# RibOSE – effects of glucose ingestion during resistance exercise training on ribosomal biogenesis in skeletal muscle

*Project leader*: Professor Stian Ellefsen, Inland Norway University of Applied Sciences

Muscle growth-responses to resistance training vary extensively between humans, with as many of 10-15 % of individuals showing severely impaired growth [1, 2]. In such individuals, cellular plasticity is compromised [3, 4], and they experience reduced functional and health-beneficial outcomes of training [3, 4]. While this impairment is likely to be of multifactorial causation that includes epigenetic, genetic and physiological variables, recent studies suggest that such individuals display a common feature [5]. They lack the ability to produce novel ribosomes in response to training [5-7], and hence display impaired increases in protein synthesis and decreased growth rates [5]. This makes ribosomal content in muscle a plausible proxy marker for training-associated muscle hypertrophy.

In a recent study, we showed that increasing the resistance training volume is associated with more pronounced muscle growth in untrained young individuals [6], a trait that was associated with increased ribosomal biogenesis [6]. Despite of this, ~50 % of the participants did not exhibit a true beneficial effect of increased training volume [6], which in turn coincided with impaired abilities to accumulate ribosomes [6]. In such individuals, means other than training volume are likely necessary to circumvent the impairing effects of genetic and epigenetic predispositions. At present, nutrient supplementation stand out as a promising therapy [e.g. 8, 9, 10]. However, our knowledge is limited to a selected few nutrients and we are largely ignorant to their effects on ribosomal biogenesis. The only exception is protein ingestion, for which we know that adequate intake is essential for achieving optimal muscle growth [8], potentially being interconnected with ribosomal synthesis [11]. For other nutrients, we know little about their effects on muscle plasticity and ribosomal biogenesis.

In cell types such as kidney cells, the most efficient mean for increasing ribosomal biogenesis (and growth rates) seems to be exposure to super-physiological levels of glucose [12], as based on experiments performed in cell culture. These experiments suggest that glucose is an important signaling molecule for increasing ribosomal production *per se*, perhaps acting as a ligand for signaling proteins or simply acting to increase energy availability. In the human body (as opposed to cultured cells), glucose may also exert growth-stimulating effects by increasing the levels of insulin in blood [13]. It thus remains plausible that glucose intake during resistance training stimulates ribosomal biogenesis and exerts beneficial effects on muscle plasticity in humans, perhaps acting in an additive manner to protein supplementation, affecting signaling pathways such as the AMPK pathway [12, 14], in turn interacting with the positive effects of protein ingestion. At present, we do not know if this is the case, though previous studies have suggested glucose ingestion in connection with acute bouts of resistance training reduces training-induced muscle damage without affecting within-session work output (i.e. volume) [15]. This lack of knowledge is utterly surprising given the long-standing appreciation of the beneficial effects of glucose intake on endurance performance, whereby it acts to delay muscular fatigue [16].

## Aims

***The aim of the study is to investigate the effects of ingesting glucose during five bouts of resistance exercise on ribosomal content (i.e. total RNA content) in m. vastus lateralis of moderately trained individuals (20-45 years of age, n=20)***

Secondary aims:

1. *To investigate the accumulated effects of ingesting glucose during five bouts of resistance exercise on rRNA and mRNA abundances (markers of muscle growth, including ribosomal proteins) in m. vastus lateralis*
2. *To investigate the accumulated effects of ingesting glucose during five bouts of resistance exercise on protein abundances (e.g. ECM and fiber-specific proteins) in m. vastus lateralis*
3. *To investigate the accumulated effects of ingesting glucose during five bouts of resistance exercise on rates of protein synthesis in m. vastus lateralis (measured using deuterium)*
4. *To investigate the effects of ingesting glucose during resistance exercise sessions on endocrine variables (such as insulin, c-peptide, testosterone, growth hormone, cortisol and inflammatory markers) and glucose concentrations in blood (measured in a rested state before and after the five main resistance training bouts, as well as before and after the sixth training session, see Figure 1)*
5. *To investigate the progressive effects of ingesting glucose during five bouts of resistance training on muscle strength (measured as isokinetic knee extension torque and isometric knee extension force)*
6. *To investigate the effects of ingesting glucose during a resistance training session on rates of muscular recovery (measured as temporal changes in isokinetic knee extension torque and isometric knee extension force after the sixth training session, see Figure 1)*

For overview of the intervention, see Figure 1. For detailed overview of outcome measures, see Table 1.

## Methodology

**Participants**

For flow chart of the intervention, see Figure 2. Twenty male and female participants (20-45 years of age) will be recruited to the study, with eligibility criteria being non-smoking and moderately trained (i.e. having performed 2-8 resistance training sessions per 14 days for the last six months). Criteria for exclusion are previous injury resulting in impaired strength, inability to perform resistance training and symptoms, and medical record of metabolic disorders including hyperglycemia, i.e. fasting venous plasma glucose ≥6.1 mmol/L and/or 2-hour glucose tolerance ≥7.8 mmol/L, and/or HbA1c >42mmol/mol.

**Study overview**

For graphical overview of the study, see Figure 1. For overview of outcome measures, see Table 1 (including allocation of tasks/responsibilities between study personnel). The study will be conducted as a 12-day intervention, with each day containing concomitant dietary intervention and resistance training (RT). Overall, the dietary intervention consists of alternating days of ingestion of glucose (GLU) and placebo (PLAC) in connection with training, accompanied by ingestion of protein (PRO) to ensure a growth-friendly physiological milieu (8). Likewise, training consists of alternating days of resistance training of the two legs using identical training protocols, with Day 1 involving training of the first leg (RT#1), Day 2 involving training of the second leg (RT#2), Day 3 involving training of RT#1, etc. Hence, six training session will be performed for each leg. In this way, each of the two dietary interventions will be associated with training of one leg, allowing within-subject comparisons of the two treatments and hence removing biological diversity between individuals as a confounding factor. The order in which participants perform the two intervention blocks will be determined in a planned randomized fashion. Half the participants (n=10) will commence the intervention with GLU on Day 1, while the other half will commence with PLAC. For participants starting with GLU, half will perform training on their dominant leg, while the other half will perform training on their non-dominant leg. The same will be the case for participants starting with PLAC.

RT will be performed as unilateral resistance training using knee extension and leg press (three sets of 10 repetitions maximum, 10RM). Muscle biopsies will be sampled from m. vastus lateralis at four time points (on four separate days): time point 1 (T1, pre RT#1), T2 (pre RT#2), T3 (post RT#1, before RT on day 11), and T4 (post RT#2, before RT on day 12) (Figure 1). Blood will be collected at the same time points. Physical performance tests (Test) will be performed prior to onset of the intervention (T0, days -7 and -5) and after finalization of the intervention (RT#1, T3, Day 11; RT#2, T4, Day 12; Day 13) (Figure 1), measured as unilateral isokinetic knee extension torque/isometric knee extension force and unilateral one repetition maximum (1RM) in knee extension and leg press of the two legs press. Body mass composition will be measured prior to onset of the intervention (Day -1), allowing lean body mass-based ingestion of deuterium (D2O, heavy water), which in turn will enable measurement of rates of RNA/protein synthesis in muscle during the intervention (i.e. heavy hydrogen isotopes will be incorporated into RNA/proteins, which can be traced in analyses of muscle biopsies). D2O will be ingested on a daily basis throughout the intervention, starting with a large bolus at Day -1 (5.25 ml ⋅ kg lean body mass-1), followed by smaller top-up volumes at days 1-12 (0.53 ml ⋅ kg lean body mass-1). This will ensure sustained physiological levels of D2O, and will be

Et bilde som inneholder innendørs, overvåke, skjermbilde, skjerm

Automatisk generert beskrivelse

**Figure 1.** **A)** Overview of the intervention, consisting of 12 days of concomitant dietary intervention and resistance training (RT), preceded by 7 days of testing (Test), dual-energy x-ray absorptiometry (DXA) scanning and biopsy/blood sampling, and followed by one day of testing. The dietary intervention consists of alternating days of glucose (GLU) and placebo (PLAC) ingestion in connection with training (see panel B for details). On each day, this will be accompanied by protein (PRO) ingestion to ensure a growth-friendly physiological milieu [8]. Training consists of alternating days of resistance exercise of the two legs (knee extension and leg press; three sets of 10 repetitions maximum, 10RM), starting with training of one leg on Day 1 (RT#1), followed by training of the other leg on Day 2 (RT#2), followed by training of leg RT#1 on Day 3, etc. In this way, each of the two dietary interventions will be associated with training of one particular leg, enabling within-subject comparisons of the two treatments. Muscle biopsies will be sampled from m. vastus lateralis at four time points during the intervention: T1, time point 1 (*pre* RT#1 leg), T2 (*pre* RT#2 leg), T3 (*post* RT#1 leg, before RT on day 11), and T4 (*post* RT#2 leg, before RT on day 12). Blood samples will be collected at the same time points. Physical performance tests (Test) will be performed prior to onset of the intervention (T0, days -7 and -5) and after the intervention (T3/T4, Day 11/Day 12; Day 13), measured as unilateral isokinetic knee extension torque/isometric knee extension force, and one repetition maximum (1RM) in knee extension and leg press of each legs. In addition, isokinetic knee extension torque/isometric knee extension force will be measured ~30 min after training on Days 3, 4, 5, 7, 8 and 9 on both legs. Body mass composition (DXA) will be measured prior to onset of the intervention (T0, Day -1), allowing lean body mass-based ingestion of deuterium (D2O, heavy water). D2O will be ingested on a daily basis throughout the intervention, starting with a large bolus at Day -1 (5.25 ml ⋅ kg lean mass-1, between 0815 hrs and 1115 hrs), followed by smaller top-up volumes at days 1-12 (0.53 ml ⋅ kg lean mass-1, ingested together with GLU/PLAC). This will ensure sustained physiological levels of D2O, which will be validated through analyses of saliva samples (Spit), collected immediately before D2O ingestion on all intervention days. **B)** Overview of days with dietary intervention and RT, including timing of GLU (upper panel) / PLAC (lower panel), PRO and D2O ingestion, as well as timing of RT. On each day, the dietary intervention spans from 0700-1200 hrs, i.e. from 2hrs before RT to ~2.5 hrs after RT (notably, for individual participants, daily onset of the intervention will vary from 0600-0800 to ensure adequate flow of participants through the study). During this time course, participants will ingest GLU/PLAC and PRO supplements only. GLU/PLAC will be ingested at three time points: 30 min prior to RT (0830 hrs, 30 g vs. 0 g glucose), immediately prior to RT (0900 hrs, 30 g vs. 0 g glucose), and immediately after completion of training (~0930 hrs, 30 g vs. 0 g glucose). PRO will be ingested at two time points: 2 hours prior to RT (0700 hrs, 25 g) and immediately after completion of training (25 g). To ensure balanced glucose intake during the entirety of GLU/PLAC days, participants will also ingest GLU or PLAC during the afternoon (between 1800 hrs and 1900 hrs; 3 x 30 g vs. 3 x 0 g glucose), wherein the allocated supplement will be the opposite of what was ingested in connection with training (i.e. between 0700 hrs and 1200 hrs). On each day, D2O will be ingested between 0830 and 0900 hrs (after ingestion of GLU/PLAC and before onset of RT), mixed with regular water to a total volume of 200 ml. Between 1200 hrs and 2200 hrs on intervention days, participants will ingest a freely chosen (registered) diet, advocated to amount to a daily energy consumption of 40 kcal ⋅ kg body mass-1. This self-chosen diet plan will be repeated on pairwise consecutive days (i.e. on days 1-2, 3-4, 5-6, etc) to ensure similar premises for training responses in the two legs.

validated by analyses of D2O in saliva samples (Spit), collected immediately before D2O ingestion on all intervention days.

**Table 1.** Core outcome domains. 1RM, one-repetition maximum; T0, prior to onset of the intervention; T1/T2, prior to onset of training for each of the two legs; T3/T4, after five training sessions RT#1 leg/ RT#2 leg (see Figure 1). PRIM, secondary outcome measure; SEC, secondary outcome measure.

|  |  |  |  |
| --- | --- | --- | --- |
| Outcome | Specific measures | Timepoint | Responsibility |
| Physiological/performance tests   1. Muscle strength | 1. 1RM knee extension and leg press, unilateral (a, SEC) 2. Isokinetic knee extension torque/isometric knee extension force, unilateral (a, SEC) | 1. T0 2. Throughout the intervention | K Lian  S Moen  H Hamarsland  D Hammarström  B Rønnestad |
| Body composition measures   1. Body mass composition | 1. Bone mineral density, fat mass (total), visceral fat mass and lean mass: DXA (a) | 1. T0 | K Lian  H Hamarsland |
| Muscle biopsy from m. Vastus lateralis   1. Ribosomal content 2. General muscle biology 3. Protein synthesis rate | 1. Total RNA levels per unit tissue weight (a, PRIM) 2. Levels of specific rRNA and mRNA; qPCR (a-b, SEC) 3. Protein abundance; Western blotting (b, SEC) 4. Protein synthesis rate, measured using D2O; chromatography and mass spectrometry (c, SEC) | 1. T1-T4 2. T1-T4 3. T1-T4 4. T3-T4 | K Lian  S Moen  S Ellefsen  H Hamarsland  D Hammarström  H Nygaard |
| Blood and spit samples   1. Glucose levels in blood with and without glucose intake/training 2. Nutrient levels 3. Endocrine variables 4. D2O | 1. Glucose in blood (a, SEC) 2. Amino acids in blood (b, SEC) 3. Hormones in blood (e.g. insulin, c-peptide, testosterone, growth hormone, cortisol) (c, SEC) 4. Inflammatory markers in blood (e.g. IL-6, CRP, TNF, NFκB) (c, SEC) 5. D2O enrichment in spit, used as a marker for RNA and protein synthesis rates in muscle (d, SEC) | 1. T1-T4 2. T1-T4 3. T1-T4 4. T1-T4 5. Throughout the intervention | K Lian  S Moen  H Nygaard  H Hamarsland  M Husøy |

**Dietary intervention**

During days with dietary intervention, the dietary intervention will span from 0700-1200 hrs, i.e. from 2hrs before RT to ~2.5 hrs after RT. During this time, participants will ingest GLU/PLAC and PRO supplements only. GLU/PLAC will be ingested at three time points: 30 min prior to RT (0830 hrs, 30 g vs. 0 g glucose), immediately prior to RT (0900 hrs, 30 g vs. 0 g glucose), and immediately after completion of training (~0930 hrs, 30 g vs. 0 g glucose). PRO will be ingested at two time points: 2 hours prior to RT (0700 hrs, 25 g) and immediately after completion of training (25 g). Notably, for individual participants, the daily onset of the dietary intervention (defined as time point for intake of PRO) will vary from 0600 to 0800 hrs to ensure adequate flow of participants through the protocol (i.e. to allow multiple participants to complete the protocol on the same day). For each participant, onset of the intervention will be the same on all days. To ensure balanced glucose intake during the entirety of GLU/PLAC days, participants will also ingest GLU or PLAC during the afternoon (between 1800 hrs and 1900 hrs; 3 x 30 g vs. 3 x 0 g glucose), wherein the allocated supplement will be the opposite to what was ingested in connection with training (i.e. between 0700 hrs and 1200 hrs). Between 1200 hrs and 2200 hrs on intervention days, participants will ingest a freely chosen diet, advocated to amount to a daily energy consumption of 40 kcal ⋅ kg body mass-1. This self-chosen diet plan will be registered using MyFitnessPal and will be repeated on pairwise consecutive days (i.e. on days 1-2, 3-4, 5-6, etc), thus ensuring similar premises for training responses in the two legs. Between 2200 hrs and 0700 hrs (alternatively 2100-0600 or 2300-0800), participants will remain in a fasted state.

**Et bilde som inneholder skjermbilde

Automatisk generert beskrivelse**

**Figure 2.** Flowchart of the intervention. The order in which participants perform the two intervention blocks will be determined in a planned randomized fashion. Half the participants (n=10) will commence the intervention with GLU on Day 1, while the other half (n=10) will commence with PLAC. Moreover, for participants starting with GLU, half will perform training on their dominant leg, while the other half will perform training on their non-dominant leg. The same will be the case for participants starting with PLAC.

One bolus of GLU will be ingested as 30 g Glucose (Glucosum monohydricum, Merck KGaA, Darmstadt, Germany) mixed with 300 ml Fun Light saft/juice (Orkla, Oslo, Norway). One bolus of PLAC will be ingested as 100 mg Stevia powder (Steviosa, Soma Nordic AS, Oslo, Norway), containing the natural sweetener erythritol in amounts equivalent to the sweetness of 30 g glucose, mixed with 300 ml Fun Light saft/juice (Orkla, Oslo, Norway). Hence, GLU and PLAC supplements will have identical chemical composition, except for their content of glucose/Steviosa. Stevia powder and erythritol are suitable placebo sweeteners, as they neither increases glucose levels in blood nor leads to insulin responses [17, 18]. Neither Stevia powder nor erythritol are defined as drugs according to Norwegian law (<https://lovdata.no/dokument/SF/forskrift/1999-12-27-1565>). The taste of the two beverages will be compared using a double-blinded crossover test, performed at Day 13, wherein participants will ingest the two beverages in a randomized order. During the test, participants will be asked to identify the beverages as either GLU or PLAC to investigate if their content can be disclosed. One bolus of PRO will be ingested as 25 g Whey Protein Isolate (Proteinfabrikken, Stokke, Norway) mixed with 150 ml H2O. After onset of training, participants are free to ingest water ad libitum.

On each intervention day, participants will receive boluses of supplements in accordance with their study ID number. The list that links this ID number to the randomization code will be kept with the person who generated the randomization code (PhD-student Knut Sindre Mølmen) until completion of data sampling and cleaning of data on main outcome measures. The study will thus be conducted as a double-blinded randomized clinical trial (RCT). Mølmen will not be involved in any aspects of data sampling or handling. None of the other project collaborators will have access to this list during the intervention or data handling.

**Resistance training protocol**

On all intervention days, resistance training will be performed as unilateral leg press and unilateral knee-extension, performed as three sets of 10RM. For each participant, the two legs will be trained on alternating days, with each leg being associated with either GLU or PLAC ingestion (Figure 1). Half the participants starting with GLU will train their dominant leg during the first training session, while the other half will train their non-dominant leg during the first session (Figure 2). The same will be the case for participants starting with PLAC (Figure 2). Participants will be instructed to perform each repetition based on a pre-defined repetition speed. Repetition maximum (failure) is achieved when the weight cannot be lifted in a controlled fashion. After each training session, participants will register their perceived rate of exertion using a 10-point scale. Trained personnel will monitor all sessions. Training volume will be logged.

**Sampling of muscle tissue and blood and analysis**

Muscle biopsies will be sampled from m. vastus lateralis at six time points during the intervention (Figure 1A): i) before the intervention from the leg performing training on Day 1 (time point T1, pre RT#1 leg), ii) before the intervention from the leg performing training on Day 2 (T2, pre RT#2 leg), iii-iv) before and after the sixth training session from the RT#1 leg (T3 and T4), and v-vi) before and after the sixth training session from the RT#2 leg (T5 and T6). All biopsies will be sampled in an overnight-fasted condition at the same time of day (0545, 0645 or 0745 hrs, depending on the daily timing of dietary intervention-onset, corresponding to 0600, 0700 and 0800 hrs, respectively), and will be sampled under antiseptic conditions and local anaesthesia (Lidokain 10 mg ml-1, Mylan Hospital AS, Oslo Norway), using the well-established, minimally invasive micro-biopsy technique [17], using a 12-14 gauge needle (Universal Plus, Mermaid medical A/S, Stenløse, Denmark) operated with a spring loaded biopsy gun (Bard Magnum, Bard Norway A/S, Oslo, Norway). Subsequent to sampling, muscle biopsies will be divided into aliquots for determination of total RNA/targeted mRNA abundances (qPCR; two aliquots) and protein abundances/phosphorylation (Western blotting) (for overview of outcome measures, see Table 1). On the first and last day of biopsy sampling from each leg (corresponding to time points T0, T3 and T5), a tissue aliquot will be allocated to measuring deuterium enrichment and rates of RNA/muscle protein synthesis (using chromatography and mass spectrometry).

Blood will be collected at four days during the intervention, coinciding with days of biopsy sampling (Day 1, 2, 11 and 12). At Day 1 and 2, blood will be collected in a rested and fasted state immediately after muscle biopsy sampling. At Day 11 and 12, blood will be collected at several time points: i) before intake of PRO supplements (e.g. 0700 hrs), ii) 30 min after intake of PRO (0730 hrs), ii) 1 hr after intake of PRO (0800hrs), iii) 1.5 hrs after intake of PRO (0800hrs, i.e. immediately before GLU/PLAC intake), iv) 2 hrs after intake of PRO (0900 hrs, i.e. immediately before training), v) immediately after training, and vi) 30 min after training. In addition, blood will be collected using fingers sticks at several time points for further investigation of blood glucose levels (i.e at 0715 hrs, 0815 hrs, after warm-up to training, in the middle of the training session, 15 min after training, 60 min after training and 2 hrs after training. Together, these blood samples/finger sticks will ensure analyses of the effects of supplement intake and training on variables such as hormones and inflammatory markers, as well as high-resolution analyses of glucose concentrations in blood (for overview of outcome measures, see Table 1). Blood samples will be collected from an antecubital vein using standard blood sampling equipment, performed by experienced personnel. Endocrine analyses will be performed at Sykehuset Innlandet Hospital Trust. Glucose analyses will be performed using in-house equipment.

**Assessment of muscle strength**

Physical performance tests (Test) will be performed on seven days for each of the two legs (Figure 1): prior to onset of the training intervention (T0, Days -7 and -5, both legs), during the intervention (Days 4, 5, 8 and 9), and after finalization of the intervention (T3/T4, Day 11, RT#1 leg; T5/T6, Day 12, RT#2 leg) (Figure 1). The complete test battery consists of unilateral isokinetic knee extension torque/isometric knee extension force and unilateral one repetition maximum (1RM) in knee extension and leg press. In connection with days 11 and 12, measurement of isokinetic knee extension torque/isometric knee extension force will be performed at four time points: i) immediately before the resistance training session, ii) 15 min after finalization of the session, iii) 2 hours after finalization of the session, and vi) 22 hours after finalization of the session. For all test time points, the highest value will be carried forward to final analyses. To ensure reliability of pre-intervention strength data, participants will be asked to refrain from training during the 3 days leading up to day -7. After commencing the intervention protocol (i.e. after day -7), participants will be asked to refrain from all other training.

**Assessments of body composition**

Body mass composition will be measured by dual-energy x-ray absorptiometry (DXA) prior to onset of the intervention (Day -1), providing data on lean body mass, fat mass and bone mineral density. Data on lean body mass will enable proper dosage of deuterium ingestion (D2O, heavy water).

**Measurement of RNA and protein synthesis rates in muscle**

D2O will be ingested on a daily basis throughout the intervention, starting with a large bolus at Day -1 (5.25 ml ⋅ kg lean mass-1, immediately after DXA measurement, between 0815 hrs and 1115 hrs, Figure 1), followed by smaller top-up volumes at days 1-12 (0.53 ml ⋅ kg lean mass-1; coinciding with GLU/PLAC intake). This will ensure sustained physiological levels of D2O. D2O levels will be measured in saliva samples (spit), collected immediately before D2O ingestion on all intervention days

D2O intake will allow measurement of rates of RNA/protein synthesis in muscle during the training intervention. Muscle RNA/protein synthesis rates in the two legs will be calculated as the relative enrichment of heavy hydrogen isotopes measured in rested-state biopsies sampled at Day 11 and 12 (Figure 1), measured using gas chromatography/mass spectrometry.

**Ethical considerations**

Ingestion of Steviosa or its ingredients is not associated with adverse effects in the prescribed doses in the selected groups of participants (young healthy), and may instead have health-beneficial effects [18, 19]. Subjects with a medical record of impaired glucose tolerance will be excluded from the study. Resistance training is not associated with risks (other than the potential occurrence of training-induced injuries). To counteract adverse event, trained personnel will carefully monitor every training session. All participants will be offered post-intervention consultation on prospective training programs.

Muscle biopsy sampling will be performed by experienced personnel, using well-established procedures. Participants will be excluded from the study if they report or experience adverse effects to local anaesthetics. Participants will be given written and oral information on post-procedure care to minimize the risk of infection. Following sampling of muscle tissue, one may experience mild soreness that usually normalizes during 1-2 days after the procedure. Other invasive methods (venous and capillary blood samples) are not associated with any risks. There are no risks associated with ingestion of heavy water in the doses used in this study. Light dizziness may occur in the hours after ingestion. Participants will be observed by the testing personnel during this period to avoid any incidents. Participants will be given access to their own test data on request. The proposed study will add basic knowledge to our understanding of optimal applications of study design to investigate responses to training, and mechanisms determining exercise training adaptations. Further understanding these basic mechanisms will aid in designing better studies and better suited exercise programs in a wide range of populations.

## Data management, study financing and dissemination

**Data management and biobank**

The data management plan is in line with the FAIR-principle. The project will be integrated into the Norwegian Services for sensitive data (TSD), allowing collection, storage, sharing and analyses of sensitive research data in a secure environment. Data will remain coded until after completion of cleaning of primary outcome data. After finalization of the project (31.12.2023), biological samples will be transferred from the project-specific biobank to the general biobank “«The TrainOME – humane cellers tilpasning til trening og miljø» (REK-ID: 2013/2041). Muscle biopsy material will be transferred to Denmark for analyses of rates of RNA and/or protein synthesis (in a coded state, remaining material will be returned to Norway/Lillehammer).

**Study financing**

The study will be financed by the Inland Norway University of Applied Sciences. The project consortium has no conflicts of interest to declare.

**Dissemination**

The results will be published in peer-reviewed international biomedical and biological journals, preferably open access. In addition, the results will be presented at scientific conferences (national and international) and will be communicated to the general public through mass media, social media, web blogs and podcasts.

## References

1. Thalacker-Mercer, A., et al., *Cluster analysis reveals differential transcript profiles associated with resistance training-induced human skeletal muscle hypertrophy.* Physiological Genomics, 2013. **45**(12): p. 499-507.

2. Álvarez, C., et al., *Interindividual responses to different exercise stimuli among insulin-resistant women.* Scandinavian Journal of Medicine & Science in Sports, 2018. **28**(9): p. 2052-2065.

3. Kumar, V., et al., *Muscle Protein Synthetic Responses to Exercise: Effects of Age, Volume, and Intensity.* The Journals of Gerontology: Series A, 2012. **67**(11): p. 1170-1177.

4. Montero, D. and C. Lundby, *Refuting the myth of non-response to exercise training: ‘non-responders’ do respond to higher dose of training.* The Journal of Physiology, 2017. **595**(11): p. 3377-3387.

5. Roberts, M.D., et al., *Physiological Differences Between Low Versus High Skeletal Muscle Hypertrophic Responders to Resistance Exercise Training: Current Perspectives and Future Research Directions.* Frontiers in Physiology, 2018. **9**(834).

6. Hammarström, D., et al., *Benefits of higher resistance-training volume are related to ribosome biogenesis.* The Journal of Physiology, 2020. **598**(3): p. 543-565.

7. Chaillou, T., T.J. Kirby, and J.J. McCarthy, *Ribosome biogenesis: emerging evidence for a central role in the regulation of skeletal muscle mass.* Journal of cellular physiology, 2014. **229**(11): p. 1584-1594.

8. Kim, J. and K.-L. Guan, *mTOR as a central hub of nutrient signalling and cell growth.* Nature Cell Biology, 2019. **21**(1): p. 63-71.

9. Alway, S.E., et al., *Resveratrol Enhances Exercise-Induced Cellular and Functional Adaptations of Skeletal Muscle in Older Men and Women.* The Journals of Gerontology: Series A, 2017. **72**(12): p. 1595-1606.

10. Jeromson, S., et al., *Omega-3 Fatty Acids and Skeletal Muscle Health.* Marine drugs, 2015. **13**(11): p. 6977-7004.

11. Figueiredo, V., et al., *High dose of whey protein after resistance exercise promotes 45 S preribosomal RNA synthesis in older men.* Nutrition, 2018. **50**: p. 105-107.

12. Tanaka, Y. and M. Tsuneoka, *Control of Ribosomal RNA Transcription by Nutrients*, in *doi:10.5772/intechopen.71866*. 2018.

13. Abdulla, H., et al., *Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown: a systematic review and meta-analysis.* Diabetologia, 2016. **59**(1): p. 44-55.

14. Goodman, C.A., et al., *The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth.* The Journal of physiology, 2011. **589**(Pt 22): p. 5485-5501.

15. Haff, G.G., et al., *Carbohydrate supplementation and resistance training.* J Strength Cond Res, 2003. **17**(1): p. 187-96.

16. Smith, J.W., et al., *Fuel selection and cycling endurance performance with ingestion of [13C]glucose: evidence for a carbohydrate dose response.* Journal of Applied Physiology, 2010. **108**(6): p. 1520-1529.

17. Tey, S.L., et al., *Effects of aspartame-, monk fruit-, stevia- and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake.* International journal of obesity (2005), 2017. **41**(3): p. 450-457.

18. Wölnerhanssen, B.K., et al., *Metabolic effects of the natural sweeteners xylitol and erythritol: A comprehensive review.* Critical Reviews in Food Science and Nutrition, 2019: p. 1-13.

19. Abbas Momtazi-Borojeni, A., et al., *A Review on the Pharmacology and Toxicology of Steviol Glycosides Extracted from Stevia rebaudiana.* Current Pharmaceutical Design, 2017. **23**(11): p. 1616-1622.