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

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Key points

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

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

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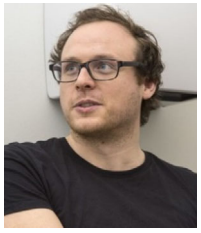

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exercise, MOD) and low-volume (1 set, LOW) resistance training in a contralateral fashion for 12 weeks (2–3 sessions per week). Muscle cross-sectional area (CSA) and strength were assessed at Weeks 0 and 12, along with biopsy sampling (m. vastus lateralis).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

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Introduction

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

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

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

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

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

Methods

Ethical approval

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

Participants and study overview

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

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

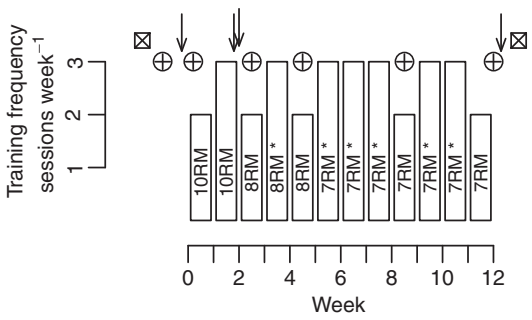


Figure 1. Study overview
Bars represent weekly training frequency with training intensity expressed as repetition maximum (RM). * indicates that one session per week was performed at 90% of prescribed RM intensities. ↓ indicates muscle biopsy: before (Week 0, $n = 34$) and after the 12 week intervention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

Table 1. Participant characteristics and habitual dietary data

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

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

Resistance-exercise training protocol

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

Muscle strength assessments

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

Maximal strength was assessed as one repetition maximum (1RM) in unilateral leg press and knee extension. The test session for each exercise started with a specific warm-up consisting of 10, 6 and 3 repetitions at 50, 75 and 85% of the anticipated maximum. Thereafter, 1RM was found by increasing the resistance progressively until the weight could not be lifted through the full range of motion. For each exercise, the highest load successfully attempted was defined as 1RM. Each participant was given four to six attempts.

At baseline, 1RM, isokinetic and isometric strength assessments were performed twice, separated by at least

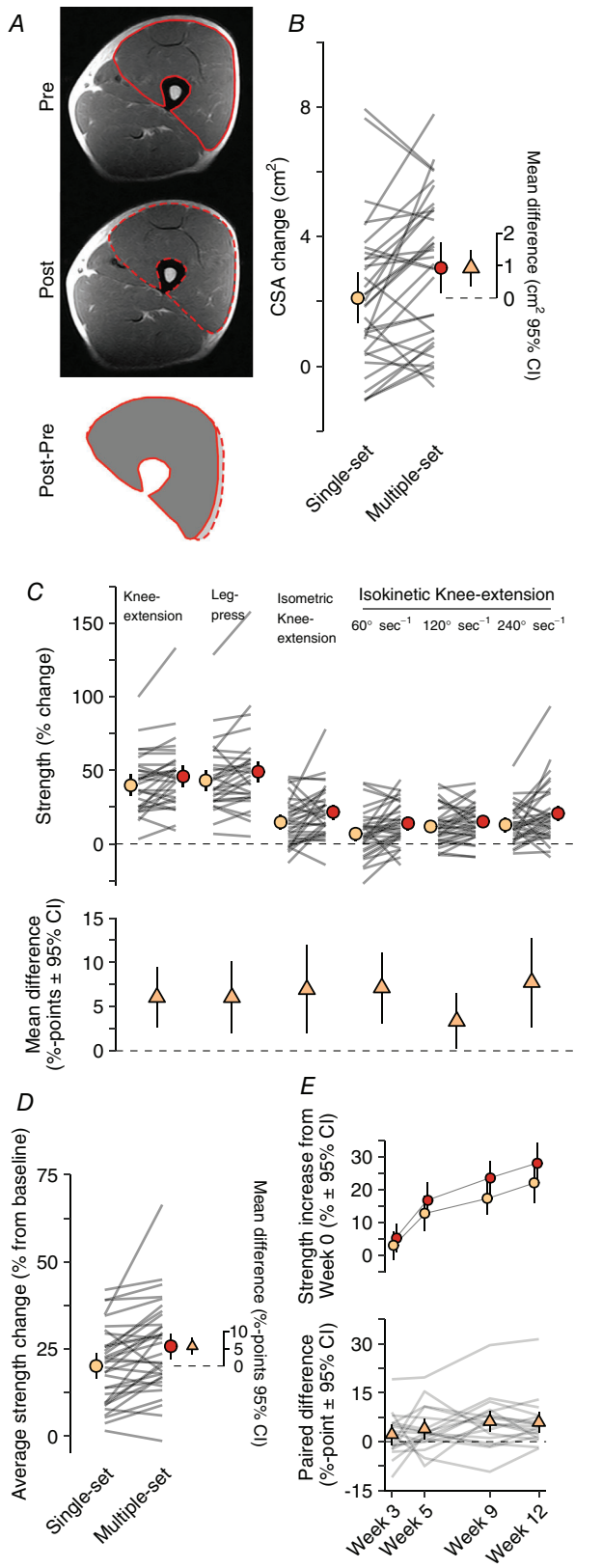


Figure 2. Volume-dependent effects on muscle mass and strength
Training volume-dependent changes in muscle mass and strength after 12 weeks of resistance training, evident as larger increases in knee-extensor muscle CSA (measured using MRI, *A* and *B*) and larger increases in one-repetition maximum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

4 days. The maximum value achieved for each of the tests was used in subsequent analysis. Strength tests were separated by at least 48 h from preceding training sessions. A combined measure of muscle strength was calculated as the average of all tests (1RM, isometric and isokinetic), wherein each test modality was given equal weight. A subset of the participants ($n = 18$) performed strength assessment during the course of the study (at Weeks 2, 5 and 9). For the remaining participants, ordinary training sessions were prioritised when participants missed training or testing due to illness or scheduling difficulties.

Muscle cross-sectional area and body composition

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

Hormonal measurements

Hormone analyses were performed on blood samples collected at five time-points: alongside muscle biopsies (Fig. 1, four sampling events) and 10 min after completion of the fifth training session. Samples were drawn from the antecubital vein into serum-separating tubes and kept

at room temperature for 30 min before centrifugation (1500 *g*, 10 min). Serum was immediately aliquoted and stored at −80°C until further processing. Serum concentrations of total testosterone, cortisol, growth hormone and insulin-like growth-factor 1 (IGF-1) were measured on an Immulite 1000 analyser, using kits from the Immulite Immunoassay System menu (Siemens Medical Solutions Diagnostics, Malvern, PA, United States), performed according to manufacturer's protocols. Serum Vitamin D (S-25-OH-D) levels were measured in samples collected before and after the intervention using a electrochemiluminescence immunoassay (Roche Cobas Vitamin D total assay, Roche Diagnostics GmbH, Mannheim, Germany) using automated instrumentation (Roche Cobas 6000 module e601, Roche Diagnostics).

Muscle tissue sampling and processing

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

Immunohistochemistry

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

Protein extraction and immunoblotting

Aliquots of muscle tissue (approximately 25 mg wet weight) were homogenised using a plastic pestle in ice-cold lysis buffer (2 mM HEPES pH 7.4, 1 mM EDTA, 5 mM EGTA, 10 mM $MgCl_2$, 1% Triton X-100) spiked with protease and phosphatase inhibitors (Halt, Thermo Fisher Scientific), incubated at 4°C for 1 h and centrifuged for 10 min at 10,000 *g* and 4°C, after which the supernatants were collected. Total protein concentrations were determined on a 1:10 dilution (Pierce Detergent Compatible Bradford Assay Reagent, Thermo Fisher Scientific). The remaining supernatant was diluted to 1.5 µg $µl^{-1}$ total protein in lysis buffer and 4X Laemmli sample buffer (Bio-Rad Laboratories AB, Oslo, Norway) containing 2-mercaptoethanol. Samples were heated to 95°C for 5 min and stored at −20°C until further processing. During analyses, protein samples (20 µg of total protein) were separated at 300 V for 30 min using 4–20% gels (Criterion TGX, Bio-Rad), followed by wet transfer to PVDF membranes (0.2 µm Immun-Blot, Bio-Rad) at 300 mA for 3 h. Gel electrophoresis and protein transfer were performed at 4°C. Membranes were then stained using a reversible total protein stain (Pierce Reversible Protein Stain, ThermoFisher Scientific) to ensure appropriate protein transfer. Primary antibodies were purchased from Cell Signaling Technology (Leiden, the Netherlands): mTOR ($mTOR^{Ser2448}$: no. 5536; pan: no. 4517), S6 kinase 1 (p85 $S6K1^{Thr412}$: no. 9206; p70 $S6K1^{Thr389}$: no. 9234; pan: no. 2708), ribosomal protein S6 ($rpS6^{Ser235/236}$: no. 4858; pan: no. 2317). Membranes were blocked for 2 h in Tris-buffered saline (TBS; 20 mM Tris, 150 mM NaCl) containing 3% bovine serum albumin and 0.1% Tween-20, followed by overnight incubation with primary antibodies targeting either the phosphorylated or non-phosphorylated epitope diluted in blocking buffer, followed by 2 h incubation with secondary horseradish peroxidase-conjugated antibodies diluted in TBS containing 0.1% Tween-20 and 5% skimmed milk. Membranes were washed in TBS containing 0.1% Tween-20 for 6 × 5 min after incubation with primary antibody, and for 8 × 5 min after incubation with

secondary antibodies. For rpS6 and mTOR antibodies, following chemiluminescence detection (SuperSignal West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific), membranes were incubated with hydrogen peroxide (15 min, 37°C) to inactivate the horseradish peroxidase (HRP), as described by Sennepin *et al.* (2009), followed by overnight incubation with primary or secondary antibodies as described above. If the phosphorylated epitope was targeted during the first incubation, antibodies for the non-phosphorylated epitope were used in the second and vice versa. HRP inactivation did not affect the phospho-specific to non-phosphorylated signal ratios. Importantly, as this technique did not involve removing the first primary antibody, antibodies from different hosts (mouse or rabbit) were used for phosphorylated and non-phosphorylated epitopes, respectively. As the antibody targeting p70 S6K1^{Thr389} had the same host as the pan-antibody, total protein was used to normalise chemiluminescent signals. All incubation and washing steps were performed at 4°C using an automated membrane processor (BlotCycler, Precision Biosystems, Mansfield, MA, USA), except for p70 S6K1 experiments, which were performed by hand at room temperature with incubations at 4°C. For mTOR and rpS6, total protein and chemiluminescence quantification was calculated as the mean value of two separate experiments. S6K1 was quantified once for each phospho-specific antibody. Total protein content was quantified using ImageJ (Rueden *et al.* 2017), and was defined as the mean grey value of the whole well with between-well values subtracted as background. Chemiluminescence signals were quantified using Image Studio Lite (LI-COR Biotechnology, Lincoln, NE, USA).

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

Approximately 25 mg of wet muscle tissue was homogenised in a total volume of 1 ml of TRIzol reagent (Invitrogen, Life technologies AS, Oslo, Norway) using 0.5 mm RNase-free zirconium oxide beads and a bead homogeniser (Bullet Blender, Next Advanced, Averill Park, NY, USA) according to the manufacturer's instructions. In order to enable analysis of target gene expression per unit tissue weight, an exogenous RNA control (λ polyA External Standard Kit, Takara Bio Inc., Shiga, Japan) was added at a fixed amount (0.04 ng ml^{-1} of Trizol reagent) per extraction prior to homogenisation, as previously described (Ellefsen *et al.* 2008, 2014*a*). Following phase separation, 400 µl of the upper phase was transferred to a fresh tube and RNA was precipitated using isopropanol. The resulting RNA pellet was washed three times with 70% EtOH and finally eluted in TE buffer. RNA quantity and purity was evaluated using a spectrophotometer; all samples had a 260 nm/280 nm ratio >1.95. RNA was stored at −80°C until further processing. In the analysis of total RNA content per unit tissue weight, one sample was excluded prior to analysis due to negative deviation from the expected value based on the relationship between sample weight and RNA content, suggesting sample loss in washing steps. RNA integrity was assessed by capillary electrophoresis (Experion Automated Electrophoresis Station using RNA StdSens Assay, Bio-Rad) with average integrity score (RNA quality indicator; RQI) 8.1 (SD = 2.1). Five hundred nanograms of RNA were reverse transcribed using anchored oligo-dT, random hexamer primers (Thermo Scientific) and SuperScript IV Reverse Transcriptase (Invitrogen) according to the manufacturers' instructions. All samples were reverse transcribed in duplicate and diluted 1:50 prior to quantitative real-time polymerase chain reaction (qPCR). qPCR reactions were run on a fast-cycling real-time detection system (Applied Biosystems 7500 fast Real-Time PCR Systems, Life Technologies AS), with a total volume of 10 µl, containing 2 µl of cDNA, specific primers (0.5 µM final concentration) and a commercial master mix (2X SYBR Select Master Mix, Applied Biosystems, Life Technologies AS). qPCR reactions consisted of 40 cycles (3 s 95°C denaturing and 30 s 60°C annealing). Melt-curve analyses were performed for all reactions to verify single-product amplification. Gene-specific primers were designed for all targets using Primer-BLAST (Ye *et al.* 2012) and Primer3Plus (Untergasser *et al.* 2012) and ordered from Thermo Scientific, except for the external RNA control, for which primers were supplied with the kit. Raw fluorescence data were exported from the platform-specific software and amplification curves were modelled with a best-fit sigmoidal model using the qpcR-package (Ritz & Spiess, 2008) written for R (R Core Team, 2018). Threshold cycles (Ct) were estimated from the models by the second-derivate maximum method with technical duplicates modelled independently. Amplification efficiencies were estimated for every reaction (as described by Tichopad *et al.* 2003; implemented in Ritz & Spiess, 2008). For every primer pair, mean amplification efficiencies (E) were utilised to transform data to the linear scale using E^{-Ct}. Primer sequences and primer characteristics (i.e. average primer efficiencies and Ct values) are presented in Table 2. Gene expression data were log-transformed prior to statistical analysis. As Ct values, but not efficiencies are related to RNA integrity (Fleige & Pfaffl, 2006), RQI scores were used in the statistical treatment of qPCR data to control for potential degradation effects on a by target basis (see below).

Data analysis and statistics

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

Table 2. Primer sequences and performance

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

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

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

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

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

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

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

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

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

Results

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

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

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

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

Table 3. Hormone measurements

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

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

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

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

Volume-dependent regulation of mTOR signalling and ribosomal biogenesis

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

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

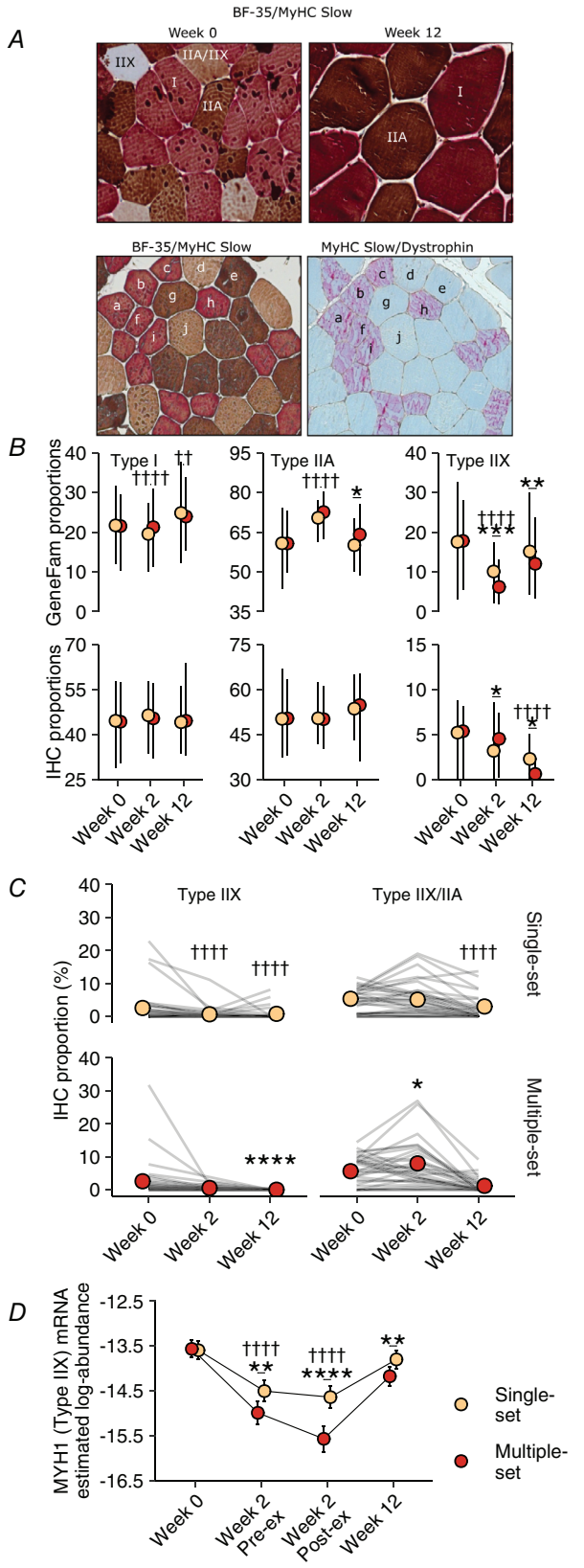


Figure 3. Fibre-type distributions
Muscle cross-sections were stained for myosin-heavy chain isoforms, Type I (MyHC Slow) and all but Type IIX (BF-35). Red staining separated Type I fibres from other fibres (*A*, lower panel). No staining was analysed as Type IIX fibres (*A*, upper panel), while weak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

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

Determinants of additional benefit of multiple-set training

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

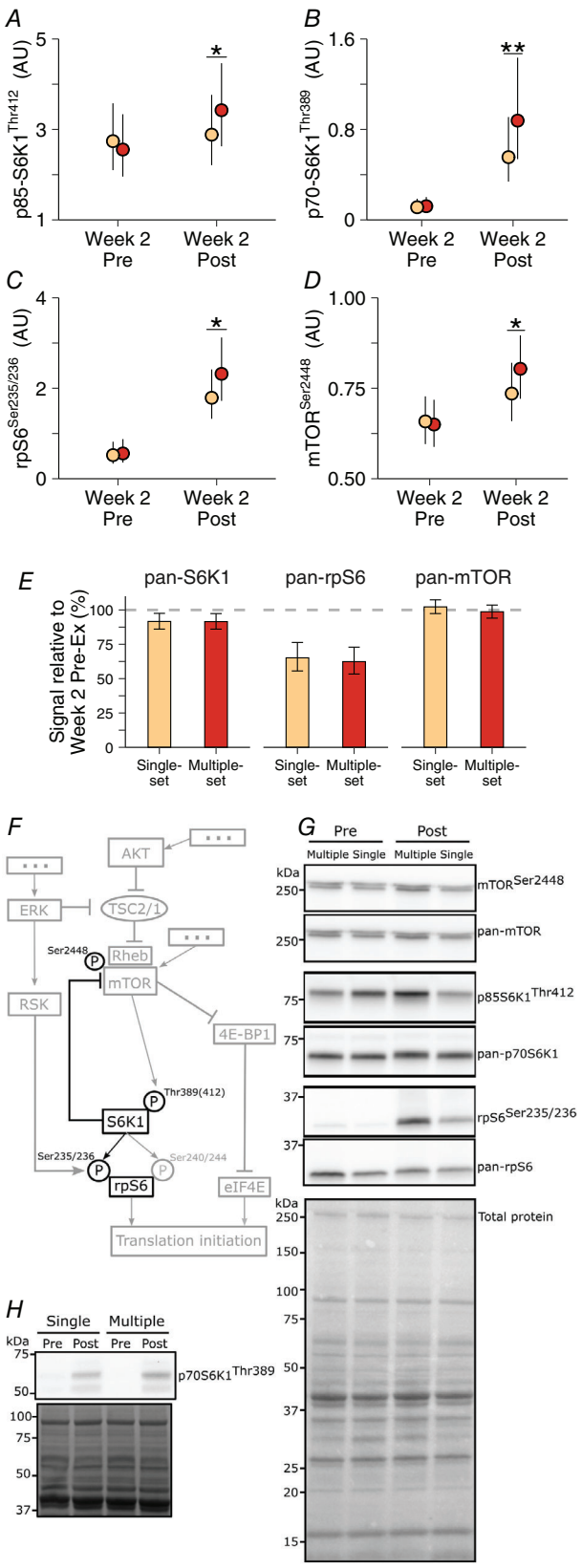


Figure 4. Western blot analysis of the mTOR signalling pathway

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

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

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

Discussion

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

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

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

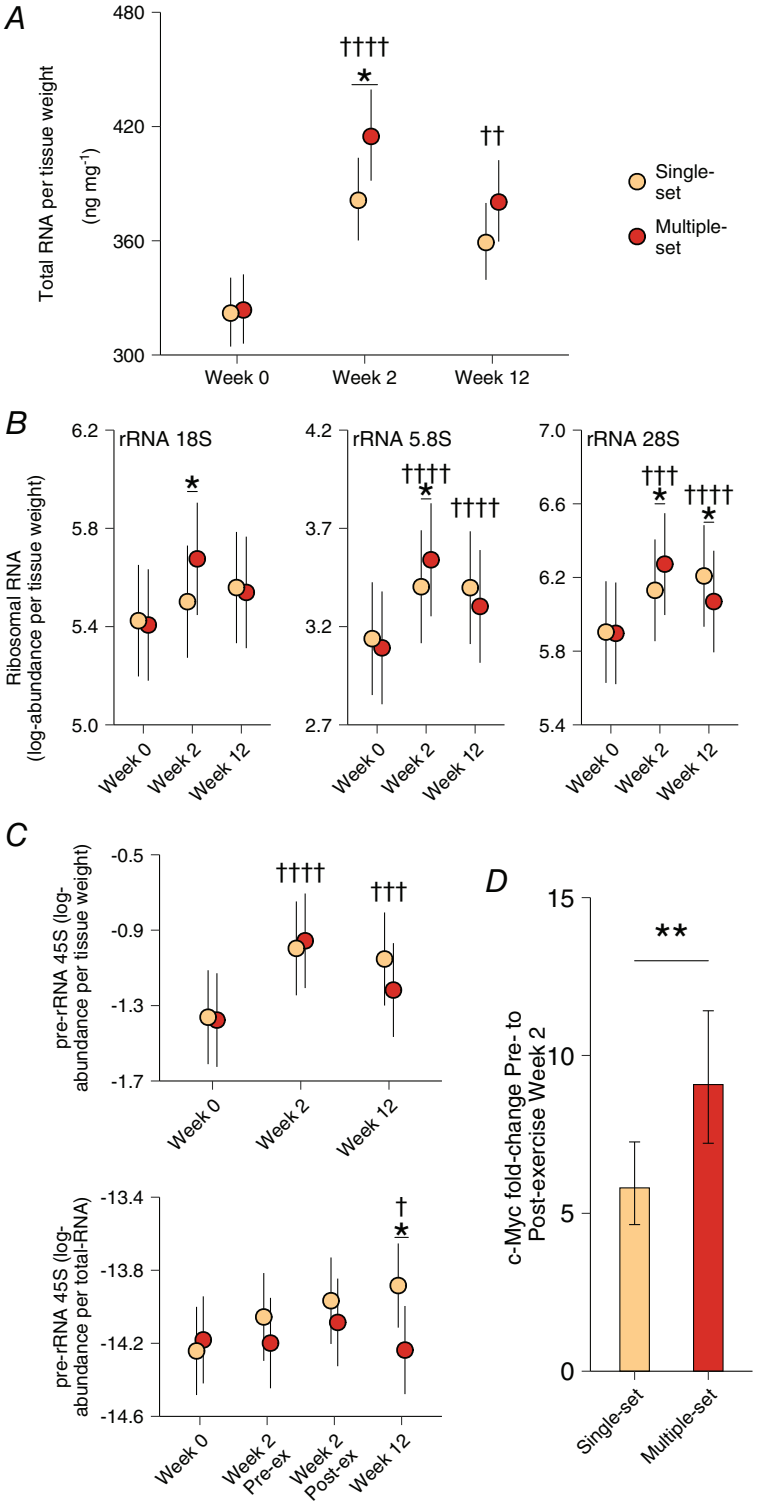
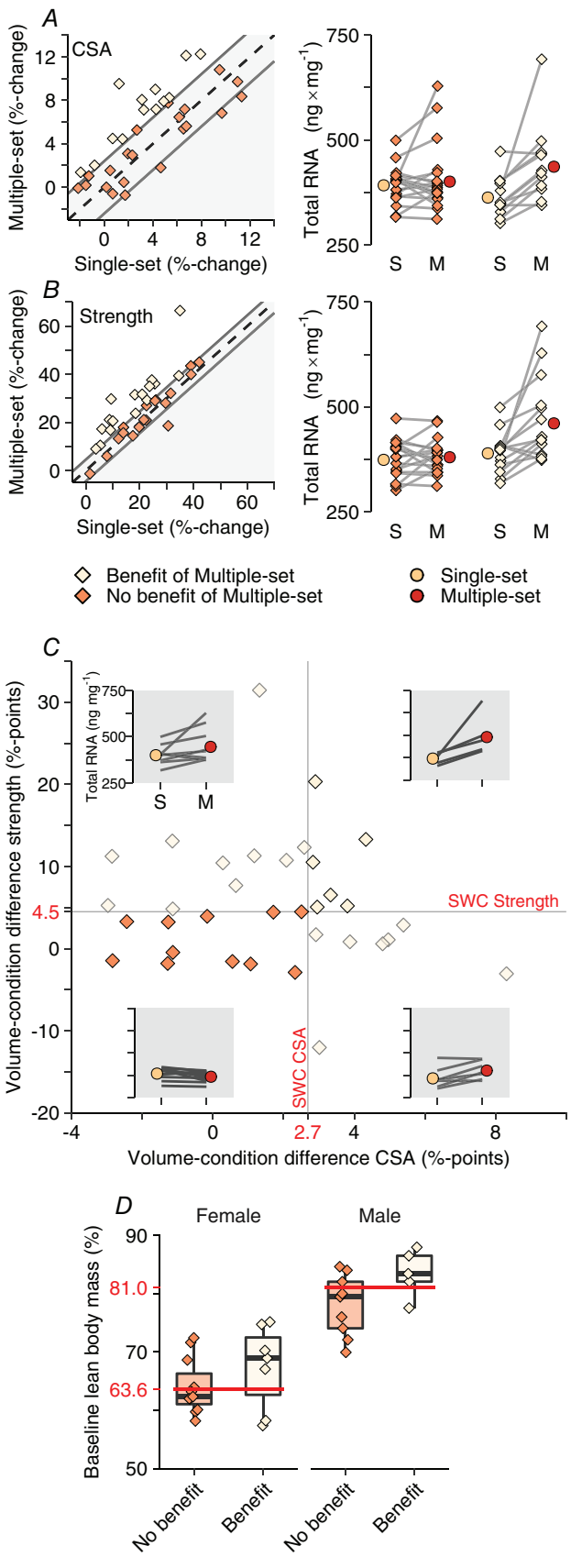


Figure 5. Total RNA and ribosomal RNA
Training volume-dependent changes in total RNA and ribosomal RNA 18S content were apparent in m. vastus lateralis after 2 weeks of resistance training (measured per unit muscle weight, Week 2, *A* and *B*). Other mature ribosomal RNA species exhibited similar expression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



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

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

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

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

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

(*Continued*)

Table 4. Continued

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Additional information

Competing interests

The authors have no conflicts of interest to disclose.

Author contributions

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

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Keywords

resistance-training, ribosome biogenesis, training-volume

Supporting information

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

Statistical Summary Document