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# Similar patterns of tendon regional hypertrophy after low-load blood flow restriction and high-load resistance training

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**Purpose:** Recent evidence indicates that low-load blood flow restriction (LL-BFR) training elicits an anabolic response in tendinous tissue. The purpose of the present study was to investigate the hypertrophic pattern induced in the Achilles tendon by LL-BFR, in comparison with the regional hypertrophy typically observed with conventional high-load (HL) resistance training.

**Methods:** $N = 40$ male participants were randomly and concealed allocated to one of two groups: LL-BFR training (20–35% one-repetition maximum/1RM) or HL training (70–85% 1RM). The training was completed three times per week for a total of 14 weeks. Before and after the training period, Achilles tendon morphology was assessed using magnetic resonance imaging along the entire tendon length. Additionally, dynamic strength measures of the plantar flexors were evaluated.

**Results:** In line with previous findings, dynamic plantar flexion strength was improved to a comparable extent in both groups (LL-BFR: 43.6%; HL: 43.5%). The results also confirmed significant increases in Achilles tendon cross-sectional area with LL-BFR (+5.2%). Moreover, they revealed that the hypertrophic pattern obtained with LL-BFR was similar to regional changes seen with conventional HL training.

**Conclusion:** The present findings point towards the notion that despite the low loads being applied, LL-BFR training induces Achilles tendon hypertrophy by potentiating anabolic responses in the same regions as with conventional high-load training. Future studies are needed to (i) focus on the potential mechanisms underlying these tendon morphology changes and (ii) apply and evaluate LL-BFR training in clinical populations to validate these results in rehabilitative settings.

KEYWORDS

Achilles tendon, blood flow restriction, cross-sectional area, muscle strength, one-repetition maximum

## 1 | INTRODUCTION

The combination of low-load resistance training and simultaneous blood flow restriction (BFR) is currently a frequently investigated synergy in the scientific community,[1–3] as the observed anabolic effects are standing in contrast to previous suggestions proposing the need of high mechanical loads during exercise.[4] Indeed, current recommendations suggest a loading of >70% of each individual's one-repetition maximum (1RM) for the induction of optimal muscular[4] as well as tendinous adaptations[5] in healthy individuals. Interestingly, previous studies using BFR in combination with low-load (LL) resistance training (20–40% 1RM) have uniformly demonstrated that the effects on muscle growth are comparable to conventional training regimens with high loading (70–85% 1RM).[6,7]

Apart from adaptive responses on the muscular level, recent data from our laboratory suggest that after 14-week of training, both LL-BFR and high-load (HL) training can effectively increase patellar tendon cross-sectional area (CSA) as well as tendon stiffness in healthy men.[8] Interestingly, no differences in the magnitude of adaptations were seen between both groups although ~1/3 of the load was used in the LL-BFR condition. In the Achilles tendon, an earlier study revealed that tendon morphology and mechanical properties can be increased following 14 weeks of LL-BFR training.[9] However, since in this study the structural adaptations were assessed with ultrasound, regional changes in CSA of the Achilles tendon could not be investigated. Due limited echo transmission of tendon morphology in some tendon areas, reliable CSA measurements with ultrasound are often not possible throughout the entire Achilles tendon length. In contrast, as the current gold standard of assessing tendon CSA, MRI allows for highly standardized evaluations of tendon morphology across the entire tendon length.

Investigating regional changes in tendon structure is particularly critical when considering the heterogeneity of hypertrophy observed following conventional HL resistance training along the tendon length. In the patellar tendon, previous studies[10,11] have shown that morphological adaptations are primarily evident within the proximal and distal regions of the tendon but not in the mid-portion. Comparable region-specific responses have been reported for the Achilles tendon.[12] Although heterogeneous stress magnitude and type have been suggested as potential mechanisms,[11] such region-specific responses are currently poorly understood. With LL-BFR training, tendon hypertrophic patterns are entirely undocumented. Since loading is lower than with HL training, LL-BFR may potentiate regional hypertrophy uniformly, regardless of stress/strain distribution.

Therefore, the main purpose of the present study was to re-evaluate the effects of 14-week of LL-BFR (20–35% 1RM) training on Achilles tendon morphology across the entire tendon length using magnetic resonance imaging (MRI). Such a detailed methodological approach was aimed at exploring the newly uncovered anabolic effect of BFR on tendon tissue.[9] Given the low mechanical stress with BFR training, we hypothesized that the low loading of LL-BFR may not elicit a region-specific hypertrophic response as delineated with HL training.

## 2 | MATERIALS AND METHODS

### 2.1 | Participants

A total of $n = 40$ adult male participants were recruited to participate in this study. The present experiments were conducted within a larger project examining the effects of LL-BFR training on muscular and tendinous adaptations, where Achilles tendon CSA was a secondary outcome. Before being included, participants were informed about the study procedures and any potential risks before giving written informed consent. Inclusion criteria were an age between 18 and 40 years, a body mass index <30 km/m$^2$, and a physical activity level of <120 min per week with no prior experience in resistance exercise. Participants were excluded if they had chronic diseases or any tendon pathologies. Study procedures were approved by the local ethics committee, and all experiments were conducted in accordance with the Declaration of Helsinki.

### 2.2 | Experimental design

To evaluate the effects of 14-week of LL-BFR and HL resistance training on Achilles tendon morphology, a single-blinded, parallel-group randomized-controlled trial with repeated measures was conducted. As further secondary outcomes, the muscle strength of the plantar flexors was assessed before and after the completion of the exercise program.

Before the start of the intervention, participants were screened during a preliminary screening visit to fit the study-specific inclusion criteria. After confirming eligibility, participants were randomly assigned to either 14 weeks of LL-BFR (20–35% 1RM) resistance training or HL resistance training (70–85% 1RM). Block randomization was implemented, and a random number generator was used for allocation sequence generation. All sessions were supervised by specifically trained personnel. Before and after the training period, serial analyses of Achilles tendon CSA (primary outcome) were assessed using MRI and 1RM testing was conducted for the plantar flexor muscle group. All analyses were conducted at the same time of the day to minimize circadian variation by group-blinded outcome assessors. Inter-assessor bias was eliminated by assigning measurements of each variable to the same investigator.

## 2.3 | Exercise protocol

Throughout the 14-week intervention, three weekly exercise sessions were performed by both groups. Two consecutive exercise sessions were separated by 1 day of rest to ensure adequate recovery. Before each exercise session, a 10-min warm-up on a cycle ergometer was completed (~50-Watt, 60–70 rpm).

### 2.3.1 | High-load training (HL)

The exact exercise protocol of the HL group is described elsewhere.[8] Briefly, to mechanically load the Achilles tendon, three sets of sitting and standing calf raises were completed. Each set was separated by a 1-min resting period, and care was taken that all exercises were performed with the full range of motion (ROM). Full ROM for calf raise exercises was defined from full dorsal flexion (~80° standing on an elevated plate) to full plantar flexion (~130°) according to Kubo et al.[13] All participants were able to manage full ROM. Training load was progressively increased every 4 weeks by 5% from 70% to 85% 1RM with the repetitions adjusted accordingly (70% 1RM = 12 repetitions, 75% 1RM = 10 repetitions, 80% 1RM = 8 repetitions, 85% 1RM = 6 repetitions). On these occasions, dynamic 1RM measurements were implemented to adjust the load to the current strength level of each participant. For means of increasing training compliance, additional exercises for the lower limbs (knee extensions, leg press) and trunk and upper body muscles (lat pull, bench press) were included following the same loading regiment.[8]

### 2.3.2 | Low-load blood flow restriction training (LL-BFR)

In the LL-BFR group, participants followed the same training protocol as the HL group, except that the training load for the lower extremities was set to 20% 1RM at the first 4 weeks and was progressively increased by 5% every 4 weeks until a final load of 35% 1RM in the final 2 weeks was reached. Similarly to the HL group, the training load was progressively adjusted every 4 weeks to the current strength level. The specific exercise protocol consisted of four sets with 30 repetitions in the first set and 15 repetitions in the remaining three sets. This protocol is in accordance with previous BFR protocols in the scientific literature.[7,14] During all lower extremity exercises, a 12-cm-wide pneumatic nylon tourniquet [Tourniquet Touch TT20, VBM Medizintechnik GmbH, Germany] was applied at the most proximal portion of each thigh to ensure proper blood flow restriction during exercise. Individual cuff pressures were based on measurements of individual arterial occlusion pressure (AOP) assessments. AOP was determined in a sitting position and the cuff was gradually increased until no arterial pulse at the posterior tibial artery was detectable by Doppler ultrasound [Handydop, Kranzbühler, Solingen, Germany]. At this point, an arterial occlusion of 100% was assumed. During the exercises, cuff pressure was continuously set to 50% AOP[7,14] and kept inflated during inter-set rest periods of 60 s. Between exercises, the cuffs were deflated for 3 min. Upper extremity exercises were performed in the same manner compared to the HL group (without BFR). All exercise sessions were supervised by specially trained personnel.

## 2.4 | Achilles tendon cross-sectional area

Magnetic resonance imaging (MRI) scans were acquired with participants in a supine position and with the knees fully extended, hips at 0° rotation and ankle fixed in a 90° position using a custom-built orthosis. Achilles tendon CSA was examined by axial MRI scans [Magnetom, Aera 1.5T, Siemens, Berlin, Germany] using the following parameters: repetition time = 620 ms, echo time = 12 ms, slice thickness = 4 mm, FOV = 200 × 200, Matrix = 448 × 358, interslice gab = 0 mm. Transversal MRI scans were obtained in the perpendicular direction to the Achilles tendon alignment from the most proximal aspect of the tuberositas calcanei to the most distal part of the soleus muscle.[12] All images were analyzed using image analysis software ImageJ [1.51, NIH, Maryland, USA], and CSA was manually outlined three times. The average value was used for statistical analyses. To investigate site-specific changes, tendon CSA was linearly interpolated at each 10% interval of tendon absolute length (from 0–100% of tendon length).[11] The CSAs of all sites were used for subsequent statistical analyses. The measurements average (across all tendon lengths) CV was 1.1%, after reanalyzing the same images twice following 72 h.

## 2.5 | One-repetition maximum assessment

Dynamic muscle strength was assessed using dynamic 1RM testing for the standing calf raise exercise.

Measurements were implemented at the beginning and after the 14-week intervention period. Before 1RM testing, participants completed a warm-up on a stationary cycling ergometer (5 min at 50 W) and subsequently performed an exercise-specific warm-up of two sets with ten repetitions of 50% estimated 1RM.[9] Then, two additional warm-up sets allowing three to five repetitions were completed.[15] The actual 1RM test consisted of single attempts, lifting the weight through the full range of motion (from maximal dorsal extension to maximal plantar flexion[9]) using the correct lifting technique. Following each successful lift, the load was gradually increased by 5–10% until participants failed to lift the weight with the specified technique through full ROM.[15,16] To ensure optimal recovery, each trial was separated by a 4-min resting period. All final 1RMs were achieved within five attempts. The average coefficient of variation (CV) was 3.4%.

## 2.6 | Lifestyle parameters

To control for additional activities outside the 14-week resistance training program, physical activity was assessed before and after the intervention using a validated questionnaire of physical activity.[17] Additionally, participants were advised to maintain their nutritional regimen throughout the study. To account for potential changes in macronutrient intake, participants completed nutritional protocols at 3 days (2 weekdays and 1 weekend day) before and after the intervention. Macronutrients were accordingly tracked with NutriGuide 4.6 software (Nutri Science, Hausach, Germany).

## 2.7 | Statistics

Statistical analysis was performed using R software.[18] After verifying statistical assumptions of variance homogeneity and normal distribution, a mixed ANOVA with the within-group factor "time" (pre vs. post) and between-group factor "group" (HL vs. LL-BFR) was conducted to test for changes in Achilles tendon CSA and muscle strength. Significant interaction effects were followed by Benjamini–Hochberg corrected post-hoc paired *t*-test. Linear associations between mean tendon CSA and maximal strength were investigated using Pearson correlation coefficients. Grubb's test was used to identify outliers[19] and truncated according to.[20] Missing values ($n = 2$) were imputed using a multiple imputation approach. Between-group differences in baseline parameters were assessed using unpaired *t*-tests.

**TABLE 1** Baseline participant characteristics.

| | HL ($n = 15$) | LL-BFR ($n = 14$) |
|---|---|---|
| Age (yrs) | 27.6 ± 4.3 | 28.4 ± 4.9 |
| Height (cm) | 181.9 ± 7.2 | 179.6 ± 6.8 |
| Weight (kg) | 79.7 ± 11.7 | 75.1 ± 7.8 |
| BMI (kg/m$^2$) | 24.2 ± 4.0 | 23.3 ± 2.3 |
| Tendon length (mm) | 51.2 ± 24.4 | 48.0 ± 15.6 |

Abbreviations: BMI, body mass index; HL, high-load training group; LL-BFR, low-load blood flow restriction training group; yrs, years.

In text and tables, all presented data are expressed as mean ± standard deviation. All data in the figures are depicted as mean ± 95% CI. The level of significance was set to $p < 0.05$. Effect sizes are calculated using partial eta-squared ($\eta_p{}^2$) with $\eta_p{}^2 = 0.01$ indicating a small, $\eta_p{}^2 = 0.06$ a medium, and $\eta_p{}^2 = 0.14$ a large effect size.

# 3 | RESULTS

In total, $n = 29$ participants successfully the 14-week intervention with $n = 5$ dropouts in the HL and $n = 6$ dropouts in the LL-BFR group. No adverse events were reported, and none of the dropouts was related to any side effects of the training program. Baseline parameters were not significantly different and are depicted in Table 1.

## 3.1 | Tendon morphology

Regarding Achilles tendon CSA, significant time effects were found for all tendon sites ($p < 0.05$) except at 0% ($F_{(1, 27)} = 2.22$, $p = 0.148$, $\eta_p{}^2 = 0.076$) and 100% tendon length ($F_{(1, 27)} = 3.60$, $p = 0.069$, $\eta_p{}^2 = 0.118$) (Figures 1 and 2). Additionally, no significant interaction effects were observed at any tendon site (Table 2). Averaging all tendon sites, percentage increases of 5.2% and 5.3% were observed for LL-BFR and HL, respectively. Post-training between-group effect sizes (d) are depicted in Figure 3.

## 3.2 | Plantar flexor strength

Dynamic muscle strength of the plantar flexors significantly increased from 114.8 ± 30.0 kg to 164.7 ± 48.8 kg in the HL and from 121.8 ± 40.7 kg to 174.9 ± 34.8 kg in the LL-BFR group (Figure 4). After calculation of a mixed ANOVA, a significant time effect ($F_{(1, 27)} = 94.86$, $p < 0.01$, $\eta_p{}^2 = 0.778$) but no interaction effect were observed ($F_{(1, 27)} = 0.09$, $p = 0.764$, $\eta_p{}^2 = 0.003$).

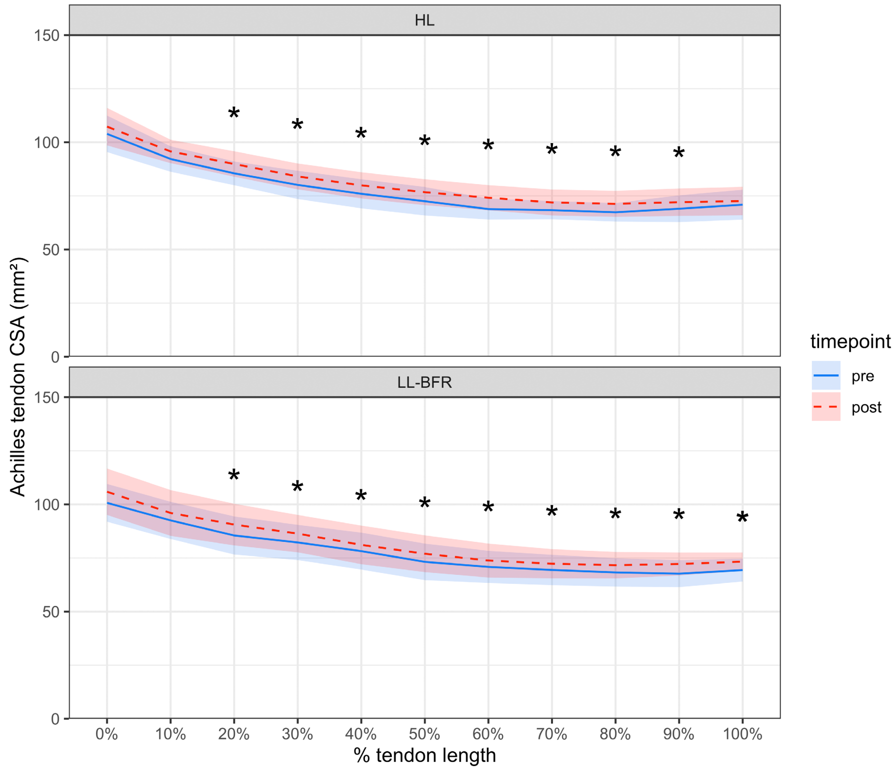


**FIGURE 1** Achilles tendon cross-sectional area (CSA) is shown at pre (solid blue) and post (dashed red) for the high-load (HL, $n = 15$) and low-load blood flow restriction (LL-BFR, $n = 14$) group. Lines represent the mean and the shaded area with 95% confidence intervals. *Indicates significant differences from baseline following adjusted paired $t$-test ($p < 0.05$).

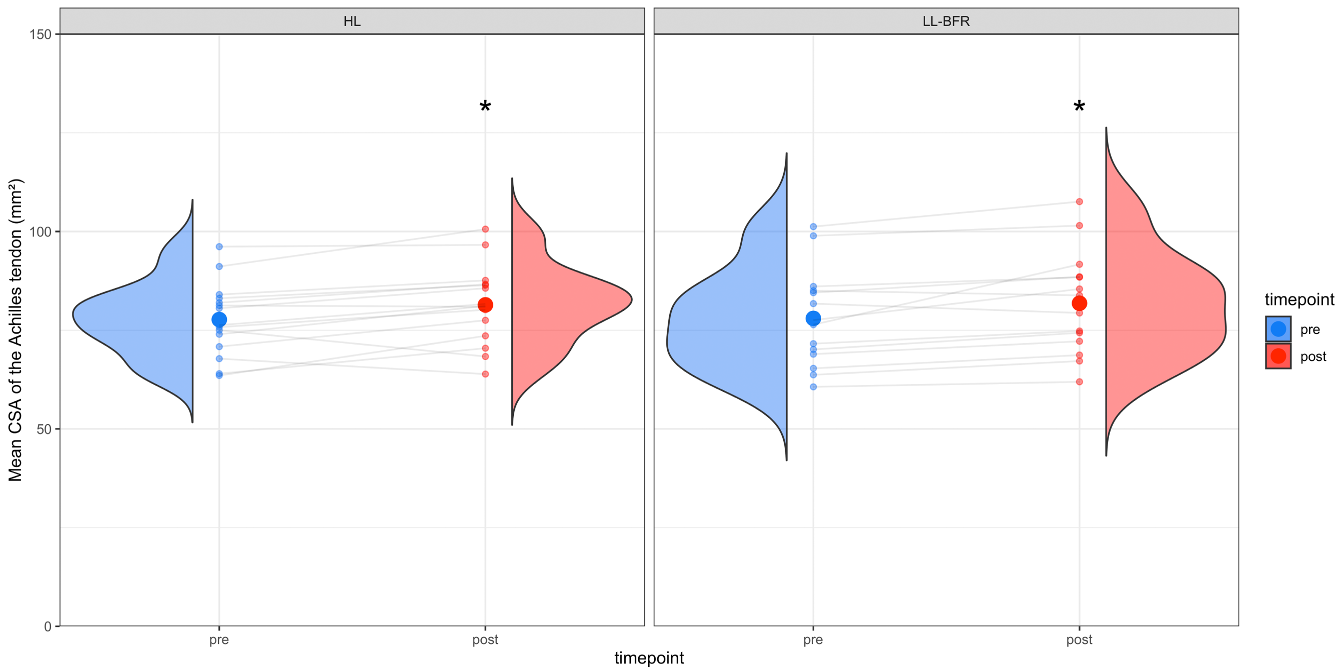


**FIGURE 2** Changes in mean Achilles tendon CSA from pre (blue) to post (red) in the HL and LL-BFR groups are depicted. Small dots indicate individual values, and big dots indicate mean values. Half violin plots show the data distribution. *Indicates significant differences from baseline following adjusted paired $t$-test ($p < 0.05$).

### 3.2.1 | Association between mean tendon CSA and plantar flexor strength

After the correlation of percentage changes in mean tendon CSA and plantar flexor strength, no significant associations were observed for HL ($r = -0.44$, $p = 0.17$) or LL-BFR ($r = 0.02$, $p = 0.95$).

## 3.3 | Lifestyle parameters

For self-reported physical activity performed outside the study, analyses using mixed ANOVAs did not reveal any significant time ($F_{(1, 27)} = 2.539$, $p = 0.124$, $\eta_p^2 = 0.092$) or interaction effect ($F_{(1, 27)} = 1.318$, $p = 0.262$, $\eta_p^2 = 0.050$).

Regarding the nutritional status, no significant interaction effects were observed for the intake of protein ($F_{(1, 27)} = 0.304$, $p = 0.586$, $\eta_p^2 = 0.011$) or carbohydrates ($F_{(1, 27)} = 1.406$, $p = 0.246$, $\eta_p^2 = 0.05$). Significantly interaction effects were observed for fat ($F_{(1, 27)} = 15.005$, $p = 0.001$, $\eta_p^2 = 0.357$), with a significantly lower fat intake in the HL group at baseline ($p < 0.05$).

**TABLE 2** Time × group interaction effects for all Achilles tendon sites.

| Tendon site | *F* | *p*-value | $\eta_p^2$ [95% CI] |
|---|---|---|---|
| 0% | $F_{(1, 27)} = 0.100$ | 0.754 | 0.004 [0.00, 0.15] |
| 10% | $F_{(1, 27)} = 0.001$ | 0.977 | 0.000 [0.00, 0.00] |
| 20% | $F_{(1, 27)} = 0.115$ | 0.737 | 0.004 [0.00, 0.16] |
| 30% | $F_{(1, 27)} = 0.006$ | 0.937 | 0.000 [0.00, 0.06] |
| 40% | $F_{(1, 27)} = 0.220$ | 0.642 | 0.008 [0.00, 0.18] |
| 50% | $F_{(1, 27)} = 0.044$ | 0.835 | 0.002 [0.00, 0.12] |
| 60% | $F_{(1, 27)} = 1.582$ | 0.219 | 0.055 [0.00, 0.28] |
| 70% | $F_{(1, 27)} = 0.158$ | 0.694 | 0.006 [0.00, 0.16] |
| 80% | $F_{(1, 27)} = 0.099$ | 0.755 | 0.004 [0.00, 0.15] |
| 90% | $F_{(1, 27)} = 0.490$ | 0.490 | 0.018 [0.00, 0.21] |
| 100% | $F_{(1, 27)} = 0.555$ | 0.463 | 0.020 [0.00, 0.21] |

## 4 | DISCUSSION

The current study provides novel evidence regarding the morphological responses along the entire Achilles tendon length following 14-weeks of low mechanical loading with BFR (20–35% 1RM) compared to high mechanical loading (70–85% 1RM) under normal blood flow conditions. Our findings expand on previous findings[9] and demonstrate that there are similar patterns in regional Achilles tendon hypertrophy between LL-BFR and HL training. Changes in tendon morphology were accompanied by comparable improvements in plantar flexor strength in both groups.

### 4.1 | Tendon morphology

In the past few decades, adaptive responses of the muscular system have been well-reported following LL-BFR training.[6,21] However, there is still considerable controversy regarding adaptations of the tendinous apparatus following this training regimen since previous research indicated the need for high mechanical loading for optimal tendon changes.[5] The overall number of studies investigating the long-term effects of LL-BFR training on tendons is small. In an early experiment by Kubo and colleagues,[22] the authors found that patellar tendon CSA (mean CSA value at 25%, 50% and 75% tendon length) remained unchanged following 12 weeks of either LL-BFR

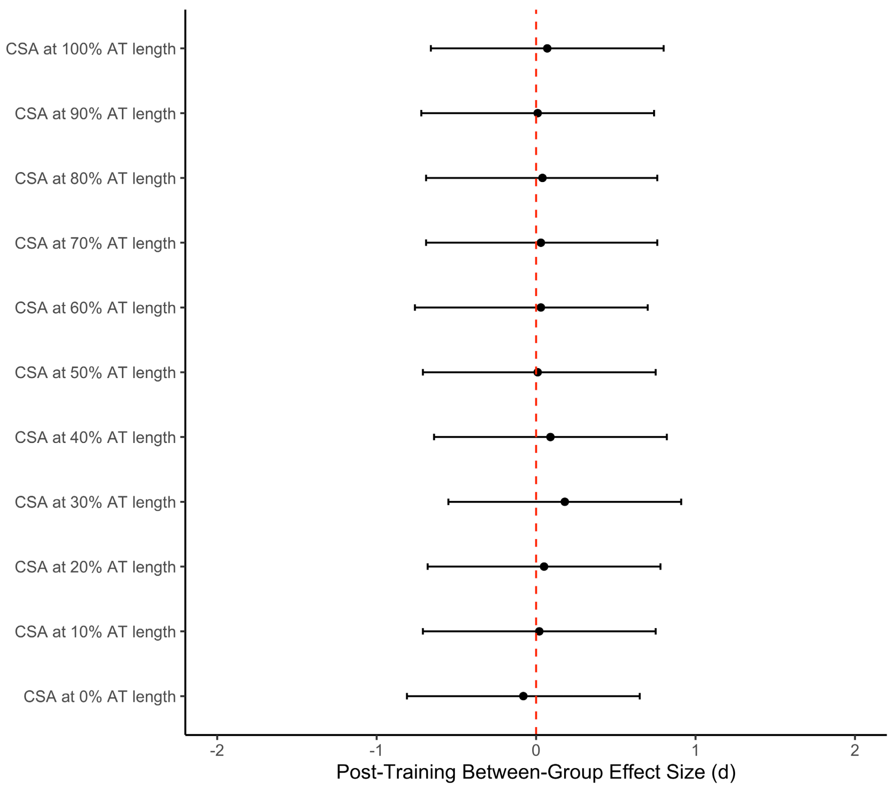


**FIGURE 3** Post-training between-group effect sizes (Cohen's $d \pm 95\%$ CI) for Achilles tendon cross-sectional area (CSA) at each specific tendon site. The red dashed line represents the line of zero effect.

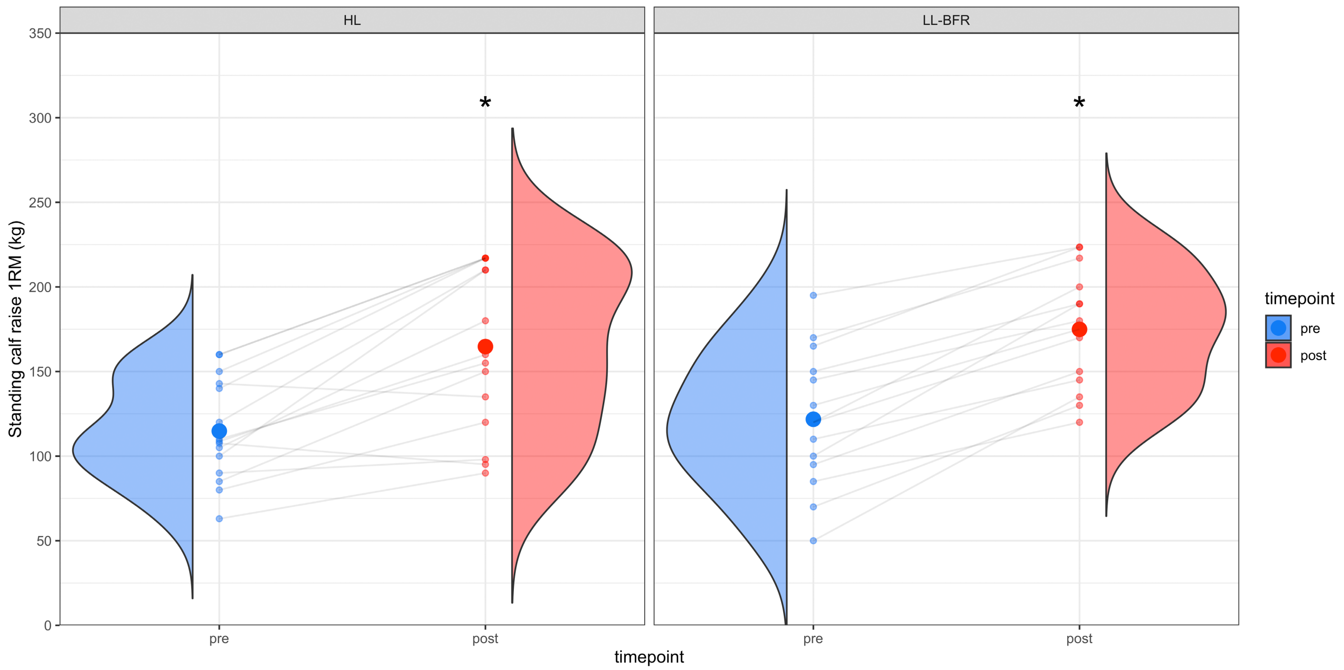


**FIGURE 4** Changes in dynamic plantar flexor strength from pre (blue) to post (red) in the HL and LL-BFR groups are illustrated. Small dots indicate individual values and big dots indicate mean values. Half violin plots show the data distribution. *Indicates significant differences from baseline following adjusted paired *t*-test ($p<0.05$).

or HL resistance training. Conversely, recent data from our laboratory indicated that both LL-BFR and HL training were able to induce increases in patellar tendon CSA following 14 weeks of training in healthy men.[8] These discrepant findings may be ascribed to different load progression paradigms as well as intervention durations, as the study by Kubo et al.[22] used 12 weeks with a progressive adjustment of training load only in the HL group by reassessing the 1RM every 4 weeks. However, the present findings and our previous experiment on the Achilles tendon[9] seem to corroborate the effect of LL-BFR training on tendon mechanical and morphological properties in male individuals. Although previous data indicate that HL training may facilitate region-specific changes in tendon CSA[10,11] potentially via heterogeneous stress magnitudes, very similar tendon hypertrophy patterns were seen in the current study when comparing LL-BFR and HL training, with uniform increases in tendon CSA at every location except at the most distal and most proximal points. The reasons for these homogeneous increases in tendon hypertrophy with LL-BFR are unclear and cannot be explained with the current study design. Speculatively, it might be argued that BFR potentiates the anabolic responses without affecting their heterogeneity as seen with conventional HL training.[10,11] This, however, needs to be further investigated in future trials focusing on the underlying physiology of the induction of collagen synthesis following LL-BFR. Although there is currently a lack of studies focusing on exact mechanisms, evidence suggests that hypoxia stimulates tendon stem cell proliferation compared to normoxic conditions.[23] Additionally, hypoxia has been demonstrated to upregulate mRNA levels of transforming growth factor-beta-1, an important mediator of collagen synthesis and fibroblast growth.[24,25] To further investigate the potential effect of hypoxia and BFR, future studies need to implement study designs which allow direct comparison between LL and LL-BFR resistance training regimens.

Given that increases in the cross-sectional area will have a strong impact on overall tendon stress reduction, these findings may be relevant for clinical populations. To date, only three case series exist which investigated the efficacy of BFR in patients with tendinopathy[26,27] or tendon rupture.[28] Interestingly, the trials showed that the utilization of BFR facilitated improvements in pain relief,[26,27] strength[26,28] and diminished tendon vascularity[27] during tendon rehabilitation.

## 4.2 | Plantar flexor strength

In the present trial, we were able to demonstrate a gain in dynamic plantar flexor muscle strength of 43.6% and 43.5% in the LL-BFR and HL groups, respectively. This is in line with our previous trial in healthy young men measuring maximal voluntary isometric contraction,[9] although the present changes in 1RM were considerably higher probably due to a better transferability from dynamic training to dynamic testing[29] or the repetitive practice of the 1RM test.[30]

The scientific literature is currently still debating on whether HL training might be superior in increasing muscular strength compared to LL-BFR. On the one hand, previous meta-analyses suggested that despite similar anabolic adaptations, HL shows greater gains in muscle strength compared to LL-BFR in both older[21] as well as mixed-aged populations.[6] On the other hand, a very recent meta-analytical study found no significant differences between both regimens.[31] The comparable increase in dynamic strength seen in the present study is at odds with previous studies about the influence of training load magnitude in the knee extensors (without BFR).[32] In the plantar flexor muscles, however, the influence of load magnitude on strength gains is less clear.[12] For instance, a study by Arampatzis and co-workers[12] found that 14 weeks of training with low- (55% MVC) and high-strain (90% MVC) repetitive isometric plantar flexor contractions induced comparable increases in plantar flexor strength (20.4% vs. 31.6%, respectively). Again, it needs to be mentioned that differences in training duration, study population or methodological approaches (e.g., mode of testing) might hamper easy comparisons between studies.

For higher explanatory power, further mechanistic studies are necessary, which elucidate potential differences on the neural level (e.g., spinal and cortical inhibition assessments) between both training strategies.

### 4.3 | Limitations

The present study design incorporated a single-blinded, parallel-group randomized-controlled trial with repeated measures in order to eliminate the bias of potential cross-transfer effects reported in previous research with BFR training.[33] Nevertheless, future studies may apply within-subject protocols which allow bigger sample sizes and minimize the impact of between-subject variation in adaptive responses. From a methodological point of view, MRI was used to assess changes in tendon morphology following 14 weeks of either LL-BFR or HL training. Although in the present study only the intra-rater reliability was evaluated, previous trials have confirmed excellent test–retest reliability scores.[34] Despite MRI still being considered as the common gold standard for assessing tendon morphology, good reliability scores can be obtained using modern ultrasound systems,[34] which might ease the tendon morphology assessments in practice.

Lastly, the findings might not be transferable to adaptations in pathological conditions (e. g., patients with tendinopathy) and further clinical trials are necessary before an evidence-based BFR protocol can be applied in these populations with the aim of increasing tendon CSA.

## 5 | PERSPECTIVE

In conclusion, the present long-term trial provides evidence that low-load blood flow restriction training is a viable tool for increasing Achilles tendon cross-sectional area following 14-week of resistance training. Region-specific tendon hypertrophy patterns were similar to that seen following HL training. Additionally, both training regimens demonstrated similar increases in ankle plantar flexor strength. Further studies are warranted to focus on the mechanisms involved in the observed adaptations and validate these findings in clinical settings.

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### CONFLICT OF INTEREST STATEMENT

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

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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