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RESEARCH ARTICLE

Effects of alternating blood flow restricted training and heavy-load resistance training on myofiber morphology and mechanical muscle function

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Hansen SK, Ratzer J, Nielsen JL, Suetta C, Karlsen A, Kvorning T, Frandsen U, Aagaard P. Effects of alternating blood flow restricted training and heavy-load resistance training on myofiber morphology and mechanical muscle function. *J Appl Physiol* 128: 1523–1532, 2020. First published April 23, 2020; doi:10.1152/japplphysiol.00015.2020.—To investigate if short-term block-structured training consisting of alternating weeks of blood flow restricted low-load resistance training (BFR-RT) and conventional free-flow heavy-load resistance training (HL-RT) leads to superior gains in mechanical muscle function, myofiber size, and satellite cell (SC) content and myonuclear number compared with HL-RT alone. Eighteen active young participants (women/men: 5/13, 23 ± 1.2 yr) were randomized to 6 wk (22 sessions) of lower limb HL-RT [70–90% one repetition maximum (1-RM)] (HRT, $n = 9$) or block-structured training alternating weekly between BFR-RT (20% 1-RM) and HL-RT (BFR-HRT, $n = 9$). Maximal isometric knee extensor strength (MVC) and muscle biopsies (VL) were obtained pre- and posttraining to examine changes in muscle strength, myofiber cross-sectional area (CSA), myonuclear (MN) number, and SC content. MVC increased in both training groups (BFR-HRT: +12%, HRT: +7%; $P < 0.05$). Type II myofiber CSA increased similarly (+16%) in BFR-HRT and HRT ($P < 0.05$), while gains in type I CSA were observed following HRT only (+12%, $P < 0.05$). In addition, myonuclear number remained unchanged, whereas SC content increased in type II myofibers following HRT (+59%, $P < 0.05$). Short-term alternating BFR-RT and HL-RT did not produce superior gains in muscle strength or myofiber size compared with HL-RT alone. Noticeably, however, conventional HL-RT could be periodically replaced by low-load BFR-RT without compromising training-induced gains in maximal muscle strength and type II myofiber size, respectively.

NEW & NOTEWORTHY The present data demonstrate that periodically substituting heavy-load resistance training (HL-RT) with low-load blood flow restricted resistance training (BFR-RT) leads to similar gains in type II myofiber CSA and muscle strength as achieved by HL-RT alone. Furthermore, we have for the first time evaluated myonuclear content and myonuclear domain size before and after training intervention across separate fiber size clusters and found no within-cluster changes for these parameters with training.

hypertrophy; myonuclei; quadriceps muscle; resistance training; satellite cells

INTRODUCTION

Heavy-load resistance training (HL-RT) is a highly effective exercise modality for improving mechanical muscle function and increasing skeletal muscle size (e.g., 1, 13, 32, 35, 43, 47, 49). However, in conditions where HL-RT is not feasible due to the high exercise loads imposed on muscles, tendons, and joints, low-load (20–30% of maximal loading, 1-RM) resistance training (LL-RT) represents a less stressful and equally effective exercise regime (30) and consequently finds use during deloading or tapering periods (50). However, LL-RT must be performed to voluntary failure if intending to evoke muscle hypertrophy and/or strength gains (14, 26, 30). When concurrent blood flow restriction (BFR) is added to LL-RT, muscle hypertrophy and gains in maximal strength can be achieved with substantially less mechanical work (fewer repetitions, less contractile work) compared with performing free-flow LL-RT to failure (14). Thus, BFR resistance training using low training loads (BFR-RT) has been found to elicit robust improvements in skeletal muscle size and mechanical muscle function, respectively (7, 8, 14, 33, 34, 45). Although previous meta-analysis reports have indicated muscle strength adaptation to be attenuated with BFR-RT compared with HL-RT (10, 27), more recent meta-analysis focused on healthy individuals (20–80 yr) indicates that strength gains may be similar between BFR-RT and HL-RT (19). However, whether a combination of BFR-RT and HL-RT results in superior physiological adaptations compared with single-mode training has only been sparsely examined so far. Initial reports suggest that the combination of BFR-RT and HL-RT yields similar gains in muscular strength and whole muscle cross-sectional area (CSA) compared with performing HL-RT alone when matched for duration and training volume (49). More recently, however, competitive power lifters were exposed to 2 wk of BFR-RT merged (*weeks 1* and *3*) into a 7 wk training period of conventional HL-RT, leading to elevated type I fiber CSA and increased myonuclear content in type I fibers accompanied by increased anatomical CSA of the knee extensors compared with 7 wk single-mode HL-RT (7). Given these conflicting data, it is possible that block-structured training as implemented by Bjørnsen et al. (7) may result in superior strength and hypertrophy responses compared with that achieved with

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single-mode HL-RT. In addition, protocols of block-combined HL-RT and low-load BFR-RT might provide a more tolerable (less stressful) training regime due to the periodized intervals (alternating weeks) of deloading (BFR-RT blocks) administered throughout the training program.

Muscular hypertrophy, governed by myofibrillar protein synthesis, is influenced by the transcriptional capacity of the myofiber residing myonuclei (9). Myonuclear accretion alongside muscular hypertrophy has been reported following both conventional HL-RT (28, 39, 42) and BFR-RT (7, 8, 34). Currently, there are indications of the existence of a linear relationship between myofiber size and myonuclear number accompanied by a nonlinear relationship between myofiber size and myonuclear domain size, revealing disproportionately smaller myonuclear domains in smaller compared with larger myofibers (24, 40). However, whether this disproportionate relationship is maintained during training-induced gains in myofiber CSA remains unsolved (40).

Upon activation and proliferation satellite cells (SC) act as myonuclear donors of nonmitogenic myonuclei (21, 42) to support both postnatal muscle growth and postinjury myofiber regeneration (36, 41). Hence, SCs are considered important for the maintenance and hypertrophic plasticity of skeletal myofibers and to play a significant role in muscle repair upon damage and for extracellular matrix remodeling during load-induced muscle hypertrophy (13, 18, 31, 36, 41).

Increases in SC content (~20–40%) along with concomitant myofiber hypertrophy have previously been reported following 4–12 wk HL-RT type exercise intervention (5, 16, 22, 29, 44, 46). In addition, a positive relationship between the training-induced increase in SC number and myofiber size (44, 46) or whole muscle size (5) has been reported following HL-RT. Similar relationships have been observed following short-term (3 wk) high-frequency (twice daily) BFR-RT intervention resulting in large increases (~140%) in SC content (34), underpinning the potential role of BFR-RT to facilitate myocellular growth. However, the effect of combined conventional heavy-load resistance training and low-load BFR training (HL-RT and BFR-RT) on SC proliferation has not previously been investigated.

The purpose of the present study, therefore, was to examine if HL-RT alternated by blocks of BFR-RT would result in superior gains in maximal muscle strength and myofiber size accompanied by more pronounced increases in satellite cell content and myonuclear number compared with HL-RT alone. It was hypothesized that amplified gains in muscle size and mechanical muscle function would be demonstrated in response to alternating BFR-RT and HL-RT. In addition, it was hypothesized that combined BFR-RT and HL-RT would yield more pronounced SC proliferation and myonuclear addition, respectively, compared with HL-RT alone.

MATERIAL AND METHODS

Participants. Eighteen active and injury-free men ($n = 13$) and women ($n = 5$) [age: 23 ± 1.2 yr, height: 178.6 ± 6.9 cm, body mass: 80.8 ± 10.2 kg (means ± SD)] volunteered for the study. The study was approved by The Regional Committees on Health Research Ethics for Southern Denmark (S-20160149) and conformed to the Declaration of Helsinki. All participants gave their written informed consent before participation.

Stratified for sex and 1-RM (one repetition) leg press strength, participants were randomized to perform either low-load blood flow restricted resistance training combined with conventional heavy-load resistance training (BFR-HRT) ($n = 9$, 3 women/6 men) or conventional heavy-load resistance training alone (HRT) ($n = 9$, 2 women/7 men). Randomization was performed following baseline testing using stratified randomization procedures.

Protocol overview. Testing and muscle biopsy sampling were performed before the training intervention (pre) and following the 6 wk intervention period (post), except from the repetition maximum (RM) test, which was performed before intervention only.

To identify the target training loads at baseline, a bilateral 5-RM test was conducted, from which each participant's 1-RM was estimated (4). The 5-RM test was conducted for the leg press and leg extension exercise in weight-stacked machines (ARTIS, TechnoGym International).

Muscle biopsy sampling and isolated muscle strength testing (isokinetic dynamometer, described in detail below) were conducted for the dominant leg, which was defined as the participant's preferred kicking leg. Preintervention biopsy samples were collected 12–13 days before the intervention, while muscle strength testing was performed 3–6 days before intervention start. Postintervention biopsy samples were collected 5–7 days after cessation of training, respectively. Maximal muscle strength was tested 7–8 days after cessation of training.

Participants were instructed to maintain their usual diet and refrain from other exercise activities throughout the intervention period and also to refrain from any strenuous activities at least 48 h before muscle biopsy sampling and 24 h before all other tests.

Training protocol. Participants underwent 6 wk of resistance training (BFR-HRT or HRT) with four sessions performed per week to comprise a total of 22 training sessions (cf. last week involved only two training sessions).

All training sessions were supervised by experienced staff, who ensured maximal level exercise effort through strong verbal encouragement, proper execution of the exercises, and application of the designated cuff pressure during BFR-RT weeks.

Training in both groups consisted of unilateral leg press and leg extensions using weight-stacked training machines (ARTIS, TechnoGym International). The leg press exercise was performed horizontally with a semireclined seat. The two exercises were separated by ~5 min of rest.

BFR-HRT training consisted of alternating weeks of low-load BFR-RT (*weeks 1*, *3*, and *5*) and progressively adjusted heavy-load resistance training (*weeks 2, 4*, and *6*), while the HRT group performed progressive heavy-load resistance training throughout the entire intervention period (Fig. 1). All BFR-RT was performed using a 20 cm-wide pneumatic cuff (Heine Gamma G5 cuff M-000.09.615) inflated to an effective pressure of 110 mmHg. The cuff remained inflated throughout the four exercise sets and deflated immediately after the last repetition in the fourth set.

During BFR-RT sessions the 1st set was unilaterally performed until concentric failure using an exercise load corresponding to ~20% of 1-RM with successive sets performed until concentric failure using the same load, with sets interspaced by 30 s rest periods. During the weeks of heavy resistance training, the BFR-HRT group performed an exercise program that was identical to that of the HRT group, consisting of heavy-load resistance training with number of sets increasing from two to four during the time course of the study, interspaced by 2 min of rest, while the number of repetitions ranged between three and eight using training loads of 3-RM to 8-RM, respectively (Fig. 1).

HRT training consisted of progressive resistance training with number of sets increasing from two to four, interspaced by 2 min of rest, number of repetitions ranged between three and 10 using training loads ranging from 3-RM to 12-RM (i.e., ~70–90% 1-RM). In *weeks 3, 4*, and *5*, which consisted of heavy maximal-effort training (3–

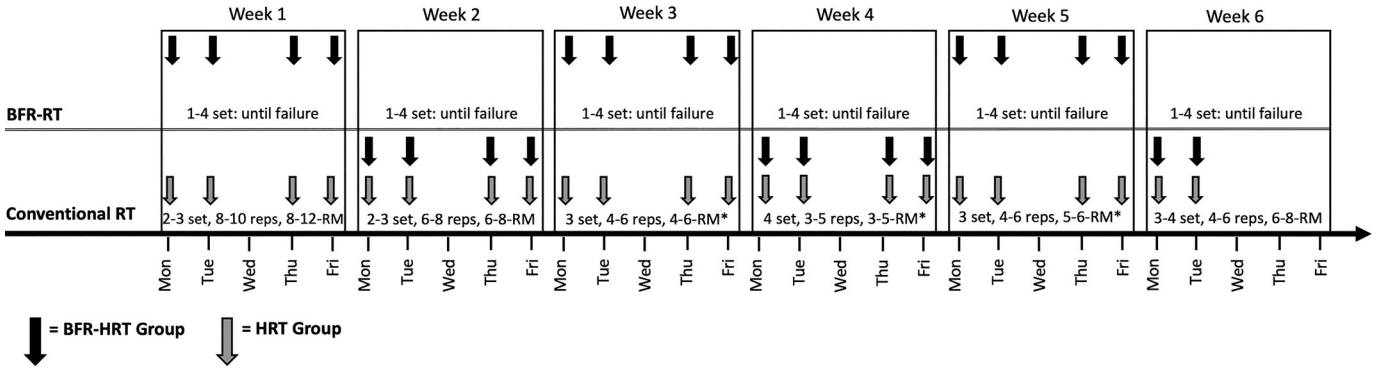

Fig. 1. Schematic illustration of the training performed in the two intervention groups: BFR-HRT, low-load blood flow restricted resistance training in alternating weekly block combination with heavy-load resistance training; HRT, heavy-load resistance training alone. *Tuesday, deload day: 3 sets, 8 reps, 12-RM loads.

6-RM training), a deload day was employed to facilitate recovery and avoid overreaching (cf. Fig. 1).

Muscle strength assessment (quadriceps maximal voluntary contraction). Quadriceps maximal voluntary contraction (MVC) was measured during maximal isometric knee extensor contractions performed at a knee joint angle of 70° (0° = full extension) in an isokinetic dynamometer (Kinetic Communicator 500H, Chattecx Corp.) (2). As described in detail previously (2, 43) participants were placed in the dynamometer in a seated position, with the lateral femoral epicondyle of the knee visually aligned with the rotational axis of the dynamometer powerhead. The hip and distal thigh were fastened with a belt and strap to the dynamometer, and the lower leg was attached to the lever arm 3 cm above the medial malleolus. The length of the dynamometer lever arm and body weight were noted for later calculations of maximal knee extensor torque normalized to body mass.

Participants performed a brief standardized warm-up in the dynamometer consisting of 10 submaximal isokinetic contractions at a 90–10° range of motion (0° full knee extension) with an angular speed of 60°/s followed by two submaximal and four maximal contractions with 30°/s. After being carefully instructed to contract as "fast and forcefully" as possible, participants performed five isometric knee extensor MVCs (70° knee joint angle, 0° = full extension) interspaced by 30 s rest. Participants were verbally encouraged, and online visual feedback of the force exerted was provided on a computer screen throughout the test.

Trials with visible countermovement (abrupt force drop) were discharged and repeated. All recorded torque values were corrected for gravity of the lower limb and normalized to body mass (kg) (2), and the trial with the highest MVC was chosen for further statistical analysis.

Muscle biopsy sampling. Muscle biopsies were obtained from muscle vastus lateralis (VL) using a percutaneous 5 mm Bergström biopsy needle (6). Approximately 100–200 mg tissue was obtained from the VL muscle under local anesthesia. All muscle samples were mounted in Tissue-Tec (4583, Sakura Finetek, Alphen aan den Rijn, The Netherlands) and frozen in isopentane precooled in liquid nitrogen, and subsequently stored at −80°C for later analysis. Transverse serial sections (~8 μm) of the embedded muscle biopsy were cut at −22°C (HM560; Microm, Walldorf, Germany) and mounted on glass slides (Thermo Scientific SuperFrost Plus Slides).

Immunohistochemical methodology was slightly modified from Nielsen et al. (34). In brief, immunohistochemical stains were fixed for 10 min at room temperature in a 4% formaldehyde fixation buffer, containing 0.05% Triton X-100 (Sigma-Aldrich, St. Louis, MO) followed by protein blocker (DAKO, X0909) for 10 min following two sequences of 60 min incubation with primary and secondary antibodies diluted in PBS with 1% BSA.

Analysis of muscle fiber area, myonuclear number, and SC content. Antibodies against myosin fast (M4276, Sigma-Aldrich; 1:2,000), Laminin (Z0097, Dako, Glostrup, Denmark; 1:1,000), Pax7 (Hybridoma Bank, Iowa City, IA; 1:100) and DAPI (62248, Thermofischer) followed by mounting medium (H-5501, Vector) were added for distinction of the myofiber type II (fast), basal lamina, myogenic SC, and DNA content.

Immunofluorescence and histochemical stains were visualized on a computer screen with a microscope (Axio Imager M1, Carl Zeiss, Germany) and a high-resolution digital camera (AxioCam, Zeiss). Digital images were captured using ×10 objectives and standardized exposure. Morphometric analysis was performed using a digital analysis program (AxioVision 4.6, Carl Zeiss).

All visible and intact myofibers in sample cross-sections were classified into type I (unstained) and type II (stained) fibers and analyzed. CSA was quantified by tracking the inner part of the basement membrane in type I and type II myofibers, respectively. Mean myofiber CSA (MFA; type I and type II fibers pooled) as well as type I and type II myofiber CSA were calculated as previously described (34). A mean of 151 ± 70 type I myofibers and 129 ± 88 type II myofibers were analyzed per biopsy. The relative proportion of myofibers in 1,000 μm^2 size intervals was calculated to determine myofiber area distribution (frequency; percentage occurrence of type I and II fibers in respective CSA size groups) and enabling visualization in frequency histograms (Fig. 4).

Myogenic SC were defined as Pax7 and DAPI positive, located in a sublaminar position, i.e., residing inside the laminin-defined border of the fiber (24, 34). SC abundance were quantified for type I and type II myofibers separately and normalized to number of type I and type II fibers counted (24, 34).

Myonuclei were defined as DAPI-positive and Pax7-negative nuclei, located inside the laminin-defined basement membrane of the myofiber.

Fiber size cluster analysis. All analyzed fibers were allocated into three clusters based on their CSA (see below), following which a subgroup of 15 myofibers per cluster were randomly selected for counting of myonuclei.

A fiber size-specific cluster analysis was performed as described previously (24). Three size clusters were defined for type I and type II myofibers separately; small fiber CSA cluster (CL:S): 0–4,000 μm^2, medium fiber CSA cluster (CL:M): 4,000–7,000 μm^2, large fiber CSA cluster (CL:L): >7,000 μm^2. Myonuclei per myofiber and myonuclear domain [defined as the ratio between mean fiber area (MFA) and number of myonuclei per fiber] was analyzed separately in each of the designated fiber size clusters (24).

Statistical analysis. A mixed linear model was used for statistical analysis of pre- to post changes with subject ID as a random effect and time (pre, post) and group (BFR-HRT, HRT) as fixed effects (20, 34). Within-cluster differences were analyzed with subject ID as a random effect and cluster size (small, medium, and large), time (pre, post), and group (BFR-HRT, HRT) as fixed effects. Values are presented as

Table 1. *Training progression*

	Reps, number/session		Load, kg/session		Volume, kg/session	
	LE	LP	LE	LP	LE	LP
C1						
BFR-HRT	68.6 ± 12.6**	79.4 ± 15**	14.2 ± 4.7	30.2 ± 11.4	972.7 ± 359.8	2,463.6 ± 1230.6**
HRT	20.1 ± 6.0	20.1 ± 6.0	47.0 ± 13.5**	96.8 ± 26.9**	900.3 ± 259.1	1,855 ± 486.4
C2						
BFR-HRT	17.5 ± 5.0*	17.6 ± 5.0*	49.4 ± 12.8	93.7 ± 22.2	849.7 ± 281.8	1,619.1 ± 531.1
HRT	15.6 ± 5.2	15.7 ± 5.1	52.9 ± 12.9	106 ± 23.7**	830.5 ± 328.3	1,608.2 ± 481.2
Whole intervention						
BFR-HRT	45.4 ± 27.3**	51.0 ± 33.0**	30.3 ± 19.9	59.3 ± 36.1	916.6 ± 331.4	2,076.6 ± 1059.8**
HRT	18.1 ± 6.1	18.1 ± 6.0	49.7 ± 13.5**	101.2 ± 25.8**	868.6 ± 293.8	1,743 ± 498.3

Values presented as means ± SD. C1, *week 1, 3*, and *5* combined; C2, *week 2, 4*, and *6* combined. BFR-HRT, low-load blood flow restricted resistance training alternating with heavy-load resistance training; HRT, heavy-load resistance training alone; LE, leg extension exercise; LP, leg press exercise. Between group differences: $^{**}P < 0.001$, $^{*}P < 0.05$.

means ± SD unless otherwise stated. Linear correlation analysis was performed using the Pearson Product-Moment method.

Data were analyzed according to intention-to-treat principles.

The level of statistical significance was set at $P \leq 0.05$ (two-tailed testing). All statistical analysis was performed using STATA 15.1 (StataCorp, College Station, TX).

RESULTS

Study participants (BFR-HRT $n = 9$, HRT $n = 9$) completed the scheduled training with an absence of fewer than two training sessions (mean attendance rate BFR-HRT: 97%, HRT: 99%) with exception of one subject (BFR-HRT group), who missed five complete training sessions and in one session performed a single exercise only (leg extensions). None of the participants reported any adverse events from the intervention.

No between-group difference was observed at baseline for any of the measured parameters.

Training progression. Due to the block-structured nature of the resistance training program (cf. Fig. 1), training repetitions, loads, and volumes were compared between BFR-HRT and HRT in *weeks 1, 3*, and *5* combined (C1) and in *weeks 2, 4*, and *6* combined (C2), respectively. Training progression was quantified in repetitions (reps), load (kg), and total volume (repetitions × load) per session (Table 1). In C1 (weeks of BFR-RT), the BFR-HRT group performed markedly more repetitions (LE and LP: $P < 0.001$) with a lower load (LE and LP: $P < 0.001$) and a higher volume (LE: $P = 0.09$, LP: $P < 0.001$) compared with the HRT group. In C2 (weeks of HL-RT only), a slightly higher number of repetitions (LE and LP: $P = 0.01$) with comparable or marginally lower loads (LE: $P = 0.07$, LP: $P < 0.001$) and matching volume (LE and LP: NS) were performed by the BFR-HRT group compared with the HRT group. Averaged across the entire 6 wk of intervention, the BFR-HRT group performed a higher number of repetitions (LE and LP: $P < 0.001$) with overall lower training loads (LE and LP: $P < 0.001$) and with comparable or higher total volume compared with the HRT group (LE: $P = 0.13$, LP: $P < 0.001$) (Table 1).

Maximal muscle strength (MVC). Quadriceps MVC increased with BFR-HRT training from 2.98 ± 0.65 to 3.34 ± 0.76 Nm/kg (+ 12.2%, $P = 0.0001$) and with HRT training from 3.13 ± 0.67 to 3.36 ± 0.72 Nm/kg (+ 7.2%, $P < 0.01$), with no significant difference in % delta change between groups. No between-group differences in MVC were observed before or after the intervention period (Fig. 2).

Muscle fiber type composition. Myofiber type composition (VL) did not change with training. BFR-HRT pre: type I; 55.7 ± 15.2%; type II: 44.3 ± 15.2%; BFR-HRT post: type I; 57.2 ± 15.5%; type II 42.8 ± 15.5%; HRT pre: type I; 56.2 ± 12.6%; type II: 43.8 ± 12.6%; HRT post: type I; 53.8 ± 6.8%; type II 46.2 ± 6.9%.

Muscle fiber CSA. Type II myofiber CSA increased following BFR-HRT (5,622 ± 1,693 μm^2 vs. 6,521 ± 1,866 μm^2, +16.0%, $P = 0.01$) and HRT (5,981 ± 1,157 μm^2 vs. 6,959 ± 1,257 μm^2, +16.4%, $P = 0.007$) (Fig. 3*B*). Type I myofiber CSA increased following HRT (5,507 ± 886 vs. 6,160 ± 711, +11.9%, $P = 0.048$), while remaining unchanged following BFR-HRT (5,192 ± 701 vs. 5,321 ± 857, $P = 0.69$) (Fig. 3*A*). MFA (type I and type II myofibers pooled) increased with HRT training (5,707 ± 962 vs. 6,488 ± 810, +13.7%, $P = 0.02$) but remained unaltered following BFR-HRT (5,320 ± 1,049 vs. 5,759 ± 1,059, $P = 0.21$) (Fig. 3*C*).

Our frequency histogram analysis indicated a uniform hypertrophy response across all fiber size intervals for type II myofibers following BFR-HRT and HRT (Fig. 4, *B* and *D*) as was observed for type I myofibers following HRT only (Fig. 4).

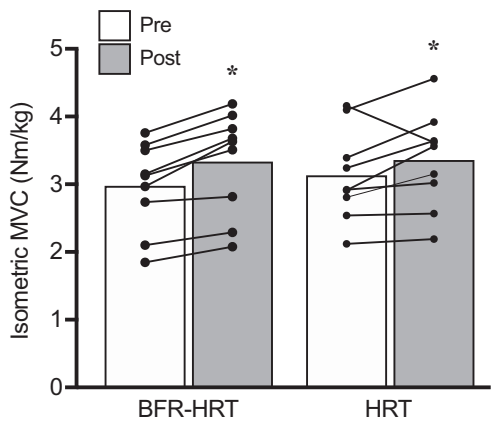

Fig. 2. Maximal isometric knee extensor strength (MVC) measured before (pre, open bars) and after (post, gray bars) 6 wk of combined BFR-RT and HL-RT (BFR-HRT) or HL-RT alone (HRT). BFR-RT, low-load blood flow restricted resistance training; HL-RT, free-flow heavy-load resistance training. *Pre vs. post ($P < 0.05$).

Myogenic SC content. SC number remained unchanged in type I muscle fibers following BFR-HRT (0.093 ± 0.04 vs. 0.078 ± 0.04, $P = 0.25$) and HRT (0.088 ± 0.05 vs. 0.088 ± 0.06, $P = 0.77$). In contrast, elevated SC content was observed in type II fibers following HRT (0.075 ± 0.04 vs. 0.119 ± 0.07, + 58.8%, $P = 0.01$) while failing to reach statistical significance in BFR-HRT, despite a substantial relative change in SC content (0.079 ± 0.05 vs. 0.112 ± 0.09, + 42.3%, $P = 0.15$).

Myonuclear number. No pre- to posttraining changes in myonuclear content expressed as number of nuclei per fiber were observed for type I myofibers following BFR-HRT (2.29 ± 0.38 vs 2.19 ± 0.45, $P = 0.60$) or HRT (2.48 ± 0.39 vs. 2.57 ± 0.61, $P = 0.68$). Likewise, myonuclear number remained unchanged in type II myofibers following BFR-HRT (2.50 ± 0.50 vs. 2.42 ± 0.58, $P = 0.86$) and HRT (2.56 ± 0.43 vs. 2.75 ± 0.78, $P = 0.38$).

Myonuclear domain size of type II myofibers increased following BFR-HRT (2,236 ± 457 vs. 2,819 ± 112 μm^2/nuclei, +26%, $P = 0.02$) with no change following HRT (2,336 ± 315 vs. 2,639 ± 595 μm^2/nuclei, $P = 0.22$). Myonuclear domain size remained unaltered in type I myofibers following BFR-HRT (2309 ± 407 vs. 2,496 ± 523 μm^2/nuclei, $P = 0.15$) and HRT (2,232 ± 313 vs. 2,461 ± 320 μm^2/nuclei, $P = 0.07$).

Fiber size cluster analysis. No pre- to postintervention changes were detected in myonuclear content or myonuclear domain size in the three main size clusters (small, medium, or large), respectively. When data (pre- and postintervention) from all participants were pooled (due to no group effect), the cluster analysis revealed that myonuclear content of type I myofibers was higher in myofibers of larger size in the order of the small fiber size cluster (1.81 ± 0.49) to the medium size cluster (2.39 ± 0.44, $P < 0.001$) to the large size cluster (2.97 ± 0.75, $P < 0.001$) (Table 2, Fig. 5). An identical pattern was observed for type II myofibers, where a higher myonuclear content was observed from the smallest fiber size cluster (2.08 ± 0.9) to the medium size cluster (2.47 ± 0.50, $P = 0.026$) to the large size cluster (3.35 ± 0.87, $P < 0.001$) (Table 2, Fig. 5). Myonuclear content within each fiber size cluster did not differ between type I and II fibers.

Myonuclear domain size also increased with increasing myofiber size in the order of small fiber size cluster (type I fibers: 1,937 ± 514 μm^2/nuclei, type II fibers: 1,726 ± 530 μm^2/nuclei) to medium size cluster (type I fibers: 2,365 ± 430 μm^2/nuclei, $P < 0.001$; type II fibers: 2,335 ± 428 μm^2/nuclei, $P < 0.001$) to large size cluster (type I fibers: 2,984 ± 763 μm^2/nuclei, $P < 0.001$; type II fibers: 2,794 ± 736, $P < 0.001$).

Positive relationships were observed between myofiber size and myonuclear content (type I fibers: r = 0.67, $P < 0.001$; type II fibers: r = 0.59, $P < 0.001$) and also between myonuclear domain and myofiber size (type I fibers: r = 0.59, $P < 0.001$; type II fibers: r = 0.61, $P < 0.001$) (Fig. 6).

DISCUSSION

The present study investigated the effect of 6 wk of alternating (1 wk block structured) low-load blood flow restricted resistance training and free-flow heavy-load resistance training on muscle strength, myofiber size, myogenic muscle stem cell content (satellite cells: SCs), and myonuclear number. The main finding was that maximal muscle strength (MVC) and type II myofiber CSA increased to a similar extent following BFR-HRT compared with HRT alone. Furthermore, type I myofiber CSA increased following HRT but not BFR-HRT. Finally, no changes in myonuclear number were evident post intervention in either intervention group despite that SC content increased in type II myofibers in response to HRT alone.

Adaptive changes in maximal muscle strength (MVC). Knee extensor MVC increased to a similar extent following 6 wk (22 sessions) of BFR-HRT (+ 12%) or HRT alone (+ 7%). Comparable increases in MVC have been reported following more prolonged periods (12–16 wk) of conventional HL-RT

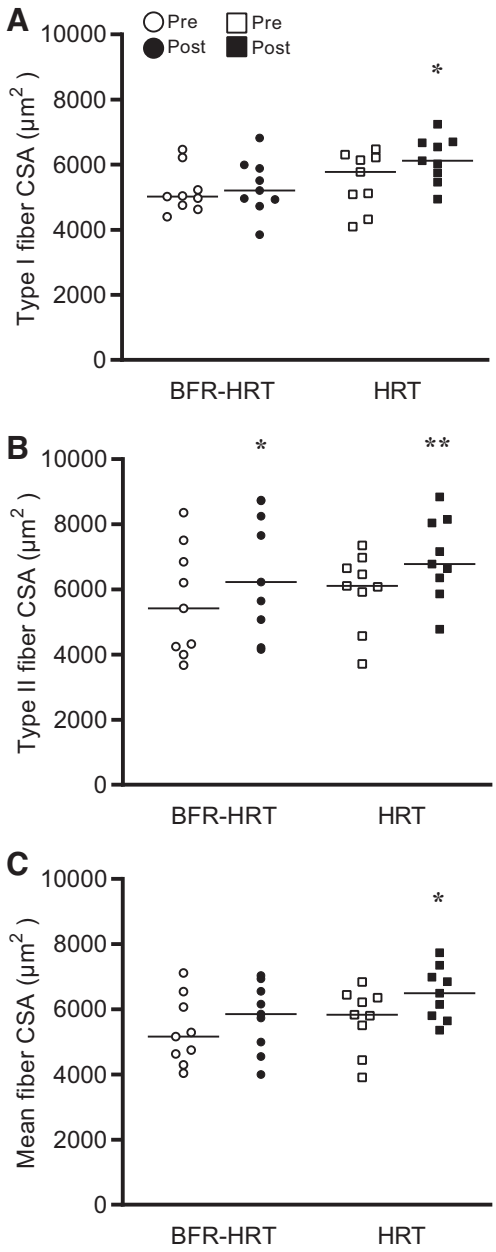

Fig. 3. Changes in myofiber cross-sectional area (CSA) (group mean ± SD). *A*: type I fibers, *B*: type II fibers, *C*: type I and II fibers pooled. Circles, BFR-HRT group; squares, HRT group. Open symbols, preintervention values; closed symbols, postintervention values. Pre to post differences: $*P < 0.05$, $**P < 0.01$.

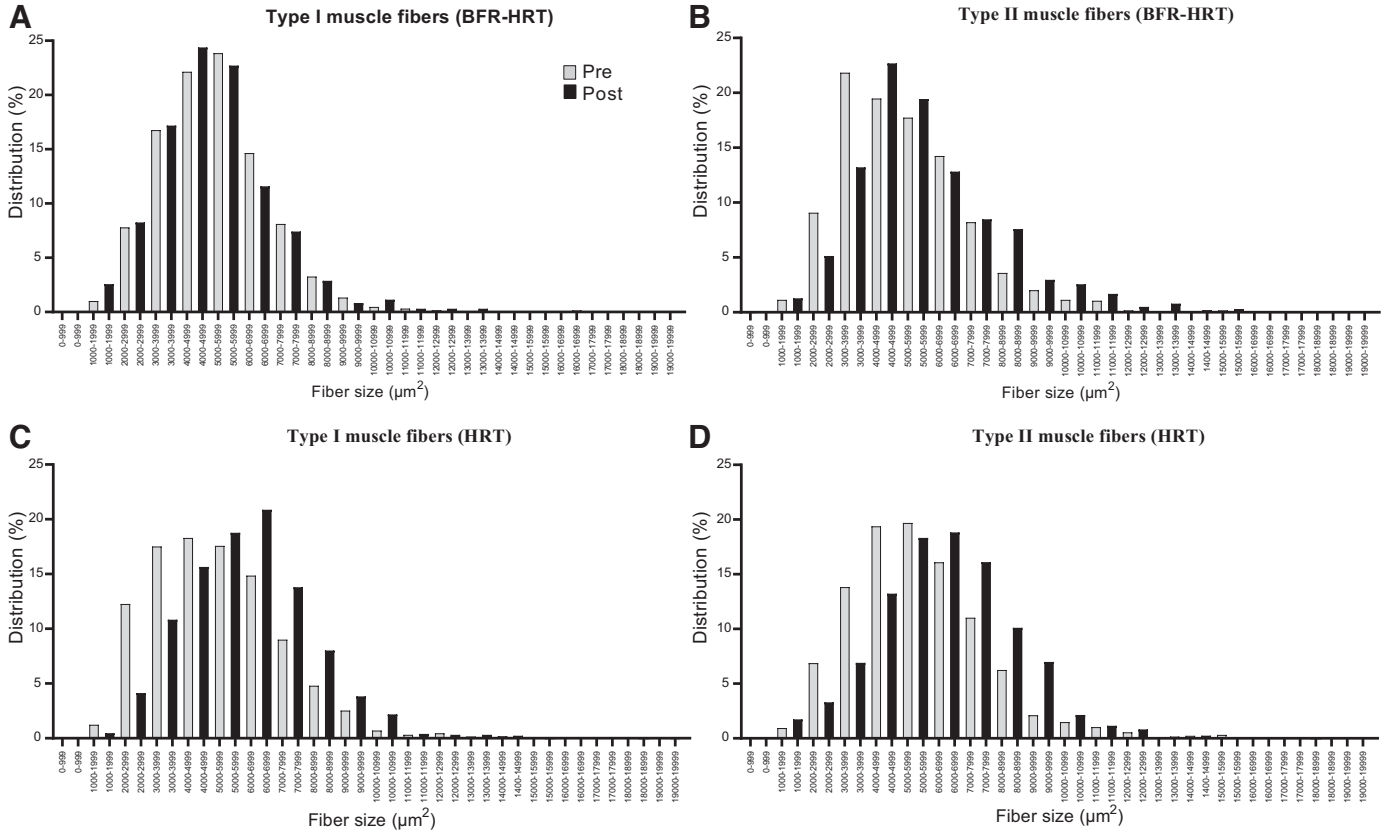

Fig. 4. Frequency histograms of muscle fiber size (cross-sectional area) before (gray bars) and after (black bars) 6 wk of training intervention. *A*: distribution of type I muscle fiber area in BFR-HRT. *B*: distribution of type II muscle fiber area in BFR-HRT. *C*: distribution of type I muscle fiber area in HRT. *D*: distribution of type II muscle fiber area in HRT.

(~+15%) (1, 17, 35, 38), illustrating that the present block-structured training regime of combined HL-RT and BFR-RT was highly effective in stimulating mechanical muscle function.

Yasuda and coworkers (49) examined the physiological effects of combined HL-RT and BFR-RT training and observed similar gains in muscle strength in response to 6 wk of weekly mixed BFR-RT (2 sessions/wk) and HL-RT (1 session/wk) compared with volume matched HL-RT alone (3 sessions/wk). In the present study we used a block-structured combination of HL-RT and BFR-RT (BFR-HRT), and in line with the results by Yasuda and colleagues (49), we found no indication of an inferior effect on MVC following 6 wk (22 sessions) of BFR-HRT (+12%) compared with HRT alone (+7%). Thus, the present data indicate that short-term (6 wk) alternating BFR-RT and HL-RT can be applied without compromising muscle strength gains compared with conventional (i.e., nonalternating) HL-RT.

Table 2. *Fiber size cluster analysis*

	BFR-HRT		HRT	
	Pre	Post	Pre	Post
Myonuclei/fiber				
Type $I_{CL:S}$	1.78 ± 0.27	1.59 ± 0.40	1.90 ± 0.14	1.97 ± 0.85
Type $I_{CL:M}$	2.32 ± 0.42	2.24 ± 0.52	2.55 ± 0.37	2.45 ± 0.46
Type $I_{CL:L}$	3.03 ± 0.97	2.70 ± 0.73	3.06 ± 0.50	3.11 ± 0.78
Type $II_{CL:S}$	2.01 ± 0.48	1.72 ± 0.46	2.30 ± 0.70	2.22 ± 1.51
Type $II_{CL:M}$	2.49 ± 0.38	2.29 ± 0.49	2.46 ± 0.41	2.63 ± 0.68
Type $II_{CL:L}$	3.24 ± 0.69	3.59 ± 1.19	3.37 ± 0.76	3.21 ± 0.88
Myonuclear domain (μm^2/nuclei)				
Type $I_{CL:S}$	1,933 ± 352	2,139 ± 424	1,713 ± 147	1,960 ± 852
Type $I_{CL:M}$	2,393 ± 468	2,508 ± 576	2,205 ± 273	2,352 ± 364
Type $I_{CL:L}$	2,951 ± 964	3,245 ± 915	2,817 ± 436	2,924 ± 692
Type $II_{CL:S}$	1,762 ± 450	1,839 ± 469	1,504 ± 346	1,824 ± 769
Type $II_{CL:M}$	2,229 ± 329	2,495 ± 514	2,300 ± 324	2,316 ± 530
Type $II_{CL:L}$	2,750 ± 474	2,843 ± 1104	2,637 ± 456	2,945 ± 839

Values presented as means ± SD. CL:S, small cluster 0–4,000 μm^2, CL:M, medium cluster 4,000–7,000 μm^2, CL:L, large cluster >7,000 μm^2.

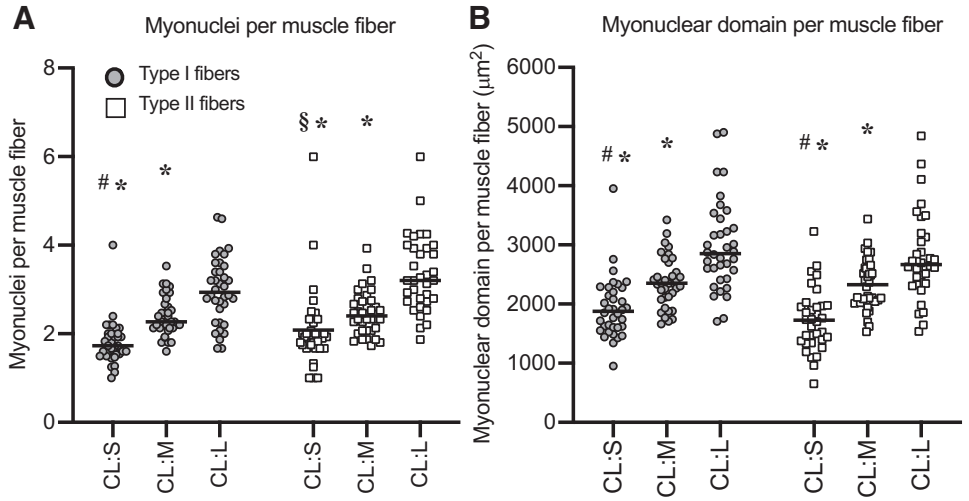

Fig. 5. Myonuclear content per fiber and myonuclear domain per fiber in small-sized (CL:S; 0–4,000 μm²), medium-sized (CL:M, 4,000–7,000 μm²) and large-sized (CL:L; ≥7,000 μm²) fiber clusters. Intervention groups and time points (pre and post) are pooled. Gray circles denote type I myofibers, open squares denote type II myofibers. #Different from medium-sized fiber cluster ($P < 0.001$), §different from medium-sized fiber cluster ($P < 0.05$), *different from large-sized fiber cluster ($P < 0.001$).

Adaptive changes in myofiber CSA. Separated into fiber types, a gain of ~16% in type II myofiber CSA was observed in both BFR-HRT and HRT, respectively, whereas type I myofiber CSA increased ~12% following HRT alone while remaining statistically unaltered with BFR-HRT. MFA increased following HRT only (+14%), at a magnitude consistent with previous reports on gains in MFA following 3–4 mo of conventional progressive HL-RT (1, 22, 39).

The substantial magnitude of training-induced gain in myofiber size observed in the present study is noteworthy considering the short duration of training (6 wk). While BFR-HRT participants demonstrated a slightly higher training volume (repetitions × load), half of their training sessions were performed using considerably lower external loads (~20% 1-RM) compared with HRT participants (~80% 1-RM). Nevertheless, BFR-HRT participants reached similar increases in type II myofiber CSA as HRT, indicating that block-structured low-load BFR-RT can effectively substitute alternating weeks of heavy-load resistance exercise training without compromising the hypertrophy response of type II myofibers.

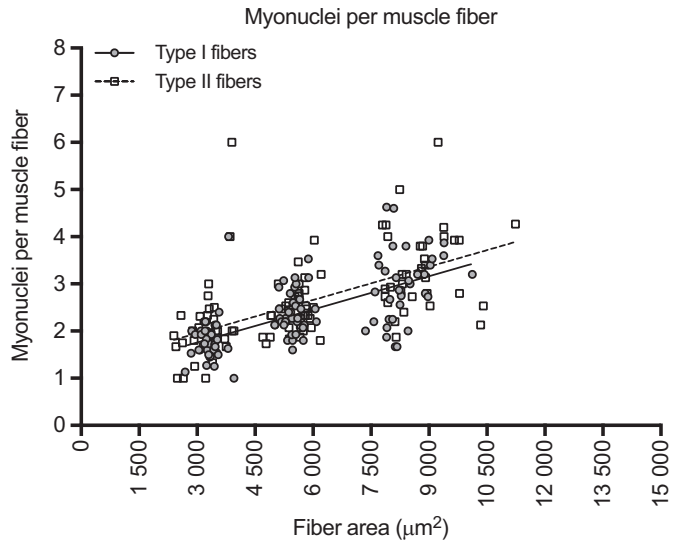

Fig. 6. Myonuclei per myofiber. Groups and time (pre and post) are pooled. Gray circles, type I myofibers; open squares, type II myofibers.

Increases in myofiber CSA have previously been reported in response to conventional heavy-load resistance training. Specifically, type II fiber hypertrophy of 16–22% were reported following 10–14 wk of high-load (> 70% 1-RM) resistance training (1, 3, 11, 15, 25). Interestingly, low-load BFR-RT per se also appears capable of producing skeletal muscle hypertrophy, verified both at the macroscopic (whole muscle) (7, 14, 27) and microscopic (myofiber) (7, 8, 34) levels.

The present frequency histogram analysis indicates that type II myofibers experienced a uniform hypertrophy response across all fiber size intervals irrespectively of training protocol (cf. Fig. 4, *B* and *D*). In contrast, for type I myofibers a similar pattern was evident only following HRT and not with BFR-HRT (cf. Fig. 4*C*). This indicates a differential response in type I myofibers following short-term combined BFR-HRT vs HRT alone, suggesting that the hypertrophy response of type I fibers may require a relatively larger training volume compared with type II fibers. Contrary to the present observations, marked type I myofiber hypertrophy previously have been reported in response to high-frequency BFR-RT protocols (8, 34).

Adaptive changes in myogenic SC content. SCs associated with type I fibers remained unaltered with training in both intervention groups. In contrast, type II myofibers demonstrated elevated SC content following HRT alone (+59%) while remaining unchanged following BFR-HRT (+43%). SC content has previously been reported to increase in response to acute BFR-RT (48) as well as following short-term (2–3 wk) high-frequency BFR-RT (8, 34). It may be speculated, therefore, that BFR-RT should be repeated in multiple daily sessions (i.e., accumulating extensive myofiber stress) rather than using two to four weekly sessions, to elicit robust SC accumulation.

Notably, the present finding of an increased number of SCs in type II myofibers following 6 wk of single-mode HRT extends previous reports of SC proliferation following more prolonged periods (10–16 wk) of heavy-load resistance training (13, 35, 37, 46).

Adaptive changes in myonuclear content. In the present study there was no increase in myonuclear number with either training mode. Thus, the present data could indicate that the existing population of myonuclei were capable of increasing transcriptional efficiency to allow for myofibrillar protein syn-

thesis to control a greater volume of cytoplasmic area. However, a recent meta-analysis concluded that myonuclear addition can occur also with smaller degrees of myofiber hypertrophy (<10%), while more robust hypertrophy seems to be required before changes in myonuclear content may converge toward statistical significance (12). One plausible explanation for the present and previously reported [cf. (22)] lack of myonuclear addition despite measurable myofiber hypertrophy could be if the relative increase in myonuclear content were substantially smaller than the relative increase in fiber size, making it more difficult to detect increases in myonuclear content, particularly with moderate increases in fiber size. In support of this notion and in line with previous observations (23, 24, 40), the present cluster analysis demonstrated a positive correlation between fiber size and myonuclear content, which predicted that a ~100% larger fiber CSA (e.g., 4,500 vs. 9,000 μm^2) would be accompanied by a ~50% increased myonuclear content (e.g., 2 vs. 3 myonuclei) (cf. Fig. 6). Scaled to the current setting, the 12–16% gains in myofiber CSA observed with HRT and BFR-HRT would be expected to require a ~6–8% increase in myonuclear number, which may have been difficult to detect by our statistical analysis given the relatively low sample size ($n = 9$) included in the present intervention groups.

As further implicated by the disproportionate relationship between fiber CSA and myonuclear number (Fig. 6), myonuclear domain was found to be systematically larger from the smallest to the largest fiber size cluster (CL:S < CL:M < CL:L) (cf. Fig. 5) as reported by others (23, 24, 40). These data strongly suggest that myonuclear content is regulated with respect to fiber size, but at the same time larger fibers have larger myonuclear domains. Interestingly, in the present study we have for the first time evaluated myonuclear content and myonuclear domain size before and after training intervention (HRT, BFR-HRT) across fiber clusters with different size intervals and found no within-cluster changes for these parameters with training. Thus, the present data suggest that the strict relationship between fiber size and myonuclear content previously demonstrated to exist throughout a large range of fiber sizes (24, 40) is maintained after resistance training intervention resulting in moderate hypertrophy, hence providing novel insight to the relationship between myofiber hypertrophy and myonuclear accretion with training.

However, further studies are needed to fully elucidate this aspect of myocellular plasticity, i.e., to verify if myofiber size/myonuclear content relationship indeed is maintained during conditions of more pronounced myofiber hypertrophy (>25%).

Conclusion

In conclusion, single-mode HL-RT appeared to be periodically replaceable by BFR-RT without compromising training-induced gains in muscle strength and type II myofiber size. In contrast to our initial study hypothesis, however, alternating weekly blocks of BFR-RT and HL-RT did not prove superior to single-mode HL-RT in producing gains in muscle strength and myofiber size, nor resulted in elevated SC content or increased myonuclear number.

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS

S.K.H., J.R., T.K., U.F., and P.A. conceived and designed research; S.K.H., J.R., J.L.N., C.S., and U.F. performed experiments; S.K.H., J.R., J.L.N., A.K., and P.A. analyzed data; S.K.H., J.R., J.L.N., A.K., T.K., U.F., and P.A. interpreted results of experiments; S.K.H. and J.R. prepared figures; S.K.H. drafted manuscript; S.K.H., J.L.N., C.S., A.K., T.K., U.F., and P.A. edited and revised manuscript; S.K.H., J.R., J.L.N., C.S., A.K., T.K., U.F., and P.A. approved final version of manuscript.

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