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

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Running title: Resistance training-volume and ribosome biogenesis

Key points
• For individuals showing suboptimal adaptations to resistance training, manipulation of training volume is a potential measure to facilitate responses. This remains unexplored.
• Here, 34 untrained individuals performed contralateral resistance training with moderate and low volume for 12 weeks. Moderate volume led to larger increases in muscle cross-sectional area, strength and type II fibre-type transitions.
• These changes coincided with greater activation of signalling pathways controlling muscle growth and greater induction of ribosome synthesis.
• Thirteen and sixteen participants, respectively, displayed clear benefits of moderate-volume training on muscle hypertrophy and strength. This coincided with greater total RNA accumulation in the early-phase of the training period, suggesting that ribosomal biogenesis regulates the dose-response relationship between training volume and muscle hypertrophy.
• These results demonstrate that there is a dose-dependent relationship between training volume and outcomes. On the individual level, benefits of higher training volume were associated with increased ribosomal biogenesis.
Abstract: Resistance-exercise volume is a determinant of training outcomes. However not all individuals respond in a dose-dependent fashion. In this study, 34 healthy individuals (males n = 16, 23.6 (4.1) years; females n = 18, 22.0 (1.3)) performed moderate- (3 sets per exercise, MOD) and low-volume (1 set, LOW) resistance training in a contralateral fashion for 12 weeks (2-3 sessions×week⁻¹). Muscle cross-sectional area (CSA) and strength were assessed at weeks 0 and 12, along with biopsy sampling (m. Vastus lateralis). Muscle biopsies were also sampled before and one hour after the fifth session (Week 2). MOD resulted in larger increases in muscle CSA (5.2 (3.8)% versus 3.7 (3.7)%; P <0.001) and strength (3.4-7.7% difference, all P < 0.05). This coincided with greater reductions in type IIX fibres from week 0 to 12 (MOD, -4.6; LOW -3.2%-point), greater phosphorylation of S6-kinase 1 (p85 S6K1Thr412, 19%; p70 S6K1Thr389, 58%) and ribosomal protein S6Ser235/236 (37%), greater rested-state total RNA (8.8%) and greater exercise-induced c-Myc mRNA expression (25%; Week 2, all P < 0.05). Thirteen and sixteen participants, respectively, displayed clear benefits in response to MOD on muscle hypertrophy and strength. Benefits were associated with greater accumulation of total RNA at Week 2 in the MOD leg, with every 1% difference increasing the odds of MOD benefit by 7.0% (P = 0.005) and 9.8% (P = 0.002). In conclusion, MOD led to greater functional and biological adaptations than LOW. Associations between dose-dependent total RNA accumulation and increases in muscle mass and strength points to ribosome biogenesis as a determinant of dose-dependent training responses.

Introduction

In humans, the biological adaptation to resistance training varies with exercise-training variables such as volume, intensity, rest between repetitions and sets, selection and order of exercises, repetition velocity and frequency of training sessions (Ratamess et al., 2009). In addition, genetic and epigenetic disposition and environmental factors play a role in variations in adaptations (Timmons, 2011; Seaborn et al., 2018; Morton et al., 2018). As time constraints often hinder participation in exercise training-programs (Choi et al., 2017), numerous studies have searched for the minimal required exercise dose to promote beneficial adaptations. Within-session volume has received particular attention, and although a handful of studies have shown that low-volume training provides similar gains in strength and muscular mass as moderate-volume training (Cannon & Marino, 2010; Ostrowski et al., 1997; Mitchell et al., 2012), meta-analyses conclude in favor of moderate volume protocols (Rhea et al., 2003; Krieger, 2009, 2010; Schoenfeld et al., 2016). This apparent discrepancy
of specific studies to demonstrate benefits of increased training volume is likely due to a combination of small sample sizes and substantial variation in training responses between individuals and experimental groups. In theory, within-participant designs should alleviate these limitations.

Individual response patterns to resistance training, including muscle strength and mass, correlate closely with muscle cell characteristics, measured in both rested-state and acute training-phase conditions (Thalacker-Mercer et al., 2013; Raue et al., 2012; Stec et al., 2016; Terzis et al., 2008). In this context, molecular signatures conveyed by the mechanistic target of rapamycin complex 1 (mTORC1) has been in particular focus. Inhibition of mTORC1 impairs protein synthesis in humans (Drummond et al., 2009) and activation of its associated downstream target S6 kinase 1 (S6K1) correlates with increases in muscle protein synthesis and subsequent muscle growth (Burd et al., 2010; Terzis et al., 2008). In line with this, surplus exercise volume leads to greater phosphorylation of S6K1 (Burd et al., 2010; Terzis et al., 2010; Ahtiainen et al., 2015) and is accompanied by increases in myofibrillar protein synthesis (Burd et al., 2010), fitting the notion that increased training volume provides more pronounced adaptations through repeated episodes of increased protein synthesis.

Recent observations in humans are challenging this view by indicating that translational capacity is a limiting factor for training-induced muscle hypertrophy. First, increased abundances of rRNA in response to resistance training, measured as total RNA per-weight-unit muscle tissue, correlate with muscle hypertrophy (Figueiredo et al., 2015). In accordance with this, training-induced increases in rRNA are larger in muscle hypertrophy high-responders than in low-responders (Stec et al., 2016; Mobley et al., 2018). Secondly, elderly participants typically show blunted ribosome biogenesis, coinciding with attenuated hypertrophic responses (Stec et al., 2015; Brook et al., 2016). Collectively, these observations suggest that muscle growth depends at least in part on increased translational capacity, making it a prime candidate for explaining the diverse response patterns seen in resistance training with different volume in different individuals. To date, no study has investigated the association between training volume, ribosome biogenesis and regulation, and gross training adaptations.

Muscle fibre composition is another potential determinant of muscular responses to resistance training. Type II fibres have greater growth potential compared to type I fibres (Stec et al., 2016; Jespersen et al., 2011), and readily switch from IIX to IIA phenotypes in response to mechanical loading (Andersen & Gruschy-Knudsen, 2018; Widrick et al., 2002; Ellefsen et al., 2014b), suggesting that these fibres display greater plasticity in response to resistance training.

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

**Methods**

**Ethics statement**

All participants were informed about the potential risks and discomforts associated with the study and gave their informed consent prior to study enrollment. The study design was pre-registered (ClinicalTrials.gov Identifier: NCT02179307), approved by the local ethics committee at Lillehammer University College, Department of Sport Science (nr 2013-11-22:2) and all procedures were performed in accordance with the Declaration of Helsinki.

**Participants and study overview**

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

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

**Resistance-exercise training protocol**

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

**Muscle strength assessments**

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

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

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

**Muscle cross-sectional area (CSA) and body composition.**

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

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

Muscle tissue sampling and processing.

Muscle biopsies were obtained bilaterally from m. vastus lateralis under local anesthesia (Xylocaine, 10 mg × ml⁻¹ with adrenaline 5 μg × ml⁻¹, AstraZeneca AS, Oslo, Norge) using a 12-gauge needle (Universal-plus, Medax, San Possidonio, Italy) operated with a spring loaded biopsy instrument (Bard Magnum, Bard, Rud, Norway). For each participant, resting samples were collected at the same time of day at all time-points and all sampling was done in the morning after a standardised breakfast. Participants were instructed to standardise meals during the last 24 h leading up to the sampling and to refrain from strenuous physical activity the last 48 h. **Biopsy sampling prior to the fifth sessions was performed in the morning two days after session four. Post-intervention biopsy sampling was performed three and six days after the last training bout and strength testing session, respectively.**

Samples were obtained within 10 minutes from both legs at all time-points. The first biopsy was sampled 1/3 of the distance from the patella to anterior superior iliac spine, subsequent biopsies were sampled ~ 2 cm proximal to the previous sample. The tissue was quickly dissected free of blood and visible connective tissue in ice-cold sterile saline solution (0.9% NaCl). Samples for immunohistochemistry (~ 15 mg) were transferred to a 4% formalin solution for fixation 24-72 h, before further preparation. Samples for protein and RNA analyses (~ 60 mg) were blotted dry, snap-frozen in isopentane cooled to -80°C and stored at -80°C until further analyses.

Immunohistochemistry

Formalin-fixed muscle biopsies were processed for 2.5 h using a Shandon Excelsior ES (Thermo Scientific, USA), paraffin-embedded and sectioned into 4 μm transverse sections. For determination
of muscle fibre types, sections were double-stained using BF-35 (5 μg × ml⁻¹, Developmental Studies Hybridoma Bank, deposited by Schiaffino, S.) and MyHCSlow (1:4000, catalog M8421L, Sigma-Aldrich Norway AS, Oslo, Norway). The primary staining was visualised using BMU UltraView DAB and UltraView Red (Ventana Medical Systems, Inc. Tucson, USA). Muscle fibres were counted as either Type I (red), Type IIA (brown), Type IIX (unstained) or hybrid fibres Type IIA/IIX (light-brown) (for representative image, see Figure 5B). Fibres identified as hybrid fibres were analysed as 0.5 × Type IIA and 0.5 × Type IIX.

**Protein extraction and immunoblotting**

Aliquots of muscle-tissue (approximately 25 mg wet weight) were homogenised using a plastic pestle in ice-cold lysis buffer (2 mM HEPES pH 7.4, 1 mM EDTA, 5 mM EGTA, 10 mM MgCl₂, 1% Triton X-100) spiked with protease and phosphatase inhibitors (Halt, Thermo Fischer Scientific, Life Technologies AS, Oslo Norway), incubated at 4°C for 1 hr and centrifuged for 10 min at 10 000 g and 4°C, after which the supernatants were collected. Total protein concentrations were determined on a 1:10 dilution (Pierce Detergent Compatible Bradford Assay Reagent, Thermo Fischer Scientific). The remaining supernatant was diluted to 1.5 μg × μl⁻¹ total protein in lysis buffer and 4X Laemml sample buffer (Bio-Rad Laboratories AB, Oslo Norway) containing 2-Mercaptoethanol. Samples were heated to 95°C for 5 min and stored at -20°C until further processing. During analyses, protein samples (20 μg of total protein) were separated at 300 V for 30 min using 4-20% gels (Criterion TGX, Bio-Rad), followed by wet transfer to PVDF membranes (0.2 μm Immun-Blot, Bio-Rad) at 300 mA for 3 h. Gel electrophoresis and protein transfer were performed at 4°C. Membranes were then stained using a reversible total protein stain (Pierce Reversible Protein Stain, ThermoFischer Scientific) to ensure appropriate protein transfer. **Primary antibodies were purchased from** Cell Signaling Technology (Leiden, The Netherlands): mTOR (mTOR：<sup>Ser2448</sup>; #5536; pan: #4517), S6 kinase 1 (p85 S6K1<sup>Thr412</sup>: #9206; p70 S6K1<sup>Thr389</sup>: #9234; pan: #2708), ribosomal protein S6 (rpS6<sup>Ser235/236</sup>: #4858; pan: #2317). Membranes were blocked for 2 h in tris-buffered saline (TBS, 20 mM Tris, 150 mM NaCl) containing 3% bovine serum albumin and 0.1% Tween-20, followed by over-night incubation with primary antibodies targeting either the phosphorylated or non-phosphorylated epitope diluted in blocking buffer, followed by 2 h incubation with secondary horseradish peroxidase-conjugated antibodies diluted in TBS containing 0.1% Tween-20 and 5% skimmed milk. Membranes were washed in TBS containing 0.1% Tween-20 for 6 × 5 min after incubation with primary antibody, and for 8 × 5 min after incubation with secondary antibodies. **For rpS6 and mTOR antibodies, following** chemiluminescent detection (SuperSignal™ West Femto Maximum Sensitivity Substrate, ThermoFischer Scientific), membranes were incubated with hydrogen peroxide (15 min, 37°C) to inactivate the horseradish peroxidase (HRP), as described by Sennepin et al. (2009), followed by over-night incubation with primary or
secondary antibodies as described above. If the phosphorylated epitope was targeted during the first incubation, antibodies for the non-phosphorylated epitope were used in the second and vice versa. HRP inactivation did not affect the phosphospecific to non-phosphorylated signal ratios. Importantly, as this technique did not involve removing the first primary antibody, antibodies from different hosts (mouse or rabbit) were used for phosphorylated and non-phosphorylated epitopes respectively. As the antibody targeting p70 S6K1 Thr389 had the same host as the pan-antibody, total-protein was used to normalise chemiluminescent signals. All incubation and washing steps were performed at 4°C using an automated membrane processor (BlotCycler, Precision Biosystems, Mansfield, MA, USA), except for p70 S6K1 experiments, which were performed by hand at room temperature with incubations at 4°C. For mTOR and rpS6, total-protein and chemiluminescence quantification was calculated as the mean value of two separate experiments. S6K1 was quantified once for each phospho-specific antibody. Total-protein content was quantified using ImageJ (Rueden et al., 2017), and was defined as the mean grey value of the whole well with between-well values subtracted as background. Chemiluminescence signals were quantified using Image Studio Lite (LI-COR Biotechnology, Lincoln, Nebraska USA).

**Total RNA extraction, quantitative real-time reverse transcription polymerase chain reaction (qPCR)**

Approximately 25 mg of wet muscle-tissue was homogenised in a total volume of 1 ml of TRIzol reagent (Invitrogen, Life technologies AS, Oslo, Norway) using 0.5 mm RNase-free Zirconium Oxide beads and a bead homogeniser (Bullet Blender, Next Advanced, Averill Park, NY, USA) according to the manufacturer’s instructions. In order to enable analysis of target gene-expression per-unit tissue weight, an exogenous RNA control (λ polyA External Standard Kit, Takara Bio Inc, Shiga, Japan) was added at a fixed amount (0.04 ng × ml⁻¹ of Trizol reagent) per extraction prior to homogenisation, as previously described (Ellefsen et al., 2008, 2014a). Following phase-separation, 400 μl of the upper phase was transferred to a fresh tube and RNA was precipitated using isopropanol. The resulting RNA pellet was washed three times with 70% EtOH and finally eluted in TE buffer. RNA quantity and purity was evaluated using a spectrophotometer, all samples had a 260/280 nm ratio > 1.95. RNA was stored at -80°C until further processing. In the analysis of total RNA content per-unit tissue weight, one sample was excluded prior to analysis due to negative deviation from the expected value based on the relationship between sample weight and RNA content suggesting sample loss in washing steps. RNA integrity was assessed by capillary electrophoresis (Experion Automated Electrophoresis Station using RNA StdSens Assay, Bio-Rad) with average integrity scores (RQI) 8.1 (SD = 2.1).

Five-hundred nanograms of RNA were reverse transcribed using anchored Oligo-dT, random hexamer primers (Thermo Scientific) and SuperScript IV Reverse Transcriptase (Invitrogen).
according to manufacturer’s instructions. All samples were reverse transcribed in duplicates and diluted 1:50 prior to real-time polymerase chain reaction (qPCR). qPCR reactions were run on a fast-cycling real-time detection system (Applied Biosystems 7500 fast Real-Time PCR Systems, Life technologies AS), with a total volume of 10 μl, containing 2 μl of cDNA, specific primers (0.5 μM final concentration) and a commercial master mix (2X SYBR Select Master Mix, Applied Biosystems, Life technologies AS). qPCR reactions consisted of 40 cycles (three seconds 95°C denaturing and 30 seconds 60°C annealing). Melt-curve analyses were performed for all reactions to verify single-product amplification. Gene-specific primers were designed for all targets using Primer-BLAST (Ye et al., 2012) and Primer3Plus (Untergasser et al., 2012) and ordered from Thermo Scientific, except for the external RNA control, for which primers were supplied with the kit. Raw fluorescence data was exported from the platform specific software and amplification curves were modelled with a best-fit sigmoidal model using the qpcR-package (Ritz & Spiess, 2008) written for R (R Core Team, 2018). Threshold cycles (Ct) were estimated from the models by the second-derivate maximum method with technical duplicates modeled independently. Amplification efficiencies were estimated for every reaction (as described by Tichopad et al., 2003; implemented in Ritz & Spiess, 2008). For every primer pair, mean amplification efficiencies (E) were utilised to transform data to the linear scale using \(E^{-\Delta Ct}\). Primer sequences and primer characteristics (i.e. average primer efficiencies and Ct values) are presented in Table 2. Gene expression data was log-transformed prior to statistical analysis. As Ct-values, but not efficiencies are related to RNA integrity (Fleige & Pfaffl, 2006), RQI scores were used in the statistical treatment of qPCR data to control for potential degradation effects on a by target basis (see below).

**Data analysis and statistics**

All descriptive data are presented as mean and standard deviation (mean (SD)) unless otherwise stated. A priori sample-size calculations indicated that 40 participants was sufficient to detect ~ 3% and 5%-point differences in the primary outcomes, muscle cross-sectional area and maximal voluntary strength, respectively, between volume conditions. Sample-size calculations were based on a desired 80% power, assuming differences between volume condition corresponding to effect sizes of 0.47-0.51, as estimated from previous studies (Ronnestad et al., 2007; Mitchell et al., 2012). To assess the effect of volume-conditions (number of sets) on muscle hypertrophy and strength, linear mixed-effects models were specified with relative changes from baseline as the dependent variable and number of sets as the main fixed effect. Baseline values were used as a co-variate together with sex. The interaction between sex and number of sets were explored for all hypertrophy and strength outcomes. Training-effects on molecular characteristics (Total-RNA and western-blot data) were also assessed using linear mixed-effects models specified with time and the time to exercise-volume interaction as fixed effects. Models were specified with random intercepts for participants and when appropriate, random slopes for time and exercise-volume on the level of participants. Model
simplification was performed through reduction of random-effects parameters based on likelihood-ratio tests. Plots of residual and fitted values were visually inspected to assess uniformity of variance over the fitted range. Whenever deviations from these assumptions were identified, data were log-transformed and models were re-fitted.

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

Tests against the null-hypotheses of no differences between volume-conditions and no effect of time were performed on model-parameter estimates resulting from LMM and GLMM. LMM were fitted using the nlme-package (Pinheiro & Bates, 2000), binomial GLMM models using the lme4-package (Bates et al., 2015) and beta GLMM using glmmTMB-package (Magnusson et al., 2019) written for R.

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

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

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

**Results**

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

Overall, twelve weeks of resistance training led to a 25% (95% CI: [20, 29], $P<0.001$) increase in average muscle strength and a 4.4% ([3.2, 5.6], $P<0.001$) increase in muscle mass (mean values of both volume conditions). Adherence to the protocol was 96 (5) of the prescribed 31 sessions (range 81-100%), which gives an efficiency for developing muscle strength and mass equivalent to 0.84 (0.42)% and 0.15 (0.12)% per session, being within the expected range of training-induced changes (Ahtiainen \textit{et al.}, 2016).
Training had no effect on serum levels of cortisol and testosterone (Table 3). IGF-1 decreased ~ 5.4 % from week zero to week two, and increased ~ 3.6 % from pre- to post-exercise in week two. Growth hormone concentrations increased in response to acute exercise, with patterns differing between sexes (Table 3). Vitamin D levels were different at baseline between males (76.6 (16.4) nmol × L⁻¹) and females (100.0 (33.4) nmol × L⁻¹, P = 0.006) and were similarly reduced from week zero to twelve in both sexes (63.1 (19.8) and 91.4 (31.7) nmol × L⁻¹ for males and females respectively, time-effect P <0.001).

The difference in number of sets per exercise between multiple- and single-set conditions resulted in a ratio of performed work (number of repetitions × external resistance) between legs corresponding to 2.9 (0.3) in knee extension and 3.0 (0.5) in leg press. This was accompanied by higher ratings of perceived exertion in response to multiple sets than single sets (7.09 (1.95) vs. 6.22 (1.82), P<0.001). Concomitantly, multiple-set resistance-training led to greater increases in muscle strength over the course of the intervention than single-set training (all variables P < 0.05, Figure 2C and D). This difference in strength gain gradually increased over the first nine weeks of the study (Figure 2E). In line with this, multiple-set training led to greater increases in knee extensor CSA (mean percentage-point difference 1.62, [0.75, 2.50], P <0.001, Figure 2B). There was no difference between sexes in relative muscle strength and mass gains, and sex did not interact with responses to different volume conditions. There were strong correlations between responses to multiple-set and single-set conditions with respect average strength gain (r = 0.80, [0.64, 0.90], P <0.001, Figure 6B) and muscle hypertrophy (r = 0.75, [0.55, 0.87], P <0.001, Figure 6A). Increases in muscle strength correlated with increases in mass (r = 0.41, [0.08, 0.66], P = 0.016) assessed as averaged effects of the two volume conditions.

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

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

In line with these data, multiple-set training resulted in 8.8% [1.5, 16.6] greater total RNA abundance per-weight-unit muscle tissue at week two than single-set training. This difference was also evident at week twelve, albeit less extensive (5.9% [-1.0, 13.3], Figure 5A). Accordingly, the multiple-set leg showed greater abundances of mature rRNA transcripts at week two (18S, 19.0% [3.9, 36.4]; 28S, 15.3% [2.7, 29.4]; 5.8S 14.7% [1.8, 29.2], Figure 5B). The abundances of these rRNA subspecies remained elevated at week twelve with a tendency towards greater levels in the single set condition, an effect most pronounced in 28S (Figure 5B). The rRNA precursor transcript 45S also increased from baseline to week two when measured per-weight-unit muscle tissue with no clear differences between volume conditions (Figure 4C, upper panel). When measured per-unit total-RNA, levels of 45S pre-rRNA showed a clear increase only at week twelve compared to baseline values (43.1% [4.9, 95.0] in the single-sets condition) with multiple-set remaining near baseline levels (-29.8% [-48.5, -4.2] of single-set, Figure 5C lower panel). Overall, these data suggest that resistance training-induced increases in ribosomal content depend on training volume. Further supporting this view, mRNA expression of the transcription factor c-Myc, which is important for initiating rRNA transcription (Riggelen \textit{et al.}, 2010), increased 1.58 [1.14-2.17]-fold more in response to multiple-set training than to single-set training (Figure 5D, measured before and after the fifth training session).

Determinants of additional benefit of multiple-set training

Thirteen and sixteen participants showed clear benefits of multiple-set over single-set for increases in CSA and strength, respectively, defined as differences in training-induced changes greater than the
SWC in favour of multiple-set (SWC CSA, 2.7%; SWC strength, 4.5%, Figure 6A and B). In contrast, only three participants showed additional benefit of single-set training on CSA and one participant showed additional benefit of single-set training for strength. To identify determinants of multiple-set benefit, we performed logistic regression analyses with purposeful selection of variables. Variables initially selected for modelling are listed in Table 4. After variable selection, total RNA content measured at rest in the multiple-set leg at week two (expressed as percentage of the single-set leg), remained as the only predictor for additional benefits of moderate volume for both CSA and strength (Table 5). Total RNA content was elevated in the multiple-set trained leg in participants with clear benefits of multiple-set (Figure 6A and B). For every percentage-point increase in total RNA in the multiple-set leg (compared to the single-set leg), the odds of multiple-set benefit increased by 1.07 [1.00, 1.15] and 1.1 [1.01, 1.19] for muscle CSA and strength, respectively (CSA-model #6 and strength-model #4, Table 5). Notably, lean body-mass also remained a significant predictor of benefit of moderate training-volume on muscle CSA after variable selection: baseline lean body-mass proportions lower than the sex-specific median reduced the odds of benefit of multiple-set to 0.21 [0.04, 1.17] (CSA-model #6, Table 5). The association between benefit of moderate volume on CSA and total RNA levels at week two was independent of baseline lean body-mass.

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

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

**Discussion**

In the present study, multiple-set resistance training led to greater increases in muscle strength and mass than single-set training. This is in agreement with results from meta-analyses concluding in favour of moderate- compared to low-volume training (Krieger, 2009, 2010; Schoenfeld et al., 2016).
The greater effect of multiple-set training coincided with greater responses in muscle biological traits indicative of hypertrophic response (Andersen & Aagaard, 2000; Goodman et al., 2011; Terzis et al., 2008; Luo et al., 2019; Stec et al., 2016), including greater transition from Type IIX to IIA muscle fibres, greater post-exercise phosphorylation S6K1 and ribosomal protein S6, greater post-exercise expression of c-Myc and greater rested-state levels of total RNA and ribosomal RNA. While most of these variables are already assumed to be volume sensitive, such as muscle mass and strength (Krieger, 2009, 2010; Schoenfeld et al., 2016) and mTOR-signalling (Burd et al., 2010; Terzis et al., 2010), this is the first study to suggest that the IIX → IIA fiber switch is also volume sensitive. Importantly, this adaptation is a hallmark of resistance training adaptations (Andersen & Aagaard, 2000). This study also suggests that the volume-sensitive increase in ribosomal content is essential for beneficial effects of increases in training volume on muscle growth and strength, as shown by thirteen and sixteen of the participants respectively. Arguably, the biological resolution of the present data was high due to the use of a within-participant training model, facilitating disclosure of volume-dependent effects. Indeed, previous studies have typically used between-participants models to assess the volume-dependency of muscle development (e.g. Starkey et al., 1996; Ronnestad et al., 2007; Rhea et al., 2002). This makes their interpretations prone to the large individual-to-individual variation in exercise adaptability (seen in e.g. Ahtiainen et al., 2016), which has been linked to variation in genetic and epigenetic predisposition (Timmons, 2011; Seaborne et al., 2018), and may potentially explain the long-standing lack of consensus (Carpinelli & Otto, 1998; Krieger, 2010).

In the present study, a large range of changes was evident for both muscle strength and muscle mass. The observed variation in muscle hypertrophy (SD of average %Δ CSA ~ 4%) was comparable to that seen in larger cohorts (Ahtiainen et al., 2016). The strong correlation between responses to the two volume-conditions (see Figure 6A-B) highlights the importance of within-participant analyses: if the response to one training protocol was strong, the response to the other protocol was also strong. Consequently, our contralateral protocol resulted in lower estimates of differences between volume-conditions on the population level, expressed as relative gains in muscle mass per weekly set, compared to a previous meta-analysis (~ 1.6 vs. ~ 2.5% estimated from Table 3 in Schoenfeld et al., 2016). Notably, in the present study, this comparison was prone to systemic contralateral adaptions to training, which would diminish differences between volume conditions. However, this effect is likely negligible as non-trained limbs typically do not show increased protein synthesis, hypertrophy or muscle fibre type transitions (Brook et al., 2016; Wilkinson et al., 2006). Instead, it is plausible that the overall effect of added training-volume, as reported in Schoenfeld et al. (2016) is overestimated due to small sample sizes, a known weakness in meta analyses (Nüesch et al., 2010). Comparing our study to the similarly designed study by Mitchell et al. (2012) is not straightforward. The present study used two exercises to activate knee extensor muscles instead of one, resulting in a doubled training volume compared to Mitchell et al. (2012). It remains unclear if this discrepancy could
explain the dissimilar between-conditions effect (~ 1.6 vs. ~ 3.8%-point differences in CSA change). This perspective is clouded by the fact that strong within-participant correlations were not accounted for in Mitchell et al. (2012).

Arguably, contralateral designs improve comparisons of responses to different training volumes and regimes by accounting for inter-individual differences in training responses. Failing to account for within-participant correlations could lead to biased conclusions.

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

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

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

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

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

To our knowledge, this is the first study to show that muscle fibre transitions from Type IIX to IIA depend on resistance training volume. Moderate volume resulted in 1.5%-point greater reductions in Type IIX fibre expression from baseline to post intervention compared to low volume, presumably driven by more pronounced reductions in mRNA expression of the MYH1 (Myosin heavy chain IIX) gene (-61% vs. -31%). Previous studies have not compared this transition directly between volume protocols. However, Pareja-Blanco et al. (2017) observed blunted IIX → IIA transitions in response to non-exhaustive high-load resistance training compared to load-matched training to volatile failure. Together with our data, this makes exercise volume and subsequent metabolic stress and dosage of neuromuscular activity plausible candidates for regulation of IIX → IIA reprogramming, as opposed to mechanical stimuli. Indeed, in rodents, mechanical load does not affect fibre-type transitions (Eftestol et al., 2016), which is instead linked to neural activation. Interestingly, after two weeks of training, the volume effect on IIX → IIA transitions was opposite to our main finding after twelve weeks, with low-volume resistance training resulting in more pronounced decreases in IIX on the cellular level, accompanied by lower abundances of IIX/IIA hybrid fibres. This seemingly early benefit of single-set training on overall IIX levels was not observed at the mRNA level, with MYH1 being more heavily suppressed in the moderate volume condition. Instead, at week two, there seemed to be a disconnection between MYH1 mRNA and IIX protein adaptations in the multiple-set leg compared to the single-set leg. Whether this phenomenon was caused by increased need for tissue-repair in the moderate-volume condition at this time-point (Kim et al., 2005; Damas et al., 2016) or other causality, rather than myofibril-specific adaptations remains unclear. Regardless of causality, these data underline the importance of optimising exercise volume to achieve optimal training progression, such as making use of progressive volume protocols. Although such protocols remain largely unexplored, previously untrained individuals will likely benefit from careful calibration of training volume during early phases of resistance training. Too large or too small a training volume may lead to suboptimal adaptations.

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

Additional information

Competing interests

No conflicting interests.

Author contributions

Data collection was done in the Sport Science Laboratory at Inland University of Applied Sciences and the Hospital for Rheumatic Diseases with molecular analyses partly performed at Åstrandlaboratoriet, The Swedish School of Sport and Health Sciences and Innlandet Hospital Trust. DH, SE, BRR designed the study; DH, SJØ, LK, MH, SE and WA performed experiments; DH analysed the data; DH and SE interpreted the results; DH drafted the manuscript; DH, SJØ, LK, MH, BRR, EB, WA, JEW, IH and SE edited and revised the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Figure 1. Study overview. Bars represent weekly training frequency with training intensity expressed as repetition maximum (RM). * indicates that one session per week was performed at 90% of prescribed RM intensities. ↓ indicates muscle biopsy: Before (Week 0, n=34) and after the 12-wk intervention (Week 12, n=34), as well as before and after (1h) the fifth exercise session (Week 2 Pre-Ex and Post-Ex, n=33). ⭐ indicates strength test: before the intervention (Week 0, n=34), during week 3, 5 and 9 weeks of training (n=18), and after finalization of the intervention (Week 12, n=34). Baseline strength was determined as the highest value obtained during two test sessions performed prior to the intervention. Body composition was measured prior to the intervention (Week 0) and after its finalization (Week 12, n=34) using full-body DXA and knee-extensor muscle MRI ( disapointed).
Figure 2. Volume-dependent effects on muscle mass and strength. Training volume-dependent changes in muscle mass and strength after 12 weeks of resistance training, evident as larger increases in knee-extensor muscle CSA (measured using MRI, A and B) and larger increases in one-repetition maximum knee-extension and leg-press, isometric isokinetic knee-extension strength in the multiple-set leg (C). A weighted average of all strength measures (D) was used to study the time course of strength changes (n=18), showing a gradually increasing difference between volume conditions (in favour of multiple-set training) until Week 9, with no further increase to week 12 (E). Summary values (circles) are estimated means ± 95% CI. Triangles signifies mean paired differences ± 95% CI.
**Figure 3. Fiber-type distributions.** Muscle cross-sections were stained for myosin-heavy chain isoforms, Type I (MyHC Slow) and all but Type II X (BF-35). Red staining separated Type I fibres from other fibres (A lower panel). No-staining was analysed as Type IIX fibres (A, upper panel), while weak brown staining was analysed as Type IIX/IIA hybrids. Volume-dependent changes in muscle fibre-type distribution was evident in m. Vastus lateralis after 2 and 12 weeks of multiple and single-set resistance training, measured as relative cell counts using immunohistochemistry (IHC) and gene family profiling (GeneFam)-normalized myosin heavy-chain mRNA expression (B). Volume-dependent effects were identified for proportions of Type IIX fibres and IIX/IIA hybrid fibres (C). Volume-dependent effects were also evident at the transcript level, measured as surplus reductions in Type IIX mRNA (MYH1) abundance in the multiple-set leg at all time points (D). Values are mean ± 10 - 90th percentile in B and, individual values and means in C and estimated means ± 95% CI in D. † represent difference from Week 0, †††† for P < 0.05 - P < 0.0001; * represent differences between sets * - *** * for P < 0.05 - P < 0.0001."
Figure 4. Western-blot analysis of the mTOR-signaling pathway. Training-volume dependent phosphorylation of S6K1 (p70, A; p85, B), rpS6 (C) and mTOR (D) proteins was evident in m. Vastus lateralis after the fifth training session. Measured phosphorylation-sites are shown in context (F) where phosphorylation of S6K1 (Thr389) is indicative of mTOR activity, S6K1 mediates negative feedback to mTOR through phosphorylation of the Ser2448 site. mTOR and MEK-ERK signalling converges on rpS6 as both pathways phosphorylates Ser235/236. Representative blots and total-protein stains are shown in G and H. Values are mean ± 95% CI. * represents differences between volume conditions, * * for $P < 0.05 - P < 0.01$. 
Figure 5. Total-RNA and ribosomal RNA. Training-volume dependent changes in total RNA and ribosomal RNA 18S content were apparent in m. Vastus lateralis after 2 weeks of resistance training (measured per-unit muscle weight, Week 2, A and B). Other mature ribosomal RNA species exhibited similar expression patterns without reaching statistical significance (B). Increases in c-Myc mRNA abundance, measured 1h after the fifth session, also showed volume-dependency (C). Ribosomal pre-RNA 45S, expressed relative to total RNA showed greater relative abundances at Week 12 than Week 0 in the single-set leg (D). Values are estimated means ± 95% CI. * represents difference between volume conditions for $P < 0.05$. † represents difference from Week 0, ††† for $P < 0.05$ - $P < 0.0001$. 

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

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### Table 1. Participant characteristics and habitual dietary data.

|                | Female | Male |
|----------------|--------|------|
| **Included**   |        |      |
| N              | 18     | 16   |
| Age (y)        | 22.0 (1.3) | 23.6 (4.1) |
| Mass (kg)      | 64.4 (10.4) | 75.8 (10.7) |
| Stature (cm)   | 168 (7) | 183 (6) |
| Body fat (%)   | 34.1 (5.6) | 20.4 (6.0) |
| MVC (Nm kg⁻¹)  | 3.1 (0.5) | 3.7 (0.6) |
| **Excluded**   |        |      |
| N              | 4      | 3    |
| Age (y)        | 22.9 (1.6) | 24.3 (1.5) |
| Mass (kg)      | 64.6 (9.7) | 88.2 (22.4) |
| Stature (cm)   | 166 (8) | 189 (5) |
| Body fat (%)   | 28.8 (8.7) | 24.3 (15.3) |
| MVC (Nm kg⁻¹)  | 3.6 (0.5) | 3.9 (0.7) |

**Dietary survey**

|                | Kcal day⁻¹ | Protein kg⁻¹ day⁻¹ | Fat kg⁻¹ day⁻¹ | CHO kg⁻¹ day⁻¹ |
|----------------|-------------|-------------------|---------------|----------------|
|                | 1994 (839)  | 1.33 (0.40)       | 1.10 (0.44)   | 3.36 (1.17)    |

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

### Table 2. Primer sequences and performance

| Gene symbol | Full name | Accession⁺ | Primer sequence (forward and reverse) | Ct mean (SD) | E    |
|-------------|-----------|------------|--------------------------------------|--------------|------|
| MYH7        | Myosin heavy chain 7 (MyHC-1) | NM_000257.3 | 5'-AGGAGCTCACCTACCAGACG-3' 5'-TGCACCTTGCTTACAGGTC-3' | 21.70 (0.77) | 1.88 |
| MYH2        | Myosin heavy chain 2 (MyHC-2A) | NM_017534.5 | 5'-CCAGGTCACGGGAGCT-3' 5'-TCACTCGCCCTCTCATGTTTG-3' | 17.65 (0.62) | 1.92 |
| MYH1        | Myosin heavy chain 1 (MyHC-2X) | NM_005963.3 | 5'-GCCCAGGTTTGTGAACCTT-3' 5'-TGCGTAGACCCCTCTGACAGC-3' | 23.33 (1.94) | 1.88 |
| c-Myc       | v-myc avian myelocytomatosis viral oncogene homolog | NM_002467.4 | 5'-GGGTAGTGGAAACACAGCAG-3' 5'-TCCTCGTCGCAGATAAGAC-3' | 30.23 (2.03) | 1.93 |
| rRNA5.8S    | 5.8S ribosomal RNA | NR_003285.2 | 5'-ACTCTTAGGCTTGTCTG-3' 5'-GTCACCTTGATACAGGAT-3' | 15.64 (0.45) | 1.88 |
| rRNA28S     | 28S ribosomal RNA | NR_003287.2 | 5'-TGCAGCCGATGTGATTG-3' 5'-TAGATGCCAGAGCATTTG-3' | 12.39 (0.66) | 1.78 |
| rRNA18S     | 18S ribosomal RNA | NR_003286.2 | 5'-TGACATGGCCGTCTTATG-3' 5'-AAGGCCACTTTGTCCTTAA-3' | 13.16 (1.45) | 1.81 |
| rRNA45S     | 45S pre-ribosomal RNA | NR_046235.1 | 5'-GCTCTCTTCGAGTCTGAGG-3' 5'-CCATAACGGAGGCGAGACA-3' | 25.60 (1.75) | 1.76 |
| Lambda PolyA | External Standard Kit | | Proprietary sequences | 23.96 (0.82) | 1.98 |

Average threshold cycles (Ct) and priming efficiencies (E) were calculated from all qPCR reactions. *⁺, NCBI Reference Sequence.
Table 3. Hormone measurements.

| Week 2 (Fifth session) |
|------------------------|
|                        |
|                        |
|                        |

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

Differences between resting samples (week zero, week two pre-exercise and week twelve), between rest and post acute-exercise in week two and between males and females were tested in mixed-effects models where * denotes significant main effect of sex; †, resting samples different from week zero; ‡ acute samples different from week two pre-exercise; ¥, change from week two pre-exercise different between men and women, all P<0.05. Missing values in growth hormone and testosterone are measurements below the detection limit (0.05 μg L⁻¹ and 0.69 nmol L⁻¹ for growth hormone and testosterone respectively). Due to small number of detectable testosterone samples in females, statistical tests were carried out in males only.
Table 4. Univariate analysis of predictors of additional-benefit of Multiple-sets on training induced muscle hypertrophy and strength

| Variable                        | Classifica | Mean (SD) | Model coefficients | Mean (SD) | Model coefficients |
|---------------------------------|------------|-----------|--------------------|-----------|--------------------|
|                                 |            |           |                    |           |                    |
| **Ribosome biogenesis**         |            |           |                    |           |                    |
| Total-RNA Week 2 (% of single-sets) | No benefit | 3.2 (15)  | 18                 | 6.2       | 2.9                | 0.00 7         | 0.00 7         | 2.2 (11)       | 16             | 6.5             | 2.4             | 0.02 1         |
|                                | Benefit    | 22 (21)   |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| Total-RNA Week 12 (% of single-sets) | No benefit | 5.7 (15)  | 5.5                | 7.1       | 0.78               | 0.44 4         | 0.44 4         | 7.7 (20)       | 2.6             | 7.3             | 0.36            | 0.72 0         |
|                                | Benefit    | 11 (26)   |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| **mTOR signalling**             |            |           |                    |           |                    |
| S6K1(T188)(fold of single-sets)  | No benefit | 1.40 (0.59) | 0.20              | 0.3       | 0.61               | 0.54 8         | 0.54 8         | 1.77 (1.01)    | -0.73           | 0.3             | 0.24            | 0.02 3         |
|                                | Benefit    | 1.62 (1.26) |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| **Endocrine parameters**        |            |           |                    |           |                    |
| Cortisol (Mean Week 0-2)        | No benefit | 544 (145) | 13                 | 48        | 0.27               | 0.79 2         | 0.79 2         | 625 (196)      | -84             | 47              | -1.81           | 0.08 0         |
|                                | Benefit    | 417 (54)  |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 577 (197) |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 402 (100) |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| Testosterone (Mean Week 0-2)    | No benefit | 0.67 (0.47) | -1.15             | 0.8       | 1.43               | 0.16 3         | 0.16 3         | 0.42 (0.46)    | 0.79             | 0.8             | 0.95            | 0.35 0         |
|                                | Benefit    | 15 (3.1)  |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 0.75 (1.62) |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 12 (2.8)  |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| Growth hormone (Mean post-exercise Week 2) | No benefit | 4.0 (2.0)  | 1.03               | 0.7       | 1.46               | 0.15 6         | 0.15 6         | 4.7 (2.3)      | -0.037           | 0.7             | 0.05            | 0.96 0         |
|                                | Benefit    | 1.44 (1.36) |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 4.3 (1.93) |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 3.4 (2.5)  |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| IGF-1 (Mean pre-exercise Week 0-2) | No benefit | 20 (5.2)  | 0.38               | 1.8       | 0.21               | 0.83 8         | 0.83 8         | 19 (4.8)       | 1.10            | 1.8             | 0.59            | 0.56 0         |
|                                | Benefit    | 20 (4.7)  |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| IGF-1 (Mean post-exercise Week 2) | No benefit | 19 (5.7)  | 1.42               | 1.9       | 0.72               | 0.47 8         | 0.47 8         | 19 (4.8)       | 2.0             | 1.9             | 1.02            | 0.31 5         |
|                                | Benefit    | 20 (4.5)  |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| Vitamin D (Mean Week 0 and 12)  | No benefit | 100 (39)  | -12                | 9.5       | -1.24              | 0.22 6         | 0.22 6         | 101 (34)       | -10             | 9.7             | -1.08           | 0.28 9         |
|                                | Benefit    | 74 (18)   |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 90 (15)   |                    |           |                    |                 |                 |                 |                |                 |                 |                |
|                                | Benefit    | 60 (14)   |                    |           |                    |                 |                 |                 |                |                 |                 |                |
| **Baseline characteristics**    |            |           |                    |           |                    |
| Baseline                       | No benefit | 6.4       | 0.41               | 0.3       | 1.17               | 0.25 6.8 (1.11) | 0.43 0.3       | 1.24 0.22      |                |                |                |                |

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| strength (kg\(^{-1}\), AU) |  | (1.10) | 5 | 0 | 5 | 6 |
|---|---|---|---|---|---|---|
| Benefit | 7.7 (0.76) | 0.25 0 | 8.1 (0.88) | 0.25 0 | 6.2 (0.89) | 0.25 0 | 7.9 (0.98) |
| Baseline lean mass (%) | No benefit | 64 (4.8) | 4.3 | 1.9 | 6 | 5 | 5 |
| Benefit | 78 (5.3) | 0.03 7 | 65 (5.9) | 0.03 7 | 82 (4.4) | 0.03 7 | 65 (6.2) |
| Benefit | 83 (4.1) | 0.03 7 | 76 (6.3) |

**Muscle fibre-types**

| Type IIA (% of total MHC) | No benefit | 65 (7.3) | 0.64 | 2.7 | 0.23 | 51 (7.5) | -0.69 | 2.8 | -0.25 | 0.80 |
| Benefit | 64 (7.2) | 0.81 7 | 50 (7.8) |
| Type IIX (% of total MHC) | No benefit | 34 (2.2) | 3.1 | 1.6 | 7 | 1.84 | 4.0 (3.9) | 0.74 | 1.7 8 | 0.41 | 0.68 |
| Benefit | 6.4 (7.0) | 0.07 6 | 5.0 (5.8) |
| Type I (% of total MHC) | No benefit | 46 (8.1) | -3.7 | 3.4 | -1.10 | 45 (8.8) | -0.053 | 3.5 | -0.01 | 0.98 |
| Benefit | 43 (11) | 0.28 0 | 45 (10) |

**Pre-study training habits**

| Pre-study training habits (n sessions >0/0) | No benefit | n = 13/8 | -0.32 | 0.7 | 1 | -0.45 | 0.65 4 | n = 10/8 | 0.27 | 0.7 | 0 | 0.38 | 0.70 | 2 |
| Benefit | n = 7/6 | 0.65 4 | n = 10/6 |
| Pre-study strength training (strength-type training, yes/no) | No benefit | n = 6/15 | 0.12 | 0.7 | 7 | 0.16 | 0.87 4 | n = 5/13 | 0.16 | 0.7 | 5 | 0.21 | 0.83 | 1 |
| Benefit | n = 4/9 | 0.87 4 | n = 5/11 |

**Training characteristics**

| Supervised sessions (100% <100%) | No benefit | n = 9/12 | -0.16 | 0.7 | 2 | -0.22 | 0.82 3 | n = 9/9 | -0.74 | 0.7 | 1 | -1.03 | 0.30 | 1 |
| Benefit | n = 5/8 | 0.82 3 | n = 5/11 |
| Total number of sessions (100% <100%) | No benefit | n = 12/9 | -0.42 | 0.7 | 1 | -0.59 | 0.55 5 | n = 8/10 | 0.69 | 0.7 | 0 | 0.99 | 0.32 | 3 |
| Benefit | n = 6/7 | 0.55 5 | n = 10/6 |

**Dietary data**

| Protein kg 'day' | No benefit | 1.34 (0.46) | -0.01 5 | 0.1 8 | -0.08 3 | 0.93 | 1.34 (0.46) | -0.18 1 | 0.1 8 | -1.05 | 0.31 |
| Benefit | 1.32 (0.36) | 0.93 | 1.32 (0.36) |
| Kcal day\(^{-1}\) | No benefit | 2169 (1036) | -334 | 368 | -0.91 | 0.38 | 2169 (1036) | -227 | 373 | -0.61 | 0.55 |
| Benefit | 1835 (620) | 0.38 | 1835 (620) |

* Model coefficients from univariate analysis using linear regression with benefit groups as the independent variable for continuous data and logistic regression with benefit groups as the dependent variable for dichotomous data. Sex was included in all models to account for sex differences. \(^1\) Sex-specific mean and SD are reported when significantly different between sexes. \(^2\) Dichotomous variable, logistic regression model used to determine association. \(^3\) Dietary data on n=21, not used in variable selection.
| Variable | Estimate $|$ SE | Z-value | P-value | LRT P-value |
|----------|----------|-------|--------|---------|-------------|
| **Muscle CSA** | | | | | |
| **Model 1** | | | | | |
| Intercept | -0.61 | 1.39 | -0.44 | 0.662 | |
| Sex (Male) | 0.67 | 0.98 | 0.68 | 0.495 | |
| Total-RNA Week 2 (% of single-set) | 0.054 | 0.034 | 1.57 | 0.115 | |
| Testosterone (Mean Week 0-2) | -1.02 | 0.93 | -1.09 | 0.274 | |
| Growth hormone (Mean post-exercise Week 2) | 0.18 | 0.23 | 0.80 | 0.422 | |
| Baseline lean mass (%) | -1.32 | 0.90 | -1.47 | 0.142 | |
| Type 2X (% of total MHC) | -0.27 | 0.95 | -0.29 | 0.775 | |
| **Model 2** | | | | | Model 1 vs. 2 P=1.000 |
| Intercept | -0.85 | 1.16 | -0.73 | 0.463 | |
| Sex (Male) | 0.75 | 0.98 | 0.76 | 0.446 | |
| Total-RNA Week 2 (% of single-set) | 0.058 | 0.034 | 1.67 | 0.095 | |
| Testosterone (Mean Week 0-2) | -1.14 | 0.91 | -1.26 | 0.209 | |
| Growth hormone (Mean post-exercise Week 2) | 0.21 | 0.22 | 0.95 | 0.344 | |
| Baseline lean mass (%) | -1.34 | 0.90 | -1.49 | 0.137 | |
| **Model 3** | | | | | Model 2 vs. 3 P=0.292 |
| Intercept | -0.10 | 0.86 | -0.12 | 0.907 | |
| Sex (Male) | 0.44 | 0.91 | 0.48 | 0.629 | |
| Total-RNA Week 2 (% of single-set) | 0.065 | 0.035 | 1.86 | 0.062 | |
| Testosterone (Mean Week 0-2) | -1.03 | 0.88 | -1.18 | 0.239 | |
| Baseline lean mass (%) | -1.35 | 0.89 | -1.52 | 0.128 | |
| **Model 4** | | | | | Model 3 vs. 4 P=0.197 |
| Intercept | -0.59 | 0.76 | -0.77 | 0.439 | |
| Sex (Male) | 0.44 | 0.88 | 0.50 | 0.617 | |
| Total-RNA Week 2 (% of single-set) | 0.068 | 0.035 | 1.93 | 0.054 | |
| Testosterone (Mean Week 0-2) | -1.51 | 0.88 | -1.71 | 0.087 | |
| **Model 5** | | | | | Model 4 vs. 5 P=0.043 |
| Intercept | -1.34 | 0.66 | -2.0 | 0.043 | |
| Sex (Male) | 0.51 | 0.84 | 0.61 | 0.545 | |
| Total-RNA Week 2 (% of single-set) | 0.063 | 0.031 | 2.1 | 0.039 | |
| **Model 6** | | | | | Model 4 vs. 6 P=0.653 |
| Intercept | -0.38 | 0.61 | -0.61 | 0.539 | |
| Sex (Male) | 0.68 | 0.036 | 1.91 | 0.057 | |
| Total-RNA Week 2 (% of single-set) | -1.58 | 0.89 | -1.78 | 0.075 | |
| **Muscle Strength** | | | | | |
| **Model 1** | | | | | |
| Intercept | 1.59 | 1.56 | 1.02 | 0.308 | |
| Sex (Male) | -0.90 | 0.98 | -0.92 | 0.356 | |
| Total-RNA Week 2 (% of single-set) | 0.086 | 0.043 | 1.99 | 0.047 | |
| S6K1 $^{\text{Thr389}}$ (fold of single-set) | -1.43 | 0.95 | -1.51 | 0.132 | |
| Cortisol (Mean Week 0-2) | -0.003 | 0.004 | -0.83 | 0.407 | |
| **Model 2** | | | | | Model 1 vs. 2 P=0.333 |
| Intercept | 1.56 | 1.46 | 1.07 | 0.285 | |
| Sex (Male) | -0.88 | 0.96 | -0.92 | 0.359 | |
| Total-RNA Week 2 (% of single-set) | 0.090 | 0.043 | 2.1 | 0.036 | |
| S6K1 $^{\text{Thr389}}$ (fold of single-set) | -1.43 | 0.89 | -1.60 | 0.110 | |
| **Model 3** | | | | | Model 2 vs. 3 P=0.011 |
| Intercept | -0.67 | 0.62 | -1.07 | 0.282 | |
| Sex (Male) | -0.36 | 0.86 | -0.42 | 0.671 | |
| Total-RNA Week 2 (% of single-set) | 0.076 | 0.037 | 2.1 | 0.037 | |
| **Model 4** | | | | | Model 2 vs. 4 P=0.261 |
| Intercept | 0.79 | 1.15 | 0.69 | 0.493 | |
| Total-RNA Week 2 (% of single-set) | 0.093 | 0.041 | 2.3 | 0.022 | |

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Variables not linear in the logit were transformed to meet assumptions: a Testosterone dichotomised to above and below the detection limit (0.69 nmol L\(^{-1}\)) in females and above and below the median in males (13.5 nmol L\(^{-1}\)). b % lean body-mass dichotomised to the sex-specific median (females, 63.6; males, 81.0). c % Type IIX fibres dichotomised above and below the median (3.7%).

| S6K1\(^{fold of single-set}\) | -1.16 | 0.78 | -1.49 | 0.136 |