Høgskolen i Innlandet

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Master thesis

Exploring the association between rDNA dosage and muscular responses to resistance training in young adults

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Forord

5 år med studier avsluttes med å levere denne masteroppgaven. Reisen har vært lang, med mange opp- og nedturer, men først og fremst har jeg lært vanvittig masse, og hver uke har gitt ny inspirasjon og glede.

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Stein Olaf Olsen,
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Abstract

Muscle plasticity is affected by physical activity and inactivity, and resistance training has been shown to prevent and reverse negative consequences of inactivity and disuse, such as loss of muscle strength, function, and quality. Ribosomal biogenesis has been shown to be an important factor in understanding the mechanisms behind muscle growth, where total RNA (~ 80-85% rRNA) is an important proxy marker. Resistance training-induced accumulation of total RNA / rRNA has been demonstrated, it is unclear whether the amount of rDNA dose in the genome is decisive for this accumulation. 46 healthy young adults (females: 24.9 (3.8) years; men: 25.0 (4.24) years) were included, and completed 8 weeks of strength training divided into three training modalities, 0 sets (control), 3 sets, and 6 sets; with a training frequency of 3 times/week. Strength tests, ultrasound, DXA scan, blood tests, and muscle biopsy were performed to map physiological, molecular, and, phenotypic changes. A valid qPCR-based method was developed to estimate the rDNA dose in muscle and blood tissues. Total RNA increased significantly from T2 to T3, and an association was found between rDNA dose and exercise-induced accumulated total RNA at T3 ($r = 0.470$; $p = 0.004$), but no significant association was found at T2 and post-intervention. rDNA dose did not predict the observed muscle growth measured post-intervention. Contralateral resistance training showed muscular response through increased muscle strength and muscle thickness, but it was not volume-dependent (moderate versus high). In conclusion, rDNA dose appears to be associated with exercise-induced total RNA accretion. More research is needed to determine whether rDNA dose may be a determining factor for resistance training-induced responses to ribosomal biogenesis.

Content

1. Background

Skeletal muscles undergo gradual muscular plasticity due to activity and inactivity (Ferrando et al., 1996), where inactivity and disuse lead to reduced muscle function, strength, and quality, which causes increased physical disability and mortality risk (Evans & Lexell 1995; Janssen et al., 2002). To sustain muscle mass and optimal muscle function, muscle fibers require stimuli, where resistance training has been proven to counteract disuse-induced muscle atrophy in healthy untrained people (Seaborne et al., 2021) and individuals with serious lifestyle diseases, like Chronic Obstructive Pulmonary Disease (COPD) (Mølmen et al., 2021), cancer (Al-Majid & McCarthy, 2001) and diabetes (Sparks et al., 2013). Muscular response to training can vary through manipulating training intensity, volume, and frequency. Exercise volume is a key variable for influencing training-induced response in humans, where it is observed a dose-response relationship (Figueiredo et al., 2018). There is individual variability related to the effects of different training volumes on muscle growth due to some components involved in ribosomal biogenesis (Hammarström et al., 2020), where the mechanism behind is not fully understood yet. Continuous resistance training shows an increased effect on muscle mass and strength, leading to better health across the life span.

Molecular and genetic markers emerge and indicate individual responses to resistance training, where differences in observed total RNA and ribosomal RNA (rRNA) accumulation that occur after training have been demonstrated (Hammarström et al., 2020; Figueiredo et al., 2021; Hammarström et al., 2022). Resistance training with moderate volume has demonstrated higher total RNA and rRNA levels in human skeletal muscle compared to low volume, and this response is heterogeneous (Hammarström et al., 2020; Hammarström et al., 2022). Further, humans demonstrate a high variability of ribosomal DNA (rDNA) copy number (Ganley & Kobayashi et al., 2013; Panov et al., 2021; Hall et al., 2022), suggesting rDNA dose is a possible determinant for ribosomal biogenesis in association to resistance training. Interestingly, a single bout of resistance exercise promotes an acute response, where rDNA transcription is positively associated with rDNA dose (Figueiredo et al., 2021). To summarize, rRNA accumulation and rDNA dosage seem to be associated with the heterogeneous response to resistance training in untrained, where rDNA dosage could be a potential genetic factor related to observed hypertrophy induced by resistance training.

1.1 Adaptations to inactivity – the reversing effect of resistance training

The population has become increasingly sedentary and physically inactive, which can be a potential cause for the development of serious lifestyle diseases (e.g., cancer, cardiovascular disease, type 2 diabetes, and obesity (Tremblay, et al., 2010) and reduced health span. Physical activity and inactivity affect muscular plasticity, further can inactivity occur voluntarily (increased sedentary behavior, bed rest) and involuntarily (microgravity, joint immobilization due to fracture) (Graham et al., 2021). Skeletal muscle is the most abundant tissue in the human body with extensive plasticity, where inactivity leads to muscle atrophy, described as a loss of muscle mass, therefore maintenance of its function is important for health across the lifespan (Hackney & Ploutz-Snyder, 2012; Dirks et al., 2014;). In situations with reduced neural input (muscle disuse and unloading) is muscle atrophy even observed in healthy individuals (Phillips et al., 2009).

Several studies have used joint immobilization as a model to observe the consequence of inactivity in short (days) and long terms (weeks). Four days with a knee brace led to a decrease in muscle mass, and daily reductions in muscle protein synthesis compared to the control leg (Willis, et al., 2021). In a study with 11 weeks of contralateral resistance training, where the participants trained one leg and the other leg was untrained, the heterogeneous response shows a 9% reduction in muscle mass on m. vastus lateralis, and the trained leg increased muscle mass by 8.1% at the group level (Stokes, et al.,

2020). Furthermore, it has been shown that lean mass in untrained individuals with a training period of 7 weeks followed by 7 weeks of detraining increases lean mass after the training period but ends up back to baseline values after a period of unloading (Seaborne, et al., 2018). The latter highlights the importance of chronic resistance training.

1.1.1 Effects of resistance training on muscle growth

Resistance training is considered a promising intervention for reversing negative effects observed with muscle atrophy, like loss of function, muscle mass, and strength. The morphological adaptations observed with resistance training interventions, such as hypertrophy (increase in cell size due to swelling), are strongly associated with training variables, like training intensity, weekly training frequency, and training volume (Figueiredo et al., 2018) In a stressful daily life, a "time-efficient" program has become popular (e.g., high-intensity interval training), where volume is often neglected and reduced in favor of intensity (Gibala, 2007; Thompson, 2016). However, adaptations observed from similar resistance training protocols appear to be similar when equated to total volume, including manipulation of the frequency (Candow & Burke, 2007) and intensity of training (Mitchell et al., 2012; Mangine et al., 2015). Therefore, training volume is thought to be a key determinant for manipulating exercise response in humans (Figueiredo et al., 2018). A dose-response relationship between training volume and the degree of muscle hypertrophy, where higher volume is associated with higher potential for muscle growth in untrained individuals has been demonstrated (Krieger, 2010). Furthermore, the same association was confirmed in young adult men, where higher training volume (low volume vs. moderate volume) showed a greater effect on increased muscle mass in m. vastus lateralis (Mitchell et al., 2012). Further evidence confirmed with untrained, where moderate training volume shows a larger increase in muscle mass compared to a low volume training prescription (Sooneste et al., 2013; Hammarström et al., 2020). A further increase in resistance training volume (10 sets) as exercise prescription didn't find any benefit compared to moderate volume (Amirthalingam, 2017). If confirmed, this may indicate that high training volume (> 6 sets per exercise per session) does not provide a further increase in muscle growth compared with < 6 sets. A potential plateau, where 3-5 sets could be a favorable volume if the training frequency is 2-3 times a week. In the meantime, the observed heterogeneous, where some individuals don't respond phenotypically (Bamman et al., 2007; Thalacker-Mercer et al., 2013; Hammarström et al., 2020, Hammarström et al., 2022), and lack of knowledge about the underlying molecular and cellular mechanisms may indicate non-consensus on the optimal exercise prescription for maximal muscle growth. Despite the reduced knowledge, the relationship between muscle protein synthesis (MPS) and muscle protein breakdown (MPB) is considered a determinant factor (Figueiredo., 2019), with MPB being relatively higher than MPS during periods of inactivity.

1.2 Muscle plasticity of training-induced muscle hypertrophy

Skeletal muscle is a postmitotic tissue and does not undergo significant cell replacement throughout life in mammalians. An efficient method for cell repair is therefore required to maintain skeletal muscle mass. This is carried out through the dynamic balance between MPS and MPB because muscle hypertrophy occurs when protein synthesis exceeds protein breakdown, resulting in a net increase in protein if nutrition is sufficient (Tipton et al., 2001; Moore et al., 2015). A single session of resistance training stimulates MPS, which is maintained for up to 48 hours after (Phillips et al., 1997). Furthermore, repeated sessions over time induce cumulative periods of positive net protein balance, which requires the rate of MPS exceeds the rate of MPB resulting in an elevated muscle protein pool (Burd et al., 2009). Changes in MPS are a significant factor that drives the anabolic response after exercise stimuli, and further, promote an increase in the protein

content in muscles. Furthermore, a potentially rate-limiting step for MPS, and thereby, muscle hypertrophy is the translational capacity (ribosomal density) and rate of messenger RNA (mRNA) translation (ribosomal efficiency) (Mobley et al., 2018). The protein complex mechanistic target of rapamycin (mTOR) complex 1 regulates c-Myc, known as the master regulator of ribosomal biogenesis, during chronic resistance training. Both contribute to the regulation of signaling pathways related to ribosomal biogenesis, where c-Myc is implicated in the coordination of MPS by upregulating the expression of rRNA, and further through regulation of UBF transcription and other transcription factors (TIF-1A, Pol I, Pol II, Pol III) (Solsona et al., 2021). Interestingly, c-Myc was expressed higher pre-training in moderate and extreme responders compared with low responders to resistance training (Thalacker-Mercer et al., 2013).

Muscle hypertrophy leads to an expansion in the muscle cross-sectional area, observed phenotypically as increased muscle size, thus increased myofiber volume. This development is visible after a few weeks in the untrained, and the expansion is linear with progressive resistance training, demonstrated through 6-9% increased muscle size in the quadriceps after a few months of strength training (Mitchell et al., 2012). Increased stimuli (e.g., mechanical stress, metabolic stress) during activity induce activation and proliferation of satellite cells, specialized stem cells located around the muscle, and fusion with an existing myofiber result in an increased number of myonuclei (Petrella et al., 2006; Solsona et al., 2021). Furthermore, the number of myonuclei proves to be a critical determinant for protein synthesis capacity by providing the amount of DNA necessary to sustain gene transcription (Blaauw & Reggiani, 2014). Training-induced hypertrophy occurs through increased amounts of proteins and myonuclei in an individual fiber, resulting in the synthesis and accumulation of new contractile myosin filaments that expand the myofiber volume (Alway et al., 1988). As described above, ribosomal density and ribosomal efficiency are responsible for protein synthesis, and ribosomal biogenesis is crucial for MPS and therefore is potentially potent for muscle hypertrophy.

1.3 The role and biology of ribosomes: biogenesis and cell growth

The ribosome is a piece of macromolecular machinery located in the cytoplasm, responsible for the translation of mRNA into protein, in which amino acids are assembled in a sequence to form polypeptide chains (Figueiredo & McCarthy, 2019). A ribosome consists of a small and a large subunit, respectively 40S and 60S, each subunit contains rRNA and ribosomal proteins (r-proteins). The large subunit, the 60S, is formed by rRNA 28S, 5.8S, and 5S, with associated r-proteins, with the smaller subunit, 40S, being composed of rRNA 18S and associated r-proteins (Ban et al., 2000; Khatter et al., 2015), respectively. Cell growth requires a remarkable number of ribosomes, to extend protein synthesis (e.g., form new contractile elements in skeletal muscle) (Bamman et al., 2018). Ribosomal biogenesis is the process where new ribosomes are produced, in eukaryotic cells this takes place in the nucleus and cytoplasm. Ribosomal biogenesis mainly occurs in the substructure of the nucleus, nucleolus, where rRNA is synthesized, processed, modified, and assembled into ribosomal subunits (Drygin, Rice & Grummt, 2010). Synthesizing of ribosomes is a coordinated activity with three different RNA polymerases (Pol I, II, III).

The rDNA genes are present with ~ 400 copies (individual range from 61 to 1590 copies) in the human genome, present as clusters of tandem repeats in 5 different chromosomes; 13, 14, 15, 21, and 22 (Stults et al., 2008; Parks et al., 2018), where, interestingly, the human genome has shown recombination of rDNA copy number (Stults et al., 2009; Xu et al., 2017). De novo synthesis of ribosomes begins with the rate-limiting step where rDNA is transcribed by Pol I to generate the precursor 47S pre-rRNA (Mayer & Grummt, 2006). Initiation of rDNA transcription is controlled by a multiprotein complex containing Pol I and the assembling of the pre-initiation complex (PIC), which is formed at the rDNA promoter

(Drygin et al., 2010). PIC consists of upstream binding factor (UBF), selective factor 1 (SL1) complex, which further is a protein complex containing TATA-binding proteins (TBP) and three Pol I-specific TBP-associated factors (TAF), transcription initiating factor (TIF-) IA and RNA Pol I (Grummt, 2003; Drygin et al., 2010). After transcription is initiated, the PIC components, with UBF and the SL-1 complex will remain connected to the promoter element, until Pol I meets the terminator element of rDNA (Figueiredo & McCarthy, 2019). Based on this transcription, we are left with a 47S pre-RNA transcript that contains 18S, 5.8S, and 28S rRNA. These rRNAs are separated by the internally transcribed spacers 1 (ITS1) and 2 (ITS2), while 5 'and 3' are externally transcribed spacers (5 '-ETS and 3-ÉTS). During the action, the spacers are removed using a series of endonucleolytic and exonucleolytic cleavages (Henras et al., 2015), and we are left with mature 18S, 5.8S, and 28S rRNA. Further, 5S rRNA is produced outside the nucleolus, generated by transcription of 5S rDNA. Since it is formed outside the nucleolus, it is transported into the nucleolus by binding to the target protein L5 (Ciganda & Williams, 2011). The rRNAs and r-proteins are then assembled to 60S and 40S. In the end, the ribosomes undergo a maturation process in the nucleoplasm, before they are actively transported out into the cytoplasm where the last processing takes place and we are left with the two mature ribosomal subunits (Henras et al., 2015; Moss et al., 2007).

1.3.1 Resistance training, ribosomal biogenesis, and muscle cell growth

Since approximately 80% of the muscle cell's total RNA is estimated to be rRNA, findings indicate that de novo production of rRNA is essential for hypertrophy to occur after resistance training in humans (Figueiredo et al., 2015). Almost two decades ago, the first evidence to support the role of ribosomal biogenesis in muscle. They found that inhibiting rRNA synthesis by rapamycin prevents protein accretion and thereby myotube growth (Nader et al., 2005). As described above, Pol I transcription of rRNA genes (rDNA) is a determinant step of ribosomal biogenesis, and recent studies in vitro have shown that the pol I-specific inhibitor CX-5461 blocks rRNA synthesis, and thus rDNA transcription, protein synthesis, and the hypertrophic response in myotubes is blunted (Stec et al., 2016; Von Walden et al., 2016). Further, animal models have shown that in an early stage of hypertrophy, levels of 45S pre-RNA increased through synergist ablation (Kirby et al., 2015). The above-mentioned findings highlight the important role of ribosomal biogenesis during and after resistance training in vivo for muscle hypertrophy.

The increasing interest in ribosomal biogenesis in association with muscle hypertrophy has emerged in the last decade, and both acute and chronic training protocols have demonstrated the link. Total RNA is considered a valid proxy marker for ribosomal biogenesis (Millward et al., 1973), the content is associated with exercise-induced hypertrophy, and rRNA 28S, 18S, and 5.8S transcripts levels increase after resistance training (Figueiredo et al., 2015; Fyfe et al., 2018). A single bout with resistance training is a stimulus for rDNA and rRNA transcription, leading to increases in pre-rRNA (Nader et al., 2014; Stec et al., 2016). Repeated bouts lead to accretion of total RNA, and rRNA, interestingly, the accumulation seems to peak around training sessions 6-9 (Hammarström et al., 2020; Hammarström et al., 2022). Indications may reveal that total RNA/rRNA accumulation is highly heterogeneous across individuals (Solsona et al., 2021; Hammarström et al., 2022), and that ribosomal biogenesis is training volume-dependent, where moderate training volume provides increased response compared to low volume (Hammarström et al., 2020). It's currently unclear if high training volume or intensity gives a further accretion in total RNA, and thus ribosomal biogenesis. Interestingly, rDNA transcription is positively associated with rDNA dose after acute resistance training, where whole genome sequencing (WGS) was correlated well with the estimation of rDNA dosage via quantitative PCR (qPCR) (Figueiredo et al., 2021). This supports that the preferred qPCR-based procedures (Jack et al., 2015) are valid.

The aim of this thesis is to develop a valid method to measure rDNA dosage from human biological material, and further investigate if rDNA dosage is associated with training-associated changes in total RNA in human skeletal muscle at different time points and muscular response during 12 weeks with a contralateral resistance training protocol. The hypothesis was that rDNA dosage would be associated with total RNA accumulation, and second that the training intervention causes phenotypic changes (muscle strength and muscle growth) to chronic resistance training.

2.0 Introduction

Skeletal muscles undergo gradual muscular plasticity due to activity and inactivity (Ferrando et al., 1996) leading to reduced muscle function, strength, and quality, which causes increased physical disability and mortality risk (Evans & Lexell 1995; Janssen et al., 2002). To sustain muscle mass and optimal muscle function, muscle fibers require stimuli, where resistance training has been proven to counteract disuse-induced muscle atrophy in healthy untrained people (Seaborne et al., 2021) and individuals with serious lifestyle diseases, like Chronic Obstructive Pulmonary Disease (Mølmen et al., 2021), cancer (Al-Majid & McCarthy, 2001), and Type 2- diabetes (Sparks et al., 2013). The consensus around resistance training does not question its importance, and positive effects on muscle plasticity have been demonstrated, yet "low responders" are observed, respectively (Sallis & Patrick, 1995; Hubal et al., 2005). Currently, there is a lack of knowledge about these mechanisms that can help predict training response, improved knowledge of cellular and molecular mechanisms can give an increased efficacy to an exercise prescription, and possibly help to improve the healthspan at the individual level. In the search for genetic factors involved in determining molecular and phenotypic changes in the skeletal muscles, ribosomal biogenesis, and associated signaling pathways (e.g., mTORC1 signaling, and Wnt/β-catenin/c-myc) in connection with resistance training have received increased attention (Stec et al., 2016; Figueiredo et al., 2021; Hammarström et al., 2022). Contralateral resistance training shows a tendency to individual variability due to components related to ribosomal biogenesis within untrained young adults (Hammarström et al., 2020).

Prolonged resistance training causes changes in the balance between MPS and MPB, where muscle protein accumulates over time after repeated workouts over weeks (Burd et al., 2009). To increase muscle mass, the addition of new contractile proteins is required, thus increasing the protein synthesis rate (Tipton et al., 2001; Moore et al., 2015). Increased MPS rate has been shown to be associated with translation capacity (Robinson et al., 2017), therefore the supply of more ribosomes in the muscles is required since the ribosomes are responsible for the translation of mRNA into protein. To support this, evidence demonstrates that resistance training-induced increases in total RNA, where approx. 80-85% is rRNA, is linked to muscle hypertrophy (Figueiredo et al., 2015), and through experiments within mammalians and humans as well, the inhibition of rRNA transcription, impairs muscle cell growth (Nader et al., 2005; Von Walden et al., 2016). rRNA is transcribed from ribosomal genes (rDNA), thus the starting point for ribosomal biogenesis, rDNA transcription can be a place to start the search for possible molecular and genetic factors for muscle growth.

Humans demonstrate a huge variability of ribosomal DNA (rDNA) copy number (61-1590 copies) (Ganley & Kobayashi et al., 2013; Panov et al., 2021; Hall et al., 2022), suggesting rDNA dose as a possible determinant for ribosomal biogenesis. Interestingly, a single bout of resistance exercise promotes an acute response, where rDNA transcription is positively associated with rDNA dose, where Figueiredo and colleagues in the same study observed changes in epigenetic markers associated with acute resistance training (Figueiredo, et al., 2021). This is consistent with the observation that a huge proportion of rDNA genes are inactive in growing cells at the epigenetic layer (McStay., & Grummt., 2008; Tariq., & Östlund Farrants., 2021), and modifications to external stimuli such as exercise can possibly provoke changes and activate more genes, which is indirectly shown by upregulation of UBF after exercise (Hammarström, et al., 2022), as UBF is involved in the activation (Solsona et al., 2021). This suggests genetic and epigenetic responses in strength training, where the genetic factors may be important for ribosomal biogenesis.

Total RNA is described as a proxy marker for ribosomal biogenesis, and differences in the total RNA and rRNA accumulation after training have been demonstrated (Hammarström et al., 2020; Figueiredo et al., 2021; Hammarström et al., 2022). Resistance training with moderate volume resistance has demonstrated a higher total RNA and rRNA level in human

skeletal muscle compared to low volume, and this response has been shown to be highly heterogeneous (Hammarström et al., 2020; Hammarström et al., 2022). In summary, it will be interesting to investigate whether rDNA dose is associated with chronic exercise-induced ribosomal biogenesis and the response to muscle growth.

The aim of this thesis is to develop a valid method to measure rDNA dosage from human biological material, and further investigate if rDNA dosage is associated with training-associated changes in total RNA in human skeletal muscle at different time points and muscular response during 12 weeks with a contralateral resistance training protocol. The hypothesis was that rDNA dosage would be associated with total RNA accumulation, and second that the training intervention causes phenotypic changes (muscle strength and muscle growth) to chronic resistance training.

3.0 Methods

3.1 Ethical approval

Participants were informed about the study and were required to give their informed consent prior to the study start (Attachments 1). This thesis is part of a larger study, pre-registered at ClinicalTrials.gov (ID: NCT03795025) and approved by the local ethics committee at Inland Norway University of Applied Science (HINN) 20.12.2018.

3.2 Participants

Men and women aged 18-35 were recruited, and a total of 46 healthy young adults were included in the study, presented in Table 1. Other inclusion criteria were, non-smoking, not injured or exposed to a chronic illness, and untrained, described as less than one resistance training session per 14 days and less than 3 hours of endurance training per week in the last 6 months. 9 participants did not complete the study; pain after biopsy (n = 1), loss of contact (n = 1), fracture injury not related to the study (n = 1), personal reasons (n = 2) and closure of training facility due to Covid-19 (n = 3). One participant did not attend DXA and ultrasound.

Table 1 Participants' characteristics

	Female	Male
Age (years)	24.9 (3.8)	25.0 (4.24)
Height (cm)	172.1 (6.87)	176.8 (6.41)
Total body mass (kg)	77.7 (16.45)	78.4 (16.91)
Lean mass (kg)	45.6 (7.37)	50.8 (9.87)
Body fat (%)	37.8 (7.69)	31.6 (9.12)

Data are presented as mean ± SD.

3.3 Experimental design

The participants performed unilateral resistance training with different training volumes, further allocated into one of four experimental groups (Figure 1). Participants in training group 4 served as control after pre-tests and habituation, but without exercise during the training period, but carry out the strength training intervention and other subsequent tests after serving the control period. Participants had three weeks of habituation (9 sessions), followed by an eight-week strength training intervention with three sessions per week (24 sessions). The training groups performed unilateral leg press and unilateral knee extension. In addition, they trained the upper body (bench press and bench pull), but upper body training is

not included in this thesis and will not be described further. Micro muscle biopsy was taken, along with body composition measurements (Dual-energy X-ray absorptiometry, DXA; ultrasound) at four-time points in the study. Strength tests were performed four times before the intervention, and a further time during and twice after the intervention period. For an overview of the study protocol and details see figure 1. For note, this thesis is using 0 sets, 3 sets, and 6 sets as groups.

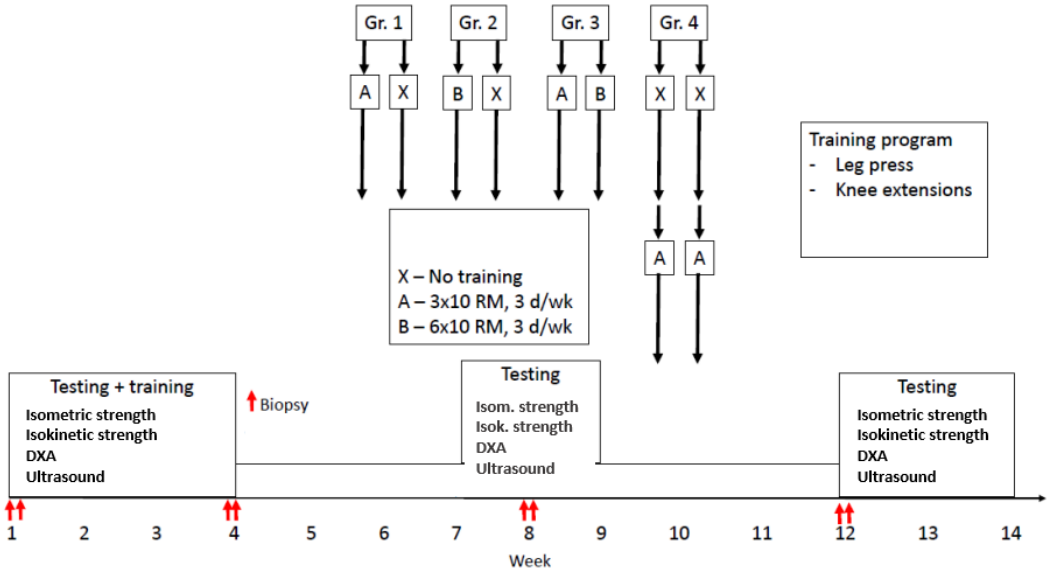

Figure 1. Study overview. Week 1-3 is the habituation period. Week 4-12 is the intervention period. Week 12-14 is the post-test period. Gr.1, Gr.2, Gr.3, and Gr.4 represent the experimental groups, whereas Gr.4 is the control group. This thesis is using 0 sets (X), 3 sets (A), and 6 sets (B) as groups. Testing at week 1 = pre-intervention, week 4 = T2, week 8 = T3 and week 12 = T4/post-intervention. DXA = Dual-energy X-ray absorptiometry.

3.4 Habituation and resistance training protocol

During the habituation period of three weeks, two warm-up sets and further two training sets were performed with 10 reps of leg pressure and 10 reps for knee extension per training session, where only the leg was supposed to train during the intervention used. The reason for three weeks of habituation was to examine the perspective of how much the strength and level of total RNA increase when performing four strength tests, and it was desirable to have a period long enough to get a total RNA response. Finally, it was also a purpose to avoid overload, especially for legs that were randomized to train in 6 sets. It is not the purpose of this thesis to investigate the effect of the habituation period. During the intervention period, participants performed a standardized warm-up consisting of five minutes of low-intensity exercise and two warm-up sets with leg pressure (10 reps) before each session. The lower body exercises were performed with 3 or 6 series, according to the randomization, all series were performed with an intensity of 10 repetitions maximum (10RM), with two-minute rest intervals between each set. Every repetition was performed with explosive muscle work in the concentric phase, while the eccentric phase was completed with a lower speed. All sessions were monitored by bachelor and graduate sports science students and, scientists from the department at HINN.

3.5 Strength tests

Muscle strength was tested through isokinetic and isometric unilateral knee extension using the HUMAC Norm dynamometer (CSMi, Stoughton, Massachusetts, USA). Standardized settings for each subject, participants were fastened to the chair, and the axis of rotation and knee joint were placed in line with the rotation axis of the dynamometer. The thigh was then fastened, and the leg was strapped two fingers above the ankle joint. All settings were noted and replicated for each test.

Maximum isokinetic torque was measured at 60° sec^{-1} and 240° sec^{-1}. Three submaximal attempts were used as familiarization at each angular velocity, then they received three attempts immediately after each other. Participants were given a 30-second break between the submaximal trials and the test, while they were given 60 seconds pause between the test and habituation to the new angular velocity. The highest value at every angular velocity was used in the analysis. Following the isokinetic tests, isometric strength was tested and measured as a maximal voluntary contraction (MVC) at a knee angle of 60°. Each participant received one habituation attempt and two attempts where they exerted maximum force for 3-4 seconds with 60 seconds of rest between each attempt. The highest measured value was used for further analyses.

3.6 Body composition and muscle thickness assessments

Prior to the determination of body composition and muscle thickness assessments, participants were asked to avoid exercise for the last 48 hours prior to the tests and to not eat 12 hours before the test was taking place. Body composition, measured as lean mass (LM, kg) and fat mass (FM, kg) was measured using DXA (Prodigy Advance PA + 302047, Lunar, San Francisco, CA, USA) according to the manufacturer's protocol. The participants were lying supine within the scanning bed, with a strap around the ankle to ensure body stabilization. The scanning was conducted in the morning when the participants were in a fast state.

M. vastus lateralis thickness (VLT) was measured using a B-Mode ultrasound with a 50-mm linear matrix transducer (L12-5, Philips, Bothell, WA, USA), the ultrasound system (HD11XE (Philips, Bothell, WA, USA) with the program echo wave II (2.7.1, Lithuania). Participants lay supine in a relaxed position for a few minutes before assessments. A mark was set on the line at 50% of the distance between the joint gap in the knee joint and the trochanter major, and a water-soluble transmission gel was applied to a transducer and placed perpendicular to the site of interest, at the most prominent parts of m. vastus lateralis (VL). When the quality of the image was acceptable, three images were taken, where the probe was relocated to the same location between each image. These images were analyzed with a plugin from ImageJ Fiji as previously described (Seynnes & Cronin, 2020). At the first test, the measuring point was marked on a plastic folder that was above the participant's VL. This plastic folder was used to find back to the same measuring point in the subsequent tests, for reliability. Ultrasound has been shown to be a reliable and valid measurement method for muscle architecture (Kwah et al., 2013).

3.7 Muscle biopsy sampling and processing

The muscle biopsies were taken bilaterally from the VL, using local anesthesia (Lidocaine 3ml, Mylan Hospital A / S, Oslo, Norway) under antiseptic conditions. A well-established micro biopsy technique (Hayot et al., 2005) was used for the invasive procedure. A spring-loaded biopsy gun was used (Bard Magnum, Bard Medical, New Jersey, USA), mounted with a 12 g needle (Universal Plus, Mermaid Medical A / S, Stenløse, Denmark). The test took place in the morning, minimum of 12 hours of fasting and 48 hours without training or intensive physical activity prior to the test. The biopsy was taken on an area ~ 1/3 of the femur length, related to the distal endpoint of the knee. When the biopsy was replicated later in the study, it was taken 1-2 cm proximal to the previous sample. The muscle biopsy was immediately washed in an ice-cold sterile saline solution (NaCl 0.9%) before blood, connective tissue, and fat were dissected away. Trying for protein, DNA and RNA assays were frozen in -80 ° C isopentane and stored in an ultra-freezer - 80°C.

3.8 Total RNA extraction, homogenization, and phase separation

300 µL TRIzol reagent (Invitrogen, Life technologies AS, Oslo, Norway) was added, with 0.5 mm RNase free (Next Advanced, Averill Park, NY, USA), further the samples were homogenized in a bullet blender (BB) (Next Advanced, Averill Park, NY, USA) for 1 min at 10 g, and further 1 min at 12 g.

700 µL TRIzol was added for a total volume of 1000 µl, followed by a 1 min shake and 5 min incubation at room temperature. Furthermore, 200 µl of chloroform was added (Sigma-Aldrich, Missouri, USA) added, before another 2-3 min incubation. The samples were then spun in Heraeus ™ Fresco ™ 21 Microcentrifuge (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 1200g for 15 min at 4 ° C, for separation of the tube content into three phases (aqueous, inter, and organic phase). Then 450 µL of the upper aqueous phase was transferred to a new tube without disturbing either the interphase or the organic phase. In the new tube, 500 µL of isopropanol (VWR International, Pennsylvania, USA) was added to accelerate the precipitation of RNA. Prior to RNA extraction, the samples were incubated for 10 min at room temperature. The interphase and the organic phase were stored at -20 ° C before further processing (protein extraction). Note, that the samples targeted for protein measurement were not used in this thesis.

For RNA extraction, the isopropanol / aqueous phase from the TRIzol extraction was centrifuged (12,000 g, 10 minutes at 4 ° C), forming a pellet containing RNA. The RNA pellet was then washed with 1000 µl 75% ethanol (-20 ° C) and centrifuged (7500g, 5 min at 4 ° C). This process was replicated before the ethanol was removed and the pellet was air-dried for 10 min. The RNA pellet was then eluted in 30 µl TE buffer and incubated at 55 ° C for 10 min, before creating a 1:3 dilution, with 8 µl TE buffer and 4 µl of the RNA dilution. RNA amount and purity were assessed and evaluated via a spectrophotometer. All samples had a 260 to 280 ratio > 1.95.

3.9 DNA extraction from blood and muscle

The protocol was given by Thermo Fischer (Thermo GeneJET Genomic DNA Purification Kit cat. No. K0722) was used for maximum DNA yield.

3.9.1 Testing the protocol

The protocol was tested due to variation in sample size from freeze-dried muscle tissue (~0.5mg - ~2mg), where some samples had slightly smaller tissue than recommended by the manufacturers. A test of critical steps was performed for verification. The incubation time on heat was recommended to vary based on the tissue weight, therefore freeze-dried muscle tissue was categorized as low (~ 0.5 mg), medium (~ 10 mg), and high (> 1.5 mg), with all samples tested 1 hour, 2 hours and 4 hours incubation time at 56°C. No significant difference was found in the extracted amount of DNA from the samples. Since some samples contained up to 2 mg of freeze-dried muscle tissue, 2 hours were set as standard according to the protocol's recommendation, for complete lysis to occur. The last step that was tested was the amount of Elution Buffer, where the protocol recommends 200 µl but specifies several solutions for maximum DNA yield. In the same model with the categorization of different tissue weights, where all the samples were tested with 50 µl, 100 µl, and 200 µl of Elution Buffer, where half of the samples <0.9 mg were performed twice. The samples with muscle tissue = <0.9 mg obtained more DNA by replication of the step, while the other categories (medium and high weight) tended to favor 50 µl and 100 µl, and thus 75 µl with Elution Buffer was set as standard. With blood, 50 µl was set as standard. The final protocol is described below.

3.9.2 The final protocol (DNA extraction from muscle and blood)

Approximately 0.6-2mg of freeze-dried muscle tissue was homogenized with 180 µl Digestion Buffer and Zirconium Oxide Beads (0.5mm RNase free, Next Advance, Inc, New York, USA). Further, 20µl proteinase K solution (Thermo Fischer Scientific) was added before an incubation time (56 degrees) of 2 hours until complete lysis occurs. 20µl RNase solution and 200µl Lysis Solution were added, before 400µl 50% ethanol was added and the prepared lysate was transferred to a DNA Purification Column inserted in a collection tube. The purified DNA was further washed twice with two different ethanol-added Wash Buffers and centrifuged between each wash (respectively; 8000g for 1 min and 12000g at 3 min) and finally eluted with 75 µl Elution Buffer, incubated at room temperature at 2 min, and finally centrifuged (8000g for 1 min). The latter step was replicated for samples below 1.0mg.

For DNA extraction from blood, almost the same procedures were conducted. 20µl proteinase K solution and 400 µl of Lysis Solution were added to 200 µl whole blood, before 10 min incubation time at 56 Celsius before 200 µl of 96% ethanol was added. After that, the prepared lysate was transferred to the DNA purification column before the exact same procedures described with muscle tissue were followed, except for one step. 50 µl with Elution Buffer was added, before the last centrifugation. All buffers and solutions were from Thermo Fischer Scientific, and further supporting information is provided in the protocol from Thermo Fischer (Thermo GeneJET Genomic DNA Purification Kit cat. no. K0722). DNA quantity and purity were evaluated using a spectrophotometer.

3.10 Estimating rDNA dose via quantitative polymerase chain reaction (qPCR)

qPCR reactions were performed with 2 µl diluted genomic DNA (gDNA) from muscle tissue, where all samples with gDNA were eluted in nuclease-free H_2O and diluted to 6.25 mg/µl. Further, SYBR-green-based master mix (PowerUp SYBR Green Master Mix, Thermo Fischer Scientific), pure water, and primers were mixed in a 10 µl reaction. After a 96-well plate was loaded in a duplicate and centrifuged, the reactions were run in a QuantStudio 5 Real-Time PCR system (Thermo Fischer Scientific) according to the manufacturers' instructions. The primers, sequence, and performance are presented in Table 2. The primers have been validated in a previous study (Gibbons et al., 2015). TP53 was used as the reference gene, according to former studies (Gibbons et al., 2015; Figueiredo et al., 2021), and rDNA 28S and rDNA 18S were normalized to TP53. Raw fluorescence data were exported from the software and estimation of quality and performance using the qpcR package (Ritz & Spiess et al., 2008).

Table 2 Primer sequences and avg. performance

Symbol	Sequence	Mean Cq	Efficiency
rDNA 28S F1R1	F: 5'-GCGGGTGGTAAACTCCATCT-3' R: 3'-CACGCCCTCTTGAACTCTCT-5'	24.18	1.948
rDNA 18S F1R1	F: 5'-GACTCAACACGGGAAACCTC-3' R: 3'-AGACAAATCGCTCCACCAAC-5'	21.66	2.000
TP53 F1R1	F: 5'-TGTCCTTCCTGGAGCGATCT-3' R: 3'-CAAACCCCTGGTTTAGCACTTC-5'	24.70	1.902

3.11 Statistics and data analysis

Descriptive data are presented as mean and standard deviation (SD). The effects of training conditions (control versus 3 set versus 6 set) on muscle strength, muscle thickness, and Total RNA was assessed using mixed models to control for fixed effects and random effects, due to the within-subject design. Pre-test and training conditions were treated as fixed

effects and an intercept with the subject was treated as random effects with muscle strength and muscle thickness. The interaction between set*time was also set as a fixed effect, including T2 and T3 in the analysis of total RNA. To further investigate the effects of total RNA, a post-hoc analysis test was completed. Before the results were evaluated assumptions checks were made through Kolmogorov-Smirnov and Shapiro-Wilk tests. Total RNA was not normally distributed and the data log-transformed with the log(10)-function in Jamovi.

Prior to correlation analysis, all variables included were checked for normal distribution. Almost all the variables did not meet the individual test requirements (Shapiro-Wilk test, Q-Q plot). When the data is nonnormal, RIN transformation is ideal, but the same study is highlighting the Spearman test as powerful, especially compared with Pearson's test (Bishara & Hittner, 2012. Due to this, Spearman's test was used. To explore the possibility of an effect between pre and post for rDNA, cohen's effects size was used. One outlier was detected and removed from rDNA data, due to a technical error.

The level of statistical significance was set to $p = 0.05$. All data analysis was done in Jamovi (The jamovi project, 2021) with R packages (Singman, 2018; Gallaucci, 2019); R Core Team, 2021), despite performance estimation of qPCR reactions (see 3.10), which was performed in R (R Core Team, 2021).

4.0 Results

4.1 Muscular response to resistance training

To confirm that the exercise prescription in the study had an effect, the % change in muscle strength and muscle thickness is presented in Figure 2A. An overall measure of strength, where isometric and isokinetic strength is combined, shows an exercise-induced increase from pre to post of 7.79% (Cl: [4.25,11.3]) and 7.75% (Cl: [3.57,11.9]) for 3 and 6 set, respectively. The exercise-induced increase is significant compared to the control leg ($p = < 0.05$) However, no volume-effect is observed. Similar outcome is observed for muscle thickness, where the pre to post demonstrated 11.6% (Cl: [8.65,14.6]) increase at 3 sets, and 11.1% (Cl: [11.1,17.8]) at 6 sets, the group difference is non-significant ($p = 0.065$). Total RNA increases in the trained legs from pre to T2, and it's accumulating further to T3 with a 7.4% (Cl: [4.93,9.88]) and 14.1% (Cl: [8.72,19.4]) increase, in 3 sets and 6 sets, respectively. The small and visible effect observed between T3 to post is not statistically significant after a post-hoc comparison. Verification of completed different training volumes between 3 and 6 sets is presented in kg in Figure 3.

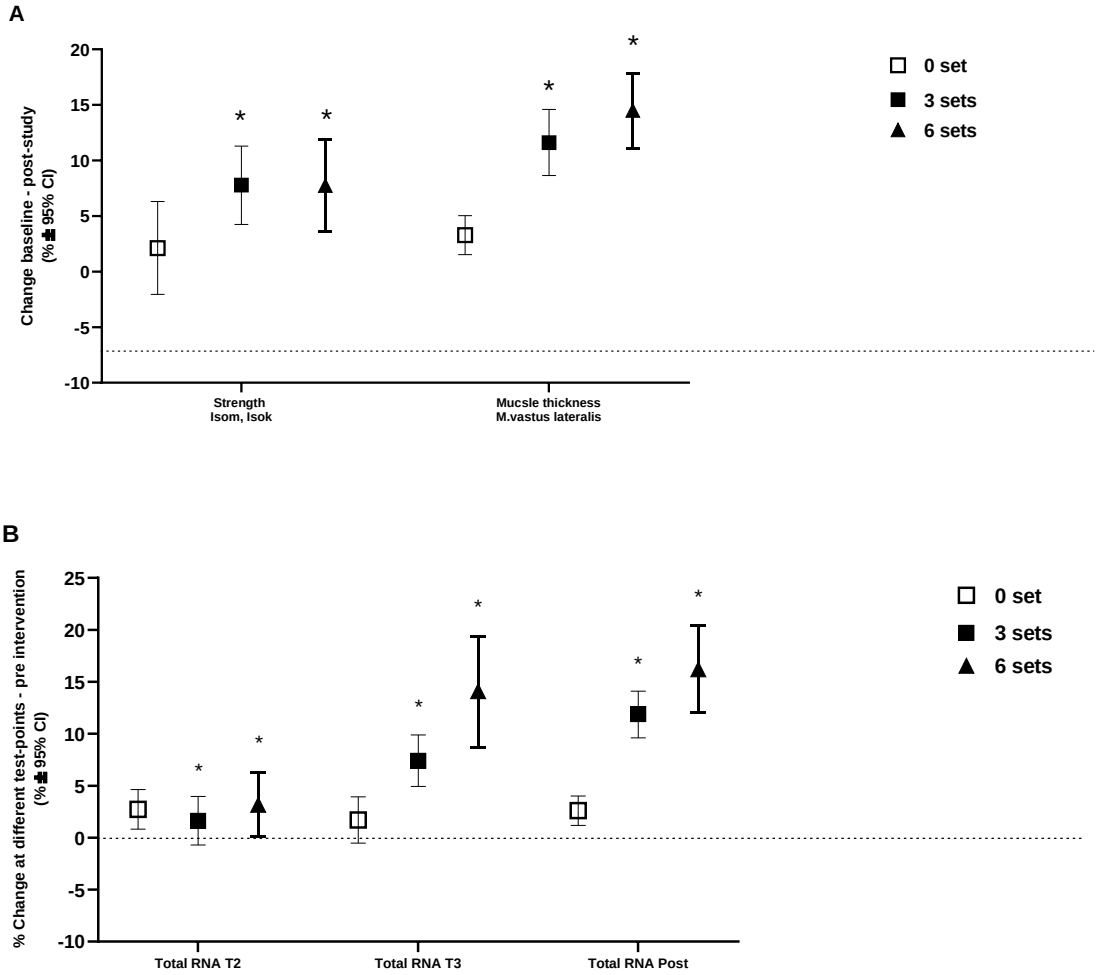

Figure 2. Muscular responses to resistance training. (A) Relative changes from pre- to post-intervention (week 1 – week 12) in muscle strength and muscle thickness in 0 sets (control), 3 sets, and 6 set legs. * = significant change in trained leg compared to control leg (0 sets). (B) Relative changes at different time points (T2, T3, and post) from baseline in 0 sets (control), 3 sets, and 6 set legs. * = significant change in a group (leg) from baseline values (ex. Total RNA post 6 set is significant from 6 set at baseline).

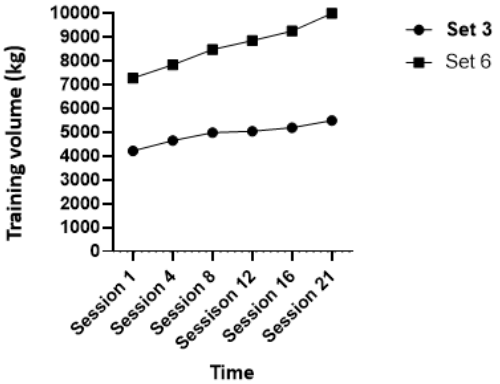

Figure 3. Training volume in the training period (T2 – post-intervention).
The habituation period is not included. Not all sessions are included. Training volume is measured in kg (repetition * weight).

4.2 qPCR estimated rDNA dosage in blood and muscle tissue

First, it was checked whether the method to estimate rDNA dose was valid, our findings show a strong correlation between relative rDNA dose in blood and muscle tissue (Figure 4A), further confirmed with a Cohen d 'effect size = 1.48 (p = 0.001), where blood shows a higher relative rDNA dose when normalized with the reference gene TP53. Moreover, quantification with qPCR of the different coding regions of the rDNA gene showed stronger inter-correlation in blood than muscle (28S versus 18S). However, the correlation is significant and strong in both tissues (Table 3). Further confirmation shows relative rDNA dose before and after the intervention was strongly correlated (r = 0.855, p = 0.001, Figure 4B), and further Cohen's d effect size showed no effect between pre and post (d = -0.06, p = 0.770). Thus, our method is valid in the presence of this study.

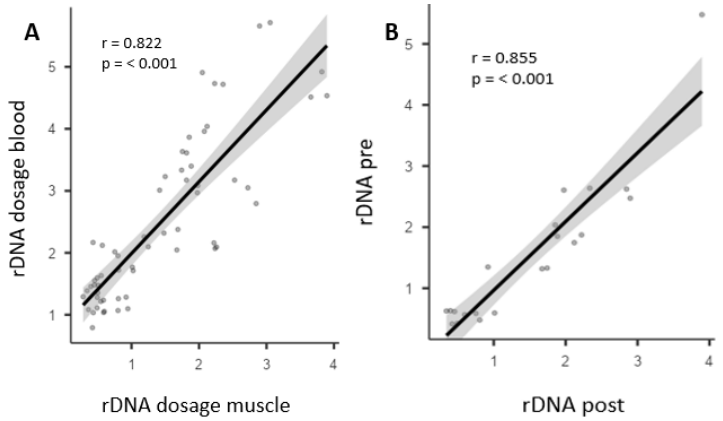

Figure 4. Spearman correlation analysis. (A) rDNA dosage in blood and muscle tissue.
Muscle samples from T1. (B) rDNA dosage pre-intervention and post-intervention. r = correlation coefficient.

Table 3 Spearman correlation analysis for rDNA 28S and rDNA 18S

Genes	Tissue	r	p-value
rDNA 28S + rDNA 18S	Muscle	0.594	0.001
rDNA 28S +rDNA18S	Blood	0.731	0.001

The correlation was made between tissue-specific rDNA 28S and 18S.
r = correlation coefficient.

4.3 Correlation analysis between rDNA and muscular response

Since rDNA copy number varies greatly between individuals and the muscular response in this study shows individual heterogeneity based on the confidence intervals, it was hypothesized that relative rDNA dose would correlate with total RNA. The observations varied at different time points, relative rDNA dose appears to be associated with exercise-induced resistance training accumulation in total RNA compared at T3 (figure 5), however, non-significant at post-intervention, and no association was found at T2. With the association at T3, it was further investigated if rDNA dose and muscle thickness in all groups (0 sets, 3 sets, and 6set) at T4, however, no correlation was found (p = > 0.5) (Figure 6).

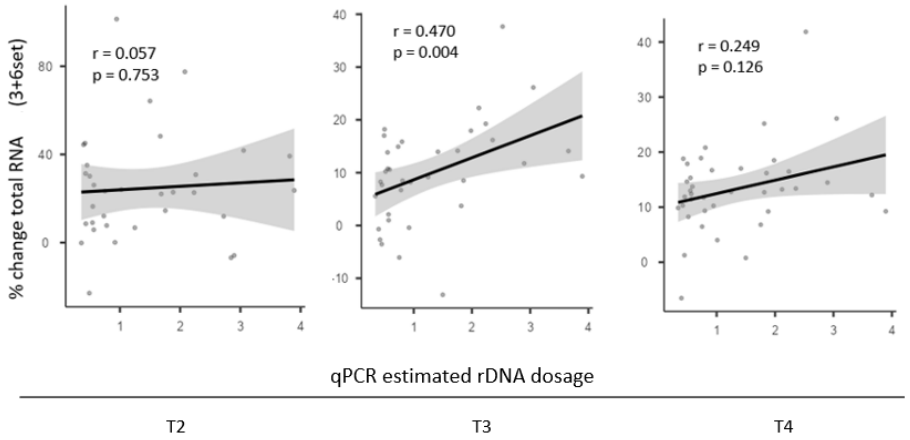

Figure 5. Spearman correlation analysis between relative training-induced changes in total RNA and rDNA dosage at T2, T3, and T4 (post). The estimated rDNA dose is 28S + 18S at T1. r = correlation coefficient.

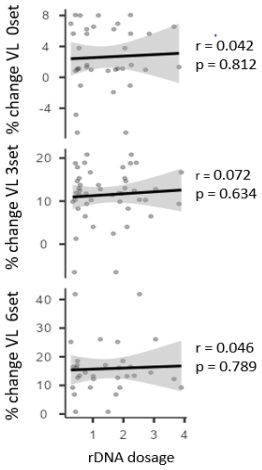

Figure 6. Spearman correlation analysis between relative change in muscle thickness at T4 (compared with baseline) and rDNA dose estimated from T1. Split by training condition into 0 sets (control), 3 sets, and 6 sets. r = correlation coefficient.

5.0 Discussion

The aim of this thesis is to develop a valid method to measure rDNA dosage from human biological material, and further investigate if rDNA dosage is associated with training-associated changes in total RNA in human skeletal muscle at different time points and muscular response during 12 weeks with a contralateral resistance training protocol. The hypothesis was that rDNA dosage would be associated with total RNA accumulation, and second that the training intervention causes phenotypic changes (muscle strength and muscle growth) to chronic resistance training.

The main findings were (1) Our qPCR-based method to estimate rDNA dosage is valid (2) rDNA dosage is associated with accumulated total RNA at T3, but not at T2, and post-intervention (3) muscle strength, muscle thickness, and total RNA increases with resistance training, with no volume-effect, and (4) rDNA dosage does not show an association with observed muscle growth post-intervention.

Our results show a clear association between rDNA and total RNA at T3, at the same time as no association was found at T2 and T4. Accumulation of total RNA at T2 may not have reached the proposed plateau observed by Hammarström and colleagues (Hammarström et al., 2022). Therefore, an assumption of the observed response so early is not clearly related to rDNA dose, as transcription of rDNA genes is still high ongoing. Although the demonstrated plateau at approximately 6-9

sessions from Hammarström et al., 2022, is contradictory to our findings, probably our first sessions in the habituation period don't have enough volume to stimulate total RNA maximum, suggesting that total RNA accumulation may be volume sensitive. Hence, based on our results (Figure 3), accumulated total RNA approaches to peak around T3. The individual variation is greatest at the T3 time-point (based on the confidence interval), indicating that the variability in rDNA dose is reflected in the heterogeneity where total RNA reaches its proposed plateau. Another explanatory model for why the association is more visible at T3 than at T2 may be due to epigenetic modifications. Remarkably, in growing cells, not all rDNA copies are in an active state, but approximately half of the genes are silenced at the epigenetic layer (Santoro & Grummt, 2005; McStay., & Grummt., 2008; Tariq., & Östlund Farrants., 2021). Previous studies have shown that chromatin remodeling complex (NoRC) recruits DNA methyltransferase and histone deacetylases to stimulate heterochromatin formation and thus silencing rDNA genes (Santoro & Grummt., 2005). Also, UBF has been shown to play a crucial role in activating rDNA genes (Sanji et al., 2008; Sanji & Hannan, 2009). UBF protein abundance is upregulated with resistance training, seen in interconnection with an increase in total RNA, which shows UBF's potential role in ribosomal biogenesis (Hammarström et al., 2022). Epigenetic modifications have been demonstrated following acute resistance training on various regions at rDNA genes along with upregulation of the c-Myc (Figueiredo et al., 2021). It could be interesting to investigate if the increased presence of UBF and c-Myc activates more rDNA genes, through chromatin remodeling.

The main objective of the thesis was to develop a method for estimating rDNA dose via qPCR to investigate whether there is an association with the accumulation of total RNA at different testing time points. Our results show that the method is valid (Figure 2), and this is consistent with previous findings showing that qPCR is a valid and preferred method (Gibbons et al., 2015; Figueiredo et al., 2021; Hall et al., 2022). One limitation is that the study design where this thesis comes from was not designed to study this relationship from the beginning. The timing of the biopsies is not adapted with respect to previous findings that show where total RNA reaches a clear plateau. This plateau is somewhat difficult to determine exactly based on the data in our study. It is suggested that a biopsy between pre-intervention - T2 and T2 - T3, in combination with higher training intensity between pre and T2 would probably have provided more accurate data on total RNA. If rDNA and total RNA would have shown a stronger association with the mentioned changes is speculative. Furthermore, it would be interesting to look at changes in chromatin remodeling between pre-T2, T2-T3, and pre-post, to investigate whether more rDNA genes get activated as mentioned previously, thus showing greater association with exercise-induced responses to total RNA.

Interesting findings have suggested possible recombination of rDNA copy number in cancer cells, suggesting dynamic plasticity in the human genome, where cancer cells have shown a loss of rDNA copy number in comparison to other healthy tissues in the same individual (Stults et al., 2009; Xu et al., 2017). The model in this study and the statistical analysis does not provide a basis for giving great answers. There is no basis for saying the same changes occur after resistance training in human skeletal muscle, based on the effect change from pre to post (Cohen's d effect size = 0.06, $p = 0.770$). In addition, the sample size is low, where several individuals had different stimuli, which gives low statistical power in this statistical analysis.

The study demonstrates muscular response, highlighted as an increase in muscle strength, muscle thickness, and total RNA A dose-response relationship is not demonstrated with higher training volumes (>6 sets), these findings are in line with two previous meta-analyses which demonstrate a plateau at 4-6 sets (Krieger, 2010; Schoenfeld et al., 2017a). Response to exercise-induced resistance training is proposed as an inverted U-shape, where the response is exponential with an increase in volume, up to a point where the response flattens out (Figueiredo et al, 2018). Changes in muscle thickness show the same tendency as muscle strength, our results demonstrate a resistance training-induced increase compared with the control leg.

Interestingly, Figure 2A shows a visual effect between 3 and 6 sets, but the results are not significant ($p = 0.065$). A meta-analysis shows a higher effect size at 4-6 sets compared to 2-3 sets (Krieger, 2010), but the result here is not significant, probably due to low statistical power. The recent meta-analysis from 2017 asks if there is still any doubt about training volume and response and refers to a dose-response relationship where a high volume of 10+ sets per muscle/muscle group per week is a minimum for maximum muscle growth (Schoenfeld et al., 2017a; Schoenfeld et al., 2017b). The question is whether the amount is enough, especially if the training frequency is 3 times per week, the number of sets will be adjusted down to 3-4 per session, which is significantly lower than the volume we defined as high in our study (6 sets per session, 18 sessions per week). If the goal of the exercise prescription is better healthspan through increased muscle strength and muscle mass, an indication from this study and a former meta-analysis reveals that untrained individuals do not rely on a high training volume for maximum effect (Kriger, 2010).

Resistance training has an effect on total RNA accretion in skeletal muscle in this study, which is consistent with previous research (Brook et al., 2015; Hammarström et al., 2019; Mobley et al., 2018), even though none of the studies have trained with high volume (>6 sets). However, it's difficult to explain the observed numeric increase observed in legs that have trained 0 sets (pre versus T2). To the author's knowledge, no such increase has been observed previously, a possible explanation is that strength testing, which took place during the habituation period is enough to stimulate a total RNA response. The highest relative change in total RNA accumulation occurs between T2 versus T3 and remains relatively stable at T4. Interestingly, the response is very heterogeneous between the individuals, especially in the group that trains with high volume (6 sets) (Figure 2B). Total RNA does not appear to have a strong association with muscle growth over the time course. It should also be mentioned that Mobley et al. (2018) find that total RNA only explains ~ 8% of the change in m. vastus lateralis, which suggests that total RNA is not such a strong predictor of muscle growth. This may help to indicate why we do not find a correlation between rDNA dose and muscle growth.

5.1 Limitations

The study did not check for gender and nutritional status, which may give inaccurate comparisons and incomplete responses, respectively. There is still a lack of understanding about the difference between women's and men's responses to exercise, but we know that the internal physiology is different (e.g., sex hormones, menopause, sex chromosome abnormalities) (reviewed by Landen et al., 2021). Nutritional status and more specified protein intake have been shown to affect rDNA transcription (Figueiredo et al., 2018), and the training response (Morten et al., 2018). Although diet registration is a weakness in the study, there is great uncertainty associated with the validity and reliability where both underestimation and overestimation can occur, which could disrupt the analyses (Hagfors et al., 2005; George et al., 2016).

6.0 Conclusion

To conclude, the qPCR-based method to estimate the rDNA dosage used in this study is valid. rDNA dose shows to have an association with resistance training-induced total RNA after accretion but failed to demonstrate an association with muscle growth. Contralateral resistance training shows an increase in muscle strength and muscle thickness, however, no volume effect when the high and moderate volume was compared.

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Attachment 1

Vil du delta i forskningsprosjektet

”ContraTRAIN – en valideringsstudie av kontralaterale treningsdesign”?

Dette er et spørsmål til deg om å delta i et forskningsprosjekt hvor formålet er å skaffe ny kunnskap om hvordan vi kan optimalisere designet på treningsstudier for å best mulig kunne undersøke effekten av trening. I dette skrivet gir vi deg informasjon om målene for prosjektet og hva deltakelse vil innebære for deg.

Formål

Fysisk aktivitet har en rekke positive effekter på menneskekroppens funksjoner og er et av våre viktigste virkemidler for å fremme folkehelsen. Ikke nok med at det gir forebygging av livsstilssykdommer som for eksempel hjerte-karsykdom, respiratoriske sykdommer og metabolske sykdommer, det gir også styrke og utholdenhet til å beherske dagliglivets utfordringer. For å forstå mekanismene bak de positive effektene av fysisk aktivitet er gode studiedesign helt sentrale. Vi ønsker i denne studien å øke kunnskapen om anvendelse av kontralaterale treningsprotokoller: trening av ett bein om gangen, med ulik type trening på de to beina.

Vanligvis sammenlignes to ulike treningsprotokoller ved at de utføres av to ulike grupper individer. Ulike individer responderer imidlertid ulikt på trening. I slike studier ser vi derfor stor variasjon i treningsresponser. Denne variasjonen gjør det vanskelig å sammenligne effektene av treningsprotokollene. Den vanligste måten å løse denne utfordringen på er å inkludere mange forsøkspersoner. I denne studien skal vi imidlertid se på hvordan vi kan gjøre sammenligningen innad i ett individ. Når samme individ gjennomfører begge treningsprotokoller forsvinner mye av variasjonen. Dette reduserer behovet for antall forsøkspersoner og styrker studiene betraktelig.

I denne studien skal vi anvende et kontralateralt studiedesign for å studere hvordan muskelstyrke, muskelvekst og relaterte cellulære mekanismer påvirkes av ulikt treningsvolum. Samtidig skal vi kartlegge hvordan slike studiedesign kan brukes i videre forskning. Økt forståelse for unilateral trening og individuelle responsmønstre vil være viktig for å utvikle individuelt tilpassede treningsprogrammer og derigjennom øke effekten av trening for den enkelte.

Dette skal undersøkes ved å studere effektene av de to treningsprotokollene på muskelstyrke, muskelmasse, samt cellebiologiske trekk som for eksempel cellers form og utseende, arvematerialets sammensetning (inklusiv DNA-sekvens og epigenetisk modifisering), proteinsyntese (ved hjelp av deuterium), proteinforekomst og -funksjon, RNA-uttrykk i beinmuskulatur til tidligere utrente individer. Vi skal også undersøke hvordan muskulaturens og blodets sammensetning forut for treningsperioden påvirker treningseffektene.

Deltakerne skal deles inn i fire grupper som skal trene som skissert i figur 1. Avhengig av hvilken gruppe du trekkes til, vil du trene ett eller begge bein i en periode på 11 uker. Du vil

også trene overkroppsmuskulaturen (på ordinær måte: to armer). Treningsprogrammet vil gjennomføres som 3 eller 6 sett med styrketrening for hver øvelse, med 10 repetisjoner maksimum i hvert sett. Totalt vil studien vare i ca 13 uker. De tre første ukene i studien vil være en kombinasjon av tilvenning til trening og testing. Fra uke 4 til 11 gjennomføres treningsintervensjonen. Testbatteriet gjentas midtveis i treningsintervensjonen (uke 7) og etter avsluttet trening (i uke 11-12). Figur 1 viser tidsplanen for studien. Gruppe 4 vil i første halvdel gjennomføre testing på samme måte som de andre gruppene, men uten trening. Etter at de andre gruppene er ferdig med sin trening gjennomfører gruppe 4 sin treningsperiode, med tilhørende testing. Studien vil derfor ha en varighet på 26 uker for deltagere i gruppe 4.

Deltakere må møte for testing og trening tre ganger i uken gjennom hele studien, med unntak av gruppe 4 som i sin første periode bare må møte til testing. Deltakere i grupper som bare trener ett bein vil få tilbud om å trene en ny periode med begge bein etter intervensjonen. All trening og testing vil foregå under veiledning på Høgskolen i Innlandet, Campus Lillehammer. Studien er et forskningsprosjekt som involverer både master- og bachelorstudenter som vil skrive sine oppgaver basert på resultatene. *Detaljert informasjon om innhold og tidsforløp vil bli gitt i informasjonsmøte hvor det og vil være mulig å stille spørsmål*.

I et uavhengig delstudium skal vi undersøke kvaliteten på våre muskelbiologianalyser. Hvis du er aktuell for delstudiet, skal du trene tung styrketrening av beina i 10 uker (valgfrie øvelser; minimum tre treningsøkter per uke). Tester av muskelstyrke og innhenting av muskelbiopsier gjennomføres før og etter treningsperioden.

Hvem er ansvarlig for forskningsprosjektet?

Høgskolen Innlandet er ansvarlig for prosjektet og Håvard Hamarsland er prosjektansvarlig.

Hvorfor får du spørsmål om å delta?

Du får dette informasjonsskrivet fordi du har vist interesse for å delta i forskningsprosjektet ContraTRAIN og oppfyller inklusjonskriteriene:

- Mellom 18 og 35 år
- Utrent (ikke har trent systematisk styrketrening mer enn to ganger per måned og ikke har trent utholdenhetstrening mer enn 3 timer per uke det sist halvåret)
- Ikke røyke
- Ikke være på medisiner som kan påvirke tilpasning til trening
- Ikke ha skader i muskel eller skjelett som hindrer deltagelse i tung styrketrening

Hva innebærer det for deg å delta?

Deltakere trekkes til 4 ulike grupper som skal trene ulike kombinasjoner av tung styrketrening på bein og overkropp (se figur 1). Deltakerne i tre av gruppene må over 13 uker møte 3 ganger per uke på Høyskolen Innlandet Campus Lillehammer for testing og trening. Deltakere i den fjerde gruppen vil først fungere som en kontrollgruppe som gjennomfører testing, men ikke trening. Etter perioden som kontrollgruppe gjennomfører også denne gruppen en treningsintervensjon med testing. For denne gruppen vil intervensjonen være 26 uker. All testing og trening vil foregå under veiledning.

Testingen vil involvere:

- Blodprøve før og etter treningsperioden.
- Måling av kroppssammensetning ved DXA. Denne testen gjennomføres fastende på morgenen før, midtveis og etter treningsperioden.
- Måling av tykkelsen av lårmuskulaturen med ultralyd.
- Maksimale styrketester i beinpress, kneekstensjon, benkpress og sittende roing, før og etter treningsperioden.
- Statisk styrke i kneekstensjon (MVC) og isokinetiske tester før, midtveis og etter treningsperioden.
- Sykkeltest med ettbeinssykling.
- Vevsprøver tatt ved mikrobiopsier (hovedstudien) eller Bergström. I hovedstudien skal det tas 4 mikrobiopsier fra hvert bein for deltakere i tre av gruppene i løpet av prosjektet, mens det i den fjerde gruppen (kontrollgruppen) skal tas 7 mikrobiopsier fra hvert bein. I delstudien (som skal validere vår bruk av laboratorieteknikker) skal det tas 2 mikrobiopsier og 2 Bergström-biopsier fra det ene beinet i løpet av prosjektet. Noen synes vevsprøvetaking er ubehagelig. Man vil typisk bli litt støl i muskelen 1-2 dager i etterkant. I svært få tilfeller vil biopsitaking kunne føre til at følelsen i huden forsvinner for en lengre periode, eller gi tydelig arrdannelse. Arrene fra microbiopsiene vil som oftest forsvinne med litt tid. Arrene fra Bergstrømbiopsiene vil være på ca en cm og forbli synlige. Biopsitaking er også forbundet med en viss infeksjonsfare. Risikoen for disse komplikasjonene er svært liten ved bruk av

prosedyrene som benyttes i dette prosjektet. Du vil få klare instrukser om hvordan du skal behandle såret i etterkant av prøvetagningen.

- For å kunne måle hvor raskt nye proteiner bygges inn i muskulaturen må du i uke 7 av prosjektet innta en dose tungtvann. Det er ingen kjente helsekonsekvenser ved inntak av de dosene som anvendes i studien, men lett svimmelhet kan forkomme. For å unngå dette vil dosen fordeles over flere inntak og du vil følges opp av testpersonalet i perioden hvor svimmelhet kan inntreffe.

Det er frivillig å delta

Det er frivillig å delta i prosjektet. Hvis du velger å delta, kan du når som helst trekke samtykke tilbake uten å oppgi noen grunn. Alle opplysninger om deg vil da bli anonymisert. Det vil ikke ha noen negative konsekvenser for deg hvis du ikke vil delta eller senere velger å trekke deg.

Ditt personvern – hvordan vi oppbevarer og bruker dine opplysninger

Vi vil bare bruke opplysningene om deg til formålene vi har fortalt om i dette skrivet. Vi behandler opplysningene konfidensielt og i samsvar med personvernregelverket.

Det er bare studenter og forskere involvert i studien som vil ha tilgang til resultatene dine. Resultatene dine vil bli lagret digitalt på en sikker forskningsserver og eventuelt i papirformat innelåst i en safe. I disse dokumentene vil ditt navn og dine kontaktopplysninger erstattes med en kode. Kodenøkkelen som binder ditt navn til resultatene vil være innelåst i en safe, adskilt fra øvrige data. Dine data vil ikke kunne gjenkjennes i de vitenskapelige publikasjonene som vil publiseres.

Hva skjer med opplysningene dine når vi avslutter forskningsprosjektet?

Prosjektet skal etter planen avsluttes 31.12.2023. Etter at studien er avsluttet vil testresultater og innsamlet biologisk materiale innlemmes i en biobank (se eget delkapittel om biobank) og destruert innen 31.12.2038. Det vil ikke være mulig å spore dine resultater eller prøver tilbake til deg fra denne biobanken. Dataene i biobanken vil danne grunnlaget for doktorgrader og vitenskapelige publikasjoner.

Biobank

Alle blod- og vevsprøver, samt øvrig informasjon som innhentes i prosjektet, inklusiv informasjon som blir utledet fra det biologiske materialet, vil bli lagret i kodet tilstand i en forskningsbiobank tilknyttet prosjektet og vil etterhvert bli overført til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» (REK-id: 213483), situert ved Høgskolen i Innlandet/Sykehuset Innlandet. TrainOME-prosjektet er igangsatt for å avdekke sammenhenger mellom individers tilpasningsevne til trening, også kalt trenbarhet, og kroppslige/cellulære særtrekk. Gjennom den generelle biobanken skal prøvene analyseres sammen med prøver fra en rekke andre prosjekter, hvor den overordnete

målsettingen er å studere faktorer som er bestemmende for generell trenbarhet. Dette innebærer generell analyse av cellebiologiske og genetiske trekk som for eksempel cellers form og utseende, arvematerialets sammensetning (inklusiv DNA-sekvens og epigenetisk modifisering), proteinsyntese, proteinforekomst og -funksjon, RNA-uttrykk og -regulering, hormonforekomst, kroppens indre miljø (metabolomet), og mange flere mål. Det biologiske materialet vil bli anonymisert innen 31.12.2038, hvorpå det vil bli destruert innen fem år. Forskningsdata som har blitt utledet av materialet vil deretter bli oppbevart i anonymisert tilstand på sikker server på ubestemt tid, sammen med øvrige data innhentet i prosjektet. Professor Stian Ellefsen er hovedansvarshavende for forskningsbiobanken.

Dine rettigheter

Så lenge du kan identifiseres i datamaterialet, har du rett til:

- Innsyn i hvilke personopplysninger som er registrert om deg
- A få rettet personopplysninger om deg
- Få slettet personopplysninger om deg
- Få utlevert en kopi av dine personopplysninger (dataportabilitet)
- Å sende klage til personvernombudet eller Datatilsynet om behandlingen av dine personopplysninger.

Hva gir oss rett til å behandle personopplysninger om deg?

Vi behandler opplysninger om deg basert på ditt samtykke.

På oppdrag fra Høgskolen Innlandet har NSD – Norsk senter for forskningsdata AS vurdert at behandlingen av personopplysninger i dette prosjektet er i samsvar med personvernregelverket.

Hvor kan jeg finne ut mer?

Hvis du har spørsmål til studien, eller ønsker å benytte deg av dine rettigheter, ta kontakt med:

- *Høgskolen Innlandet* ved *Håvard Hamarsland (tlf: 93445916, epost: havard.hamarsland@inn.no, eller Stian Ellefsen (tlf: 61288103, epost: stian.ellefsen@inn.no*
- NSD – Personvernombudet, på epost (personvernombudet@nsd.no) eller telefon: 55 58 21 17.
- Vår lokale kontaktperson for personvern i forskning: Anne Sofie Lofthus, forskningsrådgiver, Høgskolen i Innlandet, anne.lofthus@inn.no, telefon: 61288277 .

Med vennlig hilsen

Håvard Hamarsland — Prosjektansvarlig

Stian Ellefsen — (Hovedansvarshavende Biobank)

Samtykkeerklæring ContraTRAIN

Jeg har mottatt og forstått informasjon om prosjektet *(ContraTRAIN)*, og har fått anledning til å stille spørsmål. Jeg samtykker til å delta i treningsintervensjonen med tilhørende testing, herunder

- Blodprøver
- DXA
- Maksimale styrke- og utholdenhetstester
- Testing av statisk styrke i kneekstensjon med elektrisk stimulering av lårmuskulaturen
- Vevsprøver tatt ved mikrobiopsier.
- Inntak av tungtvann.

Jeg samtykker til at mine opplysninger behandles frem til prosjektet er avsluttet, ca. *31.12.2023*.

(Signert av prosjektdeltaker, dato)

FORESPØRSEL OM AVGIVELSE AV VEVS-OG BLODPRØVER TIL EN GENERELL FORSKNINGSBIOBANK

The TrainOme – humane cellers tilpasning til trening og miljø

Dette er en forespørsel til deg om du ønsker å bidra med vevs-og blodprøver i den generelle forskningsbiobanken the TrainOME.

Hva er The TrainOME?

The TrainOME er en generell forskningsbiobank som er godkjent av regional etisk komité (REK) og som legger til rette for oppbevaring av biologisk materiale som skal benyttes til forskning og kartlegging av sammenhengen mellom trenbarhet og cellulære egenskaper. Biobanken inkluderer vevs- og blodprøver fra en rekke enkeltstående forskningsprosjekt, som hver og en har blitt vurdert av regional etisk komite. Hvilke analyser som vil bli gjort på dine prøver vil i sin helhet være definert i den prosjektspesifikke prosjektprotokollen. For ytterligere informasjon, ta kontakt med hovedansvarshavende for forskningsbiobanken, Stian Ellefsen (epost: stian.ellefsen@inn.no; tlf: 61288103).

Hva skjer med prøvene og informasjonen om deg?

Prøvematerialet vil bli oppbevart i låsbar fryser på låst lagerrom, situert ved Høgskolen i Lillehammer/Sykehuset Innlandet. Alle opplysninger og prøver vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Denne vil bli oppbevart adskilt fra øvrige data, enten i låst skap lokalisert til låsbart kontor eller på sikker server tilhørende Høgskolen i Lillehammer og vil kun være tilgjengelig for autorisert personell. Det vil ikke være mulig å identifisere deg i resultatene som kommer ut av biobanken når disse publiseres. Deler av materialet vil kunne bli sendt til utlandet for analyse. Merking vil i slike tilfeller være begrenset til identifikasjonsnummer; dvs. de vil bli sendt i kodet tilstand. Ubenyttet materiale vil bli returnert til Lillehammer i etterkant av analysene. Det biologiske materialet vil bli anonymisert innen 31.12.2038, hvorpå det vil bli destruert innen fem år. Høgskolen i Lillehammer ved administrerende direktør er databehandlingsansvarlig.

Dine rettigheter

Det er frivillig om du vil la ditt biologiske materiale inngå i The TrainOME-biobanken og du kan når som helst trekke tilbake ditt samtykke uten at du trenger oppgi grunn for dette. Hvis du sier ja til innlemmelse i biobanken, har du rett til å få innsyn i opplysninger som er registrert på deg og også rett til å få korrigert eventuelle feil som oppdages. Du vil etter loven ha krav på jevnlig informasjon om hvordan materialet blir benyttet. Om du trekker ditt samtykke, vil ditt biologiske materiale samt utledete data bli slettet, med mindre opplysningene allerede inngår i analyser eller har blitt brukt i vitenskapelige publikasjoner.

Prosjektkoordinator eller øvrige prosjektmedarbeidere kan kontaktes når som helst i arbeidstiden: Stian Ellefsen (hovedansvarshavende), tlf: 61288103, epost: stian.ellefsen@inn.no

Bent Rønnestad (prosjektkoordinator), tlf: 61288193, epost: bent.ronnestad@inn.no

Gunnar Slettaløkken (prosjektkoordinator), tlf: 61288182, epost: gunnar.slettalokken@inn.no

Samtykke til deltakelse i den generelle forskningsbiobanken

Jeg bekrefter med dette å ha lest informasjonsskrivet knyttet til den generelle biobanken «The TrainOME – humane cellers tilpasning til trening og miljø» og samtykker til at mine vevs- og blodprøver kan inngå i biobanken:

Sted:…… Underskrift: …… Dato: …../…. 20…..