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# **Original Research Article**

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# Impact of early and late morning supervised blood flow restriction training on body composition and skeletal muscle performance in older inactive adults

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Abstract

**Objectives:** To investigate the impact of a supervised bloodflow restriction (BFR) training program performed at different times of the morning on body composition and muscle performance in older, inactive adults.

**Methods:** A single-arm intervention of supervised BFR resistance training was performed three times per week for six weeks. Participants (n=24; aged 65+ years) were categorized into early morning (n=13; 05:00–08:59) or late morning (n=11; 09:00–12:00) groups. Primary outcomes were changes in body composition, total work, average peak power, average peak torque, muscle strength, and physical function.

**Results:** Mixed model analysis of variance revealed a significant within-subject effect of time for all strength measures (p ranging from 0.017 to <0.001) and some physical function measures including the 30 s chair stand test, 30 s bicep curl test, and grip strength (p ranging from 0.015 to <0.001). No between-group or time by group interaction effect was observed for all outcomes.

**Conclusions:** This study showed that only six weeks of BFR training, performed at different time of the morning, did not enhance muscle mass and performance, but provided similar changes in muscle strength and some physical function tests in older adults.

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**Keywords:** aging; blood flow restriction training; body composition; performance; time of day

# Introduction

Aging is associated with several chronic conditions [1, 2] and exercise is a cornerstone to prevent and manage chronic conditions and reduce premature mortality [3]. In fact, performing 150 min of moderate-to-vigorous physical activity (PA) along with two or more days of resistance training per week has been associated with a 10-30 % reduction in overall mortality [4, 5]. However, most older adults do not reach the recommendations of PA necessary to prevent, manage, and treat chronic conditions [6]. This population may also face challenges when performing high-intensity resistance training, and the physiological adaptations to resistance training that result in increased lean mass may be compromised with aging [7]. For instance, a review on the impact of resistance training in older adults showed that only 50 % of exercise trials led to significant increases in lean body mass [8]. As such, it is essential to find solutions to improve health and resistance training outcomes in this population.

Recent interest has emerged surrounding the timing of exercise on overall health [9]. This topic is important as individuals usually exercise according to their chronotype, which refers to their circadian rhythmicity (morning individuals vs. night individuals) [10], which may impact adherence to exercise interventions. In addition, studying the timing of exercise is important since some studies suggest that training at different times of the day might impact the molecular clock of the exercising tissue, by turning on and off some genes, which can impact physiological and clinical outcomes [11]. In fact, studies suggest that skeletal muscles have an internal clock that dictates training-related adaptations and health benefits [12]. For example, individuals accumulating the most physical activity in the early morning reduce their risk of coronary artery disease by 11 % [13],

prostate and breast cancer by 26 % [14], and obesity by 26 % [15] when compared with midday or afternoon exercisers. Whereas late morning exercisers had a significant reduction in risk of stroke and ischemic stroke, by 17 and 21 %, respectively, when compared to midday exercisers [13]. However, most of these data are from observational studies, and controversy exists with intervention studies investigating the impact of exercise timing. In fact, some studies are in favor of morning exercise [16, 17] for substrate oxidation and muscle metabolism adaptation, while others suggest afternoon or evening exercise for greater muscle adaptation and performance [12, 18]. Very few studies have documented the impact of a morning dose-response on the training adaptations. Morning dose-response is of particular interest, as morning exercise has been effective at reducing body mass index (BMI) [17] and rescuing poor morning performance [19], when compared with afternoon exercise.

Despite the growing interest in exercise timing, most studies are limited by the investigation of only a few modes of exercise including aerobic exercise [16, 17], traditional resistance exercise [19, 20], or a combination of both [18]. Notably, alternative modalities to traditional resistance training, such as low-load blood flow restriction (BFR) training [21], a safe and effective method of resistance training for older adults [22], have not been investigated in relation to exercise timing. As BFR training may increase muscle-related adaptations – by promoting anabolic responses through mechanisms like hyperemia, increased metabolic stress, and increased metabolic response due to hypoxic conditions – it is particularly important to examine the effects of timing on these adaptations in older adults [22].

Therefore, research on the timing of exercise warrants further explorations as the data are 1) scarce, 2) used limited modalities of exercise, 3) did not study BFR, 4) did not focus on healthy older individuals, and 5) did not investigate if early or late morning would alter the physiological muscle adaptation. The objective of this study was to determine if BFR training performed at different times of the morning will have a different impact on the changes in body composition (lean body mass, body fat, and body fat distribution) and skeletal muscle performance, defined as total work, average power, peak torque, muscle strength, and physical function in older adults. The summary of the study is presented in Figure 1.

# Methods

#### Protocol overview

This is a sub-analysis of The BFR study, which was a parallel controlled experimental study comparing males and

females following a 6-week supervised resistance training intervention in conjunction with blood-flow restriction (Clinical Trial #: NCT05615831). Participants underwent baseline testing, separated into two visits one-week a part. Participants began six weeks of supervised BFR training within one week of the last baseline testing visit. Following the intervention, participants underwent follow-up testing no later than one week following the last exercise session. All testing and exercise sessions happened at the Cardiometabolic Exercise and Lifestyle Laboratory at the University of New Brunswick. The project was reviewed and approved by the University of New Brunswick Research Ethics Board (REB 2021-124) and all participants provided a written informed consent prior any data collection.

# Recruitment and sample

Recruitment was performed between May 2022 and October 2023 through the distribution of promotional flyers, University of New Brunswick's newsletter, posters, social media advertisements through Facebook and Instagram, and promotional booths at local markets.

#### **Inclusion criteria**

A total of 24 participants (female n=11), with a mean age of  $72 \pm 5$  years, were included in this sub-analysis. Participants were included if they were 1) aged 65+ years, 2) physically inactive, 3) did not have the presence of cardiovascular disease such as coronary heart disease, uncontrolled hypertension, peripheral vascular disease, venous thromboembolism, other blood clotting disorders, or hemophilia, 4) did not have surgery, a bone fracture, or a skin graft within the last three months, 5) were not pregnant, and 6) completed the study and have all their data for primary outcomes. Participants were deemed physically inactive if they did not meet the World Health Organization's 2020 physical activity guidelines: 150 min of moderate-vigorous physical activity (MVPA) and two muscle-strengthening activities per week. For this study an average of 10,000 steps/day was considered to be equivalent to 150 min of MVPA [23].

#### Sample size

Although this is a secondary analysis of a pre-existing trial that was not designed to answer this research question, we performed a power calculation using G-power software (version 3.1.9.4, Germany) to determine the appropriate sample size for statistical significance. Based on an alpha of

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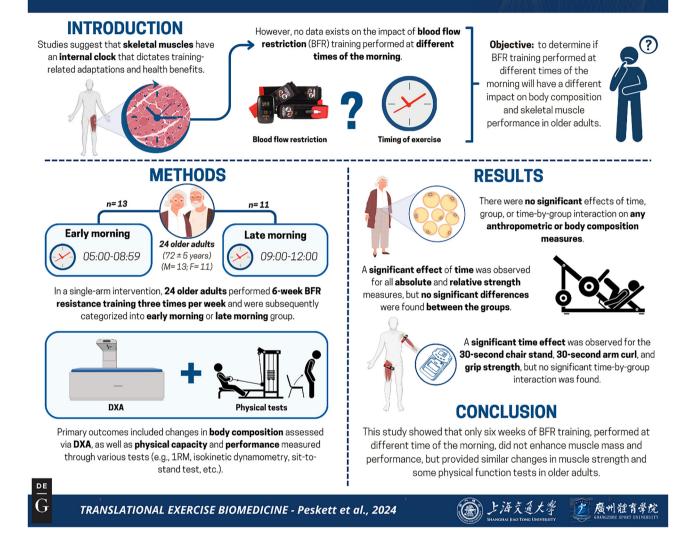


Figure 1: Graphical representation of this study. Key points: (1) This study investigated the underlying mechanisms of short-term blood flow restriction (BFR) training in healthy inactive older adults. It bridges gaps in understanding exercise timing, blood flow restriction training, and its effects on muscle mass, muscle strength, and several physical function tasks. (2) The research specifically examined how BFR training, when performed early or late in the morning, can enhance muscle mass, muscle strength, physical performance, and overall functional capacity in inactive older adults. (3) This innovative study looking at these outcomes, provides valuable insights into the timing and effectiveness of BFR training as an intervention to improve physical health in inactive older adults. Figure created with BioRender.

0.05, a power of 0.8, and an effect size of 0.5, we determined the required total sample size to be 10 participants per group for a mixed model analysis of variance (ANOVA). Nevertheless, we were able to include a total of 24 participants: early morning (n=13) and late morning (n=11) to ensure adequate ability to detect significant differences between groups.

#### Supervised exercise intervention

Participants took part in a 6-weeks of whole body BFR resistance training. The intervention consisted of three supervised exercise sessions per week. Each session consisted of five different exercises in order: chest press, seated row, leg press, knee extension, and knee flexion

(seated hamstring curl). The exercise load was individualized to 30 % of each participant's 1-RM for each exercise.

Participants completed 75 total repetitions broken into 4 sets, for each exercise. The repetition scheme was as follows: set 1: 30 repetitions; set 2: 15 repetitions; set 3: 15 repetitions; and set 4: 15 repetitions. Sets and repetitions were recorded by research staff. This protocol has previously been used in BFR research and has been found to induce muscle hypertrophy in a variety of populations which has been confirmed in a review [24, 25]. It has been suggested that BFR training, using this protocol, results in an increase in the release of Insulin growth factor-1, Testosterone, Growth Hormone, and inhibits muscle growth inhibitors, such as Myostatin [25]. At the first exercise session of week 4, participants had their 1-RM reassessed to adjust the 30 % 1-RM exercising loads. Following the 1-RM reassessment participants performed two sets per exercise (set 1: 30 repetitions; set 2: 15 repetitions) using the newly adjusted working weight, before returning to the original rep scheme for their remaining sessions.

Blood flow restriction cuffs were placed at the most proximal portion of the exercising limb (just above the biceps brachii on the arm and near the inguinal crease on the thigh). Blood flow restriction was achieved using the KAATSU C3 device (KAATSU Global, Inc., Huntington Beach, CA, USA). Cuffs were inflated to 60 % of each individual's total limb occlusion pressure, by research staff. Each participant's total limb occlusion was estimated using equations developed by Loenneke et al. [26]. Cuffs remained inflated during the rest in between the sets of each exercise and deflated for the rest between each exercise. The set rest was 60 s and the rest between exercises was 4 min, all rest was timed by research staff.

#### Primary exposure variable

#### Time of day

The research facility was open from 04:00 to 23:00, allowing participants to self-select their preferred training time within this schedule. Research staff recorded the start time of each participant's exercise session. For this analysis, participants were divided into two groups: early morning (04:00–08:59) and late morning (09:00–12:00), and group assignment was based on a pre-established cut-off of 50 % as used by Schumacher et al. [27]. As such, if a participant performed >50 % of their training sessions within a given time period, they were assigned to the corresponding group. On average, participants in the early morning group began their sessions at 08:26 and completed 83 % of their training within their time window. Those in the late morning group started on average at 10:05 and completed 84 % of their sessions within their assigned time frame.

#### **Primary outcomes**

#### **Body composition**

Lean mass was estimated using dual-energy X-ray absorptiometry (DXA) prior to the 6-week BFR training intervention, and again following the intervention. Body composition was estimated using a Hologic Horizon® DXA System (Hologic Canada ULC, Mississauga, ON, Canada). Visceral adipose tissue (VAT) was estimated from an automatically positioned android region. VAT estimated by DXA is strongly correlated (r<sup>2</sup>=0.957 (95 % CI: 0.968–0.985) to the gold standard computed tomography (CT) measurements and the Bland-Altman bias of 56 cm<sup>3</sup> (95% CI: -355 to +468 cm<sup>3</sup>) has been observed making the two measurements in a strong agreement [28]. Relative lean mass (RLM) was determined by dividing lean mass (kg) by height squared (cm<sup>2</sup>). Participants presented to the laboratory following a 12-h fast and were asked to refrain from exercise for a 24-h period prior to testing. Participants were instructed to wear loose-fitting clothing with no metal (buckles, zippers, buttons, etc.) and then instructed to lie supine on the scanner's table and remain still for the duration of the scan. Arms were placed at the participants' sides with palms facing medially and thumbs pointed upwards. For individuals too large for the width of the table, they were positioned with one arm outside of the scan area and results of the scanned arm were duplicated. The coefficient of variation in our lab for lean mass is 0.6 % and for body fat percentage is 0.7 %. This was performed on 33 people (males, n=10) with a mean age of 23.4 years and a mean BMI of 25.6.

#### **Isokinetic measures**

Muscular endurance of the dominant knee extensors and flexors was assessed using a Humac® NORM isokinetic dynamometer system (Computer Sports Medicine, Inc., Stoughton, MA, USA). Prior to testing, participants performed a 5-min walking warmup. The participants were seated and secured to the device using straps across the trunk and thighs. The positioning of the seat was adjusted to the comfort level of the participant, so long as the approximate axis of the knee (through the lateral femoral epicondyle) was aligned with the dynamometer's mechanical axis, and the setting were recorded to be used following the intervention. Range of motion was then prescribed on an individual basis (0° corresponds to full knee extension). Prior to testing, participants performed a set of five maximal repetitions at 120°/s. Upon completion of the warm-up, participants were given a two-minute recovery period before testing commenced. The testing protocol consisted of 30 reciprocal maximal contractions of the knee extensors and flexors performed at 180°/s as previously described [29]. Total work, average peak power, and average peak torque were recorded. The Humac® NORM isokinetic dynamometer system has an inter-class correlation for test-retest ranging between 0.83 and 0.96 and a percentage change and standard error measurement of 8.5 and 10.4 % respectively for community-dwelling older adults [30].

### Muscle strength

Strength was assessed by 1-RM for the five exercises used during the intervention. 1-RM was measured during the second baseline testing visit, during the first exercise session of week four of the intervention, and again during the second testing visit in the post-testing. Each participant's 1-RM was determined using the following protocol: one set of 6–10 repetitions, followed by one set of 3–5 repetitions, followed by small incremental increases for one repetition until a failure is achieved within seven attempts. If no failure was achieved within seven attempts, the 1-RM for that exercise was redone prior to their first exercise session. The use of 1-RM testing has been found to be a valid test for maximal strength of the lower and upper body in older adults [31, 32]. In addition, a systematic review showed that 1-RM testing has a good to excellent test-retest reliability regardless of age, sex, exercises selection, training experience, or the number of familiarization sessions reinforcing its use in older adults to quantify muscle strength [33]. Relative muscle strength was reported by dividing 1-RM by total body weight for each tested exercise.

#### **Physical function**

Physical function was assessed using the 30 s chair stand, 30 s bicep curl, and the 8-ft timed-up-and-go (TUG) tests from the seniors fitness test [34], and grip strength. For the 30 s chair stand test participants were instructed to sit with their feet shoulder distance apart and arms crossed over their chest. When told to "go" they began performing as many chair stands as possible in 30 s. For the 30 s bicep curl test participants were instructed to remain seated and hold the dumbbell in their dominant hand. Men were given an 8 lbs dumbbell and women were given a 5 lbs dumbbell. When told to "go" they began performing as many strict form bicep curls (straight upper arm, no swinging, full extension for eccentric phase) as possible in 30 s. For the 8-ft TUG participants were instructed to sit down, with their toes on a marked starting line. A cone was placed 8 ft from the starting line. The participant was instructed to stand, walk as fast as they can (without running) around the cone, and then return to sitting. A timer was started when the tester said "go" and was stopped when the participant returned to sitting on the chair. Grip strength was measured using a hand grip dynamometer. Participants were instructed to hold the dynamometer with their palm facing down, have their arm a 45° angle, take a deep breath in and then exhale and squeeze the dynamometer, without bringing the arm closer to their body. Three grip strength measurements were taken and averaged.

#### Statistical analysis

General characteristics of the sample are presented as mean (95% confidence interval) for continuous variables and n (%) for categorical variables. Normality was assessed using the Kolmogorov-Smirnoff test and a visual inspection of the data. Independent samples t-tests or Mann-Whitney U tests were used to detect between group differences at baseline. Main effects, time effects, and group by time interaction effects were analyzed using mixed model ANOVA. Analyses of covariance (ANCOVA) were used to adjust for potential cofounders of the primary outcomes. Effect sizes were reported as Eta-Squares for each main outcome. Data management and statistical analyses were performed using IBM SPSS Statistics Version 29.02.0. A p≤0.05 was considered significant.

# Results

Table 1 describes the general characteristics of our sample, stratified by early and late morning training groups. There were no significant differences at baseline between the early and late morning training groups except for average training time and average steps per day. The average adherence to training time was 82.7  $\pm$  14.96 % for early morning and 83.8  $\pm$  13.78 % for late morning.

Table 2 shows the impact of six-weeks of BFR training on anthropometric and body composition measures. No significant within-subject effect of time, time by group interaction or between-group effect was observed for any anthropometric or body composition measures. These results persisted when adjusting for baseline differences in average steps per day, fat mass, and BMI.

A significant within-subject time effect was found for all absolute and relative strength measures (p ranging from 0.017 to <0.001) (Table 3 and 4). However, no significant time by group interaction were observed for leg press (absolute: F=3.1,  $\eta^2=0.129$ , p=0.09; relative: F=1.644,  $\eta^2=0.073$ , p=0.21), knee extension (absolute: F=0.166,  $\eta^2$ =0.007, p=0.69; relative: F=0.039,  $\eta^2=0.002$ , p=0.85), knee flexion (absolute: F=0.825,  $\eta^2$ =0.036, p=0.37; relative: F=0.763,  $\eta^2$ =0.034, p=0.39) chest press (absolute: F=0.255,  $\eta^2$ =0.011, p=0.62; relative: F=0.222,  $\eta^2$ =0.010, p=0.64), or seated row (absolute: F=0.084,  $\eta^2$ =0.004, p=0.78; relative: F=0.282,  $\eta^2$ =0.013, p=0.60). The only muscle

**Table 1:** General characteristics of the sample.

	Early morning (n=13)	Late morning (n=11)	p-Value
Age, years	72.6 (68.8, 76.4)	71.18 (67.8, 74.52)	0.55
Female	5 (38.5 %)	6 (54.5 %)	0.45
Average TOD,	08:26 (8:14, 8:38)	10:05 (9:40, 10:29)	< 0.001
hr:min			
Average steps/Day	8,058 (7,288, 8,827)	6,030 (4,734, 7,325)	0.006
Weight, kg	77.6 (70.6, 84.7)	89.0 (69.4, 108.6)	0.25
Height, cm	169.5 (163.9, 175.1)	166.8 (160.2, 173.3)	0.49
WC, cm	100.8 (96.9, 104.6)	109.6 (91.2, 128.0)	0.32
BMI, kg/m <sup>2</sup>	26.9 (25.6, 28.3)	31.8 (25.4, 38.3)	0.06
Fat Mass, kg	24.7 (21.3, 28.1)	33.9 (22.0, 45.8)	0.09
Lean Mass, kg	48.7 (43.0, 54.3)	50.3 (39.9, 60.6)	0.76
Body fat, %	32.8 (28.9, 36.6)	37.8 (30.6, 45.1)	0.17
VAT area, cm <sup>2</sup>	184 (157.6, 210.3)	212.8 (147.3, 278.3)	0.35
RLM, kg/m <sup>2</sup>	16.8 (15.8, 17.7)	17.8 (15.2, 20.4)	0.44

Data are presented as mean  $\pm$  standard deviation for continuous variables and N (%) for categorical variables. p-values are between-group differences, p $\leq$ 0.05 is considered to be significant. WC, waist circumference; TOD, time of day; BMI, body mass index; VAT, visceral adipose tissue; RLM, relative lean mass; TOD (hr:min  $\pm$  min).

performance measure that had a significant within-subject time effect was summed work of knee extensors and flexors, with no significant time by group interaction (F=0.031,  $\eta$ <sup>2</sup>=0.001, p=0.863). No time by group interactions were observed for all strength and muscle performance measures. Following adjustment for baseline differences in average steps per day, fat mass, and BMI these results persisted in all strength and muscle performance measures.

Table 5 shows the impact of six weeks of BFR training on physical function in older adults. A significant within-subject time effect was observed for the 30 s chair stand, 30 s arm curl, and grip strength (p ranging from 0.017 to <0.001). However, no significant time by group interaction was observed for 30 s chair stand (F=0.95,  $\eta$ <sup>2</sup>=0.042, p=0.34),

30 s bicep curl (F=2.63,  $\eta^2$ =0.107, p=0.12), or grip strength (F=0.07,  $\eta^2$ =0.003, p=0.79). No within-subject time effect, between-group effect, or time by group interaction was observed for any other physical function measures. Following adjustments for baseline differences in average steps per day, fat mass, and BMI the results persisted for all physical function measures.

# **Discussion**

The objective of this study was to determine if BFR performed at different times of the morning would differently impact body composition, muscle strength and performance, and physical function in older adults. The current study is, to the best of our knowledge, the first study investigating timing of BFR training on skeletal muscle mass, performance, and physical function in older adults and has several important findings on exercise timing and BFR training for older adults. First, we found that there was no within-subject time effect on any anthropometric or body composition measures. Second, a significant within-subject time effect showed improvement in absolute and relative muscle strength, but did not improve measures of muscle performance as measured by isokinetic dynamometer. Finally, a within-subject time effect was observed for some of the physical function measurement. These results are important as they shed light on our understanding of BFR training timing in older adults.

## **Body composition**

Neither the early nor late morning exercisers changed their lean mass following six weeks of BFR training. This result was surprising since a meta-analysis of BFR training in older

Table 2: The impact of 6 weeks of BFR training on anthropometric and body composition measures.

	Early morning (n=13)		Late morning (n=11)		Eta-squares	Time	Group	Interaction
	Pre	Post	Pre	Post				
Weight, kg	77.6 ± 11.7	78.0 ± 11.5	89.0 ± 29.1	89.1 ± 28.7	0.014	0.397	0.214	0.581
WC, cm	$100.8 \pm 6.3$	$99.5 \pm 9.2$	$109.6 \pm 27.4$	109.6 ± 18.2	0.013	0.231	0.242	0.598
BMI, kg/m <sup>2</sup>	$26.9 \pm 2.3$	27.1 ± 2.2	$31.8 \pm 9.6$	$31.9 \pm 9.6$	0.005	0.348	0.090	0.755
Fat Mass, kg	$24.7 \pm 5.6$	$24.7 \pm 5.8$	33.9 ± 17.7	33.8 ± 17.7	0.005	0.778	0.092	0.735
Lean Mass, kg	$48.7 \pm 9.4$	$48.8 \pm 9.3$	50.3 ± 15.5	50.6 ± 15.1	0.008	0.447	0.740	0.675
Body fat, %	$32.8 \pm 6.4$	$32.7 \pm 6.6$	$37.8 \pm 10.8$	37.6 ± 10.6	0.006	0.385	0.171	0.712
VAT area, cm <sup>2</sup>	$184 \pm 43.6$	178.5 ± 43.8	$212.8 \pm 97.4$	212.3 ± 107.4	0.028	0.348	0.325	0.438
RLM, kg/m <sup>2</sup>	$16.8 \pm 1.6$	$16.8 \pm 1.63$	$17.8 \pm 3.9$	$17.9 \pm 3.8$	0.016	0.378	0.376	0.560

Data is presented as mean ± standard deviation. WC, waist circumference; BMI, body mass index; VAT, visceral adipose tissue; RLM, relative lean mass.

**Table 3:** The impact of six weeks of BFR training on performance outcomes.

	Early morning (n=13)		Late morr	Eta-squares	Time	Group	Interaction	
	Pre	Post	Pre	Post				
Leg press, kg	102.5 ± 24.5	112.9 ± 29.3	118.2 ± 68.2	120.8 ± 62.7	0.129	0.008	0.569	0.093
Knee extension, kg	$50.7 \pm 17.0$	$60.7 \pm 18.3$	$48.1 \pm 22.4$	$60.1 \pm 31.7$	0.007	< 0.001	0.860	0.688
Knee flexion, kg	$41.4 \pm 13.6$	$45.0 \pm 13.3$	$40.9 \pm 22.2$	$46.8 \pm 21.8$	0.036	0.002	0.925	0.374
Chest press, kg	$45.6 \pm 17.3$	$47.8 \pm 17.8$	$48.6 \pm 27.9$	$51.9 \pm 30.6$	0.011	0.017	0.717	0.618
Seated row, kg	$76.6 \pm 19.0$	$80.5 \pm 18.8$	81.9 ± 42.5	$86.5 \pm 41.4$	0.004	0.004	0.665	0.775
KF total work, W	$729.4 \pm 402.6$	931.3 ± 405.2	$676.8 \pm 539.5$	$790.2 \pm 572.9$	0.014	0.061	0.595	0.585
KE total work, W	$1935.6 \pm 808.3$	$2068.2 \pm 703.5$	$1984.5 \pm 986.6$	2,260.3 ± 1,041.4	0.023	0.053	0.732	0.480
Total work sum, W	2,665.0 ± 1,081.9	$2,999.5 \pm 975.3$	2,661.4 ± 1,494.5	3,050.5 ± 1,584.7	0.001	0.030	0.693	0.863
KF peak torque, NM	35.9 ± 13.4	37.2 ± 15.4	$33.1 \pm 6.4$	$35.1 \pm 20.0$	0.001	0.448	0.720	0.873
KE peak torque, NM	$75.8 \pm 28.2$	$73.2 \pm 23.7$	83.5 ± 41.9	$86.2 \pm 39.3$	0.054	0.994	0.453	0.276
KF peak power, N	$53.5 \pm 22.0$	58.1 ± 22.2	$52.0 \pm 35.5$	$54.4 \pm 35.9$	0.003	0.418	0.816	0.797
KE peak power, N	116.8 ± 44.9	114.8 ± 39.5	$120.8 \pm 62.4$	$126.8 \pm 62.0$	0.028	0.695	0.703	0.436

Data are presented as mean  $\pm$  standard deviation. KE, knee extensor; KF, knee flexor.

**Table 4:** The impact of six weeks of BFR training on standardized performance outcomes.

	Early morning (n=13)		Late morning (n=11)		Eta-squares	Time	Group	Interaction
	Pre	Post	Pre	Post				
Leg press, kg/kg	1.31 ± 0.2	1.43 ± 0.3	1.31 ± 0.5	1.37 ± 0.5	0.073	0.003	0.867	0.214
Knee extension, kg/kg	$0.65 \pm 0.2$	$0.77 \pm 0.2$	$0.56 \pm 0.2$	$0.69 \pm 0.3$	0.002	< 0.001	0.310	0.846
Knee flexion, kg/kg	$0.53 \pm 0.1$	$0.57 \pm 0.1$	$0.47 \pm 0.2$	$0.54 \pm 0.1$	0.034	0.003	0.494	0.392
Chest press, kg/kg	$0.58 \pm 0.2$	$0.61 \pm 0.2$	$0.54 \pm 0.2$	$0.58 \pm 0.3$	0.010	0.012	0.686	0.642
Seated row, kg/kg	$0.98 \pm 0.2$	$1.03 \pm 0.2$	$0.90 \pm 0.3$	$0.97 \pm 0.3$	0.013	0.003	0.480	0.601

Data are presented as mean ± standard deviation. KE, knee extensor; KF, knee flexor. All exercises are standardized to total body weight.

**Table 5:** The impact of six weeks of BFR training on physical function.

	Early morning (n=13)		Late morning (n=11)		Eta-squares	Time	Group	Interaction
	Pre	Post	Pre	Post				
30s chair stand, reps	17.1 ± 4.8	19.8 ± 5.2	17.2 ± 7.0	18.8 ± 6.5	0.042	<0.001	0.858	0.339
30s arm curl, reps	$19.8 \pm 3.3$	$21.8 \pm 2.7$	$19.1 \pm 6.5$	$22.3 \pm 3.6$	0.107	< 0.001	0.900	0.119
8 ft TUG, s	$5.9 \pm 1.0$	$5.8 \pm 0.8$	$6.6 \pm 1.7$	$6.5 \pm 2.0$	0.003	0.567	0.244	0.788
Grip strength, kg	$30.8 \pm 9.7$	$33.3 \pm 10.8$	$30.8 \pm 11.6$	$32.7 \pm 13.9$	0.007	0.015	0.947	0.705
SPPB balance score	$4.0 \pm 0$	$3.7 \pm 0.8$	$3.4 \pm 1.3$	$3.8 \pm 0.6$	0.139	0.720	0.316	0.073
SPPB gait score	$4.0 \pm 0$	$4.0 \pm 0$	$3.8 \pm 0.4$	$3.9 \pm 0.3$	0.051	0.287	0.141	0.287
SPPB gait speed, s	$2.6 \pm 0.4$	$2.8 \pm 0.4$	$2.9 \pm 0.6$	$2.8 \pm 0.8$	0.057	0.424	0.463	0.259
SPPB chair stand score	$3.6 \pm 0.7$	$4.0 \pm 0$	$3.4 \pm 1.2$	$3.6 \pm 0.9$	0.005	0.067	0.284	0.746
SPPB chair stand time, s	$9.5 \pm 3.3$	$8.4 \pm 2.0$	$10.6 \pm 4.7$	$9.4 \pm 4.4$	0.001	0.076	0.469	0.906
SPPB total score	$11.6 \pm 0.7$	$11.7 \pm 0.8$	$10.5 \pm 2.8$	11.4 ± 1.2	0.075	0.122	0.232	0.196

Data is presented as mean ± standard deviation. TUG: Timed-up-and-go. SPPB, short physical performance battery.

adults that included studies of at least 8 weeks observed an increase in lean mass [21]. However, all included studies that reported significant change were of longer duration (12 weeks). Our observations align with an 8-week BFR training intervention in older adults that used resistance bands [35]. Considering that lean mass decreases by 3-8%

every decade after the age of 30 and faster after 60 [36], the preservation of lean mass should be seen as a positive outcome. In fact, our data supports that only six weeks of low load BFR training in older adults is enough to prevent the loss in lean mass. This is of great concern considering that lean mass is positively correlated with muscle strength [37],

which is a strong predictor of mortality and physical function in older adults [38].

One variable of interest that was observed to have no change was VAT. VAT was of particular interest because for every increase of 1 standard deviation of volume, males and females are 4.2 and 4.7 times more likely of having Metabolic Syndrome [39], putting them at a greater risk of cardiovascular diseases and type 2 diabetes. Arciero et al. [40] found a significantly larger reduction in abdominal fat in morning exercisers when compared to afternoon exercisers [40]. Our data, adds to this study by showing that six weeks of BFR resistance training in the morning does not decrease VAT in older adults. Contrary to our results, a randomized controlled trial observed a greater reduction in the highintensity interval training and BFR training group compared to the high-intensity interval training alone [41]. However, our differing results could be explained by our measurement tool. In fact, although DXA scans are gold-standard for body composition (fat mass and lean mass), DXA appears to underestimate longitudinal changes in VAT [42]. Nevertheless, future research should examine VAT changes in older adults after BFR training at different times of the morning to clarify these results.

# Strength and performance

The largest effect of time for muscle strength measured by 1-RM, following BFR training, was observed for knee extension (*F*=20.85,  $\eta^2$ =0.487, p<0.001) and knee flexion  $(F=13.01, \eta^2=0.372, p=0.002)$ . This result was expected since the leg muscles were actively being occluded, as opposed to other muscle groups, and used specific movements to engage isolated muscles. Therefore, the improvements in strength may indicate that the most benefits from BFR training will be found in muscles that were actively being occluded while performing isolated movement. The improvements in strength are important results, as it shows that exercising at any time of the morning will still produce strength gains in older adults in the absence lean mass gains, which could be potentially explained by increased motor unit recruitment, as previously shown with BFR [43]. These results continue to add to the increasing amount of data in favour of using low-load BFR as a potential alternative for strength training, especially in older adults.

As for muscle performance, only combined total work of knee flexors and extensors had a significant effect of time. Few studies have investigated the effects of BFR training on isokinetic measures in older adults. A meta-analysis by Yang et al. [44] found that BFR training was able to improve isokinetic torque and power [44]. However, only one study

included in the meta-analysis, that investigated isokinetic measures, had participants older than 30 years of age. That study found that six weeks of treadmill walking with BFR can improve isokinetic torque for knee flexors, but not knee extensors, at 180°/s for adults aged 60–78 [45]. However, a review by Clark [46] could explain the lack of improvement in torque or power in the present study as they saw minimal effects after 8 weeks of traditional resistance training with a dose-response between adaptation and duration of training [46]. Therefore, it is possible that a longer duration of BFR training would have generated a greater adaptation on the isokinetic measures in our older adults.

# Physical function and aging

A significant effect of time was observed for total reps in the 30 s chair stand and 30 s bicep curl test, and grip strength. We hypothesize that this is due to increased neural drive, in older adults, following BFR training. This hypothesis is strengthened by the fact that there was no increase in lean mass. These results are important because a 40.1 % decline in physical function occurs between 60 and 90 years of age [34] and is associated with increased risk of mortality [47] and falls [48]. However, since our study did not use electromyography, we do not have the data to verify this hypothesis. One potential explanation for improvement in these physical function outcomes could be the motivation factor. In fact, several studies suggest that motivation for physical activity varies significantly across the day and impacts subsequent behaviours [49]. Although motivation for physical activity is lower in the morning, individuals who are motivated in the morning perform more high-intensity exercise [49], which could translate in better physical function [50].

# **Strengths and limitations**

This study does have some limitations that need to be acknowledged. First, this study is a sub-analysis of a larger trial and therefore participants were not randomized into early or late morning groups and chronotype was not assessed. Second, the design of the current study also did not have a control group, which limits the conclusions that can be drawn from the study. Third, the BFR study had a relatively short duration of only six weeks. It is possible some of the group differences were masked by the short duration of the intervention. Fourth, although we used validated tests that were reliable, we did not have familiarization for these tests, and did not perform multiple testing to calculate our

own coefficient of variation of our sample. Therefore, it is possible that some of the observed changes are within the day-to-day or measurement error. Finally, our study has a very small sample size. However, the study is strengthened by a very high participants' compliance. The exercise sessions were also supervised by a limited number of research staff to maximize compliance and control the environment. All testing sessions were performed by the same experimenters to minimize error and used gold-standard measure of body composition.

#### Conclusions

This study showed that only six weeks of BFR training, performed at different time of the morning did not enhance muscle mass and performance, but provided similar changes in muscle strength and some physical function tests in older adults. Future studies using low-load BFR training in older adults with a stronger design and a bigger sample size should replicate this study to understand if exercise timing has an impact on adaptations to BFR training.

**Research ethics:** The project was reviewed and approved by the University of New Brunswick Research Ethics Board (REB 2021-124). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

**Informed consent:** Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Trial Registration: Clinical trial registration number is: NCT05615831.

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