

Original Article

Temporal Dynamics of Achilles Tendon Vascular Signals After Acute Hopping Exercise: A Comparison of Microvascular Imaging and Power Doppler

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Abstract

Objectives: To characterize the temporal dynamics of intratendinous Achilles tendon vascular signals after acute hopping exercise and to compare microvascular imaging (MVI) and power Doppler (PD) across analytical metrics. **Methods:** In this repeated-measures acute exercise study, 20 healthy adult men without regular exercise habits (38 legs) performed six sets of 30 single-leg hops. Vascular signals were assessed with MVI and PD at six time points spanning exercise, using signal count, presence/absence, and grading (0–4). Inter-rater reliability was assessed using ICC(2,1). **Results:** Signal count, presence, and grade showed significant overall effects of method and time (all $P < 0.05$), without significant method-by-time interactions. Signal count was the highest at 5 min post-exercise and exceeded pre-exercise, inter-set, immediate post-exercise, and 15-min values after Holm adjustment. Overall, MVI yielded lower vascular signal outcomes than PD. The grade at 5 min was higher than pre-exercise, immediate post-exercise, and 15-min values. Signal count showed more statistically significant and detectable temporal contrasts than grade or presence. ICC(2,1) was 0.753 for PD and 0.834 for MVI. **Conclusions:** Vascular signal count was highest at the 5-min post-exercise measurement, yielding more statistically detectable temporal contrasts than presence or grade. MVI showed lower vascular signal outcomes than PD across measurement time points.

Keywords: Intratendinous blood flow, Mechanical loading, Reactive hyperemia, Transient vascular response, Ultrasonography

Introduction

Achilles tendinopathy is a common disorder affecting individuals ranging from recreational exercisers to elite athletes, particularly in activities involving repetitive tensile loading such as running and jumping¹. Recurrence rates reach up to 27% after diagnosis², and residual functional deficits persist in approximately 25% of

patients despite symptomatic recovery³. Therefore, identifying the physiological responses of the Achilles tendon to mechanical loading may contribute to the early detection and prevention of Achilles tendinopathy.

Ultrasonography is commonly used to assess structural abnormalities, such as hypoechoic areas and tendon thickening, as well as intratendinous blood flow^{4,5}. Its potential clinical relevance is supported by prospective evidence showing that preseason sonographic abnormalities of the Achilles tendon, including tendon thickening, hypoechogenicity, and neovascularity, were associated with an 18.8-fold increased risk of subsequent time-loss injury in collegiate athletes⁶. Blood flow assessment is noninvasive and repeatable and may provide information about the physiological and pathological responses of tendon tissue to mechanical loading. In addition, injured tendons show a prolonged increase in blood flow after running⁷. Blood flow may also change

The authors have no conflict of interest.

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Edited by: G. Lyritis

Accepted 4 September 2026



Table 1. Physical characteristics of participants.

Characteristics	Value (n=20)
Age (years)	23.8±2.7
Height (cm)	173.8±6.8
Weight (kg)	69.7±8.3
Body fat percentage (%)	15.3±5.5
BMI (kg/m ²)	23.1±2.3
<i>Values are expressed as mean ± standard deviation. BMI, body mass index.</i>	

dynamically within a single bout of exercise: mechanical compression can transiently restrict flow during loading, while subsequent vasodilation and reactive hyperemia may increase perfusion afterward. Accordingly, reported responses vary depending on the timing of measurement, ranging from an immediate reduction after brief exercise⁸ to a prolonged increase after longer-duration running⁹. The finding that upper-limb exercise did not alter Achilles tendon blood flow further supports a local, mechanically driven mechanism rather than a systemic hemodynamic effect¹⁰.

Despite these findings, the temporal course of intratendinous blood flow after acute repetitive loading remains unclear in asymptomatic individuals without regular exercise habits, as previous studies have mainly examined injured tendons or athletes at limited post-exercise time points. Whether asymptomatic Achilles tendons in these individuals show an immediate or delayed vascular response, and how quickly this response resolves, is therefore uncertain. Studying healthy individuals without regular exercise habits may reduce the influence of habitual training and pre-existing tendon pathology and may help characterize the acute vascular signal response to a standardized loading task. Capturing such subtle responses also requires ultrasound techniques that are sensitive to fine and low-velocity flow. Conventional Doppler ultrasonography has limited sensitivity and is prone to motion artifacts¹¹, which may partly explain inconsistent findings linking blood flow, exercise, and tendon pathology^{12,13}. Microvascular imaging (MVI) offers a higher frame rate and improved motion-artifact suppression¹⁴, potentially allowing clearer visualization of low-velocity flow than conventional Doppler^{15,16}. However, few studies have applied MVI to the Achilles tendon, and its detection and visualization characteristics relative to power Doppler (PD), as well as the responsiveness of different analytical metrics (signal count, presence, and grading), remain insufficiently understood^{9,17}.

The primary objective of this study was to characterize the temporal course of intratendinous vascular signals in

asymptomatic Achilles tendons after an acute single-leg hopping protocol. We hypothesized that loading would induce a transient change in vascular signals and that the magnitude of this change would vary depending on the measurement timing. As secondary objectives, we compared the detection and visualization characteristics of MVI and PD and examined how signal count, signal presence, and grading reflected changes observed following exercise. We further hypothesized that signal count would capture subtle temporal changes more clearly than the other metrics and that MVI would provide clearer vascular visualization with fewer motion artifacts than PD.

Materials and Methods

Participants

This study was conducted at the University of Tsukuba between August and October 2025 in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Tsukuba (Approval No. TAI O25-17). Participants were recruited from students affiliated with the University of Tsukuba. Twenty healthy adult men who had not engaged in specific exercise three or more times per week during the previous year were enrolled. Before testing, current medical conditions, previous injuries, and surgical history were assessed by interviewing them for medical-history, followed by body-composition measurement. Participants reported no current Achilles tendon symptoms. A standardized physical examination and baseline structural assessment using B-mode ultrasonography were not performed; therefore, the included Achilles tendons were considered asymptomatic rather than structurally normal.

Twenty participants included 40 potentially eligible legs. One leg in each of two participants, one left leg and one right leg, was excluded before the hopping protocol because a previous non-Achilles ankle injury was judged to preclude safe performance of the task. The contralateral leg of each participant was eligible and was assessed. Therefore, 38 legs from 20 participants were included in the study (Table 1).

Experimental Protocol

Participants were instructed to avoid strenuous exercise for 24 h before the experiment. After the medical-history interview and body-composition measurement (InBodyDial H20, InBody Japan), participants rested for 15 min before the pre-exercise measurement (Pre). Subsequently, a single-leg hopping exercise task (30 repetitions × 6 sets) was performed, and vascular signal measurements were obtained between sets 3 and 4 (Mid), immediately after the exercise task (Post0), and at 5-, 10-, and 15-min post-exercise (Post5, Post10, and Post15). Both legs were assessed sequentially on the same day. The first-tested leg was selected at random. After completion of the full protocol through Post15 for the first leg, participants

rested for 15 min before the protocol was initiated for the contralateral leg. Thus, the protocol was designed such that there was an interval of at least 30 min between completion of hopping with the first leg and initiation of the contralateral-leg protocol.

Exercise Protocol

The exercise task consisted of single-leg hopping, which allows easy standardization of load conditions while applying the repetitive tensile loads known to cause Achilles tendon injury. Before the 15-min rest period preceding the pre-exercise measurement, participants were familiarized with the single-leg hopping technique and the prescribed 2-Hz rhythm. Familiarization consisted of instructions and practice to confirm the prescribed hopping technique and rhythm. No separate formal warm-up was performed. The familiarization and hopping task were performed barefoot on the laboratory floor. The task consisted of 6 sets of 30 repetitions with a 30 s rest between sets. However, a 3 min rest was taken between the third and fourth sets to perform the Mid measurement. The hopping tempo was fixed at 2 Hz using a metronome, and participants were instructed to keep their hands on their hips, avoid letting their heels touch the ground, and minimize ground contact time. An investigator remained beside each participant throughout the protocol to supervise task performance and provided verbal feedback when necessary to maintain the prescribed tempo and technique. All participants completed the prescribed protocol without failed repetitions or protocol deviations. No Achilles tendon pain, injury, or other adverse events were reported during the protocol.

Intratendinous Vascular Signal Measurement

An ultrasound diagnostic system (LOGIQ Fortis, GE Healthcare Japan) and a linear-array transducer (L3-12-D) were used. Separate fixed acquisition presets were used for PD and MVI. For PD, the principal settings were: frame rate, 11 frames/s; Doppler frequency, 8.0 MHz; PRF, 0.8 kHz; wall-filter setting, 66; and Doppler acoustic output, 100%. For MVI, the corresponding settings were 44 frames/s, 8.2 MHz, 0.18 kHz, 5, and 100%, respectively, with a smoothing-filter setting of 3. In both methods, the B-mode frequency was 12 MHz, B-mode gain was 70 dB, imaging depth was 3.0 cm, and B-mode acoustic output was 100%. Color gain was determined separately for PD and MVI during Pre-measurement for each leg, which was held constant at all subsequent time points for that leg; gain ranged from 16 to 20 dB for PD and from 19 to 20 dB for MVI. Fixed system presets were used to control other mode-specific processing settings, and these were not manually changed during data acquisition. The color box was positioned to include the full thickness of the Achilles tendon at each anatomical location, and its size and position were maintained during the paired PD and

MVI acquisitions. Measurements were performed with participants in the prone position and the ankle fixed at 90°. The entire Achilles tendon, from the calcaneal insertion to the soleus musculotendinous junction, was systematically scanned in the longitudinal plane by moving the probe to cover the full length. The acquisition order of PD and MVI was randomized at each measurement time point. At each anatomical location, paired 5-s PD and MVI video sequences were acquired in the same imaging plane without moving the probe between methods. Each video sequence was recorded with the probe held stationary. After acquiring both sequences, the probe was moved to the next anatomical location, and this procedure was repeated to cover the tendon's entire length. When vascular signals were detected at multiple anatomically distinct locations, separate paired video sequences were recorded at each location. Excessive probe pressure was avoided to minimize vascular compression. For each stored video sequence, one representative frame was selected on the basis of image stability and the absence or minimal presence of visible artifacts, rather than the maximum number of visible signals. During frame selection, the assessor was blinded to participant-identifying information; however, the measurement time point remained visible in the video information. After frame selection, the selected images were relabeled to conceal participant identity and measurement time point during vascular signal counting and grading. Blinding to imaging method was not feasible during frame selection and analysis because PD and MVI images were visually distinguishable.

Analysis of Intratendinous Vascular Signals

To perform quantitative and qualitative evaluations of intratendinous vascular signals, the following three indices were calculated from all representative static ultrasound images obtained during the whole-tendon assessment. When vascular signals were identified at multiple anatomical locations, findings from the corresponding images were combined at the tendon level. First, for the number of vascular signals, the total number of non-duplicated, spatially distinct detected color-signal regions depicted in the Achilles tendon parenchyma was counted. Spatially distinct color-signal regions were counted individually. Clearly separated punctate signal regions were counted as separate signals. Signal regions judged to represent a continuation of the same signal based on their anatomical location and morphology were counted as one signal. Signals appearing in overlapping fields of view were identified by their anatomical location and morphology and were counted only once. Thus, vascular signal count was regarded as an imaging surrogate and not a direct measurement of the number of anatomical vessels, blood-flow volume, or blood-flow velocity. Annotated examples of the signal-counting criteria are shown in Figure 1. Second, the presence or absence of

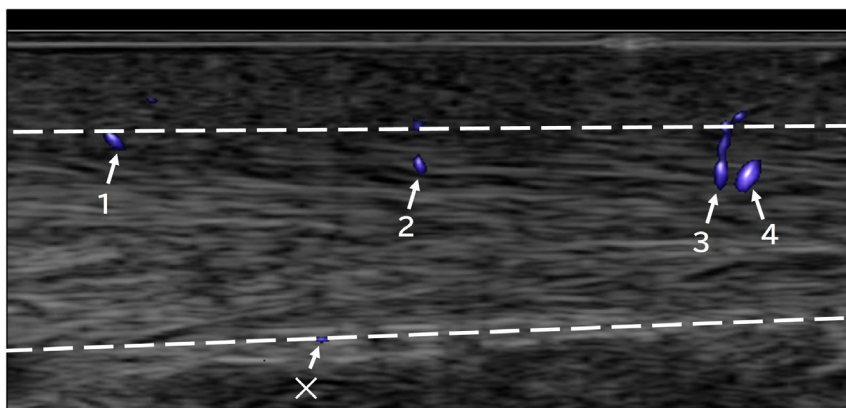


Figure 1. Annotated examples of the vascular signal counting criteria. The dashed lines indicate the defined boundaries of the Achilles tendon. Numbered arrows indicate spatially distinct detected color-signal regions included in the vascular signal count. Clearly separated punctate regions within the tendon boundaries were counted as separate signals. Color-signal regions located on or outside the tendon boundaries were excluded from the count. In this example, four vascular signal regions were counted. The image illustrates the operational counting criteria and does not establish the anatomical identity of individual vessels.

vascular signals was evaluated as binary data (0: no signal detected; 1: one or more signals detected) throughout the whole-tendon assessment. Third, vascular signals were graded with reference to criteria from previous research¹⁸. Grading was performed on a five-point scale based on the detection range and number of vascular signals, from Grade 0, in which no signals were recognized, to Grade 4, in which mesh-like signals were present throughout the entire tendon. The grade was assigned at the tendon level using information from all anatomical locations assessed.

Statistical Analysis

All analyses were performed using R (version 4.4.3). As the outcome variables were count, binary, and ordinal, formal normality testing was not applicable. The data were clustered, with bilateral legs nested within participants and repeated measures within legs. To account for this non-independence, all primary mixed-effects models included random intercepts for participant and for leg within participant.

Vascular signal count was analyzed using a generalized linear mixed model (GLMM) with a negative binomial (type 1) distribution and log link, selected based on comparison of candidate count models and diagnostic checks. Vascular signal presence was analyzed using a binomial GLMM with a logit link. Vascular signal grade was analyzed using a cumulative-link mixed model (CLMM) with a logit link and flexible thresholds, limited to the observed categories (Grades 0–2). As a nominal-effects test was not available

for the CLMM, the proportional-odds assumption was checked using a supplementary fixed-effects cumulative link model. For each outcome, imaging method, time, and their interaction were included as fixed effects. The method-by-time interaction was tested using the likelihood-ratio test; when not significant, it was dropped and a reduced model including the main effects of method and time was used for final inference. Main effects were tested using likelihood-ratio tests. Estimated marginal contrasts were used to derive incidence rate ratios (vascular signal count) and odds ratios (vascular signal presence and grade), with 95% confidence intervals. Where the effect of time was significant, all 15 pairwise comparisons across the six time points were performed, with P values adjusted within each outcome using the Holm method.

For sensitivity analysis, all models were refitted using a single leg per participant: for the 18 participants with bilateral data, the randomly selected first-tested leg was included; for two participants with only one eligible leg, the available leg was used, resulting in total of 20 participants and 20 legs.

Outcome data were complete for all 38 included legs using both imaging methods and all six time points; therefore, no imputation of missing outcome data was required. No observations from the included legs were excluded based on their outcome values. Vascular signal detection rates were calculated as the proportion of signal-positive legs, with exact 95% confidence intervals obtained using the Clopper–Pearson method.

Count and binary GLMMs were fitted with the `glmmTMB` package (version 1.1.14), and CLMMs with the ordinal

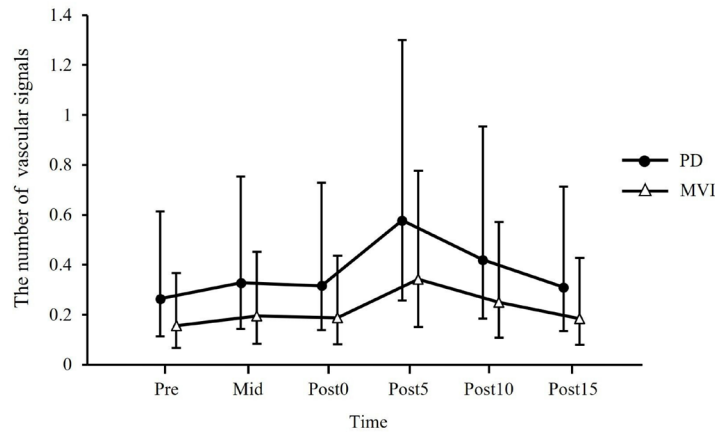


Figure 2. Changes in vascular signal count. Model-estimated marginal means and 95% confidence intervals for power Doppler (PD) and microvascular imaging (MVI) at each measurement time point are shown. The method-by-time interaction was not significant ($P = 0.918$). The overall effects of imaging method and time were significant (both $P < 0.001$). After Holm adjustment, vascular signal count at Post5 was significantly higher than at Pre, Mid, Post0, and Post15 when averaged across imaging methods. Pre, pre-exercise; Mid, inter-set measurement between sets 3 and 4; Post0, immediately post-exercise; Post5, 5 min post-exercise; Post10, 10 min post-exercise; Post15, 15 min post-exercise.

Table 2. Overall effects of imaging method on vascular signal outcomes.

	Effect estimate, MVI vs PD	95% CI	χ^2 (df)	P value
Vascular signal count	IRR = 0.59	0.47-0.75	20.12 (1)	< 0.001**
Vascular signal presence	OR = 0.46	0.26-0.80	7.76 (1)	0.005**
Vascular signal grade	OR = 0.40	0.25-0.65	13.99 (1)	< 0.001**

CI, confidence interval; IRR, incidence rate ratio; OR, odds ratio; PD, power Doppler; MVI, microvascular imaging. Effect estimates are expressed as MVI relative to PD and are averaged over the six measurement time points. An IRR or OR below 1 indicates a lower estimated vascular signal count, lower odds of vascular signal presence, or lower odds of a higher vascular signal grade with MVI than with PD. P values were obtained from likelihood-ratio tests of the overall imaging-method effects. The method-by-time interaction was not significant for any outcome. **: $P < 0.01$.

package (version 2025.12.29). Estimated marginal contrasts were obtained using emmeans (version 2.0.3), and model fit was evaluated using simulation-based residual diagnostics implemented in the DHARMa package (version 0.4.7). Statistical significance was set at $P < 0.05$.

To assess inter-rater reliability of vascular signal counts, images from eight randomly selected participants were used. For each participant, the first-tested leg was included, resulting in 96 images in total (48 each for PD and MVI). The primary rater, a physical therapist with 5 years of experience in musculoskeletal ultrasonography, selected the representative static frames. The same preselected frames were independently evaluated by the

primary rater and a second rater trained in ultrasound image assessment. Before the reliability assessment, the images were relabeled and presented in randomized order so that participant identity and measurement time point were concealed from both raters. Both raters had been trained in the predefined vascular signal counting criteria, and the second rater was blinded to the primary rater's results. Blinding to imaging method was not feasible because PD and MVI images were visually distinguishable. Reliability for each method was evaluated using the intraclass correlation coefficient (ICC [2,1]), following established protocols for counting scores¹⁹.

Results

Temporal Changes in Intratendinous Vascular Signal Counts

The overall analysis of signal counts showed a significant main effect of time (Time: $\chi^2[5] = 22.35$, $P < 0.001$). Signal counts were highest at Post5, and post hoc tests indicated that the number of signals at Post5 was significantly higher than that at Pre (IRR = 2.19, 95% CI, 1.49–3.23; Holm-adjusted $P = 0.001$), Mid (IRR = 1.76, 95% CI, 1.23–2.54; Holm-adjusted $P = 0.027$), Post0 (IRR = 1.83, 95% CI, 1.27–2.63; Holm-adjusted $P = 0.015$), and Post15 (IRR = 1.87, 95% CI, 1.29–2.70; Holm-adjusted $P = 0.012$), regardless of measurement method. No significant method-by-time interaction was observed (Method $\times$ Time: $\chi^2[5] = 1.46$, $P = 0.918$), indicating no evidence that the temporal patterns differed between PD and MVI (Figure 2). Regarding differences between imaging methods, a significant main effect of measurement method was observed (Method: $\chi^2[1] = 20.12$, $P < 0.001$). Across measurement time points, MVI detected fewer signals than PD (IRR = 0.59, 95% CI, 0.47–0.75; $P < 0.001$; Table 2).

Temporal Changes in Vascular Signal Presence

Regarding the presence of vascular signals, the logistic mixed-effects model showed significant main effects of measurement method and time, whereas no significant method-by-time interaction was observed (Method: $\chi^2[1] = 7.76$, $P = 0.005$; Time: $\chi^2[5] = 14.00$, $P = 0.016$; Method $\times$ Time: $\chi^2[5] = 1.12$, $P = 0.953$; Table 3). Across measurement time points, the odds of vascular signal presence were lower with MVI than with PD (OR = 0.46, 95% CI, 0.26–0.80; Table 2). Although the main effect of time was significant, no pairwise comparisons among the six measurement time points remained significant after Holm adjustment. Observed vascular signal detection rates with exact 95% confidence intervals are presented in Table 3.

Temporal Changes in Vascular Signal Grades

Vascular signal grade showed a significant main effect of time (Time: $\chi^2[5] = 19.90$, $P = 0.001$). Post hoc tests revealed that the vascular signal grade at Post5 was significantly higher than that at Pre (OR = 4.70, 95% CI, 1.33–16.65; Holm-adjusted $P = 0.005$), Post0 (OR = 3.46, 95% CI, 1.01–11.81; Holm-adjusted $P = 0.039$), and Post15 (OR = 4.14, 95% CI, 1.20–14.28; Holm-adjusted $P = 0.011$). No significant method-by-time interaction was observed (Method $\times$ Time: $\chi^2[5] = 0.63$, $P = 0.987$; Table 4), indicating no evidence that the temporal patterns differed between PD and MVI.

Regarding differences between imaging methods, a significant main effect of measurement method was observed (Method: $\chi^2[1] = 13.99$, $P < 0.001$). Across measurement time points, MVI yielded lower grades than

PD (OR = 0.40, 95% CI, 0.25–0.65; $P < 0.001$; Table 2). Notably, all observed data were Grade 2 or lower.

Sensitivity Analyses

In the one-leg-per-participant sensitivity analyses, the main effect of measurement method remained significant for vascular signal count, presence, and grade, while the method-by-time interaction remained non-significant for all three outcomes. The main effect of time remained significant for vascular signal count ($\chi^2[5] = 16.01$, $P = 0.007$) but was not significant for vascular signal presence ($\chi^2[5] = 5.43$, $P = 0.366$) or grade ($\chi^2[5] = 7.48$, $P = 0.188$).

Qualitative Image Characteristics

In the image findings (Figure 3), PD depicted vascular signals as multiple intermittent punctate signals, whereas MVI showed greater continuous tubular signal patterns. Furthermore, visible motion artifacts were more frequently observed in the representative PD images than in the corresponding MVI images.

Inter-rater Reliability

The inter-rater reliability for counting vascular signals showed moderate to good agreement for both methods. The intraclass correlation coefficient (ICC [2,1]) was 0.753 (95% CI, 0.651–0.828) for PD and 0.834 (95% CI, 0.761–0.886) for MVI.

Discussion

The principal finding of this study was that intratendinous vascular signals in asymptomatic Achilles tendons changed transiently after acute repetitive loading. Although no clear increase in signal counts was observed immediately after exercise, they were highest at 5 min post-exercise and subsequently decreased toward the pre-exercise level during the early recovery period. The absence of a significant method-by-time interaction indicated that there was no evidence of a difference in this temporal pattern between PD and MVI. Among the three analytical metrics, signal count captured more temporal differences than signal presence or grading. Regarding the imaging methods, PD yielded higher signal counts, whereas representative MVI images showed more continuous vascular signal patterns and fewer visible motion artifacts. The overall method effects and the absence of method-by-time interactions were retained in the one-leg-per-participant sensitivity analyses. However, time effects for signal presence and grade were not retained, whereas those for signal count remained significant.

Acute Intratendinous Vascular Signal Response to Exercise

The delayed increase in vascular signals, with no clear increase immediately after exercise but a higher signal

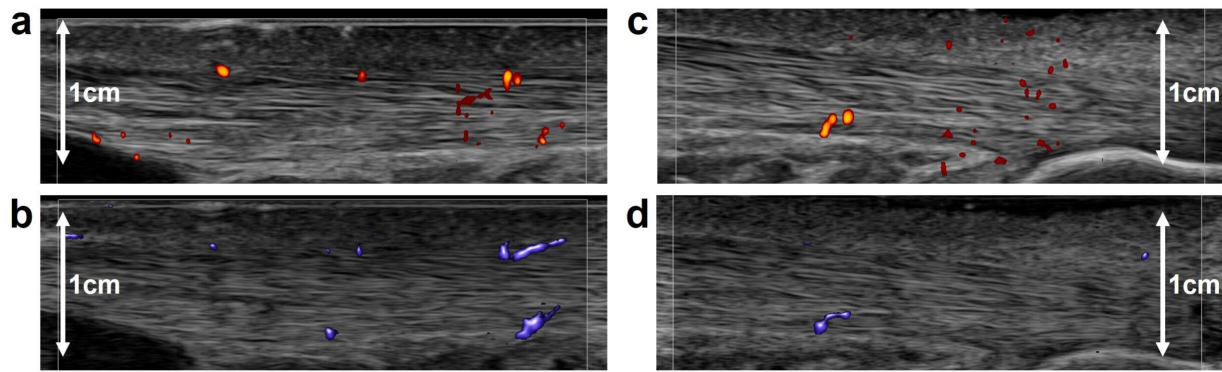


Figure 3. Corresponding images of the Achilles tendon obtained by PD and MVI from the same participant at 5 min post-exercise. Images (A) and (B) show the midportion, while (C) and (D) show the distal insertion. In each pair, the MVI images (B, D) show more continuous vascular signal patterns and fewer visible motion-related artifacts than the corresponding PD images (A, C) in the same imaging plane. A 1-cm scale bar is shown in each panel. These images are illustrative examples and were not used to establish anatomical accuracy or method superiority.

Table 3. Observed vascular signal detection rates with exact 95% confidence intervals.

Time Point	PD, n/N (%) [95% CI]	MVI, n/N (%) [95% CI]
Pre	14/38 (36.8) [21.8-54.0]	11/38 (28.9) [15.4-45.9]
Mid	16/38 (42.1) [26.3-59.2]	12/38 (31.6) [17.5-48.7]
Post0	15/38 (39.5) [24.0-56.6]	11/38 (28.9) [15.4-45.9]
Post5	20/38 (52.6) [35.8-69.0]	17/38 (44.7) [28.6-61.7]
Post10	17/38 (44.7) [28.6-61.7]	16/38 (42.1) [26.3-59.2]
Post15	15/38 (39.5) [24.0-56.6]	10/38 (26.3) [13.4-43.1]
Fixed Effect Analysis	χ^2 (df)	P
Method	7.76 (1)	0.005**
Time	14.00 (5)	0.016*
Interaction (Method $\times$ Time)	1.12 (5)	0.953

CI: confidence interval, PD: power Doppler, MVI: microvascular imaging. Pre: pre-exercise, Mid: inter-set measurement between sets 3 and 4, Post0: immediately post-exercise, Post5: 5 minutes post-exercise, Post10: 10 minutes post-exercise, Post15: 15 minutes post-exercise. Detection rates were observed proportions, and exact 95% confidence intervals were calculated using the Clopper-Pearson method. *: $P < 0.05$. **: $P < 0.01$.

count at Post5, may reflect changes in tendon circulation during loading and early recovery. Previous research has shown that Achilles tendon blood flow decreases during isometric plantar-flexion contractions but increases during recovery after repeated contractions²⁰. Repetitive tensile loading and increased tissue pressure during hopping may have temporarily compressed the intratendinous microvasculature, whereas the release of this compression and subsequent vasodilation may have increased tendon

perfusion during recovery. Previous research showed that in healthy Achilles tendons, intratendinous vessels that were absent at rest were detected at the 5-min post-exercise assessment after 30 min of running, indicating that exercise can induce a transient physiological vascular signal response during early recovery⁹. Thus, the increase at Post5 in the present study may represent an early vascular response during recovery from mechanical loading. However, we assessed detected vascular signals

Table 4. Observed distribution of vascular signal grades.

Time Point	Method	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Pre	PD	24 (63.2%)	11 (28.9%)	3 (7.9%)	0 (0.0%)	0 (0.0%)
	MVI	27 (71.1%)	10 (26.3%)	1 (2.6%)	0 (0.0%)	0 (0.0%)
Mid	PD	22 (57.9%)	12 (31.6%)	4 (10.5%)	0 (0.0%)	0 (0.0%)
	MVI	26 (68.4%)	9 (23.7%)	3 (7.9%)	0 (0.0%)	0 (0.0%)
Post0	PD	23 (60.5%)	12 (31.6%)	3 (7.9%)	0 (0.0%)	0 (0.0%)
	MVI	27 (71.1%)	8 (21.1%)	3 (7.9%)	0 (0.0%)	0 (0.0%)
Post5	PD	18 (47.4%)	12 (31.6%)	8 (21.1%)	0 (0.0%)	0 (0.0%)
	MVI	21 (55.3%)	13 (34.2%)	4 (10.5%)	0 (0.0%)	0 (0.0%)
Post10	PD	21 (55.3%)	10 (26.3%)	7 (18.4%)	0 (0.0%)	0 (0.0%)
	MVI	22 (57.9%)	14 (36.8%)	2 (5.3%)	0 (0.0%)	0 (0.0%)
Post15	PD	23 (60.5%)	11 (28.9%)	4 (10.5%)	0 (0.0%)	0 (0.0%)
	MVI	28 (73.7%)	9 (23.7%)	1 (2.6%)	0 (0.0%)	0 (0.0%)
Fixed Effect Analysis		χ^2 (df)			P	
Method		13.99 (1)			<0.001**	
Time		19.90 (5)			0.001**	
Interaction (Method $\times$ Time)		0.63 (5)			0.987	

PD: power Doppler, MVI: microvascular imaging. Pre: pre-exercise, Mid: inter-set measurement between sets 3 and 4, Post0: immediately post-exercise, Post5: 5 minutes post-exercise, Post10: 10 minutes post-exercise, Post15: 15 minutes post-exercise. Values are presented as the number of legs, with percentages in parentheses. Percentages were calculated for each imaging method and measurement time point and rounded to one decimal place; therefore, the totals may not equal exactly 100% because of rounding. Grades 3 and 4 were not observed in our dataset. **: $P < 0.01$.

rather than blood-flow volume or velocity, and the physiological mechanism underlying the observed signal changes was not directly measured.

Importantly, this transient increase should not be interpreted as pathological neovascularization. Exercise-induced Doppler activity has been observed in asymptomatic Achilles tendons, indicating that the presence of intratendinous vascular signals alone does not necessarily represent tendon pathology^{7,9,21}. The timing and persistence of the response may therefore provide more relevant information than signal presence alone.

The present findings also emphasize the importance of measurement timing. Measurements obtained only immediately after exercise may fail to capture a delayed response, whereas later measurements may underestimate the greatest observable change. Under the present loading conditions, the 5 min measurement captured the clearest increase in signal count. However, 5 min should not be regarded as universally optimal because the temporal response may vary with exercise mode, intensity, duration, and study population. Furthermore, because measurements were obtained only at predefined time points, the precise timing of the maximum physiological response could not be determined. The interval between exercise and ultrasound assessment should therefore be standardized and clearly reported.

Duration of the Post-exercise Vascular Signal Response

Signal counts decreased after Post5, suggesting that the observed vascular signal response was relatively brief under the present loading conditions. This may partly reflect the short duration of active hopping time, approximately 1.5 min per leg, excluding the inter-set rest periods and the 3-min measurement interval. In contrast, longer-duration exercise produces more immediate or sustained responses. Achilles tendon microvascular volume increased immediately after a 1 h run but remained unchanged after arm cycling, supporting an association with local tendon loading¹⁰. Following 30 min of running, blood flow returned to baseline by 30 min in most healthy responding tendons but remained elevated for up to 120 min in some tendinopathic tendons⁷.

Exercise mode may also influence the response. Ten minutes of running and plyometric exercise increased Achilles tendon blood flow immediately after exercise, whereas static stretching and eccentric heel-drop exercise produced no significant changes²². Collectively, these findings suggest that the timing and duration of the vascular response depend on the mode, intensity, and duration of loading. Under the present conditions, a transient vascular signal pattern was observed following short-duration hopping, which was highest at the Post5 measurement and decreased subsequently.

Vascular Signal Detection and Visualization Characteristics of PD and MVI

PD yielded higher signal counts than MVI. One possible explanation is the difference in the detectable flow-velocity range. Under the present settings, PD could depict velocities above 10 cm/s, whereas the upper limit of MVI was 10 cm/s. MVI may therefore have failed to depict some higher-velocity components. Other modality-specific acquisition and signal-processing characteristics may have contributed to the observed differences.

However, a higher signal count does not necessarily indicate more accurate visualization. In the representative paired images, PD tended to depict vascular signals as multiple punctate signals, whereas MVI showed more continuous vascular signal patterns. Visible motion artifacts were also less evident in the representative MVI images^{15,23}. The methods should therefore be regarded as having different characteristics rather than one being uniformly superior. PD may depict signals over a broader velocity range, whereas MVI may provide a different representation of vascular signal continuity and morphology. Because no independent anatomical or flow-reference standard was included, we could not determine the imaging method that more accurately represented the underlying vasculature.

Signal-count reliability was moderate to good for PD and good for MVI, with ICCs of 0.753 and 0.834, respectively²⁴. These values were lower than those previously reported in tendinopathic tendons with abundant vascularity¹⁹. The sparse signals and limited variability in the present healthy population may have increased the influence of minor inter-rater disagreement. Nevertheless, the observed ICCs indicate moderate-to-good inter-rater reliability of vascular-signal counting in the present asymptomatic population. Higher ICC for MVI may partly reflect differences in the appearance and continuity of vascular signals and lower frequency of visible motion artifacts in the representative images. However, no direct statistical comparison of reliability was performed between the two methods.

Responsiveness of Vascular Signal Analysis Metrics

Among the three analytical metrics, signal count captured more temporal differences than signal presence or grading. After Holm adjustment, signal count identified differences between Post5 and Pre, Mid, PostO, and Post15, whereas grading identified differences between Post5 and Pre, PostO, and Post15. Although signal presence showed a significant overall time effect in the primary analysis, no individual time-point comparison remained significant after Holm adjustment.

Binary assessment does not reflect changes in signal number unless the classification changes between absence and presence. Similarly, grading requires a change large enough to cross a category threshold. Signal count retains information about individual signals and

may therefore provide greater granularity when vascular signals are sparse. The restricted grade distribution in the present study, in which all values were Grade 2 or lower, may also have reduced the responsiveness of grading. Signal counting may therefore be better suited to subtle physiological responses in healthy tendons, whereas grading may remain useful for tendinopathic tendons with abundant vascularity^{17–19,25}. Because statistical sensitivity was not directly compared among the metrics, the present findings indicate only that signal count captured more temporal differences under the current experimental conditions. This interpretation is supported by sensitivity analyses, wherein the overall time effect was retained for signal count but not for signal presence or grade.

Study Limitations

This study has several limitations. First, as noted above, there were constraints in the detectable flow velocity range of the device; specifically, the upper limit of MVI was 10 cm/s, which may have led to underestimation of high-velocity flow components after exercise. Differences in acquisition settings and signal processing between PD and MVI may have influenced the observed vascular signal outcomes. Second, the participants were limited to non-habitual exercisers. Response characteristics of vascular signals and the findings obtained using MVI may differ in athletes exposed to high daily loads or in individuals with a history of tendon injury; therefore, comparative verification in these groups is needed in the future. Third, the participants were limited to young adult men, which restricts the generalizability of the findings to women, trained athletes, older adults, younger populations, and patients with Achilles tendinopathy. Fourth, a standardized physical examination and baseline B-mode structural assessment of the Achilles tendon were not performed. Therefore, included tendons were classified as asymptomatic rather than structurally normal. Leg dominance, medication use, vascular disease history, caffeine intake, food and fluid intake, hydration status, room temperature, and time of day were not systematically recorded or standardized. Fifth, both legs were assessed sequentially on the same day; therefore, residual systemic or contralateral carryover effects cannot be excluded, although the overall imaging-method effects were retained in the one-leg-per-participant sensitivity analyses. Sixth, although an a priori sample-size calculation was conducted during study planning, the original calculation records and assumptions were not available and could not be verified retrospectively. Consequently, the adequacy of the sample size cannot be established. The study may have had limited power to detect smaller effects, particularly for vascular signal presence and grade; therefore, non-significant findings for these outcomes should be interpreted cautiously. Finally, we did not include a no-exercise control condition, systemic hemodynamic measurements, or an independent anatomical or flow-

reference standard. Therefore, the observed temporal changes cannot be attributed exclusively to local tendon loading, and the differences between PD and MVI cannot be interpreted as evidence of the anatomical accuracy or superiority of either method.

Conclusion

The primary finding of this study was that intratendinous vascular signals in asymptomatic Achilles tendons changed transiently after acute hopping exercise, a loading mode not previously examined in this context. Signal counts were highest at 5 min post-exercise and subsequently decreased toward the pre-exercise level during early recovery, indicating a measurable acute change in detected vascular signals following a short-duration, high-intensity hopping task, consistent with previous findings after running-based exercise. These findings indicate that measurement timing is important when evaluating tendon vascular signal changes observed following exercise and that assessment at 5 min may be useful for capturing the largest observed change following the short-duration hopping protocol used in this study.

Among the evaluated analytical metrics, signal count captured more temporal differences than signal presence or grading and provided greater granularity for monitoring subtle physiological changes. The time effect for signal count was also retained in the one-leg-per-participant sensitivity analysis, but those for signal presence and grade were not.

In the comparison between imaging methods, PD yielded higher signal counts, whereas representative MVI images showed more continuous vascular signal patterns and fewer visible motion artifacts. Thus, PD and MVI may provide complementary information: PD may depict a broader range of vascular signals, while MVI may provide a different representation of vascular signal continuity and morphology. However, because no independent anatomical or flow-reference standard was used, we could not establish the anatomical accuracy or superiority of either imaging method in this study.

These findings provide a methodological basis for future studies investigating physiological and pathological tendon vascular signal responses. Comparisons among asymptomatic individuals, trained athletes, and patients with Achilles tendinopathy are required to determine whether the magnitude and duration of the post-exercise vascular signal response can help distinguish physiological adaptation from tendon pathology.

Ethics approval

The study protocol was approved by the Ethics Committee of the University of Tsukuba (Approval No. TAI 025-17). The study was conducted in accordance with the Declaration of Helsinki.

Consent to participate

All participants provided written informed consent before their participation.

Authors' contributions

NI contributed to the study design, data acquisition, statistical analysis, and drafting of the manuscript. KT contributed to the data acquisition and interpretation of data. HT contributed to the conception and design of the study, supervision, and critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Funding

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 23K27955.

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Supplementary Material

Supplementary Table S1. Model specifications and diagnostic results.

A. Final model specifications				
Outcome	Distribution / link	Participant variance	Leg variance	Convergence
Vascular signal count	Negative binomial type 1 / log	2.271	0.693	Converged
Vascular signal presence	Binomial / logit	5.069	3.090	Converged
Vascular signal grade	Cumulative logit; flexible thresholds	5.224	2.405	Converged
All final models included imaging method and time as fixed effects and random intercepts for participant and leg nested within participant. Non-significant method-by-time interactions were removed. Models were estimated by maximum likelihood with the Laplace approximation. All final models converged with positive-definite Hessian matrices.				
B. Candidate distribution comparison for vascular signal count				
Candidate model	df	AIC	Delta AIC	Convergence
Negative binomial type 1, NB1	15	883.06	0.00	Converged
Poisson	14	884.71	1.65	Converged
Negative binomial type 2, NB2	15	886.71	3.65	Converged
Zero-inflated negative binomial type 2, ZINB2	16	888.71	5.65	Converged
AIC, Akaike information criterion; ΔAIC, difference in AIC relative to the model with the lowest AIC; NB1, negative binomial type 1; NB2, negative binomial type 2; ZINB2, zero-inflated negative binomial type 2. All candidate models converged with positive-definite Hessian matrices. The NB1 model was selected because it had the lowest AIC.				
C. Simulation-based residual diagnostics				
Outcome / model	Uniformity P	Dispersion P	Zero-inflation P	Outlier P
Vascular signal count / NB1	0.256	0.296	0.612	1.000
Vascular signal presence / binomial	0.155	0.782	0.848	1.000
Simulation-based residual diagnostics were performed using 1,000 simulations. None of the uniformity, dispersion, zero-inflation, or outlier tests was significant for either model. NB1, negative binomial type 1.				
D. Assessment of the Proportional-Odds Assumption for Vascular Signal Grade				
Term	Likelihood-ratio statistic	df	P value	
Imaging method	1.874	1	0.171	
Time	1.178	5	0.947	
The proportional-odds assumption was assessed using nominal-effects tests in a supplementary fixed-effects cumulative-link model because a corresponding test was not available for the cumulative-link mixed model. Neither imaging method nor time showed significant evidence of non-proportional odds.				

Supplementary Table S2. Primary and one-leg-per-participant sensitivity analyses.

Outcome	Effect	Primary analysis, χ^2 (df)	P value	Sensitivity analysis, χ^2 (df)	P value
Vascular signal count	Imaging method	20.12 (1)	<0.001	19.35 (1)	<0.001
	Time	22.35 (5)	<0.001	16.01 (5)	0.007
	Method $\times$ Time	1.46 (5)	0.918	0.87 (5)	0.972
Vascular signal presence	Imaging method	7.76 (1)	0.005	4.26 (1)	0.039
	Time	14.00 (5)	0.016	5.43 (5)	0.366
	Method $\times$ Time	1.12 (5)	0.953	1.39 (5)	0.926
Vascular signal grade	Imaging method	13.99 (1)	<0.001	9.92 (1)	0.002
	Time	19.90 (5)	0.001	7.48 (5)	0.188
	Method $\times$ Time	0.63 (5)	0.987	1.30 (5)	0.935
The primary analysis included 20 participants and 38 legs. The sensitivity analysis included one leg per participant, resulting in 20 participants and 20 legs. For the 18 participants with two eligible legs, the randomly selected first-tested leg was included; for the two participants with only one eligible leg, the available leg was included. χ^2 and P values were obtained from likelihood-ratio tests. Imaging method and time were tested using the reduced models containing both main effects, whereas the method-by-time interaction was tested by comparing models with and without the interaction term.					

Supplementary Table S3. Pairwise temporal contrasts in the primary analyses.

A. Vascular-Signal Count				
Contrast	IRR	95% CI	Unadjusted P	Holm-adjusted P
Pre vs Mid	0.80	0.52–1.24	0.324	1.000
Pre vs Post0	0.83	0.54–1.29	0.412	1.000
Pre vs Post5	0.46	0.31–0.67	<0.001	0.001
Pre vs Post10	0.63	0.42–0.95	0.026	0.288
Pre vs Post15	0.85	0.55–1.33	0.474	1.000
Mid vs Post0	1.04	0.69–1.57	0.864	1.000
Mid vs Post5	0.57	0.39–0.82	0.002	0.027
Mid vs Post10	0.78	0.53–1.15	0.210	1.000
Mid vs Post15	1.06	0.70–1.61	0.782	1.000
Post0 vs Post5	0.55	0.38–0.79	0.001	0.015
Post0 vs Post10	0.75	0.51–1.11	0.152	1.000
Post0 vs Post15	1.02	0.67–1.55	0.916	1.000
Post5 vs Post10	1.38	0.99–1.92	0.061	0.614
Post5 vs Post15	1.87	1.29–2.70	<0.001	0.012
Post10 vs Post15	1.36	0.92–2.01	0.125	1.000
B. Vascular-Signal Presence				
Contrast	OR	95% CI	Unadjusted P	Holm-adjusted P
Pre vs Mid	0.70	0.27–1.82	0.467	1.000
Pre vs Post0	0.89	0.34–2.31	0.807	1.000
Pre vs Post5	0.24	0.09–0.65	0.005	0.073
Pre vs Post10	0.39	0.15–1.03	0.056	0.618
Pre vs Post15	1.00	0.38–2.60	1.000	1.000
Mid vs Post0	1.27	0.49–3.28	0.628	1.000
Mid vs Post5	0.35	0.13–0.92	0.032	0.389
Mid vs Post10	0.56	0.22–1.45	0.230	1.000
Mid vs Post15	1.42	0.55–3.70	0.467	1.000
Post0 vs Post5	0.27	0.10–0.73	0.010	0.124
Post0 vs Post10	0.44	0.17–1.15	0.094	0.846
Post0 vs Post15	1.13	0.43–2.93	0.807	1.000
Post5 vs Post10	1.60	0.62–4.18	0.333	1.000
Post5 vs Post15	4.10	1.54–10.95	0.005	0.073
Post10 vs Post15	2.56	0.98–6.69	0.056	0.618
C. Vascular-Signal Grade				
Contrast	OR	95% CI	Unadjusted P	Holm-adjusted P
Pre vs Mid	0.66	0.18–2.35	0.335	1.000
Pre vs Post0	0.74	0.21–2.63	0.481	1.000
Pre vs Post5	0.21	0.06–0.75	<0.001	0.005
Pre vs Post10	0.37	0.11–1.31	0.021	0.236
Pre vs Post15	0.88	0.25–3.16	0.771	1.000
Mid vs Post0	1.12	0.32–3.90	0.790	1.000
Mid vs Post5	0.32	0.10–1.10	0.007	0.082
Mid vs Post10	0.57	0.17–1.93	0.174	1.000
Mid vs Post15	1.34	0.38–4.69	0.494	1.000
Post0 vs Post5	0.29	0.08–0.99	0.003	0.039
Post0 vs Post10	0.51	0.15–1.72	0.103	0.924
Post0 vs Post15	1.20	0.34–4.18	0.675	1.000
Post5 vs Post10	1.75	0.54–5.70	0.163	1.000
Post5 vs Post15	4.14	1.20–14.28	<0.001	0.011
Post10 vs Post15	2.36	0.69–8.12	0.041	0.408

Effect estimates are expressed for the first time point relative to the second time point in each contrast. IRRs are reported for vascular-signal count, and ORs are reported for vascular-signal presence and grade. Within each outcome, all 15 pairwise comparisons among the six measurement time points were treated as one multiplicity family, and P values were adjusted using the Holm method. Unadjusted and Holm-adjusted P values are both presented. CI, confidence interval; IRR, incidence rate ratio; OR, odds ratio; Pre, pre-exercise; Mid, inter-set measurement between sets 3 and 4; Post0, immediately post-exercise; Post5, 5 min post-exercise; Post10, 10 min post-exercise; Post15, 15 min post-exercise.