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ABSTRACT

PURPOSE: To investigate the effects of blood flow restricted resistance exercise (BFRRE) on myofiber areas (MFA), number of myonuclei and satellite cells (SC), muscle size and strength in powerlifters. **METHODS:** Seventeen national level powerlifters (25±6 yrs [mean±SD], 15 men) were randomly assigned to either a BFRRE group (n=9) performing two blocks (week 1 and 3) of five BFRRE front squat sessions within a 6.5-week training period, or a conventional training group (Con; n=8) performing front squats at ~70% of one-repetition maximum (1RM). The BFRRE consisted of four sets (first and last set to voluntary failure) at ~30% of 1RM. Muscle biopsies were obtained from *m. vastus lateralis* (VL) and analyzed for MFA, myonuclei, SC and capillaries. Cross sectional areas (CSA) of VL and *m. rectus femoris* (RF) were measured by ultrasonography. Strength was evaluated by maximal voluntary isokinetic torque (MVIT) in knee extension and 1RM in front squat. **RESULTS:** BFRRE induced selective type I fiber increases in MFA (BFRRE: 12% vs. Con: 0%, $p<0.01$) and myonuclear number (BFRRE: 17% vs. Con: 0%, $p=0.02$). Type II MFA was unaltered in both groups. BFRRE induced greater changes in VL CSA (7.7% vs. 0.5%, $p=0.04$), which correlated with the increases in MFA of type I fibers ($r=0.81$, $p=0.02$). No group differences were observed in SC and strength changes, although MVIT increased with BFRRE ($p=0.04$), whereas 1RM increased in Con ($p=0.02$). **CONCLUSION:** Two blocks of low-load BFRRE in the front squat exercise resulted in increased quadriceps CSA associated with preferential hypertrophy and myonuclear addition in type 1 fibres of national level powerlifters.

KEYWORDS: Ischemic training; Kaatsu; Myogenic stem cells, Myonuclear addition, Myonuclear domain, Athletes.

INTRODUCTION

Resistance training is often recommended for the muscular development and performance of athletes (1). Heavy-load strength training (>70 % of one repetition maximum [1RM]) is traditionally recommended for muscle growth and maximal strength development (1). However, low-load (20-50 % 1RM) resistance training combined with blood flow restriction (BFR) is used by sport and fitness practitioners (2). Low-load blood flow restricted resistance exercise (BFRRE) can increase strength, muscle size, and sports performance in a variety of athletes (i.e. track and field athletes, American football and rugby players) (2-4). An important benefit of BFRRE is that low-loads can be used to achieve hypertrophic and strength responses, similar to traditional high-load unrestricted strength training (5). This has applications for individuals who may not be able to tolerate the mechanical stresses associated with higher-load strength training, such as older and clinical populations, or athletes during rehabilitation from an injury (5). Furthermore, low-load BFFRE could potentially serve as a method to facilitate muscular development without adding substantially to the total training dose experienced, or as a supplement for athletes already well accustomed to heavy-load strength training. Although, the impact of low-load BFRRE has not yet been investigated in highly specialized strength athletes, such as powerlifters.

The precise mechanisms involved in muscle adaptations to BFRRE are not fully known. BFRRE has been shown to increase protein synthesis accompanied by mTOR pathway activation (6) and reduced proteolysis-related gene expression (7). Yet hypertrophy is complex and involves mechanisms that include non-coding microRNA and ribosomal biogenesis (8,9). To our knowledge, no study has yet investigated changes in miRNA abundance and ribosomal responses after BFRRE. In addition to the elevated protein synthesis and decreased expression of

proteolytic genes, activation and proliferation of satellite cells have recently been implicated in the hypertrophic response observed with low-load BFRRE (6,10). Nielsen et al. (10) reported that three weeks of low-load high-frequency BFRRE (23 sessions) resulted in large increases in satellite cell (150-300%) and myonuclei numbers (~30%) in untrained individuals. Interestingly, the satellite cell and myonuclear responses in Nielsen et al (10) appeared to plateau after one week of training, suggesting that the responsiveness to BFRRE may diminish with training. To circumvent this plateauing effect, it may be hypothesized that applying multiple short blocks of BFRRE would be effective. In support of this, previous studies have observed that hypertrophy-associated signaling pathways can to be restored (re-sensitized) after ~10 days of detraining (11).

Consequently, the aim of the present study was to investigate the effects of two one-week blocks of high-frequency low-load BFRRE during six weeks of periodized strength training in elite powerlifters. We hypothesized that two blocks of BFRRE would be superior to traditional heavy-load strength training in increasing muscle size and strength in elite powerlifters, and that these changes would be related to increases in the numbers of myonuclei and satellite cells, as well as to the noncoding miRNA and ribosomal biogenesis to elicit a coordinated regulation of protein synthesis.

METHODS

Subjects. Nineteen (16 men and 3 women) elite Norwegian Powerlifters aged 25 ± 6 years (mean ± SD) were recruited through the Norwegian Powerlifter Federation. To be included in the study, participants had to be qualified for the national powerlifting championship and competed within the last 6 months. Exclusion criteria were any injuries in the musculo-skeletal system that could prevent the participants from conducting training or testing, use of medication or anabolic steroids, and any prior experience with BFRRE. Two of the nineteen participants did not

complete the intervention for reasons not related to the study. Furthermore, one powerlifter was excluded from one repetition maximum (1RM) and maximal voluntary isokinetic torque (MVIT) tests because of un-related health concerns, and one participant was excluded from the cross-sectional area measurements of *m. vastus lateralis* due to technical error. These participants were included in all other analyses. The study complied with the standards set by the Declaration of Helsinki and was reviewed by the Regional Committee for Medical and Health Research Ethics (REC South-East) and the Norwegian Centre for Research Data. The nature and goals of the study were thoroughly explained, and all subjects provided a written informed consent.

Study design. The present study design was conducted as a randomized controlled experiment. Participants were assigned to either a BFRRE group (n=9) or a conventional group (Con, n=8). We divided into two groups (above and below average) based on 1RM measurements at baseline, from which participants were randomly selected. During six and a half weeks of periodized strength training with five bouts per week, the only difference between the BFRRE- and the Con group was ten front squat sessions during week 1 and 3 (figure 1). In week 1 and 3, the BFRRE group performed two blocks of five BFR front squat sessions, whereas the Con group performed front squats at 60-85% of 1RM. Muscle biopsies, ultrasound images of muscle size, 1RM in front squat and MVIT in knee extension were obtained two days before initiating the training period, as well as three days after the last strength training session. All tests and measurements were performed by the same test leader at each timepoint and done in the same order each time.

Training protocol. In week 1 and 3, the BFRRE group performed four sets (first and last set to voluntary failure [~30 and ~8 repetitions, respectively]; set two and three to target repetitions of 15 and 12, respectively) with 30 seconds rest between sets, whereas the Con group performed

normal front squat sessions (60-85% of 1RM, 1-6reps, and 6 or 7 sets). The six-and-a-half week strength training intervention was designed by the head national- and junior national team coaches, and was a planned part of the powerlifters annual periodization. During the strength training intervention, a variant of squat (normal, high/low bar, medium or narrow grip, with stop, shorter range of motion or slow eccentric phase [4 seconds] or front squat) and bench-press were trained five times per week; a variant of deadlift (normal, sumo, wide grip, shorter range of motion or stiff-legged) was trained two times per week, whereas bent-over barbell-rows, shoulder press, and a *biceps*- and *triceps* exercises (self-chosen) were trained once a week. For every exercise, the load increased progressively (60-85% of 1RM) during six or seven sets per exercise, with one to six repetitions per set. During the six and a half weeks, both groups performed 6 front squat sessions in addition to the 10 front squat sessions performed in week 1 and 3. The load during BFRRE was calculated by a formula used by the powerlifters to adjust for body mass: relative load (1RM × 0.4 [week 1] - 0.6 × body mass or × 0.45 [week 3]) - 0.6 × body mass; corresponding to ~24- and 31 % of 1RM during the first and third week, respectively. The conventional group trained with an average of 74- and 76% of 1RM during front squat in the corresponding weeks.

Blood flow restriction. To restrict blood flow during BFRRE, elastic knee bands (7.6 cm wide) were wrapped around the proximal part of the thigh, a method which is often referred to as practical blood flow restriction (pBFR) or practical occlusion (2,4,12), and which was first suggested for BFRRE purposes by Loenneke & Pujol (12). However, elastic wraps have been used to restrict blood flow in sports medicine and physiological settings for many decades (e.g. (13)). We used our own modified model of pBFR in which we applied the elastic wraps in a slightly overlapping manner to a total width of ~ 13-14 cm. The pBFR was applied just before

the working sets and then maintained until all four sets of front squat were completed. The powerlifters were trained to reproduce a pressure corresponding to approximately 120 mmHg before initiating BFRRE, verified with an underlying lightly inflated 6 x 83 cm pressure cuff (SC5, Hokanson, Bellevue, WA, USA) connected to a sphygmomanometer (DS400 aneroid, Welch Allyn, Hechingen, Germany). The procedure of a small underlying cuff to standardize the pressure applied with the knee wraps was adapted from the method of Thorsson et al. (13), who used a lightly inflated air bladder from a blood pressure cuff placed underneath the elastic wrappings to monitor the applied pressure. The choice of a total wrap width of 13-14 cm was based on the experience of the coaches, and on studies which have demonstrated that lower pressures are needed to achieve arterial occlusion pressure (AOP) with wider tourniquet cuffs compared with narrow cuffs, in which wider cuffs have also yielded a more narrow range in AOP (14,15). With 14.5 cm wide thigh cuffs, we have previously observed that 100 mm Hg in a seated position in young subjects resulted in a reasonably narrow range of relative pressure (~54-64%) normalized to limb occlusion pressure (Bjørnsen, Wernbom et al., unpublished). To accommodate for the larger thigh size in powerlifters compared with normal subjects, we opted for ~120 mm Hg in BFR pressure in the present study. Two powerlifting coaches conducted random checks during week 1 and 3 to supervised the BFRRE sessions and ensure that the BFRRE group achieved a pressure close to 120 mmHg, verified by the underlying pressure cuff.

Strength tests. Muscle function was evaluated by testing MVIT in knee extension using a dynamometer (HUMAC 2009NOMR CSMi, Testing and Rehabilitation System, Arizona, Phoenix, USA) and 1RM in front squat using a bar and weight plates approved for powerlifting competitions. General warm-up consisted of 5 minute cycling with a standardized watt load (100 watt) for each subject. The isokinetic torque at 60 °/sec of the knee extensors was measured over

a range of 70° (from 20°-90° when 0° is fully extended). Following four warm-up attempts with gradually increasing resistance, 2 × 3 maximal repetitions were performed and the highest value obtained from these was noted as peak torque. Participants were strapped to the dynamometer chair with two belts crossing over their chest. Hands were placed on these belts to ensure isolation of the knee extensor muscles. The specific warm-up to the 1RM test in front squat consisted of sets with 5 repetitions, 4 repetitions, 3 repetitions, 2 repetitions and 1 repetition with gradually increasing resistance. After warm-up, single repetitions with increasingly heavier loads were performed until the 1RM load was found, i.e. the highest load that could be lifted throughout the range of motion. Three minutes rests were given between each 1RM attempt. The lift was valid if the body was lowered until the top surface of the thighs at the hip joint was lower than the top of the knee. The subjects were allowed to use lifting belt and magnesium during the 1RM test and strong verbal motivation was given during the test from the same test leader.

Muscle cross sectional area and muscle thickness. Muscle thickness in *m. rectus femoris* (RF), *m. vastus lateralis* (VL), *m. vastus medialis* (VM) and *m. vastus intermedius* (VI) were assessed by ultrasonography (Philips HD11XE Ultrasound system, Eindhoven, Netherlands). Panoramic imaging was applied to measure cross sectional area (CSA) of RF and VL. All participants lay on a bench in a supine position. Measurements were obtained at a distance equal to 40 % of the femur length and probe position was recorded for each measurement as previously described in Bjørnsen et al. (16). Muscle thickness was measured as the shortest distance between the upper and lower aponeuroses. For each muscle, this variable was obtained as an average of three measurements at 25%, 50%, and 75% of the probe width (40 mm probe width). The experienced examiner that performed all ultrasound measurements analyzed the images blindly in a random order with the software ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD,

USA), but was not blinded for group randomization. Test-retest measurements from two following days revealed a coefficient of variation (CV) of 0.3-0.6 % in muscle thickness measurements (VL, VM, VI and RF) as well as 0.8% (RF) and 1.3 % (VL) in cross sectional area measurements.

Muscle biopsy sampling. Muscle biopsies (200-300mg) were obtained from *m. vastus lateralis* using a 6mm sterile Bergström needle (Pelomi, 6mm, Albertslund, Denmark) under local anaesthesia (Xylocain-adrenaline, 10mg /ml + 5mcg/mL, AstraZeneca, Södertälje, Sweden) and placed approximately 2-3 cm apart from each timepoint. Visible connective tissue and fat were dissected away before a bundle of fibers for later immunohistochemical analyses was mounted in OCT Embedding Matrix (Tissue-tek, O.C.T. compound, Sakura, USA) and immediately frozen in isopentane, which was pre-cooled (~ -140° C) with liquid nitrogen and stored at -80°C for later analysis. A ~20 mg piece was snap frozen in liquid nitrogen for RNA analysis.

Histochemical staining. Muscle biopsies were cut to 8 µm thick cross sections at -20°C using a microtome (CM 3050, Leica Biosystems GmbH, Wetzlar, Germany) and mounted on microscope slides (Superfrost Plus, Menzel-Glaser, Brouschweig, Germany). Satellite cells were visualized with antibodies against PAX7 (DSHB, Iowa City, Iowa, USA, 1:100), NCAM (Abcam, 153377-1, Cambridge, UK, 1:200) and laminin (Dako, 00090772, Glostrup, Denmark, 1:400) as well as DAPI-stains (for nuclear staining) (Invitrogen, 1266174, Carlsbad, CA, USA). Neighboring sections were stained for MHC-II (SC-71, DSHB, Iowa City, Iowa, USA, 1:1000) and dystrophin (Abcam, 831009, Cambridge, UK, 1:500) for identification of type II myofibers (17) and delineation of the myofiber border, respectively. Antibodies and stains (Pax7 + Laminin + DAPI, NCAM + Laminin + DAPI, and SC71 + Dystrophin + DAPI) were applied for 45 min incubation in a serum-free protein blocker (Dako, 10082504, Glostrup, Denmark) and PBS-t

solution (QC213624, Thermo Fisher Scientific, Carlsbad, CA, USA). Specific secondary antibodies (Alexa-488 [goat anti-mouse, Invitrogen, 1008801, Carlsbad, CA, USA, 1:200] and CF-594 [goat anti-rabbit, Invitrogen, 1008648, Carlsbad, CA, USA, 1:200]) were applied after each primary antibody. Sections were mounted with a fluorescent anti-fade containing DAPI solution (Invitrogen, cat.no. P36935, Carlsbad, CA, USA). Capillaries were visualized with antibodies against CD31 (M0823; Dako A/S, Glostrup, Denmark, 1:100) and incubated overnight at 4°C, followed by incubation with appropriate secondary antibodies (Alexa-594, [goat anti-mouse, Invitrogen, 1008801, Carlsbad, CA, USA, 1:200]). The sections were visualized on a computer screen using a light microscope (Olympus BX61, Tokyo, Japan) connected to a fluorescent light (X-Cite 120PCQ; EXFO Photonic Solutions Inc., Mississauga, Ontario, Canada). The microscope was connected to a digital camera (Olympus DP72, Tokyo, Japan). All morphometric analysis was performed in Cell-F (Olympus, Tokyo, Japan), TEMA (ChekVision, Hadsund, Denmark) and ImageJ.

RNA extraction and cDNA /RTPCR. Total RNA was extracted using AllPrep® DNA/RNA/miRNA Universal Kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. Total RNA concentration and purity was measured using a NanoDrop 1000 running 3.1.2 NanoDrop software (BioLab, Auckland, New Zealand). 1500ng of input RNA was used for cDNA synthesis using the High- Capacity RNA- to- cDNA™ kit (Life Technologies, Carlsbad, CA, USA), messenger RNA (mRNA) and ribosomal RNA (rRNA) were measured by RT- PCR on a LightCycler 480 II (Roche Applied Science, Penzberg, Germany) using SYBR Green I Master Mix (Roche Applied Science). Target mRNAs were Paired box 7 (*PAX7*), Neural Cell Adhesion Molecule (*NCAM*) Myogenic Differentiation 1 (*MYOD*), Myogenin (*MYOG*), CyclinD1 (*CCND1*), CyclinD2 (*CCND2*), Vascular Endothelial Growth

Factor (VEGF) and Nucleolar pre-rRNA Processing Protein (Nip7) (See Table, Supplemental Digital Content 1, mRNAs, rRNAs and miRs sequences, http://links.lww.com/MSS/B390). Mature rRNA targets included 28S, 18S, 5.8S and 5S. Pre-rRNA targets included 28S +ITS, 18S +ITS and 5.8S +ITS. mRNA primers were designed using BLAST software and rRNA primers were designed by Qiagen using the RT^2 Profile PCR Arrays (Qiagen; Venlo, Limburg, The Netherlands). The geometric mean of Endoplasmic reticulum membrane protein complex subunit 7 (EMC7), valosin-containing protein (VCP), charged multivesicular body protein 2A (CHMP2A) and chromosome 1 open reading frame 43 (C1orf43) were identified as the least variable and used as reference genes. Standard and melting curves were performed for every target to confirm primer efficiency and single product amplification.

miRNA cDNA and RT-PCR. As described in D'Souza et al. (18), 10 ng of total RNA was converted to cDNA using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific, Carlsbad, CA, USA), miR abundance were measured by RT-PCR on a QuantStudio 6 (Thermo Fisher Scientific, Carlsbad, CA, USA) using Applied Biosystems Fast Advanced Master Mix (Thermo Fisher Scientific, Carlsbad, CA, USA). Target miRNAs were miR-15a-5p, -16-5p, -1-3p, -486-5p -133a, -206, -126-3p, -499a-3p and (Thermo Fisher Scientific, Cat# A25576, Carlsbad, CA, USA) (Table 1). The geometric mean of three stable endogeneous miRs (miR-361-5p, -320a and -186-5p) were identified as the least variable and used as reference genes. The abundance of miRs and mRNA were measured using the $2^{(-\Delta\Delta CT)}$ method (18).

EMG amplitude during exercise. Six of the subjects in the BFRRE group participated in a separate sub-experiment to investigate muscle activity with surface electromyography (EMG) on *m. vastus lateralis*. After a skin preparation (shaving and alcohol rinse) two electrodes

(BluesensorM, Ambu, Ballerup, Denmark; diameter: 31 mm) were placed on *m. vastus lateralis*, according to recommendation by SENIAM (19). EMG was recorded at 1000 Hz (bandwidth 20-500 Hz) and rectified and smoothed (100 ms moving average) with the root-mean-square (RMS) algorithm (hardware and software from Ergotest, Langesund, Norway). With the instruction to move as fast as possible in the concentric phase, the participants performed two sets of three repetitions of front squat at 80 % of 1RM, separated by 2 min. After fifteen minutes of rest to ensure full recovery, four BFRRE sets of front squat were performed at 30% of 1RM (i.e., similar as under the training intervention). The peak EMG values from the average of the three first and last repetitions in each set of BFRRE were expressed relative to the average peak EMG values during the 3 repetitions at 80% of 1RM (without BFR).

Statistical analysis. With a minimum of 8 participants in each group we had 80% power to detect a mean group difference between the two groups of 11 % in muscle fiber area with an expected SD of 7 % (20) (alpha: 0.05). We considered such difference to be well within the physiological meaningful range (10). Statistical analyses were performed using Graph Pad Prism Software (GraphPad Software Inc., La Jolla, CA). Variables showed overall normal distribution (Gaussian distribution). Statistical differences between the BFRRE- and Con group were determined using an Independent Sample t-test. Paired Sample t-test was applied to evaluate differences between baseline and post-intervention measurements for each group separately, and Pearson r was used to assess correlations. Descriptive data are presented as mean ± SD, whereas results are presented as mean with 95% confidence intervals. Statistical significance level was set to 5 %.

RESULTS

All seventeen powerlifters (Table 1, 15 men and 2 women) reached the minimum adherence of 85% completed training sessions during the intervention (28 of 33 sessions). Participants in the BFRRE group (8 men and 1 woman) completed all ten BFRRE sessions, except one participant who had to abort two BFRRE sessions due to exercise-induced migraine. No significant differences between groups were detected at baseline (Table 1). The total training volume in front squat did not differ between groups during week 1 (BFFRE: 11 104 [9105, 13 104] kg vs. Con: 11 211 [9588, 12 833] kg), but the BFRRE group lifted 3995 (1097, 6894) kg (p=0.005) more than the Con group during week 3.

Muscle size. During the 6.5-weeks strength training intervention, type I muscle fiber cross-sectional area (MFA) increased more in the BFRRE group compared to the Con group (974 [402, 1547] μm^2 vs. 13 [-390, 416] μm^2; p=0.003, figure 2A). Type II MFA did not increase significantly in either of the groups, and no group differences were observed (BFRRE: 379 [-157, 915] μm^2 vs. Con: 220 [-483, 922] μm^2). The cross-sectional area (figure 3A) of VL increased more in the BFRRE group (1.64 cm^2 [0.41, 2.87]), compared to the Con group (0.12 cm^2 [-0.70, 0.93], p=0.03). The CSA of RF increased in the BFRRE group compared to baseline (0.97 cm^2 [0.01, 1.93], p=0.03), but only a tendency (p=0.09) was observed when comparing changes to the Con group (0.21 cm^2 [-0.30, 0.71]). The BFRRE group increased muscle thickness (figure 3B) of RF (BFRRE: 0.11 [0.07, 0.15] mm vs. Con: -0.04 [-0.15, 0.07] mm; p=0.01), VL (BFRRE: 0.13 [0.06, 0.20] mm vs. Con: -0.04 [-0.18, 0.10] mm; p=0.02) and VM (BFRRE: 0.10 [0.01, 0.19] mm vs. Con: -0.15 [-0.39, 0.09] mm; p=0.02) more than the Con group. No group difference was observed in VI thickness (BFRRE: 0.09 [-0.01, 0.19] mm vs.

Con: 0.00 [-0.14, 0.15] mm). The increases in CSA of VL were correlated with the increase in MFA of type I fibers (r=0.81, p=0.02) in the BFRRE group.

Maximal strength. No group differences were observed in the changes of MVIT in knee extension (figure 4A) or 1RM in front squat (figure 4B). However, the BFRRE group increased their MVIT with 9.4 (0.0, 18.7) Nm (p=0.04) during the 6.5 weeks of strength training, whereas no changes were observed in the Con group (-1.8 [-17.4, 14.0] Nm). In 1RM of front squat, the Con group increased their 1RM with 5.9 (1.2, 10.7) kg from baseline to post exercise (p=0.02), whereas only a tendency was observed in the BFRRE group (4.1 [-0.7, 8.8] kg, p=0.08). The changes in MVIT were correlated with the changes in RF and VL (summed mean) CSA (r=0.68, p=0.04) and MFA of type I fibers (r=0.79, p=0.01) in the BFRRE group. Furthermore, a tendency was observed between the changes in 1RM of front squat and the changes in MFA of type I fibers (r=0.63, p=0.07) in the BFRRE group. No other associations between the increases in strength and muscle size were identified (r<0.4, p>0.05).

Myonuclei, myonuclear domain, satellite cells. The number of myonuclei (figure 2B) in type I fibers increased in the BFRRE group (1.1 [0.5, 1.7]) compared to the Con group (0.0 [-0.7, 0.8]; p=0.02). The number of myonuclei per type II fibers did not change in either group (BFRRE: 0.5 [-0.1, 1.1] μm^2 vs. Con: 0.2 [-0.8, 1.3] μm^2). Similarly, the MFA per nucleus (myonuclear domain, figure 2C) in type I (BFRRE: -61 [-147, 25] μm^2 vs. Con: 1 [-90, 92] μm^2) and type II fibers (BFRRE: -26 [-132, 80] μm^2 vs. Con: 5 [-197, 207] μm^2) remained unchanged. NCAM/Pax7 positive satellite cells (per 100 muscle fibers, figure 2D) per type I (BFRRE: -0.8 [-3.3, 1.6] vs. Con: 2.0 [-0.9, 5.0]) and type II fibers (BFRRE: -0.2 [-1.4, 1.1] vs. Con: 1.4 [-1.0, 3.8]) also remained unchanged. The relative increase in the number of myonuclei per type 1 fiber tended to correlate with the increase in MFA of type I fibers (r=0.62, p=0.08) in the BFRRE group.

Capillarization. The number of capillaries around type I fibers (figure 2E) increased with 0.66 (0.29, 1.03) in the BFRRE group compared to baseline, and the increase tended (p=0.07) to be greater than the change in the Con group (0.00 [-0.46, 0.46]). No changes were found in the number of capillaries around type II fibers. The capillary per muscle fiber areas (capillary density) remained unchanged in both type I and type II fibers in both groups (figure 2F).

Messenger-, micro- and ribosomal RNA abundance. Pax7 and NCAM mRNA abundance (figure 5A) increased more in the BFRRE group compared to the Con group (Pax7: p=0.02 and NCAM: p=0.02). The abundance of Cyclin D1 (p=0.02), Cyclin D2 (p=0.001), Myogenin (p=0.05), VEGF (p=0.01) and Nip7 (p=0.01) mRNA increased in the BFRRE group, and the increases tended to be larger than the Con group (p=0.05-0.10).

No significant group differences were identified in changes of miR abundance (figure 5B). However, the increase in miR-206a (p=0.09) and -126 (p=0.09) tended to be higher in the BFRRE group compared to the Con group, whereas the abundance of miR486 (p=0.07), -16 (p=0.09), -15 (p=0.09) and -1 (p=0.07) tended to be lower in the BFRRE group.

The abundance of mature rRNA 5.8S increased in the BFRRE group (p=0.01), and the increase tended (p=0.09) to be larger compared to the Con group (figure 5C). We observed no group differences in abundance of mature rRNA 5S, 18S, 28S or total RNA per mg. Pre-rRNA 18S+ITS increased in the BFRRE group compared to the Con group (p=0.05). The changes in pre-rRNA 28S+ITS tended (p=0.07) to be higher in the BFRRE group compared to the Con group. No group differences were observed in pre-rRNA 5.8S + ITS.

EMG amplitude during low-load BFRRE and high-load free-flow front squat. In the subset of six participants from the BFRRE group (See Figure, Supplemental Digital Content 2, EMG activity during front squats, http://links.lww.com/MSS/B391), peak RMS was higher during

three repetitions of front squat at 80 % of 1RM (100±7%) compared to the first and last three repetitions during set one (52±8% and 57±14 %, $p<0.001$), set two (53±16% and 58±18%, $p<0.001$), set three (51±14% and 58±15%, $p<0.001$) and set four (53±16 % and 62±16%, $p<0.001$) of the low-load BFRRE.

DISCUSSION

The present study investigated the effects of implementing two one-week blocks of high-frequency low-load BFRRE during six weeks of periodized strength training in elite powerlifters. The main findings were that the BFRRE group displayed significantly larger increases in RF and VL CSA (7-8%) and muscle thickness (3-6%) in *m. quadriceps femoris* as a whole. This whole muscle hypertrophy was reflected in increased muscle fiber cross-sectional area and myonuclear number. Notably, myofiber hypertrophy and addition of myonuclei were restricted to type I fibers (12% and 18%, respectively).

Changes in muscle size during BFRRE. The robust increases in VL and RF muscle CSA (7-8%) in the BFRRE group compared to the Con group are remarkable, considering the short training period and the training status of the elite powerlifters. To the authors' knowledge, this is the first study to investigate supplementary BFRRE during heavy-load strength training in elite strength-athletes. However, several previous studies have shown that BFRRE can increase both muscle size and strength in a variety of athletes (i.e. track and field, American football, rugby and netball) (2-4). Of these, only Yamanaka et al. (4) and Luebbers et al. (2) compared the effects of low-load BFRRE to high-load strength training during a periodized training intervention. Similar to the present study, Yamanaka et al. (4) observed a greater increase in chest and arm girth (~ 3%) after 4 weeks of low-load BFRRE in American football players, whereas Luebbers et al (2) could not detect an additional effect of BFRRE in arm-, chest- or

thigh girth. However, Luebbers et al (2) excluded all high-load training in exercises targeting girths-locations for the low-load BFRRE group. Thus, it may be necessary to maintain some high-load strength training in combination with low-load BFRRE to achieve additional gains in muscle size for athletes during a periodized strength-training regime. Furthermore, effects of BFRRE on trunk and hip muscles proximal to the cuff can be very different from the effect seen on muscles distal to the cuff (21).

Preferential type I fiber hypertrophy after BFRRE. Notably, the larger gains in muscle CSA in the BFRRE group appeared to be the result of preferential hypertrophy of type 1 fibers (type I: 12% vs. type II: 4%), which seems to be in contrast with the greater hypertrophy of type II fibers observed with heavy-load strength training (22). However, the body of literature remains somewhat equivocal to whether hypertrophy of type I and type II muscle fibers are different between high- and low-load conditions (23). It is well documented that low-load (~20-30% of 1RM) BFRRE can activate and stress both type I and II fibers when sets are performed until failure, as evidenced by acute changes in creatine phosphate, inorganic phosphate and glycogen depletion (24,25). This is further supported by the finding that short-term BFRRE can cause hypertrophy of both fiber types (10). However, it is also important to note that low-load BFRRE seems to induce a greater heat shock protein response and glycogen depletion in the type I fibers than in type II fibers, as shown by Cumming et al. (24), who used an unilateral knee extension BFR training model with 5 sets to failure at 30% of 1RM. These findings suggest that type II fibers are overall less stressed than type I fibers in low-load BFRRE even with multiple sets to failure. Therefore, low-load BFRRE may serve as a novel stimulus for the type I fibers in elite powerlifters and these fibers probably have a large growth potential, as powerlifters seems to have preferentially hypertrophied type II fibers (22). In contrast to previous investigations

(26,27), we did not observe any marked increases in EMG signal amplitude during sets of low-load BFRRE to in the present study (See Figure, Supplemental Digital Content 2, EMG activity during front squats, http://links.lww.com/MSS/B391). Although greater EMG responses do not necessarily reflect greater motor unit recruitment (28), an increased EMG amplitude could indicate that increasingly larger motor units are activated. In contrast to previous single-joint exercise studies (26,27), our participants performed BFRRE with a bilateral multi-joint exercise, front squat. A greater magnitude of peripheral fatigue seems to be tolerated in unilateral single-joint exercise compared to bilateral multi-joint exercise, probably because the feedback to the central nervous system from group III/IV afferents is less due to a smaller active muscle mass (29). Thus, fatigue and pain signals from large muscle masses including both the left and right quadriceps may have induced central fatigue during the front squat, and thereby inhibited sufficient activation and stress of type II fibers in *m. vastus lateralis*. In line with this scenario, several of the subjects in the BFRRE group expressed that they were affected by pain and/or whole-body fatigue. Moreover, it was difficult for the powerlifters to keep the torso vertical at the end of each set. Consequently, difficulties in keeping the technique may have led the powerlifters to end the sets before the thigh muscles were exhausted.

Myonuclear responses. The fiber-type specific increases in MFA was associated (r=0.62) with the preferential myonuclear addition in type I fibers of the BFRRE group. It is believed that each nucleus controls the capacity for protein synthesis within a finite area (volume) of cytoplasm, referred to as the myonuclear domain (30). Consequently, skeletal muscle hypertrophy may necessitate more nuclei. However, contrary to a proposed "rigid" myonuclear domain, it is suggested that strength training can induce muscle fiber hypertrophy up to a 20-30% increase in MFA in absence of myonuclear addition (31), as well as myonuclear addition in complete

absence of hypertrophy (32). Nevertheless, the relationship between gains in MFA and myonuclear addition in the present study supports the myonuclear domain theory. Interestingly, Murach et al. (30) proposed a model for fiber type specific satellite cell dependence during hypertrophy, suggesting that type I fibers have a more stringent reliance on myonuclear accretion during hypertrophy due to their greater relative metabolic activity and protein synthesis rate, whereas glycolytic fibers may possess a more flexible myonuclear domain. Interestingly, a preferential myonuclear addition in type I fibers was also observed in a recent study on bodyweight squats combined with BFR (33). However, these investigators did not find any increases in myofiber areas or muscle strength, despite modest but significant gains in quadriceps CSA and considerable increases in endurance (ending up at ~200-300 repetitions per session). We suggest that their exercise protocol was simply too endurance-oriented to result in marked strength-type adaptations.

Despite the significant increases in myonuclear content in the BFRRE group, no increase in satellite cells was observed in the current study. Importantly, the post-biopsies were obtained ~4 weeks after the last BFFRE session. It may therefore be speculated that the BFRRE group increased the number of satellite cells during the two blocks of BFRRE, and subsequent reduced the number towards baseline levels during the 4 weeks after BFRRE due to fusion with the growing type I fibers. Indeed, the satellite cells responses reported by Nielsen et al. (10) increased during the first week of low-load BFRRE, but decreased 10 days after the intervention. Furthermore, Snow et al. (34) observed that the number of satellite cells increased concomitantly with fiber size the first week after surgical ablation in a rodent model, but the number of satellite cells decreased back to baseline levels while the muscle fiber area continued to increase during the following 23 days. Finally, powerlifters do have a greater number of satellite cells per muscle

fiber compared to untrained individuals (18), and alternatively, it could be speculated that the elite powerlifters do not expand their satellite cell pool in addition to the proliferated satellite cells that fuse into existing muscle fibers.

Regulation of satellite cell cycle/myoblast fusion. A targeted approach was made to investigate some known myogenic regulatory factors (35), as well as myogenic miRs that are vastly more abundant within skeletal muscle and observed to be differentially expressed in powerlifters versus untrained individuals (18). Satellite cells are PAX7 and NCAM positive multipotent cells resident in the stem cell niche (35), and PAX7 and NCAM mRNA expression have been shown to increase with both acute and prolonged resistance exercise (36). Despite no change in NCAM/Pax7 positive satellite cells in the present study, the expression of Pax7 and NCAM mRNA increased significantly more in the BFRRE group. Three regulators important for satellite cell differentiation and fusion to existing muscle fibers, myogenin, cyclin D1 and D2 (35), increased more in the BFRRE group in the present study, whereas no change was observed in another important myogenic regulatory factor; MyoD. Cyclin D1 and D2 directly inhibit MyoD and are themselves directly inhibited by miR-16 and miR-15 (37). The increase in Cyclin D1 and D2 in the present study, likely resulting from trending decrease in miR-16 and miR-15, could have inhibited MyoD expression. miR-1, -133a, -206 and -486 downregulate PAX7 protein concentrations (8) and all except miR-486 are transcribed in response to increased MyoD and Myogenin expression (8) to provide a negative feedback mechanism. With BFRRE there was a trend towards lower abundance of mir-486 and miR-1, whereas miR-206 tended to be elevated in BFRRE group despite the higher abundance of Pax7. Although there are some inconsistencies in our results, these changes in muscle miR and gene expression act to support an increased myogenesis and muscle remodeling in the elite powerlifters following the BFRRE protocol.

Markers of ribosomal capacity. We could not detect an increase in most of the ribosomal capacity markers in the present study, as demonstrated by the unchanged total RNA and the mature rRNAs 5S, 18S and 28S. However, the mature rRNA 5.8S and the processing factor Nip7 increased in the BFRRE group. The 5.8S rRNA is known to be important in eukaryotic protein synthesis via its critical role in the regulation of translation elongation (38). Interestingly, a recent study showed a 4-fold upregulation of 5.8S rRNA after 8 weeks of strength training (9). It could therefore be speculated that upregulation of 5.8S rRNA supports exercise-induced muscle hypertrophy via enhanced translation elongation. Nip7 is involved in the maturation of the 18S rRNA, which may suggests a greater capacity to process 18S rRNA and allowing a quicker export and incorporation into the 40S (39). In contrast to the largely unchanged ribosomal capacity, ribosomal biogenesis tended to be upregulated in the BFRRE group, evident by increased pre-rRNA 18S + ITS, and a tendency towards increase in 28S + ITS ($p=0.07$). These changes in active ribosomal biogenesis could indicate that BFRRE can induce a ribosomal response, but that the changes in ribosomal capacity are relatively small in elite powerlifters. Furthermore, the lack of significant changes in ribosomal capacity may be attributed to fiber type specific responses in the present study, as we could not separate fiber types in these analyses. Consequently, a possible increase in in ribosomal capacity in type I fibers may be masked by no change in type II fibers in our results.

Angiogenesis. Strength training can induce angiogenesis to support hypertrophy, but normally capillary density is unchanged (36). Similar to previous observations during strength training, we observed an increase in capillary number, whereas capillary per muscle fiber areas remained unchanged. The novel finding in our study was that also the capillary response was specific to type I fibers with the BFRRE protocol. The expression of the proangiogenic gene VEGF,

increased in the BFRRE group together with a decrease in the direct inhibitor of VEGF, miR-16 (37). Furthermore, miR-126, shown to increases VEGF expression (37), tended to increase in the BFRRE group. The reduction in miR 16 together with the tendency to increase in miR-126 may partly explain the increase in VEGF expression; however, the direct regulation of angiogenesis by BFRRE via miRs requires further elucidation.

Muscle strength. Previous investigations have demonstrated that low-load BFRRE can increase maximal strength and performance in elite athletes (2-4), and a few studies have observed increased maximal strength after supplementing low-load BFRRE during a high-load strength-training regime (4). Despite the larger gains in size of the *quadriceps* muscles of the BFRRE group in the present study, we could not detect any significant group differences in strength gains. Nevertheless, only the BFRRE group increased MVIT in knee extension from baseline, and the increase in type 1 MFA was strongly correlated with the gains in MVIT (r=0.79), and tended to correlate with the increases in front squat 1RM (r=0.63). As discussed by Buckner et al (40), statistical relationships like these do not prove that the changes in muscle size and changes in strength were causally related. However, although a casual relationship has not been categorically demonstrated, there are several experimental issues that make it challenging to tease out the nature of these relationships and it is generally accepted that changes in muscle size affect muscle strength (41). The fact that two different measures of muscle hypertrophy (at the cellular and whole muscle level [summed VL + RF CSA gains], respectively) correlated with strength changes in the present study adds further support to the notion that muscle hypertrophy was one of the drivers of the strength increases in the BFRRE group. The increases in front squat 1RM were similar between groups in the present study (Con: 4%, BFRRE: 3%). Notably, we had our participants perform BFRRE with front squat to stress the knee extensors during the squat

exercise, and therefore decided to measure strength in the same exercise. Unfortunately, several of the powerlifters were clearly not well familiarized with this variant of the squat exercise. Consequently, the Con group, performing ten high-load front squat sessions more than the BFRRE group (16 versus 6 high-load sessions, respectively), seemed to improve their technique and/or muscle strength of the *truncus* more than the BFRRE group (visual confirmation by the national team coaches). In support of this hypothesis, Bryanton et al. (42) observed alterations in the biomechanics of squat performance with change in intensity of load, as the ratio of hip-to-knee extensor moments increased with heavier loads. Hence, practicing front squats with the form most specific to the 1RM test may optimize motor pattern coordination and the front squat technique. In addition, high load strength training appears to induce greater neural adaptations than low-load BFRRE (43,44). In fact, several studies have reported that low-load BFRRE induces strength gains and muscle hypertrophy without any significant changes in measures of voluntary activation and neural drive (43-45). Hence, suboptimal neural adaptations could explain why the BFRRE group failed to increase significantly in strength in this movement despite the evident hypertrophy in the VL and RF muscles. Furthermore, it could be speculated that the BFRRE group could have improved their 1RM more if tested in the back squat due to their increase in knee extensor strength and muscle CSA, given a likely superior back squat vs. front squat technique.

Strengths and limitations. First of all, our statistical power may have been too low to detect generally mild/moderate effects of low-load BFRRE; type II statistical errors might have occurred. However, significant effects of BFRRE was detected for both muscle fiber hypertrophy, myonuclear addition and increases at the whole muscle level, demonstrating a clear effect of BFRRE on these variables. Second, elastic knee bands were used to restricted blood

flow; hence, the absolute applied pressure was not strictly controlled. Third, the multi-joint exercise front squat may not have exhausted the *m. vastus lateralis* enough to induced sufficient activation and stress on the type II fibers during low-load BFRRE.

In conclusion, we report herein that two one-week blocks with high-frequency low-load BFRRE implemented during six weeks of periodized strength training induced a significant increase in muscle size and myonuclear addition in elite powerlifters. Preferential hypertrophy and myonuclear addition of type I fibers appears to explain most of the overall muscle growth. Thus, low-load BFRRE in combination with traditional strength training may be of importance to optimize adaptation of both fibre types in highly strength-trained individuals. Despite the increases in muscle size, we could not observe any group differences in maximal strength. Future research should investigate if low-load BFRRE can increase maximal strength in the back squat exercise that powerlifters are well familiarized with.

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AUTHOR CONTRIBUTIONS: The contributions of the authors were as follows: conceived the study and performed experiments: TB, MW, AK, GP, LS, and TR. Analysed data: TB, MW, AK, GP, LS, LB and TR. Drafted the manuscript: TB. Critically evaluated and contributed to the manuscript: TB, MW, AK, GP, LS, LB, DCS, SB and TR. All authors have approved the final version of the manuscript.

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List of Supplemental Digital Content

Supplemental Digital Content 1. Figure that illustrates EMG activity during front squats .tiff

Supplemental Digital Content 1. Table that illustrates mRNAs, rRNAs and miRs sequences .docx

Figure legends

Figure 1. Schematic illustration of the study design

Black arrows denote traditional strength training sessions that was similar between groups (see the Methods section for more details). Grey arrows denote the ten front squat sessions that differed between the BFRRE group and the conventional training group.

Figure 2. Immunohistochemistry

Relative changes in type I and II muscle fibers are from baseline to post 6.5 weeks of strength training. Muscle fiber cross-sectional area (A). Myonuclei per muscle fiber (B). Muscle fiber cross-sectional area per nucleus (C). NCAM and $Pax7^{+}$ myogenic stem cells per muscle fiber (D). Capillaries per muscle fiber (E). Capillaries per muscle fiber area (F). * represents significant group differences ($p<0.05$), # represents significant change from baseline ($p<0.05$) Data expressed are expressed as means with 95 % confidence intervals.

Figure 3. Muscle thickness and cross sectional area

Relative change in the cross-sectional area (A) of m. *rectus femoris* (RF) and of m. *vastus lateralis* (VL), as well as muscle thickness (B) of RF, VL, m. *vastus medialis* and m. *vastus intermedius*. * represents significant group differences ($p<0.05$), # represents significant change from baseline ($p<0.05$). Data expressed are expressed as means with 95 % confidence intervals.

Figure 4. Maximal isokinetic and dynamic strength

Relative changes in maximal voluntary isokinetic torque (A) and 1 repetition maximum (B). # represents significant change from baseline ($p<0.05$). Data expressed are expressed as means with 95 % confidence intervals.

Figure 5. Messenger-, micro- and ribosomal RNA

(A) fold change in mRNA abundance of Pax7, NCAM, MyoD, Myogenin, Cyclin D1, Cyclin D2, VEGF and Nip7 mRNA. (B) fold change in miR abundance of 15a, 16, 1, 486, 133a, 206, 126, 499. (C) fold change in rRNA abundance of 5.8S, 5S, 18S, 28S, 5.8S+ITS, 28S+ITS, 18S+ITS and total RNA. mRNAs and rRNA are normalised to geomean of 4 housekeepers, whereas miRs are normalised to geomean of 3 housekeepers. * represents significant group differences ($p<0.05$), # represents significant change from baseline ($p<0.05$). Data expressed are expressed as means with 95 % confidence intervals.

Supplemental Digital Content 1. EMG activity during front squats

The circles denote (A) three repetitions at 80% of 1RM), as well as (B) the three first, middle and last repetitions during the first set, and the three first and last repetitions during the second (C), third- (D) and fourth set (E) of BFRRE at ~30% of 1RM. * represents significantly different from A ($p<0.05$). Data expressed are expressed as means with 95 % confidence intervals.

Figure 1. Schematic illustration of the study design

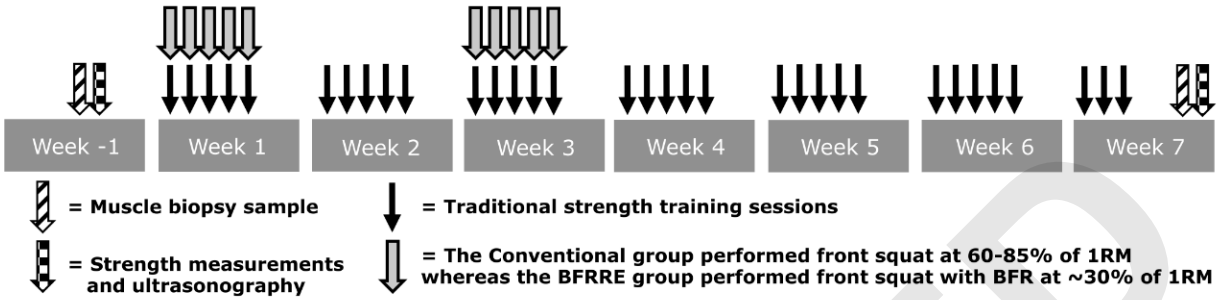


Figure 2

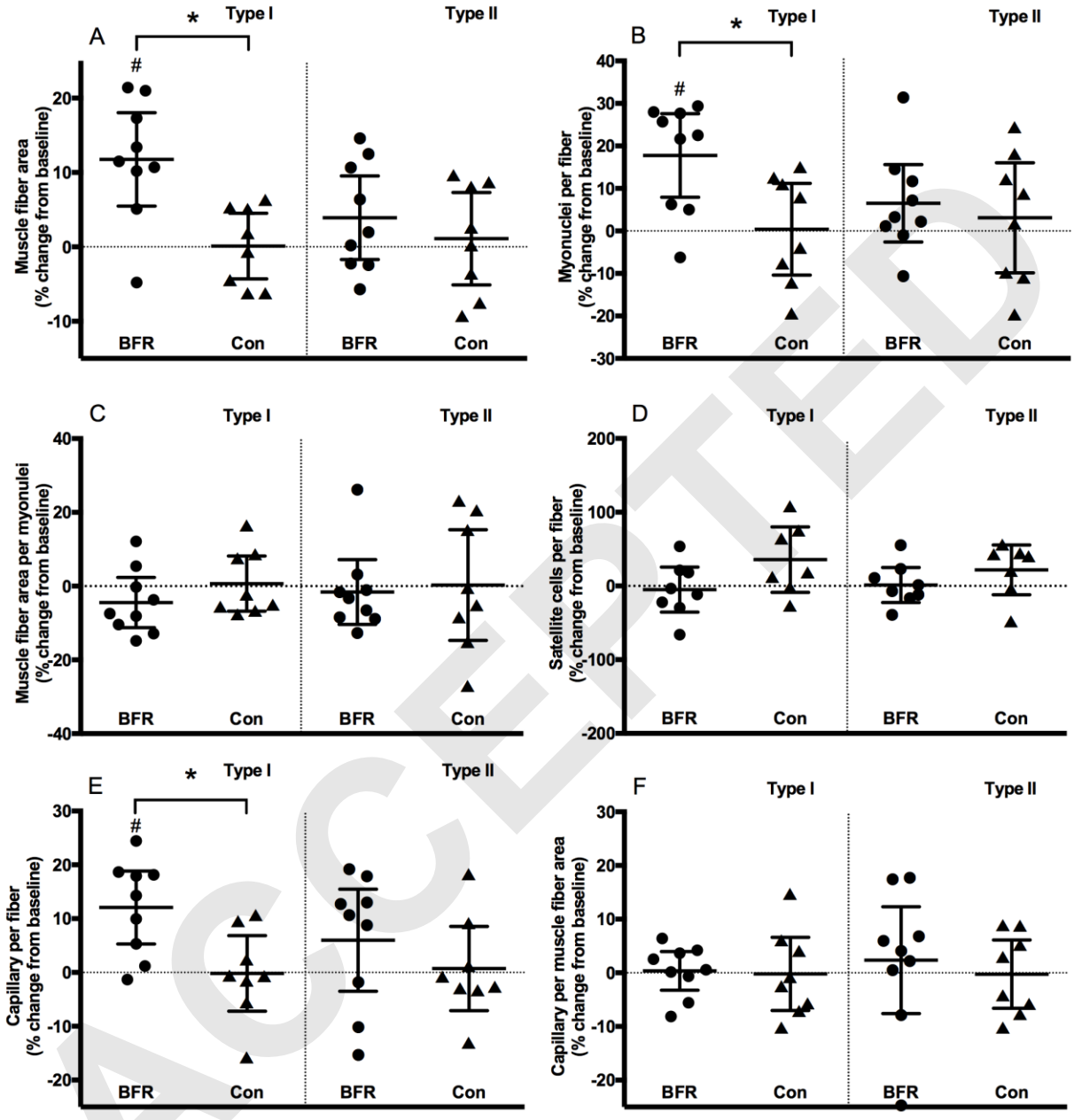


Figure 3

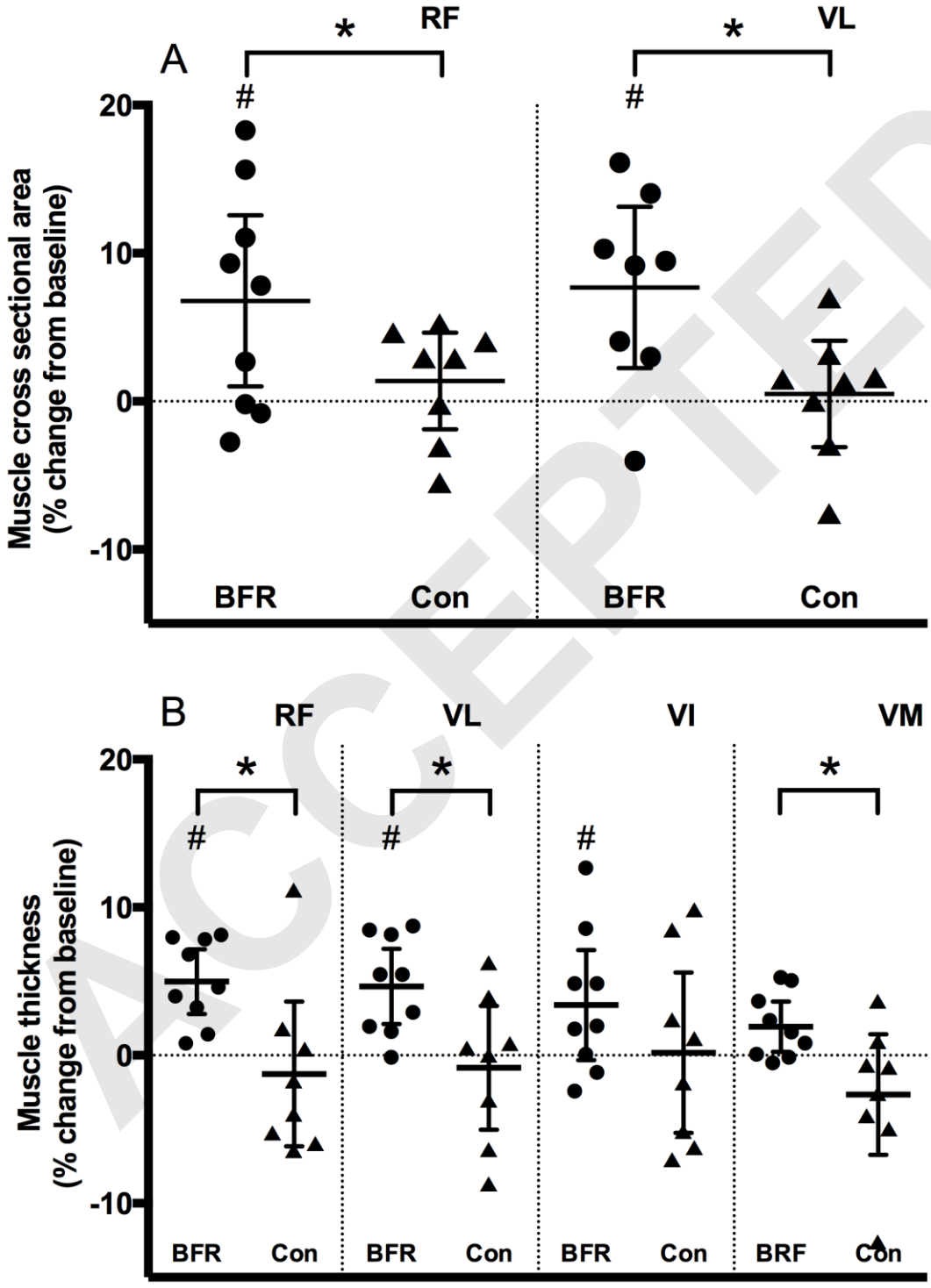


Figure 4

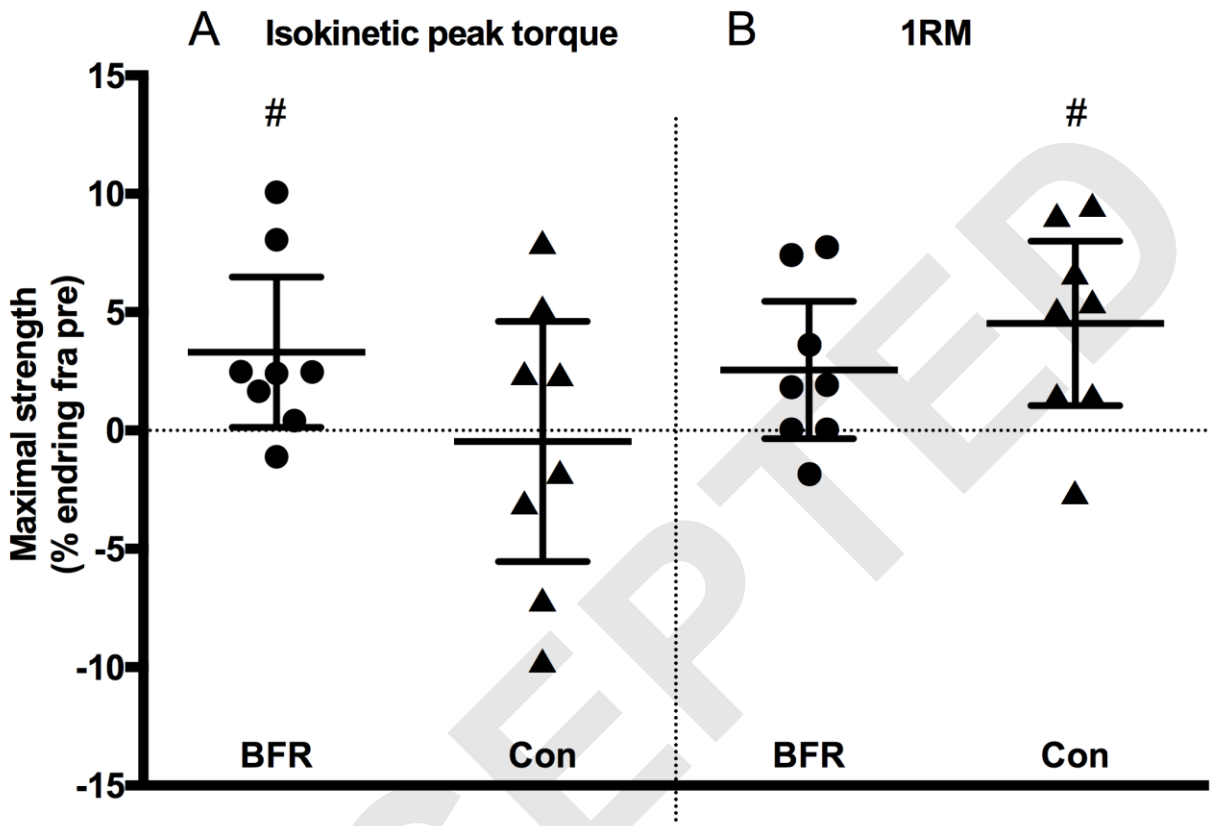


Figure 5

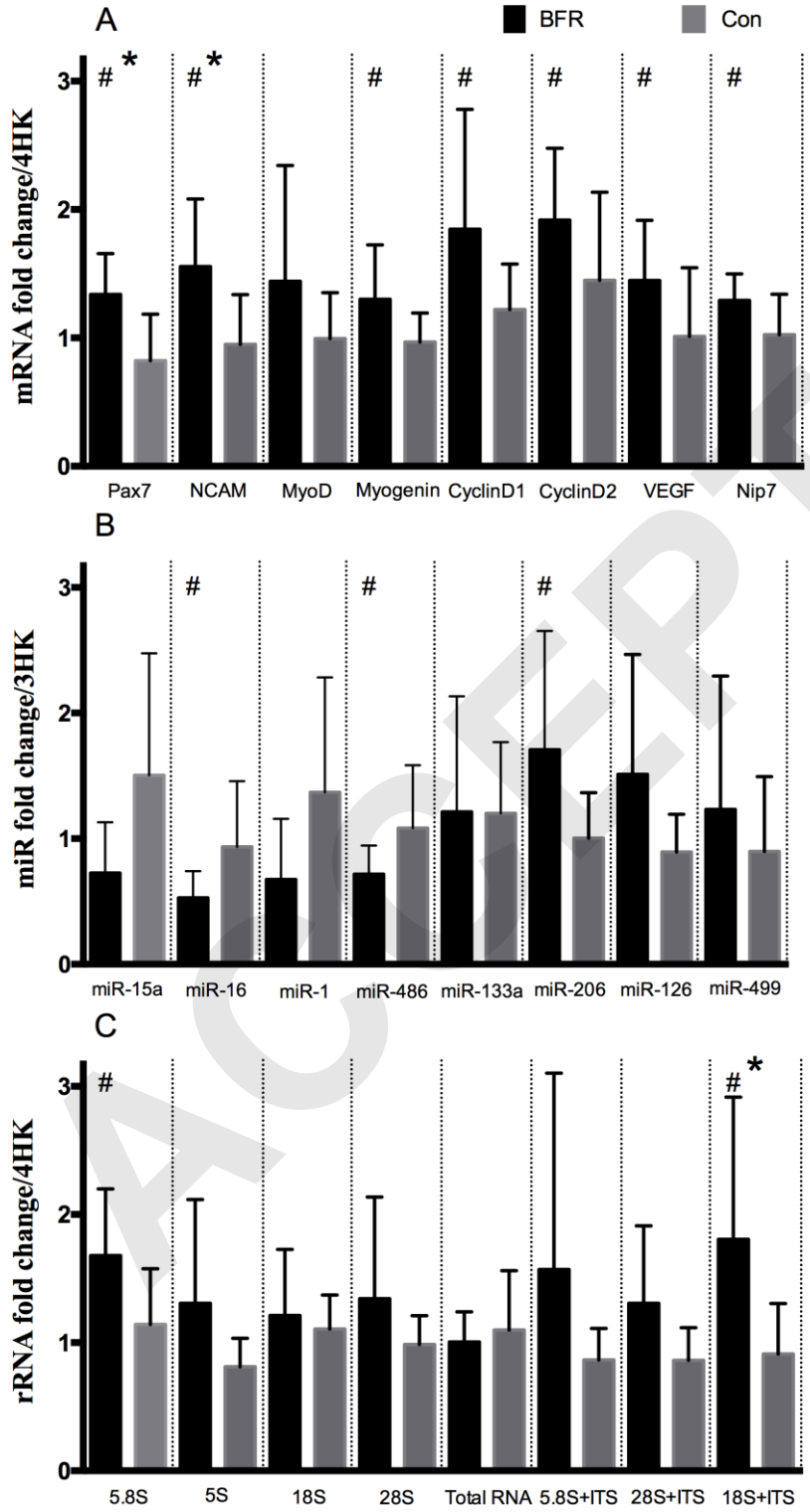
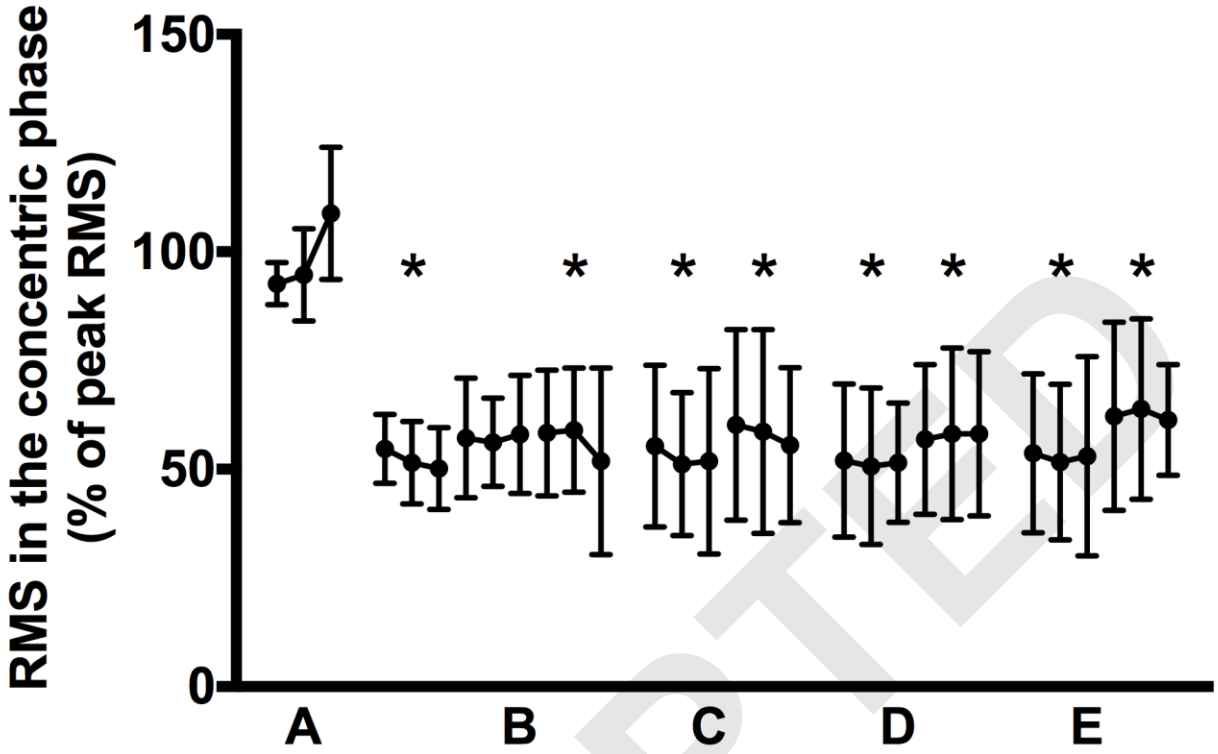


Table 1. Descriptive characteristics of the participants.

	BFRRE group (n=9)	**Conventional group (n=8)**	**Group differences at baseline (p-values)**
Age (years)	24 (3)	26 (8)	0.66
Height (cm)	176 (5)	177 (9)	0.81
Weight (kg)	89 (14)	102 (18)	0.11
Powerlifting experience (years)	4 (2)	6 (4)	0.27
Muscle strength			
MVIT in knee extension (nm)	283 (42)	315 (68)	0.25
1RM in front squat (kg)	141 (25)	151 (26)	0.46
Personal record in squat (kg)	186.7 (42)	207 (40)	0.33
Personal record in deadlift (kg)	227 (44)	244 (36)	0.40
Personal record in benchpress (kg)	135 (28)	154 (32)	0.21
Muscle size			
Rectus femoris CSA (cm^2)	12.1 (5.4)	14.8 (4.4)	0.28
Vastus lateralis CSA (cm^2)	23.5 (4.5)	24.0 (3.2)	0.80
Rectus femoris thickness (mm)	2.3 (0.4)	2.4 (0.4)	0.57
Vastus lateralis thickness (mm)	3.0 (0.4)	3.4 (0.4)	0.09
Vastus medialis thickness (mm)	5.3 (0.5)	5.3 (0.7)	0.97
Vastus intermedius thickness (mm)	2.7 (0.5)	2.7 (0.3)	0.93
MFA type I (um^2)	8700 (1262)	9058 (1538)	0.61
MFA type II (um^2)	10568 (2001)	10711 (2196)	0.89
Myonuclei			
Myonuclei per fiber type I (n)	6.8 (1.5)	6.8 (1.1)	0.97
Myonuclei per fiber type II (n)	7.6 (0.9)	8.1 (0.7)	0.17

Myonuclear domain type I (um^2)	1294 (119)	1345 (190)	0.53
Myonuclear domain type II (um^2)	1393 (185)	1327 (302)	0.60
Satellite cells			
Satellite cells per fiber type I (n)	0.063 (0.021)	0.068 (0.029)	0.71
Satellite cells per fiber type II (n)	0.054 (0.012)	0.061 (0.021)	0.46
Capillaries			
CAF type I (n)	5.8 (0.8)	5.9 (0.8)	0.74
CAF type II (n)	5.6 (0.9)	6.0 (0.7)	0.29
CD type I (mm^2)	672 (89)	672 (150)	0.99
CD type II (mm^2)	538 (109)	580 (139)	0.49

The values are presented as mean ± standard deviation (SD). No statistically significant differences were seen between the two groups at baseline. MVIT, maximal voluntary isokinetic torque; 1RM, 1 repetition maximum; CSA, cross-sectional area; MFA, muscle fiber area; CAF, capillaries per fiber; CD, capillary density; BFRRE, blood flow restricted resistance exercise.



Supplemental Digital Content 2. Table of mRNAs, rRNAs and miRs sequences.

Gene	Sequence
MYOD (Forward)	CGGCATGATGGACTACAGCG
MYOD (Reverse)	CAGGCAGTCTAGGCTCGAC
PAX 7 (Forward)	CCTTTGGAAGTGTCCACCCC
PAX 7 (Reverse)	TCGCCCATTGATGAAGACCC
CCND1 (Forward)	GCTGCGAAGTGGAAACCATC
CCND1 (Reverse)	CCTCCTTCTGCACACATTTGAA
CCND2 (Forward)	CTGCCCCCACCTAGATCATA
CCND2 (Reverse)	TCCCTTATGCTGTACTTCAAATAGG
MYOG (Forward)	GGCCAAACTTTTGCAGTGAATATT
MYOG (Reverse)	TCGGATGGCAGCTTTACAAACAAC
NCAM (Forward)	GCAGCGAAGAAAAGACTCTGG
NCAM (Reverse)	GCAGATGTACTCTCCGGCAT
VEGF (Forward)	TCTTCAAGCCATCCTGTGT
VEGF (Reverse)	CTTTCTTTGGTCTGCATTC
Nip7 (Forward)	CCGGGTGTACTATGTGAGTGAGAA
Nip7 (Reverse)	TTGTGGGTTTTAGTGAATTTTCCA
Reference genes:	
EMC7 (Forward)	GGGCTGGACAGACTTTCTAATG
EMC7 (Reverse)	CTCCATTTCCCGTCTCATGTCAG
VCP (Forward)	AAACTCATGGCGAGGTGGAG
VCP (Reverse)	TGTCAAAGCGACCAAATCGC
CHMP2A (Forward)	CGCTATGTGCGCAAGTTTGT
CHMP2A (Reverse)	GGGGCAACTTCAGCTGTCTG
C1orf43 (Forward)	CTATGGGACAGGGGTCTTTGG
C1orf43 (Reverse)	TTTGGCTGCTGACTGGTGAT
rRNA:	**Catalog number:**
5S	PPH82091A-200
5.8S	PPH82091A-200
28S5	PPH82090A-200
18S5	PPH71602A-200
28S + ITS	PPH82112A-200
18S + ITS	PPH82110A-200
5.8S + ITS	PPH82111A-200

miR:	CAT NO:	ID Number:
miR-15a-5p	A25576	477858_mir
miR-16-5p	A25576	477860_mir
miR-486-5p	A25576	478128_mir
miR-126-3p	A25576	477887_mir
miR-133a-3p	A25576	478511_mir
miR-206	A25576	477968_mir
miR-1-3p	A25576	477820_mir
miR-499a-3p	A25576	478948_mir
miR-186-5p	A25576	477940_mir
miR-320a	A25576	478594_mir
miR-361-5p	A25576	478056_mir

Forward and reverse sequences of analysed genes, as well as classification, catalogue and order identification number of rRNAs and miRs.