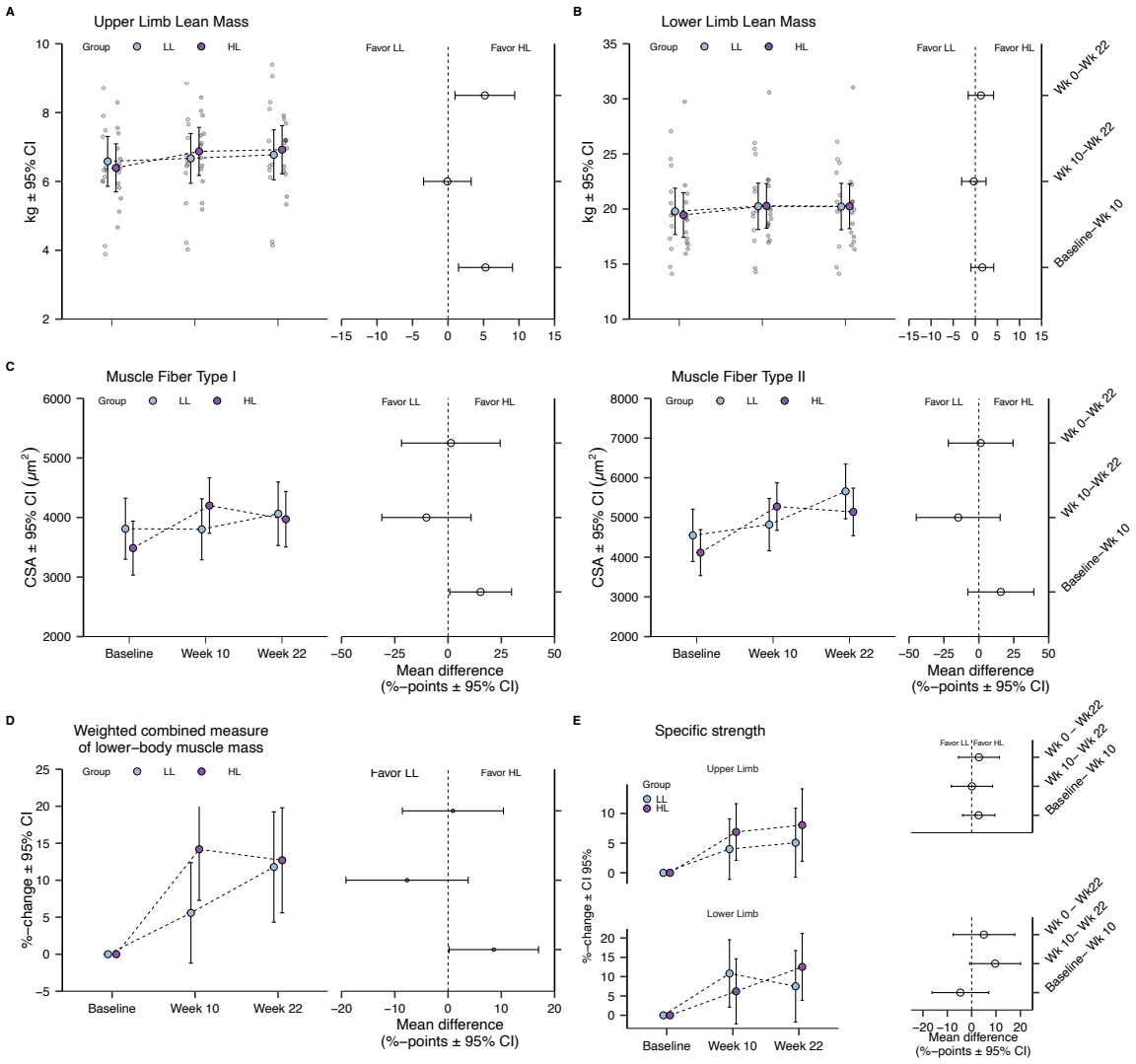
A


Week number
-1 0 5 10 15 22
† ‡ ‡ ‡ ‡ § §
Baseline
Week 10
Week 22
Test day 1
DXA & Blood
CMJ
MIHS
1RM
END
Test day 2
Agility
Test day 3
Biopsy
† Dietary survey
‡ Military leave
§ Field exercise

B


Group
LL
HL
Upper Limb
Lower Limb
Change in training load (% ± 95% CI)
95
85
75
65
55
45
35
25
15
5
0
-5
Week 2
Week 10
Week 22
Week 2
Week 10
Week 22


A
Weighted Upper Limb Strength
Group
LL
HL
Average strength ± 95% CI
Favor LL
Favor HL
Wk 0–Wk22
Wk 10–Wk 22
Baseline–Wk 10
1RM Biceps curl (kg ± 95% CI)
1RM Bench press (kg ± 95% CI)
B
Weighted Lower Limb Strength
Baseline
Week 10
Week 22
Weighted mean difference
(%−points ± 95% CI)
1RM Leg press (kg ± 95% CI)
Maximal isometric half squat (Nm ± 95% CI)


A
Upper Limb Lean Mass
Group LL HL
kg ± 95% CI
Favor LL
Favor HL
B
Lower Limb Lean Mass
Group LL HL
kg ± 95% CI
Favor LL
Favor HL
Wk 0–Wk 22
Wk 10–Wk 22
Baseline–Wk 10
C
Muscle Fiber Type I
Group LL HL
CSA ± 95% CI (μm²)
Baseline
Week 10
Week 22
Mean difference
(%–points ± 95% CI)
Muscle Fiber Type II
Group LL HL
CSA ± 95% CI (μm²)
Baseline
Week 10
Week 22
Mean difference
(%–points ± 95% CI)
D
Weighted combined measure
of lower–body muscle mass
Group LL HL
%–change ± 95% CI
Baseline
Week 10
Week 22
Mean difference
(%–points ± 95% CI)
E
Specific strength
Upper Limb
Group
LL
HL
Lower Limb
%–change ± CI 95%
Baseline
Week 10
Week 22
Wk 0 – Wk22
Wk 10– Wk 22
Baseline– Wk 10
Mean difference
(%–points ± 95% CI)

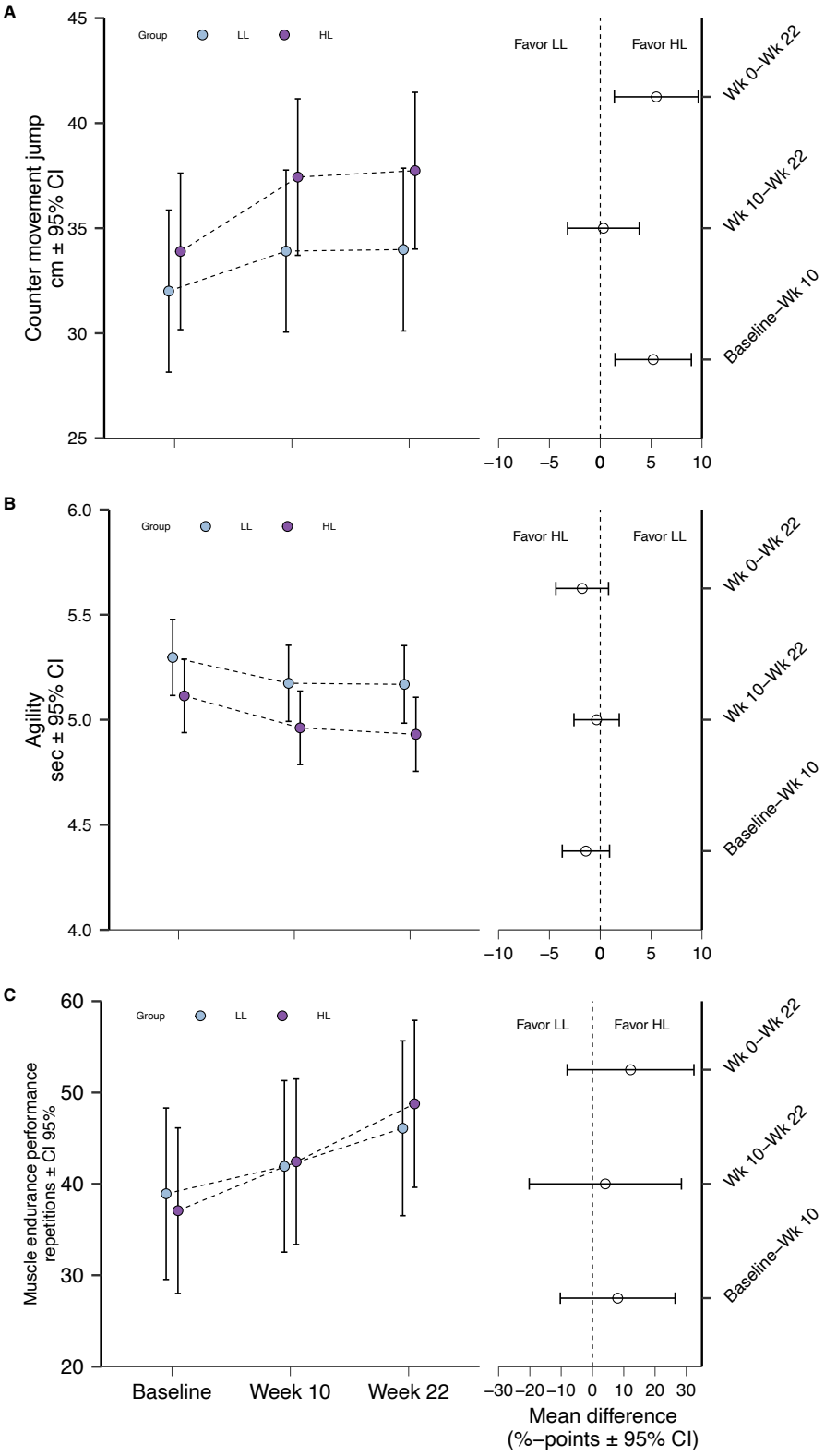

A
Group
LL
HL
Counter movement jump
cm ± 95% CI
45
40
35
30
25
Favor LL
Favor HL
Wk 0–Wk 22
Wk 10–Wk 22
Baseline–Wk 10
−10
−5
0
5
10
B
Group
LL
HL
Agility
sec ± 95% CI
6.0
5.5
5.0
4.5
4.0
Favor HL
Favor LL
Wk 0–Wk 22
Wk 10–Wk 22
Baseline–Wk 10
−10
−5
0
5
10
C
Group
LL
HL
Muscle endurance performance
repetitions ± CI 95%
60
50
40
30
20
Favor LL
Favor HL
Wk 0–Wk 22
Wk 10–Wk 22
Baseline–Wk 10
Baseline
Week 10
Week 22
−30 −20 −10 0 10 20 30
Mean difference
(%–points ± 95% CI)

Table 1 Participants characteristics and energy intake. Data are mean ± (SD)

		High-load		Low-load	
		Female (n=2)	Male (n=12)	Female (n=3)	Male (n=10)
Baseline	Age (year)	20.6 (1.1)	20.8 (0.7)	21.4 (0.4)	21.0 (1.0)
	Stature (cm)	171.0 (1.4)	183.1 (5.0)	173.5 (5.9)	186.2 (10.9)
	Body mass (kg)	67.6 (7.2)	75.4 (12.2)	71.5 (16.5)	79.2 (12.6)
	Fat mass (kg)	18.2 (6.8)	14.6 (6.3)	22.8 (8.0)	15.7 (6.5)
	Lean body mass (kg)	46.9 (0.2)	57.7 (6.3)	46.0 (8.6)	60.2 (8.6)
Week 10	Body mass (kg)	67.7 (4.5)	76.9 (12.1)	70.6 (15.5)	79.9 (12.3)
	Fat mass (kg)	16.9 (6.0)	14.0 (6.2)	21.9 (7.4)	15.0 (6.2)
	Lean body mass (kg)	48.2 (1.7)	59.8 (6.4)	46.0 (8.7)	61.6 (8.7)
Week 22[a]	Body mass (kg)	66.6 (3.5)	76.8 (11.7)	61.9 (5.3)	81.1 (11.2)
	Fat mass (kg)	15.6 (4.8)	14.0 (5.5)	17.7 (6.3)	15.0 (6.1)
	Lean body mass (kg)	48.4 (1.5)	59.7 (6.6)	41.8 (0.9)	62.8 (7.9)
		kcal day^{-1}	CHO kg^{-1} day^{-1}	Protein kg^{-1} day^{-1}	Fat kg^{-1} day^{-1}
HL	Energy[b]	2633.7 (867.6)	4.3 (2.1)	1.8 (0.7)	1.4 (0.5)
LL		2686.2 (1023.1)	4.1 (1.8)	1.6 (0.7)	1.4 (0.6)

[a]High-load: male n = 11, Low-load: female n = 2, male n = 9

[b]Data from dietary survey

Table 2 Hormone measurements

	Sex	High-load			Low-load		
		Baseline	Week 10	Week 22	Baseline	Week 10	Week 22
Testo (nmol l^{-1})	Female	0.8 (0.1)	0.7 (0.1)	0.7 (0.0)	0.7 (0.0)	0.7 (0.0)	0.7 (0.0)
	Male	13.2 (2.7)	14.5 (2.3)	12.2 (2.5)	12.7 (3.1)	12.4 (2.3)	12.1 (3.6)
IGF-1 (nmol l^{-1})	Female	29.1 (5.5)	24.6 (8.1)	27.8 (8.6)	27.3 (6.3)	25.0 (7.3)	24.5 (2.6)
	Male	23.6 (6.0)	20.9 (4.5)	22.5 (4.3)	26.4 (6.7)	23.1 (4.0)	22.7 (4.5)
GRH (µg l^{-1})	Female	2.2 (2.8)	3.4 (4.7)	2.1 (2.6)	2.5 (2.1)	6.8 (7.5)	1.4 (0.6)
	Male	0.2 (0.1)	0.2 (0.4)	0.1 (0.1)	1.4 (2.9)	0.5 (1.0)	0.1 (0.0)
Cortisol (nmol l^{-1})	Female	629.0 (97.6)	539.5 (111.0)	484.5 (48.8)	483.7 (169.5)	516.7 (66.1)	465.5 (391.0)
	Male	395.3 (106.8)	382.1 (93.1)	310.8 (111.3)	378.2 (123.4)	369.1 (115.4)	287.6 (89.9)

Testo = Testosterone, IGF-1 = Insuling growth factor, GRH = Growth hormone, data are means ± (SD)

Table 3 Muscle fiber type proportion and muscle fiber counting

Fiber	Time	High-load[a]	High-load[b]	Low-load[a]	Low-load[b]
		%- fiber proportion	mean (min,max)	%- fiber proportion	mean (min,max)
Type 1	Baseline	49.3 (12.1)	78 (20, 164)	51.8 (10.3)	87 (33, 134)
	Week 10	49.1 (12.7)	57 (17, 103)	53.4 (9.8)	72 (31, 139)
	Week 22	48.3 (6.5)	85 (23, 159)	53.0 (9.4)	82 (25, 159)
Type 2a	Baseline	47.3 (10.4)	82 (15, 157)	45.5 (10.0)	81 (24, 140)
	Week 10	50.9 (12.7)	61 (18, 149)	46.4 (10.0)	64 (21, 137)
	Week 22	51.7 (6.5)	96 (26, 223)	47.0 (9.4)	73 (27, 159)
Type 2x	Baseline	3.4 (4.8)	6 (0, 27)	2.7 (3.9)	4 (0, 12)
	Week 10	0.0 (0.0)	0 (0, 0)	0.2 (0.4)	0 (0, 2)
	Week 22	0.0 (0.0)	0 (0, 0)	0.0 (0.0)	0 (0, 0)

[a]data are mean ± (SD)

[b]fiber counts

Paper IV

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ORIGINAL ARTICLE

WILEY

No effect of increasing protein intake during military exercise with severe energy deficit on body composition and performance

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In this study, we compare the effects of isocaloric high- (HIGH: 2 g kg^{-1} d^{-1}, n = 19) and low-protein diet (LOW: 1 g kg^{-1} d^{-1}, n = 19) on changes in body composition, muscle strength, and endocrine variables in response to a 10-day military field exercise with energy deficit, followed by 7 days of recovery. Body composition (DXA), one repetition maximum (1RM) bench and leg press, countermovement jump height (CMJ) and blood variables were assessed before and after the exercise. Performance and blood variables were reassessed after 7 days of recovery. The 10-day exercise resulted in severe energy deficit in both LOW and HIGH (−4373 ± 1250, −4271 ± 1075 kcal d^{-1}) and led to decreased body mass (−6.1%, −5.2%), fat mass (−40.5%, −33.4%), 1RM bench press (−9.5%, −9.7%), 1RM leg press (−7.8%, −8.3%), and CMJ (−14.7%, −14.6%), with no differences between groups. No change was seen for fat-free mass. In both groups, the exercise led to a switch toward a catabolic physiological milieu, evident as reduced levels of anabolic hormones (testosterone, IGF-1) and increased levels of cortisol (more pronounced in HIGH, $P < .05$). Both groups also displayed substantial increases in creatine kinase. After 7 days of recovery, most variables had returned to close-to pre-exercise levels, except for CMJ, which remained at reduced levels. In conclusion, increased protein intake during 10-day military field exercise with severe energy deficiency did not mitigate loss of body mass or impairment of physical performance.

KEYWORDS
cortisol, energy deficiency, nutrient supplement, soldiers, strength, testosterone

1 | INTRODUCTION

Soldiers participating in military field exercises or warfare often operate in a state of energy deficit in a demanding environment, with little opportunity for rest, recovery, and nutritional intake.[1-4] The energy imbalance typically leads to a catabolic physiological state, accompanied by alterations in body composition, including loss of overall body mass, lean body mass (LBM), and fat mass.[1,2,5,6] The catabolic state is characterized by disturbances in endocrine functions, including reduced circulating levels of anabolic hormones such as testosterone (TESTO) and insulin-like growth factor 1 (IGF-1), and increased levels of catabolic hormones such as cortisol (COR),[3] which correlate well with observed losses in muscle mass

during military exercises of both short[7] and long[1,3,8] duration. This is in turn associated with impaired physical performance, especially strength and power performance, measured as maximal dynamic strength and vertical jump.[1,2,7,9] For military personnel, it is essential to identify strategies to avoid or minimize the loss of muscle mass and performance during periods of energy deficit and psychological and physiological stress.

Dietary intervention with increased protein intake stands out as an interesting approach for maintaining muscle mass,[10-12] ensuring amino acids availability and a sustained anabolic stimuli for muscle protein metabolism.[10,13] Indeed, intake of protein amounting 2-3 times the prevailing recommendation (0.8 g protein kg^{-1} day^{-1}, RDA) leads to preservation of lean mass and muscle strength in diet-controlled weight-loss programs.[11,14-16] Protein supplementation may thus be a potent action for sustaining muscle functions also in soldiers participating in military field exercises.

In line with this, selected studies suggest that increased protein intake attenuates LBM loss during military exercises (2.0-2.3 g kg^{-1} d^{-1} vs 1.5-1.6 g kg^{-1} d^{-1}).[5,6] In these studies, surplus protein was ingested as an addition to the regular diet, essentially meaning that the total energy intake was higher in protein-ingesting subjects than in control subjects.[5,6] Hence, they did not investigate the effect of protein supplement on muscle mass and performance per se, as energy availability is a potent modulator of these variables.[5,6] Indeed, overall energy intake and corresponding degrees of energy deficiency may be decisive for whole-body homeostasis rather than the nature of the energy source ingested (eg, protein vs carbohydrate content).[17] For example, increased protein intake (1 vs 2 g kg^{-1} d^{-1}) does not seem to hinder loss of muscle mass during 21 days of concomitant severe energy deficit (~−70%) and high altitude exposure in recreationally active men.[18] However, there is evidence to the contrary, as increased protein intake mitigates loss of muscle mass within an isocaloric diet in both resistance-trained subjects and military personnel undergoing 40% energy deficit, respectively.[11,14] The heterogeneity of available studies, with regard to aspects such as the degree of energy deficit, protein supplementation protocols, duration of the intervention and the human subpopulation of interest, thus prohibits consensus around the benefits of protein intake for maintenance of LBM and preservation of muscle performance during military exercises with severe energy deficiency. Despite these issues, a recent review concluded that the energy deficit threshold for benefiting from excessive protein ingestion on preservation of LBM resides around −40%.[19] In addition, we know little about the immediate physiological recovery from such military exercises, though 2-6 weeks seems to be sufficient to reestablish important factors for soldier readiness such as physical performance levels and endocrine variables.[2,7] Nor do we know if higher protein intake during the exercise exerts beneficial effects on these variables within such short recovery period.[20]

The aim of this study was to investigate the effect of 10-day military field exercise with severe energy deficit on changes in body composition, endocrine responses, and physical performance in soldiers. We aimed to investigate whether these variables were affected by ingestion of isocaloric diets containing either LOW (1 g kg^{-1} d^{-1}) or HIGH protein amounts (2 g kg^{-1} d^{-1}), combined with low carbohydrate intake (1.9 and 0.6 g kg^{-1} d^{-1}, respectively). We also aimed to investigate the effect of seven days of refeeding and recovery on these variables.

2 | METHOD

2.1 | Participants

Thirty-eight soldiers (age; 21.6 ± 0.8 years, height; 182 ± 9 cm, males/females ratio; 4.4) from the 2nd year at the Norwegian Defence Cyber Academy volunteered for the study. The study was approved by the local Ethics Committee at Inland Norway University of Applied Sciences and the Norwegian Centre for Research Data (ref 43901/3). Written informed consent was obtained from all participants prior to inclusion, and the study was carried out in accordance with the Declaration of Helsinki. Participants were randomly assigned into LOW (1 g kg^{-1} d^{-1}, male = 15, female = 4) or HIGH protein intake (2 g kg^{-1} d^{-1}, male = 16, female = 3) prior to the 10-day military exercise. There was no difference between the two groups for any of the characteristics or variables prior to onset of the study (Table 1).

2.2 | Experimental design

The soldiers performed a 10-day strenuous military exercise in a state of energy deficit, followed by 7 days of recovery (Figure 1). During the exercise, soldiers performed physically and cognitively demanding military tasks in a challenging outdoor environment. The exercise consisted of cyber-specific tasks, as well as marching, patrolling and physical combat conditioning training lasting for several hours. Throughout the entire exercise, the soldiers carried their personal military combat equipment (~20 kg). Most days contained activities lasting from 06.00 to 24.00 h, some days even longer. The exercise aimed to condition the participants for military combat situations, with gradual decreases in sleep and rest and gradual increases in physical and mental demands. The recovery phase (lasting for 7 days after finalization of the military exercise) was performed without restrictions in energy intake or physical activity. Pre-exercise testing was conducted 2 days prior to the exercise, which commenced toward the end of April. Post-exercise testing was conducted immediately after the exercise. Post-recovery testing was performed 7 days after finalization

TABLE 1 Absolute changes in body composition and endocrine biomarkers in LOW (1 g kg^{-1} d^{-1}) and HIGH (2 g kg^{-1} d^{-1}) supplementation groups before (pre), after 10-day military exercise (post-exercise) and following seven days of recovery (post-recovery)

	LOW			HIGH			
	Pre	**Post-exercise**	**Post-recovery**	**Pre**	**Post-exercise**	**Post-recovery**	**ES (95% CI)**
Body composition							
Body mass, scale (kg, n = 18, 19)	76.2 ± 12.2	71.6 ± 11.6*	75.3 ± 11.2$^{\$}$	75.9 ± 12.2	71.9 ± 11.9*	76.2 ± 11.2$^{\$}$	−0.38 (−1.06, 0.28)
Body mass, scale (kg, n = 12, 11)[a]	75.2 ± 11.9	70.5 ± 11.7*		77.1 ± 14.2	73.2 ± 13.3*		
Body mass, DXA (kg, n = 12, 11)	75.3 ± 11.4	71.4 ± 10.6*		78.2 ± 12.8	74.4 ± 13.2*		
Mean difference scale – DXA	0.15 ± 2.1	0.86 ± 2.0		1.06 ± 2.8	1.23 ± 0.5		
Fat mass (kg from scale)	13.8 ± 8.1	8.9 ± 7.3*		16.5 ± 6.1	11.7 ± 6.7*		−0.55 (−1.43, 0.32)
Fat-free mass, arms (kg from scale)	7.8 ± 1.5	7.7 ± 1.3		7.3 ± 1.5	7.3 ± 1.3		
Fat-free mass, legs (kg from scale)	20.8 ± 3.1	21.2 ± 2.9		20.9 ± 4.5	21.8 ± 3.9		
Fat-free mass, total body (kg from scale)	61.4 ± 8.5	61.5 ± 7.9		60.6 ± 11.7	61.5 ± 10.3		−0.37 (−1.24, 0.50)
Fat mass (kg from DXA)	13.2 ± 7.7	8.6 ± 7.0*		16.0 ± 5.6	11.3 ± 6.5*		−0.50 (−1.39, 0.37)
Fat-free mass, arms (kg from DXA)	7.4 ± 1.0.31	7.4 ± 1.1		7.1 ± 1.7	7.1 ± 1.2		
Fat-free mass, legs (kg from DXA)	19.8 ± 2.9	20.4 ± 2.6		20.3 ± 4.1	21.1 ± 3.7*		
Fat-free mass, total body (kg from DXA)	58.8 ± 7.8	59.5 ± 6.9		58.8 ± 10.5	59.7 ± 9.8		−0.12 (−0.99, 0.74)
Blood biomarkers							
TESTO (nmol L^{-1})	14.15 ± 2.63	4.44 ± 2.03*	16.21 ± 3.48$^{\$}$	13.58 ± 2.38	4.14 ± 2.14*	15.17 ± 4.19$^{\$}$	−0.06 (−0.67, 0.80)
Free TESTO	5.50 ± 1.70	0.99 ± 0.44*	4.63 ± 1.34$^{\$}$	5.20 ± 1.50	1.05 ± 0.50*	4.53 ± 1.12$^{\$}$	0.42 (−0.33, 1.17)
SHBG (nmol L^{-1})	30.30 ± 12.8	54.2 ± 20.2*	40.6 ± 12.8$^{*,\$}$	29.0 ± 8.99	41.0 ± 11.3*	37.4 ± 13.4*	1.65 (0.89, 2.42)
IGF-1 (nmol L^{-1})	23.00 ± 5.36	9.50 ± 2.49*	20.90 ± 4.51$^{*,\$}$	21.7 ± 5.53	8.98 ± 2.60*	19.1 ± 4.20$^{*,\$}$	−0.02 (−0.68, 0.63)
COR (nmol L^{-1})	397.57 ± 81.7	435.52 ± 111.32	380.55 ± 102.21	389.15 ± 148.61	529.21 ± 111.84$^{*,\#}$	362.78 ± 76.82$^{\$}$	−0.80 (−1.49, −0.12)
T3 (pmol L^{-1})	5.84 ± 0.45	4.04 ± 0.84*	5.17 ± 0.48$^{*,\$}$	6.00 ± 0.47	3.56 ± 0.86$^{*,\#}$	5.03 ± 0.53$^{*,\$}$	−0.67 (−0.01, 1.35)
T4 (pmol L^{-1})	17.36 ± 2.26	14.77 ± 3.10*	14.83 ± 2.03*	17.42 ± 2.14	13.70 ± 3.03*	14.21 ± 2.32*	−0.37 (−0.29, 1.03)
TSH (mIE L^{-1})	2.03 ± 0.72	2.04 ± 0.96	3.56 ± 1.24$^{*,\$}$	2.18 ± 0.92	2.06 ± 1.12	3.18 ± 1.90$^{*,\$}$	−0.24 (−0.41, 0.90)
CK (U L^{-1})	324.89 ± 205.72	3161.73 ± 1992.42*	129.16 ± 56.54$^{*,\$}$	421.84 ± 565.33	3876.63 ± 3786.72*	190.78 ± 144.62$^{*,\$}$	−0.40 (−1.1, 0.26)
TESTO/COR ratio	0.036 ± 0.01	0.010 ± 0.008*	0.050 ± 0.02$^{*,\$}$	0.043 ± 0.015	0.008 ± 0.004*	0.046 ± 0.016$^{\$}$	−0.43 (−0.30, 1.18)
Blood lactate							
Lactate (mmol L^{-1})	11.3 ± 1.5	7.7 ± 1.5*	10.0 ± 1.0$^{*,\$}$	11.8 ± 1.2	7.8 ± 1.7*	10.8 ± 1.0$^{*,\$}$	0.19 (−0.47 - 0.86)

Note: Lactate following Wingate 30-s sprint cycling. Effect size change score at post-exercise with confidence interval (ES (95% CI)).

Abbreviations: CK, creatine kinase; COR, cortisol; Free TESTO, free testosterone; IGF-1, insulin-like growth factor 1; SHBG, sex hormone-binding globulin; T3, Free T3; T4, Free T4; TESTO, Total testosterone; TSH, thyroid-stimulating hormone.

[a]Data from subjects with DXA measurement.

*$P < .05$ significantly different from pre.

$^{\$}P < .05$ significantly different from post-exercise.

$^{\#}P < .05$ significant change between groups

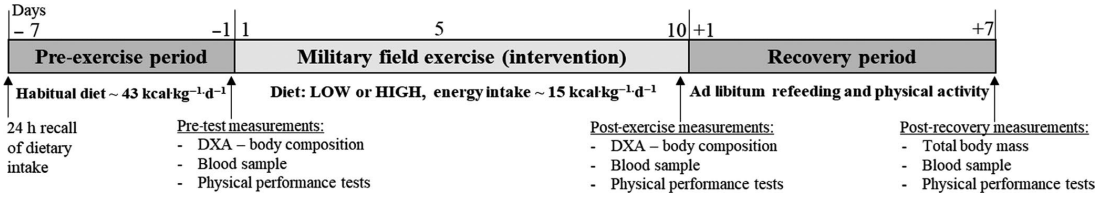

FIGURE 1 Overview of the intervention, including time points for collection of data on energy intake (24 h recall: 24 h recall of dietary intake), body mass composition (DXA; dual-energy X-ray absorptiometry), blood samples, and physical performance. During the intervention, participants were allocated to two different dietary programs, consisting of either HIGH (2 g kg^{-1} d^{-1}) or LOW (1 g kg^{-1} d^{-1}) amounts of protein, both providing 15 kcal kg^{-1} d^{-1}

of the exercise. At each time point, all tests were conducted within one test day and were supervised by trained personnel. All physical and biological tests were performed at all test days, except for measurement of body composition, fat mass, and fat-free mass (FFM), which was only performed pre- and post-exercise.

2.3 | Diet

Prior to the intervention, data on dietary intake were collected using 24 hours recall. These data were analyzed by a nutritionist using the international food database program "Dietitian Net Pro version." The reported macronutrient composition and energy intake (LOW, 3196 ± 996 kcal d^{-1}; HIGH, 3338 ± 1313 kcal d^{-1}; $P = .72$) provided the soldiers with a balanced diet with adequate levels of protein[13] (see Table 2). These estimates of energy intake corresponds well with predicted energy requirements in the two groups (LOW, 3425 ± 278 kcal d^{-1}; HIGH, 3394 ± 404 kcal d^{-1}; $P = .65$), calculated from age, sex, height, and total body mass (floor scale),[21] showing no difference from 24 hours recall data ($P = .69$). During the 10 days of exercise, the diet was restricted to ~15 kcal kg^{-1} d^{-1} (equivalent to a ~60% reduction in energy intake), which corresponds to the energy content of field rations utilized during prolonged military field exercise[1] and in weight-loss programs for athletes[14] (Table 2). In HIGH, the relative content of protein constituted a larger proportion and carbohydrate a lower proportion of the total energy intake than in LOW (Table 2). The daily energy intake for individuals were as follows: 900 kcal d^{-1} for individuals with pre-intervention weight of 56-65 kg, 1050 kcal d^{-1} for 66-75 kg, 1200 kcal d^{-1} for 76-85 kg, 1350 kcal d^{-1} for 86-95 kg, 1500 kcal d^{-1} for 96-105 kg, and 1650 kcal d^{-1} for 106-115 kg. Food was prepacked in rations to be ingested for breakfast (consumed between 08.00-10.00 h), lunch (15.00-17.00 h), and dinner (22.00-24.00 h), providing similar amounts of protein intake in every meal throughout the day. The modified rations typically contained white bread, egg, ham and 100% whey protein powder (35 g, chocolate, Proteinfabrikken, Norway). Participants were instructed to refrain from eating anything else. Rations were distributed to the soldiers every 2.5 days. The soldiers had free access to water throughout the exercise. Adherence to the provided rations was controlled through daily contact with the soldiers. Both soldiers and test personnel were blinded to supplementation group affiliation.

2.4 | Body composition and estimation of energy deficit

Lean body mass and fat mass were measured using DXA Lunar Prodigy densitometer (Prodigy Advance PA + 302 047, Lunar), using the standard scanning mode

TABLE 2 Mean and standard deviation for energy and macronutrient composition of 24 h recall conducted prior to intervention compared to the diet during the 10-day military field exercise for LOW and HIGH

	LOW (n = 18)		HIGH (n = 19)	
Time	24h recall prior intervention	Intervention diet	24h recall prior intervention	Intervention diet
Energy kcal d^{-1} (kcal kg^{-1} d^{-1})	3196 ± 996 (41.5 ± 13.7)	1183 ± 168 (15.2 ± 0.6)	3338 ± 1313 (43.4 ± 16.6)	1174 ± 170 (15.1 ± 0.6)
Carbohydrate g (g kg^{-1} d^{-1})	370.4 ± 122.9 (4.9 ± 1.8)	146.1 ± 16.1 (1.9 ± 0.1)	395.8 ± 179.6 (5.1 ± 2.2)	50.1 ± 10.6 (0.6 ± 0.0)[#]
Protein g (g kg^{-1} d^{-1})	159.2 ± 61.1 (2.1 ± 0.9)	79.2 ± 11.4 (1.0 ± 0.0)	144.8 ± 44.1 (2.0 ± 0.6)	156.5 ± 22.6 (2.0 ± 0.1)[#]
Fat g (g kg^{-1} d^{-1})	101.4 ± 48.8 (1.3 ± 0.6)	27.8 ± 6.9 (0.4 ± 0.0)	123 ± 61.3 (1.6 ± 0.8)	37.8 ± 4.4 (0.5 ± 0.0)

[#]$P < .05$ significantly different from LOW.

(13-25 cm). Analysis was performed using GE enCORE version 17.0 software (GE Healthcare). The soldiers were positioned supine within the marked lines on the scanning bed and a strap secured around the ankles to ensure standardized body position in each of the two scans, in accordance with the manufacturer. During the pre-test, soldiers were scanned in a fasted state between 07.00 and 09.00 AM, wearing limited clothing (boxer-short and sports top) and no jewelry. The post-exercise scan was performed 1-2 hours after finalization of the exercise. Before onset of each scanning session, a phantom scanning was conducted to prevent baseline drifting from affecting analyses. The same technician was used at both time points. As suggested by Nindl et al,[1] measurement of LBM should be carefully interpreted due to risk of overestimating soft tissue FFM in soldiers during extended periods of caloric deficit. Therefore, measures of FFM were calculated using a floor scale (SECA 770 Scale, Vogel & Halke) and DXA-derived percent body fat.[22] Accordingly, estimation of arm, legs, and truncus were calculated using regional mass relative to total body mass by subtracting equivalent regional percent body fat. Unfortunately, it was not possible to perform DXA scanning post-recovery, due to limited access to the equipment.

Energy deficit during the 10-day exercise was calculated based on DXA-estimated changes in fat mass and FFM using the equation from Westerterp et al[23]:

$$\text{Energy deficit (kcal d}^{-1}) = ((\Delta\text{fat mass} \times 38) * 238.846 + (\Delta\text{FFM} \times 6) * 238.846)/10 \quad (1)$$

where Δ is the change in fat mass or FFM in kg, the energy densities of fat mass and FFM are assumed to be 38 and 6 MJ d^{-1}, respectively. The factor 238 846 was used to convert megajoule into kilocalories and 10 represents the duration of the energy restriction period in days. Resting metabolic rate was calculated as described by Cunningham.[24]

2.5 | Blood samples

Fasting blood samples for hormonal analyses were obtained from an antecubital vein using serum-separating tubes, with soldiers resting in a supine position. At all three time points (pre-, post-exercise, and post-recovery), samples were taken at the same time of the day (between 08.00 and 10.00 AM). Blood samples were incubated for 30 minutes at room temperature before they were centrifuged at 1500 *g* for 10 minutes. Serum was aliquoted into Eppendorf tubes and immediately transferred to −80°C for storage until analyses. Serum concentrations of total testosterone (TESTO), cortisol (COR), insulin-like growth factor 1 (IGF-1), and sex hormone-binding globulin (SHBG) were measured using an Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics), using kits from the Immulite Immunoassay System menu (Siemens Medical Solutions Diagnostics), performed according to manufacturer's protocols. Free testosterone (Free TESTO) was calculated from testosterone and SHBG data as follows: free testosterone = 10 × testosterone/SHBG. Free triiodothyronine (T3), free thyroxin (T4), thyroid-stimulating hormone (TSH), and creatine kinase (CK) were measured using a Cobas 6000 (Roche Diagnostics/Hitachi SYSTEMS, Roche Diagnostics Norge AS). Reference intervals were as follows: TESTO (8.0-35.0 nmol L^{-1}), COR (138-690 nmol L^{-1}), IGF-1 (17-63 nmol L^{-1}), SHBG (8-100 nmol L^{-1}), T3 (3.1-6.8 pmol L^{-1}), T4 (8-20 pmol L^{-1}), TSH (0.27-4.20 mIE L^{-1}), and CK (35-400 U L^{-1}). Coefficient of variation (analytic) for the analyses were TESTO 14%, IGF-1 9%, SHBG 9%, COR 14%, T3 7%, T4 5%, TSH 4%, and CK 5%.

2.6 | Physical performance tests

Physical performance was measured using four functional tests, performed in the following order: counter-movement jump (CMJ), 1RM (one repetition maximum) leg press, 1RM bench press, and Wingate 30-second sprint power test. Each test session started with 10 minutes of general warm-up on a cycle ergometer, with intensities equivalent to 10-12 on the 6-20 Borg Rating of Perceived Exertion Scale.

Counter-movement jump height was performed on a force plate (SG-9, Advanced Mechanical Technologies, sampling frequency of 1 kHz). Throughout jumps, hands were placed on the hips and legs were placed with their individual hip width on the platform. The soldiers descended to a squat position of self-selected depth and immediately jumped upward as high as possible. If the third attempt resulted in the highest jump, an additional jump was performed. There were 30 seconds of rest between each jump. Participants were blinded to the results, and the best jump was used in data analyses.

Muscle strength of the lower and upper body was measured using 1RM. The 1RM test started with a specific warm-up, consisting of two sets with gradually increasing load (40% and 75% of expected 1RM) and decreasing number of repetitions (10 and 6). The first attempt was performed with a load approximately 5% below the expected 1RM. If a lift was successful, the load was increased by approximately 5%. For muscle strength of the lower body, a pneumatic bilateral seated leg press machine (Keiser A420, Keiser Sport Health Equipment Inc) was used. Briefly, the pneumatic equipment utilizes cylinders pressurized with air to provide different resistance. Soldiers were seated with knee and hip flexed at approximately 90°-96° and

45°, respectively. Approved 1RM efforts were defined as the maximal resistance that could be moved through the full range of motion with proper form one time. For upper-body strength, 1RM in bench press was performed. Soldiers were lying supine with their shoulders and hips kept in contact with the bench throughout the test and with their feet touching the floor. Efforts were accepted when the barbell smoothly touched the chest during the eccentric phase and the elbows were fully extended at the end of the concentric phase. Soldiers had 3-4 attempts with 2 minutes of rest between each lift for leg and bench press and the best attempt was used in data analyses. For each soldier, the same seating adjustment (leg press), body position, vocal encouragement, and supervisor were used during all tests.

Wingate 30-second sprint was performed on a cycle ergometer (Lode Excalibur Sport, Lode BV). The soldiers started pedaling at 100 W and 60 revolutions per minute for 30 seconds. Then, following a 3-second countdown, braking resistance was applied to the flywheel with a torque factor of 0.67 for females and 0.70 for males, which remained constant throughout the 30-second all-out test. Mean power output (W_{mean}) was defined as the average power output sustained throughout the 30 seconds, and peak power output was defined as the peak power (W_{peak}). Cyclists remained seated throughout the test and were given strong verbal encouragement. Cyclists were instructed to pedal as fast as possible from the start of the test and to avoid conserving energy for the last part of the test. Cyclists remained seated for one minute following the test, before blood was sampled from a fingertip and analyzed for whole blood [la^-] using Biosen C-line lactate analyzer (EKF Diagnostic BmbH, Barlebe, Germany). The seating position was adjusted according to each soldiers' preference for seat height, horizontal distance between tip of seat and bottom bracket, and handlebar position. For each soldier, identical seating positions were used at all test time points.

2.7 | Physical activity and sleep

Soldier recorded minutes spent on physical activity and sleeping on a daily basis. During the ten days of the military exercise, an average of 459 ± 273 min d^{-1} and 210 ± 111 min d^{-1} were spent on physical activity and sleep, respectively.

2.8 | Statistics

Data in text and figures are presented as mean ± standard deviation. The energy requirements from 24 hours recall were analyzed using a linear mixed-effect model,[25] with energy intake and macronutrient data acting as dependent variables and protein grouping and sex acting as main effects (fixed). To evaluate the effect of protein supplementation on body composition, physical performance, and blood markers (dependent variables), a linear mixed-effect model was utilized.[25] Interactions between groups and time points, as well as the interaction between fraction (arm, leg, truncus) for different segments of FFM, groups, sex, and time points were included as fixed effects in the model. The model included the maximal random effect structure justified by the data. Random by-subject slopes for the fraction effect were added to the model, thereby allowing fraction effect to vary by subjects. All models contained random intercept by subject. When there was an effect of time, a pairwise comparison was conducted with Satterthwaite correction. Effect size of protein supplementation was calculated with the following formula: ([HIGH mean – LOW mean]/LOW SD). The scale proposed by Rhea[26] for highly trained subjects was used to interpret the magnitude of the treatment effect; 0.0-0.24 trivial, 0.25-0.49 small, 0.5-1.0 moderate, >1.0 large. These analyses were run in R.[27] Significance level was set at $P = .05$ for all analyses.

In Wingate data, two significant outliers were detected by calculating z scores.[28] The two samples deviated by more than >3.0 standard deviations from the mean for both mean and peak power (z score −3.04, chisq $P = .0023$ and z score 3.21, $P = .001$, respectively, Figure 2). Models were therefore fitted with and without these outliers for mean power (fitted with outliers, estimate −28.08, standard error 12.94, $P = .04$; fitted without outliers, estimate −20.65, standard error 11.74, $P = .08$) and peak power (fitted with outliers; estimate −85.81, standard error 42.55, $P = .04$; fitted without outliers, estimate −75.20, standard error 42.65, $P = .08$), which resulted in a significant and non-significant interaction between the groups. Removal of the outliers were justified based on the observation exceeded the cutoff of $z \geq \pm 3.0$ standard deviation around the mean.[28]

3 | RESULTS

3.1 | Calculated energy expenditure and body composition

LOW and HIGH displayed similar total daily energy expenditure during the exercise, corresponding to 5536 ± 1305 kcal d^{-1} and 5427 ± 1029 kcal d^{-1} ($P = .86$), respectively, calculated from changes in fat mass/FFM and daily resting metabolic rates of 1699 ± 172 kcal d^{-1} and 1698 ± 222 kcal d^{-1}, respectively. With an energy intake corresponding to 1183 ± 168 kcal d^{-1} and 1174 ± 170 kcal d^{-1}, the daily energy deficit corresponded to -4373 ± 1250 kcal d^{-1} (LOW, $-77.0 \pm 1.8\%$) and -4271 ± 1075 kcal d^{-1} (HIGH, $-77.7 \pm 6.8\%$). After subtracting RMR from the total daily energy expenditure, this gives a field exercise-induced energy expenditure of 3836 ± 1290 kcal d^{-1} and

3769 ± 1106 kcal d^{-1} for LOW and HIGH, respectively ($P = .82$). This fits well with the estimated energy expenditure of the reported levels of physical activity during the exercise, amounting to 3800 kcal d^{-1} (7.6 h d^{-1} of low to moderate intensity, based on values from Tharion et al[29]).

Ten days of military field exercise led to decreased total body mass (using floor scale) and fat mass in LOW (−6.1 ± 2.4%, $P < .001$ and −40.5 ± 12.4%, $P < .001$, respectively) and HIGH (−5.2 ± 1.9%, $P < .001$ and −33.4 ± 13.3%, $P < .001$, respectively, Table 1), with no difference between groups. No changes were observed for FFM in either LOW or HIGH (0.5 ± 4.2%, $P = .79$, 1.9 ± 3.2%, $P = .20$, respectively). After 7 days of recovery, total body mass (floor scale) returned to pre-values in both LOW (−1.0 ± 3.2%, $P = .13$) and HIGH (0.6 ± 2.4%, $P = .75$, Table 1), with no difference between groups. Notably, similar estimates of body mass composition and changes thereof were seen when using DXA-based total body mass to calculate fat mass and FFM (rather than using floor scale, Table 1). The only exception was FFM of the legs, for which a significant increase was seen in HIGH only ($P < .05$, Table 1).

At baseline, male participants displayed higher body mass ($P = .02$) and higher FFM ($P < .001$) than females (independent of supplementation grouping), with FM being similar between sexes ($P = .58$, data not shown). There was no effect of sex on loss of body mass, FFM, and fat mass from pre- to post-exercise ($P = .17$, $P = .64$, $P = .15$, respectively, data not shown). Sex did not affect total daily energy expenditure ($P = .93$), exercise-induced energy expenditure ($P = .71$), or energy deficit ($P = .83$) at any time point. Female participants had significantly lower RMR (pre and post) than male ($P < .001$, data not shown).

3.2 | Blood markers

In both LOW and HIGH, 10 days of military field exercise led to decreased serum concentrations of TESTO (−68.2 ± 14.2%, $P < .001$ and −69.1 ± 15.3%, $P < .001$, respectively), free TESTO (−82.0 ± 6.42%, $P < .001$ and −78.3 ± 11.1%, $P < .001$, respectively), IGF-1 (−58.3 ± 8.7%, $P < .001$ and −58.1 ± 8.4%, $P < .001$, respectively), T3 (−30.4 ± 15.3%, $P < .001$ and −40.3 ± 13.9%, $P < .001$, respectively), T4 (−14.0 ± 18.1%, $P < .001$ and −20.0 ± 17.5%, $P < .001$, respectively), and TESTO/COR ratio (−69.6 ± 21.3%, $P < .001$ and −77.6 ± 15.2%, $P < .001$, respectively, Table 1). Similarly, both groups displayed increased concentrations of SHBG (82.2 ± 23.7%, $P < .001$ and 44.4 ± 21.9%, $P < .001$, respectively), COR (12.1 ± 33.1%, $P = .39$ and 55.6 ± 68.7%, $P < .001$, respectively), and CK (991 ± 617%, $P < .001$ and 1443 ± 1364%, $P < .001$, respectively, Table 1), with no changes for TSH (4.7 ± 43.2%, $P = .99$ and −4.9 ± 34.9%, $P = .90$, respectively, Table 1). For most variables, LOW and HIGH displayed similar changes. However, for T3 and COR, HIGH displayed a more pronounced decrease ($P = .02$) and increase ($P = .01$), respectively, compared to LOW (Table 1).

In general, after seven days of recovery, TESTO, free TESTO, SHBG, IGF-1, T3, COR, CK, and TESTO/COR ratio returned toward pre-values (or beyond) in LOW and HIGH ($P < .05$, Table 1). Only T4 remained at reduced levels compared to pre- (−14.2 ± 9.7%, $P = .99$ and −18.5 ± 7.2%, $P = .69$, respectively), resembling post-exercise levels. A couple of anomalies were detected in the post-recovery data set in both LOW and HIGH: CK was reduced to below pre-values (−49.9 ± 22.3% $P < .001$ and −23.2 ± 47.7%, $P = .006$, respectively), while TSH and the TESTO/COR ratio were increased to above pre-values (91.3 ± 76.4%, $P < .001$ and 50.3 ± 61.4%, $P = .002$, respectively; 40.3 ± 45.2%, $P = .008$ and 13.9 ± 42.6%, $P = .80$, respectively). There was no difference between LOW and HIGH for any of the blood variables at post-recovery.

3.3 | Physical performance

In both LOW and HIGH, the military field exercise led to decreased 1RM bench press (−9.5 ± 3.9%, $P < .001$ and −9.7 ± 5.4%, $P < .001$, respectively, ES = 0.04 (CI −0.62 to 0.72), Figure 3A), 1RM leg press (−7.8 ± 3.8%, $P < .001$ and −8.3 ± 4.7%, $P < .001$, respectively, ES = 0.13 (CI −0.52 to 0.79), Figure 3B), CMJ (−14.7 ± 6.7%, $P < .001$ and −14.6 ± 8.8%, $P < .001$, respectively, ES = −0.01 (CI −0.67 to 0.63), Figure 3C), Wingate mean power (−16.5 ± 5.4%, $P < .001$ and −18.8 ± 6.3%, $P < .001$, respectively, ES = 0.49 (CI −0.18 to 1.17), Figure 2A), Wingate peak power (−19.6 ± 9.5%, $P < .001$ and −25.1 ± 11.7%, $P < .001$, respectively, ES = 0.50, (CI −0.18 to 1.19), Figure 2B), and blood lactate levels after the Wingate 30-second sprint (−31.0 ± 11.6%, $P < .001$ and −33.5 ± 13.2%, $P < .001$, respectively, ES = 0.19 (CI −0.47 to 0.86), Table 1). There was no difference between LOW and HIGH for any of these variables.

After 7 days of recovery, both LOW and HIGH significantly increased strength and cycling power measurements variables toward pre-values, while CMJ remained at reduced level. (Figures 2 and 3). Compared to pre-exercise values, performance was still reduced in bench press (−5.4 ± 4.3%, $P < .001$ and −5.5 ± 5.6%, $P < .001$, respectively), leg press (−4.3 ± 4.6%, $P < .001$ and −4.8 ± 4.6%, $P < .001$, respectively), Wingate mean power (−5.2 ± 2.9%, $P = .002$ and −5.6 ± 4.1%, $P < .001$, respectively), and Wingate peak power (−8.0 ± 9.3%, $P < .01$ and −11.9 ± 7.3%, $P < .01$, respectively). This was also the case for blood lactate levels measured after Wingate 30-second sprint (−10.4 ± 11.4%, $P < .001$ and −8.2 ± 10.7%, $P < .001$, respectively). However, compared to post-exercise, performance was improved for all

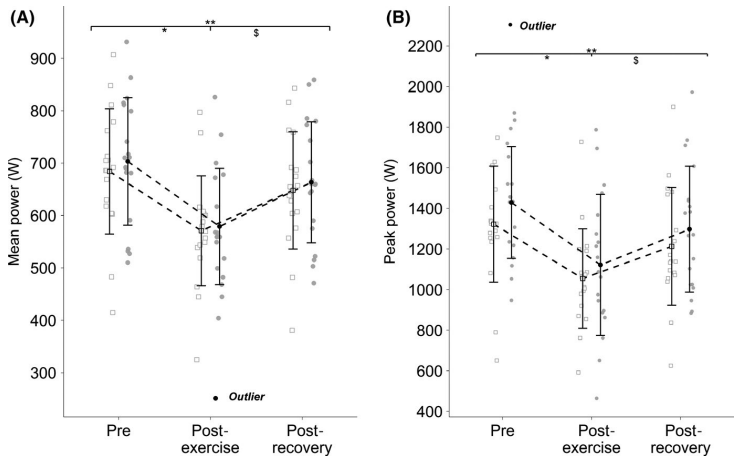

FIGURE 2 Absolute changes in Wingate 30-s sprint mean power (panel A) and Wingate 30-s sprint peak power (panel B) from before exercise (pre), after 10-day of exercise (post-exercise) and seven days of recovery (post-recovery) for LOW (white squares) and HIGH (black circles) protein supplementation groups. Mean ± SD. Additional are each participant individual data points visualized for LOW (white squares) and HIGH (black circles) at the three time points whereas the two outliers are highlighted. $P < .05$ * significantly different from pre- to post-exercise. $P < .05$ $ significantly different from post-exercise to post-recovery. $P < .05$ ** significantly different from pre

these variables ($P < .05$). In contrast, seven days of recovery had no effect on CMJ (LOW −16.8 ± 7.0%, $P = .43$; HIGH, −13.0 ± 6.4%, $P = .75$). There was no difference between LOW and HIGH for any of the performance variables at post-recovery.

At baseline, male participants displayed higher baseline levels for all performance variables than females ($P < .05$, independent of supplementation grouping, data not shown). In males, 10 days of military field exercise led to greater decline in 1RM bench press ($P < .001$), Wingate mean power ($P < .001$), Wingate peak power ($P < .001$), and CMJ ($P = .02$), and a tendency toward greater decline in 1RM leg press ($P = .06$) compared to females (data not shown). After seven days of recovery, male participants displayed less pronounced recovery in 1RM bench press ($P < .001$, normalized to pre-values) and CMJ ($P = .01$, normalized to pre-values).

4 | DISCUSSION

In this study, 10 days of military exercise with HIGH intake of protein and low intake of carbohydrate led to similar decreases in physical performance as LOW intake of protein and low intake of carbohydrate, measured as counter-movement jump height, maximal strength, and cycling sprint power. There was no benefit of ingesting more protein on muscle functionality in a setting with severe energy deficit and physical activity, supporting findings from a previous study.[14] Surprisingly, FFM remained unchanged from pre- to post-exercise in both groups. This contradicts most previous studies,[1,2,6,7,9] though it is supported by others,[5,6] a discrepancy that may be related to considerable variations in study design, including varying degrees of energy deficiency.[5,6,11,14,18] It is important to note that in the present study, FFM measurements were associated with methodological uncertainty connected to the timing of post-exercise scanning as discussed in a later paragraph. In general, HIGH and LOW led to similar declines in blood concentrations of anabolic and pro-metabolic hormones (eg, testosterone and IGF-1) and markers of muscle damage (creatine kinase), with only T3 and COR showing differential responses between groups. The relatively marked changes in blood variables are in accordance with previous studies on the physiological effects of intense military exercise.[1,3,6,7,30] Seven days of recovery led to improved performance toward pre-exercise values (eg, leg press, LOW −4.3%, HIGH −4.8%; Wingate mean power, LOW −5.2%, HIGH −5.6%), except for CMJ, which remained at reduced levels (LOW −16%, HIGH −13%). Similarly, concentrations of hormones generally returned toward or beyond resting physiological levels (eg, COR, LOW −2.8%, HIGH 3.2%; TESTO, LOW 19.2%, HIGH 11.8%), resembling pre-exercise values.

The severe level of energy deficiency experienced by the soldiers may explain the lack of beneficial effects of higher protein ingestion on performance, giving support to some studies, [14,31] but contrasting other studies in soldiers[5,6] and athletes undergoing weight loss.[15,16,32] The resulting catabolic physiological environment may have counteracted anabolic signaling events, which arguably was more pronounced in HIGH, caused by the likely higher

amino acid availability from exogenous protein intake. Importantly, HIGH experienced severe carbohydrate deficit in addition to the general energy deficit (habitual intake 5.1 g kg^{-1} d^{-1} vs diet intervention 0.6 g kg^{-1} d^{-1}). This may have impaired any positive effects of higher protein intake by further increasing the need for gluconeogenesis (ie, through amino acids) in order to sustain energy homeostasis.[31,33,34] This being said, relative levels of energy deficit seems to be more decisive for whole-body protein loss[17,18] and performance[35] than macronutrient composition in a state of severe energy deficit. However, this generalized perspective may not always be true. For example, in overweight subjects undergoing a four-day intervention with severe energy deficit (~−94%), ingestion of a sucrose-only solution (dissolved in water) led to greater preservation of leg-pedaling performance than ingestion of protein only.[31] Notably, even in LOW, carbohydrate intake was in the lower range of what is recommended (habitual intake 4.9 g kg^{-1} d^{-1} vs diet intervention 1.9 g kg^{-1} d^{-1}), suggesting that any carbohydrate-specific effects on performance and body mass should have been present also in this group. The design of the present study demanded pre-fixed protein intake (HIGH or LOW) combined with a low-energy diet (eg, 900 kcal). As we had limited access to high-protein foods, we were unable to produce food packages that contained different amounts of protein while at the same time sustaining similar amounts of carbohydrates (see Table 2).

Surprisingly, neither HIGH nor LOW displayed changes in FFM in response to the military exercise, despite substantial impairment in muscle performance. This suggests that the amount of muscle mass was unaffected by the intervention, which contrasts findings in most previous studies,[1,2,6,7,9,36,37] some of which even involved similar[36,37] or less severe energy deficit and shorter duration compared to the present study.[7,9,36,37] Conversely, our perspective data are supported by Tanskanen et al,[5] wherein 8 days of military exercise did not lead to decreases in FFM, though also in that study the intervention involved less severe energy deficit (<50%) and higher energy intake and had shorter duration. It thus seems inappropriate to draw firm conclusions based on the sustained levels of FFM in the present study. Rather, it may have resulted from methodological artefacts, such as the timing of the post-exercise DXA analysis, which was conducted immediately after finalization of the exercise. Indeed, it seems plausible that levels of physical activity toward the end of the exercise led to redistribution of body fluids to working muscle and changes in hydration status (eg, blood volume/swelling), which in turn may have violated the soft tissue coefficient, and thus the estimation of FFM obtained during DXA scanning.[38] Notably, DXA-based FFM measurements are also sensitive to depletion of carbohydrate stores in skeletal muscle, which is typically accompanied by tissue dehydration. Because our participants likely displayed severe

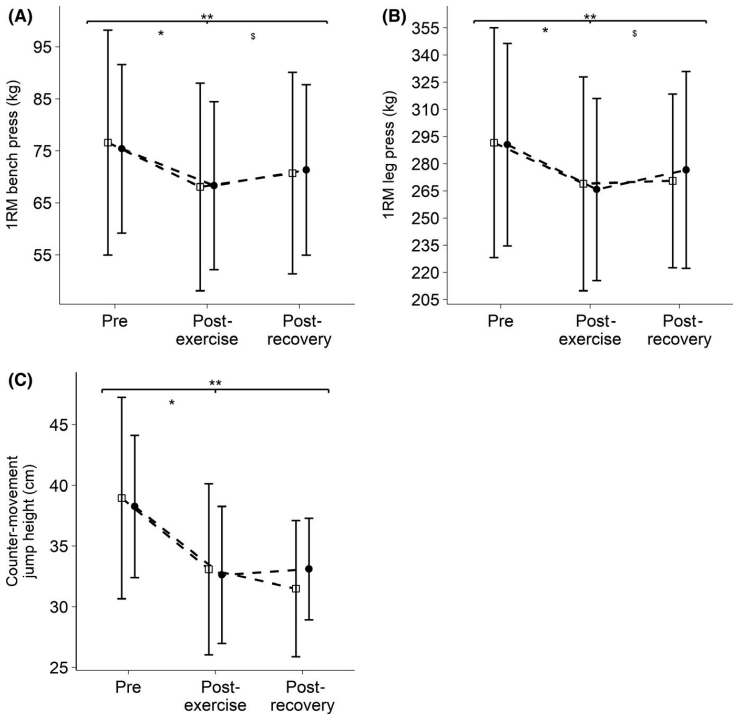

FIGURE 3 Absolute changes in 1RM bench press (panel A), 1RM leg press (panel B), and jump height (CMJ, panel C), from before exercise (pre), after 10-day of exercise (post-exercise) and 7 days of recovery (post-recovery) for LOW (white squares) and HIGH (black circles) protein supplementation groups. Mean ± SD. $P < .05$ * significantly different from pre- to post-exercise. $P < .05$ $ significantly different from post-exercise to post-recovery. $P < .05$ ** significantly different from pre

depletion of carbohydrate stores at the time point of the DXA scan this may have affected FFM data. However, this should have led to underestimation of FFM level, opposing the potential overestimation caused by the timing of DXA scanning,[39] warranting further caution upon interpretation of FFM estimates. Importantly, however, DXA-derived estimates of total body mass post-exercise did not differ from floor scale based measurements (Table 1).

Regardless of these potential pitfalls in FFM estimates, our data did not reveal a beneficial effect of increased ingestion of protein on changes in FFM (though there was a low effect size of HIGH compared to LOW). This lack of an effect may also be related to the study design, as HIGH and LOW were on equally energy-restricted diets throughout the 10-day exercise, exposing soldiers in the two groups to similar energy-dependent catabolic signaling. This perspective is supported by a recent study,[18] wherein an intervention with similar dietary groups (1 g kg^{-1} d^{-1} protein vs 2 g kg^{-1} d^{-1} protein, isocaloric) and similar levels of energy deficiency (−70%) disclosed no effect of additional protein intake on FFM. The authors thus concluded that increased protein intake during prolonged periods of negative energy balance seems to be used for energy metabolic purposes,[18] which is also supported from findings in another protein supplementation study using isocaloric energy-restricted diet.[40] Indeed, most studies that reveal beneficial effects of increased protein ingestion on FFM during prolonged periods of energy restriction intake,[5,6] involve intake of surplus protein as an additive to the regular diet, with a concomitant increase in overall energy intake.

Observed changes in endocrine variables in response to the military exercise, such as decreased levels of androgen hormones (TESTO, free TESTO, IGF-1, TESTO/COR ratio) and pro-metabolic hormones (T3 and T4) and increased levels of COR, suggest development of a catabolic physiological milieu, resembling observations made in previous studies.[1,3,6-8] This may halt cellular growth and proliferation, while allocating available energy resources toward basal metabolic demands.[3,17,41] Accordingly, during the exercise the soldiers were in a maladaptive state, with reduced ability to repair muscle tissue and sustain adequate tissue functions, providing a potential explanation for the observed impairment in physical performance. For most of the endocrine variables, there was no beneficial effect of higher protein ingestion, strengthening the notion that the severe energy deficiency was more decisive for responses to the exercise than amino acid and carbohydrate availability, as carbohydrate has a protein-sparing effect and vice versa.[42] Two observations provide further insight into this; the elevated levels of COR in HIGH and the reduced levels of T3 in HIGH (both compared to LOW). These adaptations seem counterintuitive given the potential benefit of increased protein intake for anabolic metabolism. However, these adaptations may have been necessary responses to the lowered availability of exogenous carbohydrates in HIGH (see Table 2), leading to cortisol-induced increases in gluconeogenesis through exploitation of endogenous fat stores,[3] while simultaneously lowering whole-body metabolic rate.[8]

The observed impairment in physical performance in response to 10 days of military exercise is in line with results from other studies assessing the effect of periods of near-continuous physical activity, sleep deprivation and underfeeding on muscle strength and power.[1,2,6,7,9,30] In these studies, the extent of the impaired performance co-varies with the severity of the intervention, including its length[1,2,9,30] and its degrees of energy deficiency,[5,6] as well as with differences in the timing of post-exercise testing, varying from 2 to 24 hours.[5,6,9,30] This makes it difficult to evaluate and compare results across studies. Data from the present study are in the outer-most part of the specter, despite a relatively low level of physical exhausting activities during the intervention and a relatively short duration compared to other studies.[1,2,6,7] It is thus reasonable to assume that the pronounced impairment in muscular performance was due to the substantial energy deficit, which was estimated to −4320 kcal d^{-1} (overall energy deficit: −43203 kcal, ~−77%), calculated from changes in fat mass (−4.9 ± 1.4 kg) and FFM (0.5 ± 2.2 kg), resulting in more pronounced loss of fat mass than in many other studies.[2,5,6,8,9,18,30] This is in agreement with a recent meta-analysis of data from nine military field exercise studies, which observed a decline in lower-body power and strength as an overall effect of daily energy deficit combined with exercise duration.[43] The authors concluded that the total energy deficit of military exercises/operations should not exceed −5000 to −19 000 kcal in order to limit negative effects on physical performance.[43] When energy deficit exceeds 40 000-60 000 kcal, moderate to large declines can be expected in physical performance, corroborating well with data from the present study.[43]

The association between performance and energy status is evident as two observations. First, the decreased performance during 30-second cycling sprint at post-exercise was accompanied by decreased levels of blood lactate, suggesting lowered availability of glucose.[44] It thus seems likely that muscle glycogen stores in skeletal muscle were heavily depleted. This assumption is reasonable, as military field exercise has been shown to lead to 50% reduction in CHO content of muscle tissue after only 4 days,[45] in an experimental setting involving higher energy intake than the present study (3×) and higher relative intake of CHO (65% vs 17% in HIGH and 49% in LOW). This would attenuate the ability to generate muscle tension and reduce the number of physiological contractile muscle fibers at any given time point, effectively reducing the amount of metabolic end-products and reducing the ability to generate maximal force during anaerobic performance tests.[34,35] Second, the increased rested-state

CK values post-exercise suggests an inability to sustain and repair muscle functions and may explain the overall reduction in performance. The increase in CK levels may also have affected performance in a more direct manner by inhibiting afferent neural feedback from muscle spindles, thereby reducing neuromuscular efficiency and maximal force-generation capacity.[3,9,46] As a side note, male participants displayed larger declines in performance during the military exercise than did female participants (independent of protein grouping) and also displayed a slower rate of recovery. In previous studies, this phenomenon has been associated with a larger loss of FFM in men,[47] potentially driven by a smaller metabolic contribution from fat,[47,48] and hence a larger contribution from other sources such as proteins. While this remains a potential explanation also in this study, we did not disclose sex-dependent differences in FFM changes, potentially related to methodological issues with our FFM estimates. Nor did we disclose sex-dependent differences in fat mass changes ($P = .15$), which should have been present if energy metabolism in female participants were indeed more reliant on fat. The small sample size of females in the present study ($n = 7$) and our selection of outcome measures and methods makes it difficult to conclude on this perspective.

After 7 days of recovery, body mass and most of the performance and endocrine variables had returned toward pre-exercise values. Increased protein intake during the field exercise did not affect recovery of any of the variables,[20] supporting the notion that protein dosage did not affect physiological responses to the exercise. The effectiveness of the recovery period was probably due to restoration of energy intake and rest, resembling observations made in previous studies on military exercises.[2,3,7,49] As an example, after the recovery period, the TESTO/COR ratio were actually higher than at pre-exercise, suggesting increased need for, and occurrence of, cellular growth and repair.[3] CMJ was the only variable that did not recover effectively, remaining at reduced post-exercise levels. This resembles the finding in Hamarsland et al,[7] wherein CMJ remained at reduced levels even after two weeks of recovery from an intense military hell week in military personnel. In another study, CMJ fully recovered after 5 weeks.[2] The prolonged recovery of CMJ may be due to reduced functions of muscle spindles, possibly linked to elevated CK concentrations and/or muscle fiber damage.[50,51] This may impair the stretch reflex, which is an important contributor during CMJ,[51] leading to delayed maximal shortening velocity and power.[46]

4.1 | Limitations

The present study comes with a few limitations. For example, we used dietary recall (24 hours) to provide data on dietary intake and steady-state energy requirements. Such self-report energy intake can lead to underestimation of the true energy requirement, as caused by underreporting.[52] This being said, the reported energy intake was similar to the energy requirement calculated from anthropometric data (diff: -21 ± 1033 kcal, $P = .69$). The validity of our measure of energy intake also gains support from the relationship between basal energy deficiency/physical activity levels during the exercise and the accompanying loss of fat mass, with both perspectives giving similar measures of energy deficiency. During the exercise, adherence to the diet plan was facilitated by providing the soldiers with ready-to-eat food packages. Arguably, this mitigated the need for dietary recall measurement during the intervention itself (other than whether or not they had eaten the meal), while at the same time providing a feasible manner of blinding participants (and project staff) to supplement grouping. Unfortunately, we were not able to obtain dietary data during the 7 days recovery period due to a tight school schedule. However, the substantial restoration of performance level, endocrine markers, and body mass from post-exercise to post-recovery suggests adequate levels of energy intake during this period.

Information about physical activity levels during the field exercise was also collected in a self-reported manner, as opposed to other alternatives such as using accelerometers, in turn providing suboptimal measures of energy expenditure. Again, the validity of these data gains supported from their seeming ability to explain the observed loss of fat mass during the intervention (together with the overall energy intake). Moreover, as all participants took part in the same activities, only small differences would have been present between participants, with no likely significance for comparisons between LOW and HIGH, which were the main objective of the study.

As previously discussed, the timing of DXA measurements may have compromised the validity of FFM data. Unfortunately, it was not possible to perform DXA measurements at any other time points (or at surplus time points), as we had limited access to the apparatus. However, once again, these uncertainties should not have affected LOW vs HIGH analyses. Finally, this study was conducted on a relatively small population of Norwegian soldiers. This reduces the external validity in terms of predicting future responses in other groups of military personnel, particularly for the observed differences in responses between sexes, as we only had seven female participants. There is need for more studies to elaborate on the differences in responses to military field exercises with severe energy deficit between males and females.[47]

In conclusion, 10-day of military field exercise in a state of energy deficiency led to loss of body mass, impaired physical performance and a switch toward a catabolic physiological milieu in soldiers. Increased intake of protein did not counteract these changes. Rather, the increased protein likely entered the overall energy metabolism, acting to compensate

for the substantial energy deficit, elevated energy needs and low carbohydrate availability. After seven days of recovery, most variables had returned to close-to pre-exercise levels, except for CMJ, which remained at reduced levels, suggesting impaired stretch-reflex functionality.

5 | PERSPECTIVES

This study provides novel insight into nutritional strategies for optimizing performance during strenuous military exercises. In face of substantial energy deficit, increased protein intake does not seem to counteract impairments in performance or alterations in body mass composition, at least not within the investigated timeframe. If the purpose is to maintain muscle performance, it therefore seems more pertinent to increase the total energy intake than to tweak the relative macronutrient composition of the diet (within the context of an appropriately balanced diet), ensuring the combat readiness of soldiers during prolonged military field exercises with substantial energy deficit.

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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Appendix I

Approval letter from NSD for paper I

Norsk samfunnsvitenskapelig datatjeneste AS
NORWEGIAN SOCIAL SCIENCE DATA SERVICES

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Vår dato: 04.09.2014 Vår ref: 36930 / 3 / LB Deres dato: Deres ref:

TILBAKEMELDING PÅ MELDING OM BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 07.01.2014. Meldingen gjelder prosjektet:

36930	*Kvinner og styrketrening – kan lite være nok?*
Behandlingsansvarlig	*Høgskolen i Lillehammer, ved institusjonens øverste leder*
Daglig ansvarlig	*Stian Ellefsen*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, ombudets kommentarer samt personopplysningsloven og helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, http://www.nsd.uib.no/personvern/meldeplikt/skjema.html. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, http://pvo.nsd.no/prosjekt.

Personvernombudet vil ved prosjektets avslutning, 31.12.2020, rette en henvendelse angående status for behandlingen av personopplysninger.

Vennlig hilsen

Katrine Utaaker Segadal

Lene Christine M. Brandt

Kontaktperson: Lene Christine M. Brandt tlf: 55 58 89 26
Vedlegg: Prosjektvurdering

Personvernombudet for forskning

Prosjektvurdering - Kommentar

Prosjektnr: 36930

FORMÅL, UTVALG OG INFORMASJON
Hovedformålet med studien er å sammenligne effekten av 12 uker med ett setts og tre setts tung styrketrening, på funksjonell prestasjonsevne og cellebiologiske trekk i beinmuskulatur, hos utrente kvinner. Det vil rettes særlig fokus på kartlegging av faktorer som er bestemmende for trenbarhet. Etter vedtak fra REK sør-øst 18.12.2013 faller prosjektet utenfor helseforskningsloven.

Utvalget vil bestå av kvinner, og menn vil bli inkludert som referansegruppe. Rekruttering vil i hovedsak skje blant studenter på høgskolen. Den vil foregå ved arrangering av informasjonsmøter, utsending av e-poster, opprettelse av Facebook-grupper, oppslag i lokale medier og opphenging av plakater på skolen og studentboliger. De som melder sin interesse vil få tilsendt informasjonsskriv om prosjektet, og bli invitert til særskilte informasjonsmøter der de vil få ytterligere informasjon og vil bli gitt mulighet til å stille spørsmål. Utvalget informeres altså både skriftlig og muntlig om prosjektet og samtykker til deltakelse. Informasjonsskrivet av 26.06.2014 er godt utformet, forutsatt at det er presisert at alt materialet tilhørende prosjektet slettes og anonymiseres senest ved prosjektslutt 31.12.2020, jf. epost til prosjektleder 20.06.2014.

DATAMATERIALET
Data samles inn gjennom spørreskjema, fysiske tester og målinger, samt vevsprøver fra lårmuskulatur og blodprøver. Det behandles sensitive personopplysninger om helseforhold, jf. personopplysningsloven § 2 nr. 8 c).

Personvernombudet forutsetter at det foreligger nødvendig godkjenning for bruken av det biologiske materialet i dette prosjektet.

INFORMASJONSSIKKERHET
Datamaterialet vil bli oppbevart på institusjonens forskningsserver. Herfra vil det kobles opp mot PC'er som disponeres av autorisert personell og som eies av virksomheten. PC'ene som vil kunne kobles opp mot forskningsserveren vil være bærbare ansatt-PC'er. Det biologiske materialet vil bli oppbevart i låsbar fryser situert på låst rom. Personvernombudet legger til grunn at forsker etterfølger Høgskolen i Lillehammer sine interne rutiner for datasikkerhet. Koblingsnøkkelen mellom forsøkspersonenes personalia og referansenummer vil bli oppbevart i låst skap på låst kontor (i papirformat), samt i institusjonens elektroniske saksbehandlings- og arkivsystem (Ephorte), i brukeravgrenset mappe.

PROSJEKTSLUTT
Datamaterialet anonymiseres senest innen 31.12.2020. Vi minner om at anonymisering innebærer å bearbeide datamaterialet slik at ingen enkeltpersoner kan gjenkjennes. Det gjøres ved:
- å slette direkte personopplysninger (som navn/koblingsnøkkel)
- og slette/omskrive indirekte personopplysninger (identifiserende sammenstilling av bakgrunnsopplysninger)
- å destruere biologisk materiale

Appendix II

Approval letter from NSD for paper II

Daniel Hammarström
pb 400
2418 ELVERUM

Vår dato: 13.09.2017 Vår ref: 55300 / 3 / LAR Deres dato: Deres ref:

Tilbakemelding på melding om behandling av personopplysninger

Vi viser til melding om behandling av personopplysninger, mottatt 11.08.2017.
Meldingen gjelder prosjektet:

55300	*Effekten av treningsvolum og midlertidig treningsavbrudd på muskulære tilpasninger til styrketrening*
Behandlingsansvarlig	*Høgskolen i Innlandet, ved institusjonens øverste leder*
Daglig ansvarlig	*Daniel Hammarström*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, ombudets kommentarer samt personopplysningsloven og helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database.

Personvernombudet vil ved prosjektets avslutning, 01.10.2019, rette en henvendelse angående status for behandlingen av personopplysninger.

Dersom noe er uklart ta gjerne kontakt over telefon.

Vennlig hilsen

Dokumentet er elektronisk produsert og godkjent ved NSDs rutiner for elektronisk godkjenning.

Marianne Høgetveit Myhren

Lasse André Raa

Kontaktperson: Lasse André Raa tlf: 55 58 20 59 / Lasse.Raa@nsd.no
Vedlegg: Prosjektvurdering

Personvernombudet for forskning

Prosjektvurdering - Kommentar

Prosjektnr: 55300

FORMÅL
Hensikten med denne forskningsstudien er å forstå hvordan styrketrening påvirker nydanning av ribosomer, med særlig fokus på betydningen av endringer i treningsvolum og treningsavbrudd. Studien vil øke vår forståelse for hvordan planlegge og gjennomføre styrketrening for å oppnå størst mulig treningseffekt for den enkelte.

METODER OG DATA
Data samles inn via papirbasert og elektronisk spørreskjema og fysiologiske tester, og biologisk materiale i form av styrketreningsintervensjoner, blodprøver og muskelbiopsier. Sistnevnte skal etterhvert overføres til den generelle biobanken "The TrainsOME -humane cellers tilpasning til trening og miljø" som er godkjent av REK (REK-2013/2045).

Det behandles sensitive personopplysninger om helseforhold. Det bør utøves særlig forsiktighet ved behandling av sensitive personopplysninger, både når det gjelder etiske problemstillinger, innhenting av data og informasjonssikkerhet underveis.

Det er oppgitt at det ikke skal benyttes databehandler. Vi gjør oppmerksom på at dersom det benyttes en ekstern leverandør av spørreskjema, regnes dette som en databehandler. Høgskolen i Innlandet skal i så tilfelle inngå skriftlig avtale med databehandler om hvordan personopplysninger skal behandles, jf. personopplysningsloven § 15. For råd om hva databehandleravtalen bør inneholde, se Datatilsynets veileder: http://www.datatilsynet.no/Sikkerhet-internkontroll/Databehandleravtale/

INFORMASJON OG SAMTYKKE
Utvalget informeres skriftlig og muntlig om prosjektet og samtykker til deltakelse. Informasjonsskrivet er godt utformet.

DATASIKKERHET
Personvernombudet legger til grunn at forsker etterfølger Høgskolen i Innlandet sine interne rutiner for datasikkerhet. Dersom personopplysninger skal sendes elektronisk, bør opplysningene krypteres tilstrekkelig.

VEDRØRENDE FREMLEGGELSESPLIKT FOR REK
Personvernombudet oppfatter prosjektet som å ligge i randsonen for hva som omfattes av helseforskningsloven eller ikke. På basis av opplysninger i meldeskjemaet forstår vi det imidlertid slik at det tilhører en type prosjekt som ved tidligere fremleggelsevurderinger/søknader til REK er blitt vurdert til ikke å omfattes av helseforskningsloven, slik at prosjekter med lignende formål ikke trenger å meldes til REK.

BIOLOGISK MATERIALE

Personvernombudet registrerer at Høgskolen i Innlandet (Høgskolen i Lillehammer) er gitt tillatelse til opprettelse av en generell biobank for oppbevaring av humant biologisk "The TrainsOME -humane cellers tilpasning til trening og miljø", godkjent av REK 2013/2045. Personvernombudet legger til grunn at det biologiske materiale fra prosjektet oppbevares i den generelle biobanken og anbefaler at det utarbeides dokumentasjon for oppbevaringen av det biologiske materialet. Videre anbefaler vi at Høgskolen i Innlandet orienterer REK sør-øst om at det biologiske materialet som innsamles vil inkluderes i den generelle biobanken REK sør-øst har gitt tillatelse til.

PROSJEKTSLUTT

Forventet prosjektslutt er 01.10.2019. Ifølge prosjektmeldingen skal innsamlede opplysninger da anonymiseres. Anonymisering innebærer å bearbeide datamaterialet slik at ingen enkeltpersoner kan gjenkjennes. Det gjøres ved å:

- slette direkte personopplysninger (som navn/koblingsnøkkel)
- slette/omskrive indirekte personopplysninger (identifiserende sammenstilling av bakgrunnsopplysninger som f.eks. bosted/arbeidssted, alder og kjønn)
- destruere biologisk materiale

Dersom det benyttes en databehandler, må databehandleren også slette personopplysninger tilknyttet prosjektet i sine systemer. Dette inkluderer eventuelle logger og koblinger mellom IP-/epostadresser og besvarelser.

Appendix III

Approval letter from NSD for papers III and IV

Norsk samfunnsvitenskapelig datatjeneste AS
NORWEGIAN SOCIAL SCIENCE DATA SERVICES

Harald Hårfagres gate 29
N-5007 Bergen
Norway
Tel: +47-55 58 21 17
Fax: +47-55 58 96 50
nsd@nsd.uib.no
www.nsd.uib.no
Org.nr: 985 321 884

Stian Ellefsen
Avdeling for samfunnsvitenskap Høgskolen i Lillehammer
Postboks 952
2604 LILLEHAMMER

Vår dato: 19.08.2015 Vår ref: 43901 / 3 / MHM Deres dato: Deres ref:

TILBAKEMELDING PÅ MELDING OM BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 28.06.2015. All nødvendig informasjon om prosjektet forelå i sin helhet 10.08.2015. Meldingen gjelder prosjektet:

43901	*Bedring av fysisk og mental yteevne gjennom styrke- og utholdenhetstrening*
Behandlingsansvarlig	*Høgskolen i Lillehammer, ved institusjonens øverste leder*
Daglig ansvarlig	*Stian Ellefsen*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, ombudets kommentarer samt personopplysningsloven og helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, http://www.nsd.uib.no/personvern/meldeplikt/skjema.html. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, http://pvo.nsd.no/prosjekt.

Personvernombudet vil ved prosjektets avslutning, 31.12.2023, rette en henvendelse angående status for behandlingen av personopplysninger.

Vennlig hilsen

Katrine Utaaker Segadal

Marianne Høgetveit Myhren

Kontaktperson: Marianne Høgetveit Myhren tlf: 55 58 25 29
Vedlegg: Prosjektvurdering

Dokumentet er elektronisk produsert og godkjent ved NSDs rutiner for elektronisk godkjenning.

Personvernombudet for forskning

Prosjektvurdering - Kommentar

Prosjektnr: 43901

FORMÅL
Formålet med studien er å kartlegge effekter av ulike typer styrke- og utholdenhetstrening på fysisk og kognitiv yteevne hos studenter ved Forsvarets ingeniørhøgskole, Jørstadmoen. Individuelle og gruppevise aspekter vil være i fokus.

UTVALG OG INFORMASJON
Utvalget informeres skriftlig om prosjektet og samtykker til deltakelse. Utvalget får også informasjon og forespørsel om å samtykke til at vevs- og blodprøver avgis til en generell forskningsbiobank "The TrainsOme". Informasjonsskrivene er godt utformet.

DATAINNSAMLING
Data samles inn ved spørreskjema, psykologiske/pedagogiske tester og biologiske prøver (blod- og vevsprøver). Sistnevnte skal etterhvert overføres til den generelle biobanken "The TrainsOME -humane cellers tilpasning til trening og miljø" som er godkjent av REK (REK-2013/2045). Det behandles sensitive personopplysninger om helseforhold.

DATASIKKERHET
Personvernombudet legger til grunn at forsker etterfølger Høgskolen i Lillehammer sine interne rutiner for datasikkerhet. Dersom personopplysninger skal sendes elektronisk, bør opplysningene krypteres tilstrekkelig.

VEDRØRENDE FREMLEGGELSESPLIKT FOR REK
Prosjektleder har gjennomført flere prosjekter, også med opprettelse av forskningsbiobank, som ligger i randsonen for hva som omfattes av helseforskningsloven og ikke. I den forbindelse har prosjektleder vært i dialog med REK sør-øst. Ved tidligere fremleggelsevurderinger/søknader til REK har prosjektleder fått beskjed om at prosjektene ikke omfattes av helseforskningsloven og at prosjekt med lignende formål ikke trenger å meldes til REK. Dette prosjektet er derfor ikke meldt til REK jf. telefonsamtaler 5.8. og 10.8.2015.

Denne tilbakemelding får også betydning for forskningsbiobanken som opprettes i prosjektet.

FORSKNINGSBIOBANKEN
Personvernombudet har ved en tidligere anledning forhørt seg med REK sør-øst og NEM angående hvilket regelverk som regulerer opprettelse av en forskningsbiobank til forskningsformål som ikke innebærer medisinsk og helsefaglig forskning på mennesker.

Tilbakemeldingene slik vi forstår det er at regelverket er noe uklart og at Helsedepartementet finner at det kan være hensiktsmessig å se nærmere på praktiseringen av lovverket knyttet til forskningsprosjekter som befinner seg i randsonen av helseforskningslovensvirkekområde.

Men frem til en nærmere avklaring og for at disse prosjektene ikke skal havne i et lovtomt rom, har personvernombudet mottatt epost fra NEM, 18.12.2014. Her opplyser NEM at helseforskningslovens § 25 annet ledd ikke kommer til anvendelse, og at de legger til grunn at ved opprettelse av en biobank til annet forskningsformål, vil personopplysningsloven gjelde. De skriver bl.a. annet « Det opprettes et forskningsregister. Biologisk materiale oppfattes som informasjonsbærende, og analyserbart materiale blir ”opplysninger” i registeret. Dette gjør registeret med tilhørende biobank til et konsesjonspliktig register.»

Personvernombudet har tatt tilbakemeldingen fra NEM til etterretning og er enig i at dette en akseptabel løsning frem til spørsmålet er nærmere avklart.

Personvernombudet registrerer at Høgskolen i Lillehammer er gitt tillatelse til opprettelse av en generell biobank for oppbevaring av humant biologisk "The TrainsOME -humane cellers tilpasning til trening og miljø", godkjent av REK 2013/2045. Personvernombudet legger til grunn at det biologiske materiale fra prosjektet oppbevares i den generelle biobanken og anbefaler at det utarbeides dokumentasjon for oppbevaringen av det biologiske materialet. Videre anbefaler vi at Høgskolen i Lillehammer orienterer REK sør-øst om at det biologiske materialet som innsamles vil inkluderes i den generelle biobanken REK sør-øst har gitt tillatelse til.

PROSJEKTSLUTT
Forventet prosjektslutt er 31.12.2023. Ifølge prosjektmeldingen skal innsamlede opplysninger da oppbevares med personidentifikasjon til 31.12.2038 for oppfølgingsstudier/videre forskning.