

Research article

Effects of a Manual Treatment on Lumbar Microcirculation and Tissue Stiffness Following Submaximal Eccentric Trunk Extensor Exercise: A Randomized Controlled Trial

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Abstract

Recent studies have shown that the extramuscular connective tissue (ECT) is thickened and stiffened in delayed onset muscle soreness (DOMS). However, contrarily to the normal population, severe DOMS is rare in athletes or highly trained individuals. The present randomized, controlled trial therefore aimed to investigate pain as well as microcirculation and stiffness of the ECT and the erector spinae muscle following submaximal eccentric trunk extension exercise not causing DOMS. The effect of manual treatment by a therapist (myofascial release; MFR) on these parameters was to be studied. Trained healthy participants ($n = 21$; 31.3 ± 9.6 years; > 4 h exercise per week) performed submaximal eccentric exercise of the trunk extensors. One group was manually treated ($n = 11$), while the other group ($n = 10$) received placebo treatment with sham laser therapy. Stiffness of the ECT and the erector spinae muscle (shear wave elastography), microcirculation (white light and laser Doppler spectroscopy), palpation pain (100 mm visual analogue scale, VAS) and pressure pain threshold (indentometry, PPT) were assessed before (t_0), 24 h (t_{24}) and 48 h (t_{48}) after conditions. Erector spinae muscle stiffness increased after eccentric exercise from t_0 to t_{24} (0.875 m/s) and from t_0 to t_{48} (0.869 m/s). After MFR, erector spinae muscle stiffness decreased in contrast to placebo treatment at t_{24} (-0.66 m/s), while ECT stiffness remained unchanged. Oxygen saturation increased (17 - 20.93%) and relative haemoglobin decreased (-9.1 - -12.76 AU) after eccentric exercise and MFR differed from placebo treatment at t_{48} (-3.71 AU). PPT differed after MFR from placebo treatment at t_{48} (20.69 N/mm), while VAS remained unchanged. Multiple linear regression showed that ECT stiffness and group membership predicted erector spinae muscle stiffness. MFR could have a positive effect on pain, microcirculation and muscle stiffness after submaximal eccentric exercise, suggesting better recovery, which needs to be confirmed by future work.

Key words: Shear wave elastography, connective tissue, eccentric exercise, manual treatment, myofascial release.

Introduction

There is growing evidence that eccentric exercise, in contrast to concentric exercise can lead to a variety of physiological changes, such as altered neuromotor strategies,

strain-induced modulation of actin-myosin interactions or greater cortical activation (Douglas et al., 2017). These adaptations, however, are often accompanied by muscle damage and/or inflammation (Margaritelis et al., 2021). Recent research suggests that the extramuscular connective tissue (ECT), specifically the deep fascia, may be another important player in this process, particularly when muscle soreness (DOMS) develops after an initial bout of unaccustomed exertion (Vincent and Vincent, 1997; Newton et al., 2008; Wilke et al., 2022; Tenberg et al., 2022; Brandl et al., 2023c). DOMS often occurs with a delay of a few hours after unusual, particularly eccentric exercise and reaches its peak after 24 to 48 hours with heat, sharp pain and mechanical hyperalgesia (Cheung et al., 2003; Brandl et al., 2023b). For this reason, more historical theories on the development of DOMS, e.g. sarcomere damage (Fridén et al., 1981), lactate accumulation (Gleeson et al., 1998) or the influence of free radicals (Close et al., 2004) inadequately explain DOMS-related pain. Therefore, the investigation of the deep fascia and its surroundings has gained interest in more recent studies (Lau et al., 2015; Wilke et al., 2022; Tenberg et al., 2022; Brandl et al., 2023c). The thoracolumbar fascia (TLF), as the main component of the lumbar ECT, has been focused on by some researchers as it represents a major contributor to passive spine stability and muscle force generation (Bojairami and Driscoll, 2022; Brandl et al., 2022a, 2023c; 2023b). With regard to DOMS, it is further of interest due to its rich innervation with a high percentage of free nerve endings likely serving nociceptive, proprioceptive, or autonomic regulatory functions (Mense, 2019; Schleip and Stecco, 2021). In addition to the findings on the TLF as a potential supporter of the erector spinae muscle (ES) to increase muscle strength, there is growing evidence of its role in low back pain due to densification, fibrosis and loss of shearing capacity under pathological conditions (Langevin et al., 2011; Wilke et al., 2017; Mense, 2019).

The TLF has a dense, rhomboid network of nerves and blood vessels (Hoheisel et al., 2015; Mense, 2019). Swelling could reduce blood flow and subsequently lead to

deoxygenation, which is a trigger for hypoxia-induced proteins that mediate cell inflammation (Wezenbeek et al., 2018; Calanni et al., 2021). In previous work, there was evidence that such fascial restrictions, which impede blood flow through vascular compression, could be removed by manual treatment, particularly myofascial release techniques applied by a therapist (MFR), which focus on restoring the shearing capacity of the lumbar tissues (Brandl et al., 2023c). One hypothesis was that mechanical stimuli such as MFR trigger the release of neuropeptides and neurotrophins from vascular nerve endings and promote vasodilation by increasing capillary permeability (Mense, 2019). In MFR, a mechanical shearing motion is applied to the myofascial tissue, combining compression and stretching with low force and slow speed (Ajimsha et al., 2015). This approach is thought to induce lasting changes in fascial morphology and hydration, as sustained, balanced stretching is more likely to promote fascial tissue response than intermittent, uneven loading (Warneke et al., 2024). In addition, the presence of numerous free nerve endings within the fascia, which serve as proprioceptors, nociceptors or innervating blood vessels, could trigger various neuromuscular and neurovascular reflexes during MFR treatment (Stecco et al., 2016; Wu et al., 2021).

According to a systematic review, about one third of muscle injuries are associated with structural fascial damage, possibly caused by excessive stretching during eccentric exercise (Wilke et al., 2019). In some studies, an increase in swelling of ECT was observed, which is thought to be due to micro-injury-induced edema and they also found a correlation between fascial stiffening and increased DOMS (Wilke et al., 2022; Tenberg et al., 2022; Fu et al., 2024). Brandl et al. (2023c), whose work we follow up on, were able to show that there is significant swelling of the ECT including the TLF after maximal eccentric exercise, which confirms the previous findings in the extremities for the TLF as well.

In DOMS, the recruitment strategy of the ES may be impaired, which could reduce its ability to counteract movement disorders (Abboud et al., 2021). The TLF has been found to affect the paraspinal muscles in pathological conditions, which has been hypothesized to involve stiffening of the TLF and ES, which could lead to reduced shearing capacity (Brandl et al., 2022a). Therefore, the development of therapeutic measures that prevent or counteract the influence of DOMS on these structures was widely demanded (Close et al., 2004; Nahon et al., 2021; Brandl et al., 2023c). However, in competitive sports, the extent of severe DOMS after tournaments (e.g. multiple matches in one week in soccer, martial arts competitions, athletics championships, etc.) tends to be small due to regular training and familiarization with the load (Barbas et al., 2011; Mohr et al., 2016). Besides DOMS, as mentioned above, eccentric exercise is known to elicit fundamentally different physiological responses than concentric exercises that challenge athletes in different disciplines (Douglas et al., 2017). As far as the authors are aware, no study has explicitly focused on submaximal eccentric exercise without or only light DOMS, which better reflects competitive sport scenarios. Therefore, experimental studies, as in most ran-

domized controlled trials, should rather focus on the treatment mechanisms after submaximal eccentric exercise (Mizumura and Taguchi, 2024).

The aim of this study was to investigate the effects of a submaximal eccentric exercise protocol on ECT and ES stiffening as well as on microcirculation and to determine whether a set of MFR has an effect on these mechanisms. Furthermore, the interrelation between stiffening of the ECT, ES and MFR treatment was to be investigated. Our hypothesis was that microcirculation would be reduced due to TLF alterations and muscle as well as ECT stiffness would increase. DOMS was considered not to occur, but that sensitivity to pain would be increased. It was thought that MFR alters these mechanisms to reduce pain sensitivity, increase microcirculation and reduce stiffness. We further assumed that the stiffness of the muscle and fascial tissue and the group membership of the participants would be related.

Methods

We conducted a randomized controlled trial using a repeated-measures, between-within-subjects design. All participants underwent a submaximal eccentric exercise protocol and were randomly assigned to an intervention (MFR) or placebo control group immediately after the exercise. The results of microcirculation, stiffness and pain were examined in the following days.

The study protocol was prospectively registered with the German Clinical Trials Register (DRKS00031201). It was reviewed and approved by the ethical committee of the Diploma Hochschule, Germany (Nr.1065/2023) and conducted in accordance with the declaration of Helsinki (World Medical Association, 2013). All participants provided written informed consent.

Setting and participants

The study was conducted in a university of a major city in northern Germany. The sample size was calculated based on a previous study on thoracolumbar fascia changes after eccentric exercise ($f = 0.3$, $\alpha \text{ err} = 0.05$, $1 - \beta \text{ err} = 0.8$; Brandl et al., 2023c). Assuming a drop-out rate of 10%, we enrolled $n = 21$ participants. The acquisition was carried out through direct contact and the distribution of information material at the university.

Inclusion criteria were: (a) generally healthy constitution; (b) female or male sex, (c) age between 18 to 50 years; (d) a maximum combined dermis and subcutaneous adipose tissue thickness of 7 mm in the measurement area of the ultrasound; (e) a score on the International Physical Activity Questionnaire (IPAQ; Meh et al., 2021) of more than 4 hours of strenuous exercise per week.

Exclusion criteria were: (a) contraindication to exhausting trunk extension exercises (i.e., fractures, tumors, infections, severe cardiovascular, neural, and metabolic diseases); (b) pregnancy; (c) rheumatic diseases; (d) medication affecting blood circulation, pain or mind; (e) intake of muscle relaxants; (f) skin changes (e.g. neurodermatitis, psoriasis, urticaria, decubitus ulcers, hematoma); (g) overuse disorders, surgery or other scars in the lumbar region;

(h) previous mental illness; (i) surgery in the last three months; (j) acute inflammation.

Randomization and interventions

The volunteers were first checked for eligibility by the investigator and then assigned to the MFR or placebo group using block randomization (Urbaniak and Plous, 2013). After a 10-minute resting phase on a therapy table in the prone position, the baseline measurements were taken. After a short warm-up (5 minutes of light jogging), the participants performed an eccentric exercise protocol (see below). Afterwards, they received the respective group-specific intervention from a therapist with more than 15 years of professional experience in manual therapy and a master's degree in osteopathy.

The MFR treatment (group 1) consisted of five techniques applied in sequence (Figure 1):

- Sustained manual pressure on the lateral raphe (a triangular structure that represents the connection of the transversus abdominis muscle to the TLF), performed with the therapist's fingertips 1-4 (Figure 1A).
- Lateral stretching of the TLF, performed with the therapist's hands (Figure 1B).
- Longitudinal sliding along the lumbar paravertebral muscles, performed with the therapist's open fist (Figure 1C).
- Longitudinal extension of the TLF, performed with the therapist's hands (Figure 1D).
- Unilateral longitudinal stretch of the TLF, performed with the therapist's hands (Figure 1E).

The force exerted on the tissue was moderate and between 25 and 100 N. The duration of each technique was 60 to 90 seconds, resulting in a total treatment duration of 8-10 Minutes. For a detailed description, see Brandl et al. (2023a).

The placebo group (group 2) received a sham intervention with green light imitating a laser therapy device targeting both sides of the lumbar region of the ES. The therapist and the participants had to wear protective goggles in order to increase the credibility of the placebo group treatment. Intervention duration was identical to the MFR treatment (8 - 10 minutes).

Both groups, MFR and the placebo group, filled out the Credibility and Expectancy Questionnaire, which

showed a high internal consistency of Cronbach's $\alpha = 0.86$ and a high reliability of $r = 0.75$ to 0.82 . There were no significant differences between the groups as tested with the Mann-Whitney U test ($p = .102$), indicating that both the MFR and the placebo group interventions generated the same credibility and expectancy.

Eccentric exercise protocol

The eccentric exercise protocol was previously described by Brandl et al. (2023b). The participants used a back extension bench (Finnlo Tricon, Hammer Sport AG, Neu-Ulm, Germany) to perform trunk extensions. Starting from a position parallel to the floor, they flexed their trunk to an angle of 40° for 3 seconds before quickly returning to the starting position after approximately 1 second. Each set consisted of 25 trunk extensions with 10 seconds rest between sets in flexed position. In order to achieve submaximal load, participants were instructed to repeat the exercise under time announcement until they reached a rating of perceived exertion (RPE) of 7 - 8 on Borg's CR-10 scale (Williams, 2017). The total number of repetitions was compared with a previous study (Brandl et al., 2023c) in which maximum exhaustion was induced in a similar population and reached 70.57% of the repetitions of the previous study (41 ± 9.47 vs. 58.1 ± 19.6).

Outcomes

Lumbar tissue stiffness using ultrasound elastography, myofascial lumbar microcirculation, palpation pain, and pressure pain threshold (PPT) were measured before (t_0), one (t_{24}) and two days (t_{48}) after exercise.

Lumbar stiffness

The assessment of soft tissue stiffness using shear wave ultrasound elastography (SWE) after eccentric exercise has already been described by other authors (Wilke et al., 2022; Fu et al., 2024). The method is known for its high inter-rater reliability (ICC: 0.89 to 0.95) and test-retest reliability (ICC: 0.70 to 0.97) in the assessment of musculoskeletal structures (Dubois et al., 2015; Roskopf et al., 2016; Taş et al., 2017). Briefly, SWE generates a color-coded map overlaid on the conventional ultrasound image, with blue indicating high stiffness and red indicating low stiffness, based on the raw measurement data (Taljanovic et al., 2017).

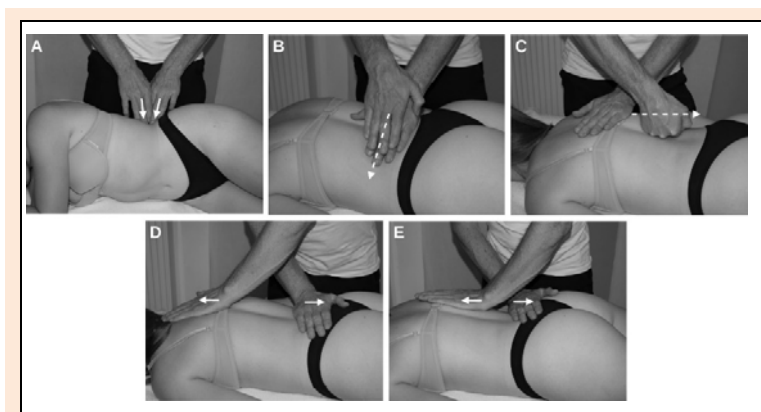


Figure 1. Myofascial release and placebo treatment at the TLF. A Manual sustained pressure on the lateral side. B Laterally extending the TLF. C Sliding longitudinally along the lumbar paravertebral muscles. D Lengthwise extension of the TLF. E Lengthwise unilateral extension of the TLF. Arrows in white indicate the direction of tissue stretching during myofascial release treatment.

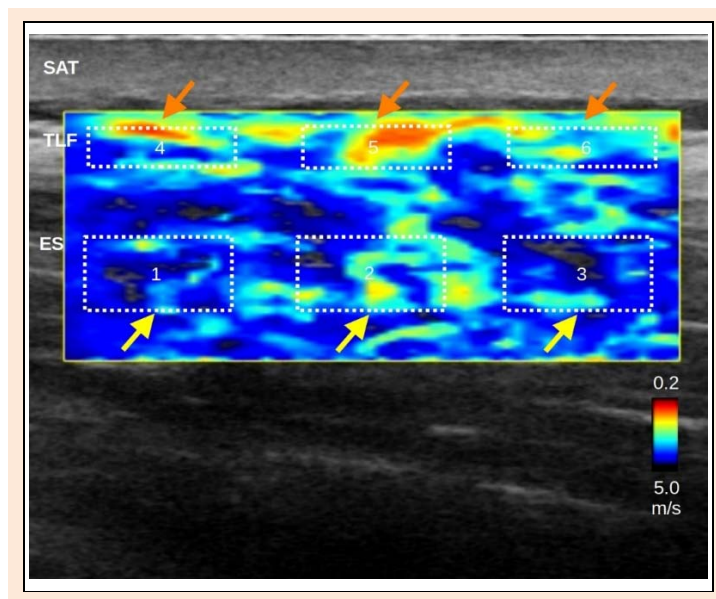


Figure 2. Ultrasound elastography. Measurements were performed in equidistant rectangular areas within the thoracolumbar fascia (orange arrows) and the erector spinae muscle (yellow arrows). SAT, subcutaneous adipose tissue; ECT, extramuscular connective tissue; ES, erector spinae muscle.

The participants lay in prone position on a treatment table for the measurement. First, the transverse process of the second lumbar vertebra was located using an SWE device (MyLabX9, Esaote, Genua, Italien) equipped with a 10L4 linear array probe (50 mm width) with a display depth of 6.5 cm. The transducer was then placed longitudinally 4 cm lateral to the spinous processes at this level, and the display depth was changed to 4 cm. A horizontal, waterproof pencil mark was made on the skin in the centre of the transducer. The side of the measurement was determined randomly beforehand (Urbaniak and Plous, 2013).

Within an area of 20 x 40 mm, three equidistant rectangular fields (3 x 10 mm) were marked out for the assessment of stiffness in the deep fascia (ECT including TLF) and three fields (7 x 10 mm) for the assessment of stiffness in the ES. Three averaged values were obtained for ES and ECT stiffness each (Figure 2).

Myofascial lumbar microcirculation

The O2C laser and white light spectroscopy (LEA Medizintechnik, Giessen, Germany) was used to measure the myofascial lumbar microcirculation in the predominantly extramuscular connective tissue of the thoracolumbar fascia at a depth of 8 mm. Therefore, the point previously marked with a pencil was used for data acquisition with a fiber probe attached to an adhesive film (LEA Medizintechnik, Giessen, Germany). The device can thus determine the blood flow, the oxygen saturation (sO_2) and the relative haemoglobin concentration (rHb) in the tissue. The method was described in detail by Brandl et al. (2023a), and the intrarater reliability was excellent with an ICC of 0.99 (Brandl et al., 2023a).

Palpation pain

Palpation pain determined with the method of Lau et al. (2015) was used to quantify DOMS. An investigator palpated the ES longitudinally on the pencil mark and applied a pressure of approximately 400 kPa with the middle and

index fingers of the right hand, which was repeated three times. Participants then used a 100-mm analogue scale (VAS) to indicate the intensity of the pressure pain, with the scale ranging from 0 for no pain to 100 for maximum pain. Prior to data collection, the experimenter was trained with a force gauge to ensure consistent application of pressure with no more than 5% variation between trials (Lau et al., 2015).

Pressure pain threshold

Algometry was used to quantify pain sensitivity (pressure pain threshold; PPT) according to the guidelines of quantitative sensory testing (Rolke et al., 2006). It was determined using the electronic IndentoPro device (IndentoPro, Fascia Research Group, University of Ulm; Institute of Human Movement Sciences, University of Chemnitz, Germany) by applying pressure to the lumbar myofascial tissue marked with a pencil until the patient reported feeling pain in addition to the mechanical sensation of pressure. This was achieved by pressing the IndentoPro plunger into the tissue with a gradually increasing force of approximately 0.5 N/mm per second (50 kPa/s) until the participant reported feeling additional pain, at which point they were instructed to say the word "now". The force at this point was recorded in N/mm. The procedure was repeated twice with a pause of 10 s each time, and the average of the measurements was used for further analysis. PPT algometry after eccentric exercise showed low standard errors of 23.3 kPa, a low coefficient of variation of 5.6% to 8.9% and a high reliability with an ICC of 0.92 to 0.98 (Chen and Nosaka, 2006; Nguyen et al., 2009).

Statistical analysis

Mean, standard deviation, and 95% confidence interval were determined for all parameters. Group differences in non-parametric epidemiological data were tested using Mann-Whitney U-test.

Mixed-repeated-measures (within-between design) ANOVA with Greenhouse-Geisser adjustment in case of

violations of sphericity or its robust equivalent (using the R-package "WRS2") for outcomes not meeting the homogeneity of variance criteria and pairwise comparisons were performed with Tukey HSD correction for outcomes. A multiple linear regression model was applied to test whether the ECT stiffness (first predictor variable) and the intervention (group membership; second predictor variable) had an effect on the stiffness of the ES (dependent variable). According to Cohen (1988), the resulting R^2 values were interpreted as "weak" (0.02 to 0.13), "moderate" (0.13 to 0.26) or "substantial" (0.26 to 1.0).

The data of two participants were missing at time t_2 (48 hours after the eccentric exercise). As the participants were unable to attend for occupational reasons, they were missing completely at random. The data were imputed using the R package "missForest" (Stekhoven and Bühlmann, 2012). Outliers were inspected manually and 90% winsorised for parametric tests (Kennedy et al., 1992). The significance level was set at $p = 0.05$. All analyses were carried out using the software R, version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

As per the sample size calculation, 7 women and 14 men took part in the study. The baseline characteristics are shown in Table 1. No adverse events or drop-outs were recorded.

Erector spinae and extramuscular connective tissue stiffness

Robust mixed-repeated-measures ANOVA (to meet the criteria of homogeneity of variance) revealed a significant interaction of ES stiffness between time and group ($F = 10.960$, $p = 0.004$). Pairwise comparisons showed a significant decrease in the MFR group versus the placebo group 24 h after eccentric exercise (-0.66 m/s, $p = 0.015$). There was a statistically significant main effect of time ($F = 19.0$, $p < 0.001$). Pairwise comparisons showed a significant increase 24 h (0.875 m/s, $p < 0.001$) and 48 h after eccentric exercise (0.869 m/s, $p = 0.002$) versus baseline (Table 2; Figure 3A).

Table 1. Baseline characteristics.

	Group	sex	N	Mean	95% Confidence Interval		SD	P-value*
					Lower	Upper		
Age	PLC	w	3	28.57	-2.17	59.30	123.715	.114
		m	7	28.37	19.May	37.69	100.783	
	MFR	w	4	31.55	24.56	38.54	43.951	
		m	7	35.27	25.37	45.17	107.057	
Height	PLC	w	3	Oca.69	Oca.49	Oca.89	0.0808	.415
		m	7	Oca.85	Oca.82	Oca.88	0.0320	
	MFR	w	4	Oca.72	Oca.68	Oca.76	0.0231	
		m	7	Oca.81	Oca.74	Oca.88	0.0743	
Weight	PLC	w	3	74.33	44.70	103.97	119.304	.062
		m	7	86.14	81.07	91.22	54.903	
	MFR	w	4	65.75	58.13	73.37	47.871	
		m	7	80.71	76.42	85.01	46.445	
BMI	PLC	w	3	25.83	20.28	31.39	22.368	.084
		m	7	26.37	22.67	30.Tem	40.003	
	MFR	w	4	21.88	18.69	25.Haz	20.006	
		m	7	24.63	22.93	26.33	18.409	

PLC, placebo group; MFR, myofascial release group; * Mann-Whitney U-test for group comparisons.

Table 2. Descriptive statistics of Elastography parameters.

	Group	Time	Mean \pm SD	95% Confidence Interval	
				Lower	Upper
Muscle Stiffness ¹ PLC n = 10 MFR n = 11 (m/s)	PLC	t_0	3.19 \pm 0.57	2.78	3.60
	MFR		3.36 \pm 0.66	2.92	3.80
	PLC	t_{24} †*	4.39 \pm 0.48	4.04	4.73
	MFR		3.77 \pm 0.64	3.35	4.20
	PLC	t_{48} †	4.12 \pm 0.61	3.68	4.56
	MFR		3.72 \pm 0.93	3.10	4.34
Fascia Stiffness ² PLC n=10 MFR n = 11 (m/s)	PLC	t_0	3.33 \pm 0.51	2.96	3.70
	MFR		3.26 \pm 0.58	2.87	3.65
	PLC	t_{24}	3.59 \pm 0.39	3.31	3.87
	MFR		3.28 \pm 0.67	2.84	3.73
	PLC	t_{48}	3.54 \pm 0.53	3.17	3.92
	MFR		3.42 \pm 0.74	2.92	3.92

PLC, placebo group; MFR, myofascial release group; VAS, visual analogue scale; SD, standard deviation; t_0 , baseline; t_{24} , 24 h after exercise; t_{48} , 48 h after exercise. ¹ Stiffness of the erector spinae muscle. ² Stiffness of the extramuscular connective tissue. † Significant time differences to t_0 at $p < .05$ level. * Significant group differences at $p < .05$ level.

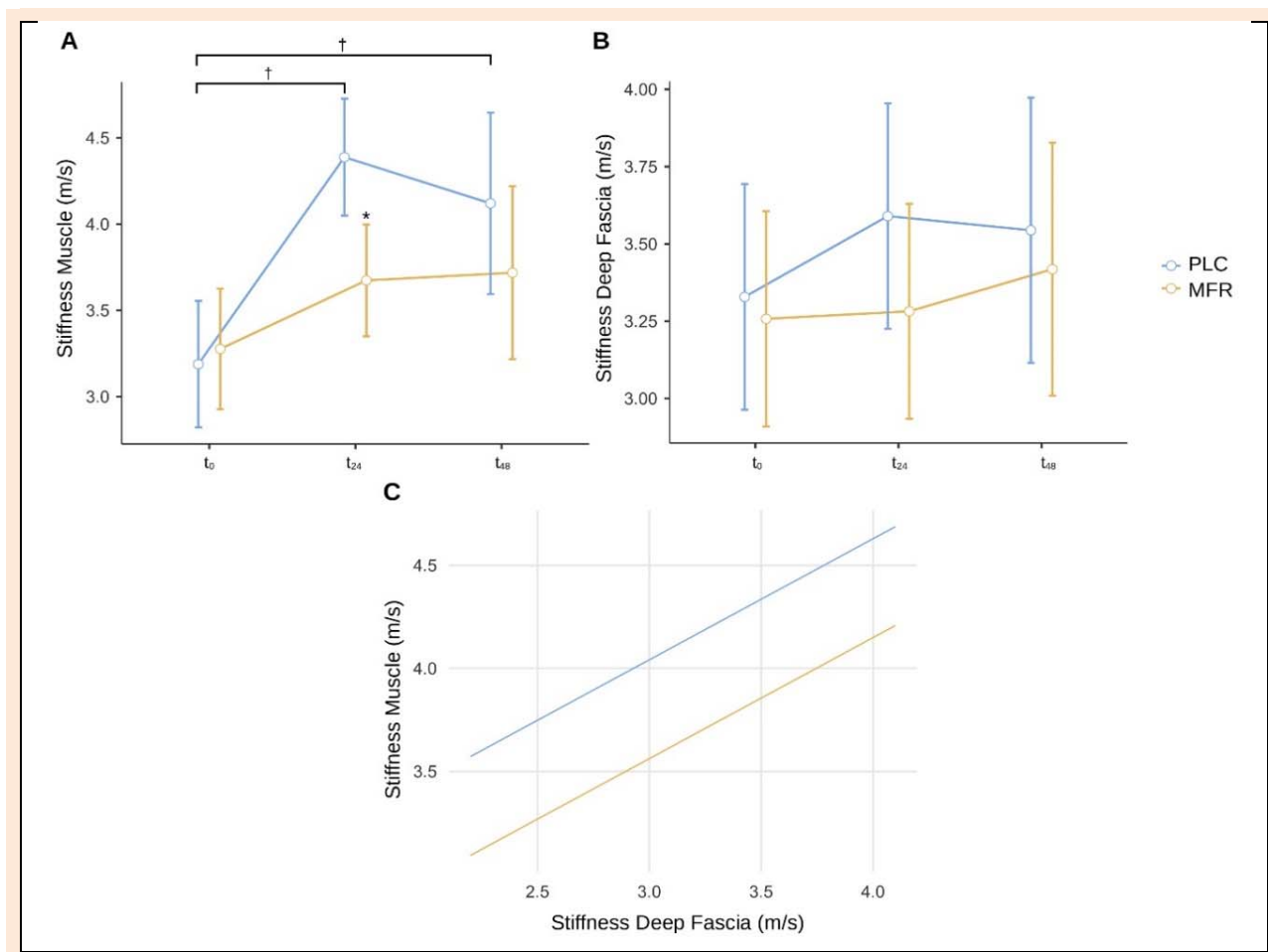


Figure 3. Elastography parameters. A Stiffness erector spinae muscle. B Stiffness extramuscular connective tissue. C Regression model. t₀, baseline; t₂₄, 24 h after exercise; t₄₈, 48 h after exercise. † Significant time differences to t₀ at p < .05 level. * Significant group differences at p < .05 level. Error bars show the 95% confidence interval.

Mixed-repeated-measures ANOVA revealed no significant interaction for the parametric outcome of ECT stiffness between time and group (the Greenhouse–Geisser adjustment was used to correct for violations of sphericity; $F(1.49, 28.39) = 0.387$, $p = 0.622$, partial $\eta^2 = 0.020$). There was no statistically significant main effect of group ($F(1, 19) = 0.729$, $p = 0.404$, partial $\eta^2 = 0.037$). There was no statistically significant main effect of time ($F(1.49, 28.39) = 0.968$, $p = 0.389$, partial $\eta^2 = 0.048$; Table 2; Figure 3B).

Myofascial microcirculation

Mixed-repeated-measures ANOVA revealed no significant interaction for the parametric outcome of blood flow between time and group ($F(2, 38) = 0.285$, $p = 0.753$, partial $\eta^2 = 0.015$). There was no statistically significant main effect of group ($F(1, 19) = 0.318$, $p = 0.579$, partial $\eta^2 = 0.016$). There was a statistically significant main effect of time ($F(2, 38) = 5.344$, $p = 0.009$, partial $\eta^2 = 0.220$). According to the Tukey-HSD, no significant differences were found (all $p > 0.05$; Table 3; Figure 4A).

Mixed-repeated-measures ANOVA revealed no significant interaction for the parametric outcome of sO₂ between time and group ($F(2, 38) = 0.464$, $p = 0.632$, partial $\eta^2 = 0.016$). There was no statistically significant main effect of group ($F(1, 19) = 0.427$, $p = 0.522$, partial $\eta^2 = 0.022$). There was a statistically significant main effect of time ($F(2, 38) = 20.221$, $p < 0.001$, partial $\eta^2 = 0.516$).

According to the Tukey-HSD, sO₂ increased significantly 24 h (17%; $p < 0.001$) and 48 h (20.93%; $p < 0.001$) to baseline (Table 3; Figure 4B).

Mixed-repeated-measures ANOVA revealed a significant interaction for the parametric outcome of rHb between time and group ($F(2, 38) = 3.70$, $p = 0.034$, partial $\eta^2 = 0.163$). According to the Tukey-HSD, rHb decreased significantly in the MFR group versus the placebo group 48 h after eccentric exercise (-3.71 AU, $p = 0.031$). There was a statistically significant main effect of time ($F(2, 38) = 20.221$, $p < 0.001$, partial $\eta^2 = 0.516$). According to the Tukey-HSD, rHb decreased significantly 24 h (-9.1 AU; $p = 0.002$) and 48 h (-12.76 AU; $p < 0.001$) to baseline, as well as from 48 h to 24 h (-3.67 AU, $p = 0.047$; Table 2; Figure 4C).

Pain

Mixed-repeated-measures ANOVA revealed no significant interaction between time and group for palpation pain ($F(2, 38) = 0.041$, $p = 0.960$, partial $\eta^2 = 0.002$). There was no statistically significant main effect of group ($F(1, 19) = 0.913$, $p = 0.351$, partial $\eta^2 = 0.046$). There was a statistically significant main effect of time ($F(2, 38) = 6.454$, $p = 0.004$, partial $\eta^2 = 0.254$). According to the Tukey-HSD, no significant differences were found (all $p > 0.05$; Table 4; Figure 5A).

Table 3. Descriptive statistics of microcirculation parameters.

	Group	Time	Mean \pm SD	95% Confidence Interval	
				Lower	Upper
Flow PLC n = 10 MFR n = 11 (AU)	PLC	t ₀	75.2 \pm 25.8	56.7	93.6
	MFR		83.4 \pm 18.1	71.3	95.5
	PLC	t ₂₄	91.8 \pm 24.1	74.6	109.0
	MFR		92.7 \pm 19.8	79.4	106.0
	PLC	t ₄₈	92.4 \pm 18.9	78.9	106.0
	MFR		95.9 \pm 21.9	81.2	110.6
sO₂ PLC n = 10 MFR n = 11 (%)	PLC	t ₀	13.80 \pm 11.57	5.52	22.1
	MFR		9.23 \pm 5.13	5.78	12.7
	PLC	t ₂₄ †	32.35 \pm 16.88	20.28	44.4
	MFR		27.02 \pm 15.57	16.56	37.5
	PLC	t ₄₈ †	31.96 \pm 17.36	19.54	44.4
	MFR		32.94 \pm 14.55	23.16	42.7
rHb PLC n = 10 MFR n = 11 (AU)	PLC	t ₀	46.0 \pm 8.93	39.6	52.4
	MFR		50.8 \pm 7.11	46.0	55.6
	PLC	t ₂₄ †	41.4 \pm 8.07	35.6	47.2
	MFR		37.2 \pm 5.71	33.4	41.1
	PLC	t ₄₈ †‡*	37.5 \pm 2.99	35.4	39.6
	MFR		33.8 \pm 1.96	32.5	35.1

PLC, placebo group; MFR, myofascial release group; AU, arbitrary units; SD, standard deviation; sO₂, oxygen saturation; rHb relative haemoglobin; t₀, baseline; t₂₄, 24 h after exercise; t₄₈, 48 h after exercise. † Significant time differences to t₀ at p < 0.05 level. ‡ Significant time differences to t₁ at p < 0.05 level. * Significant group differences at p < 0.05 level.

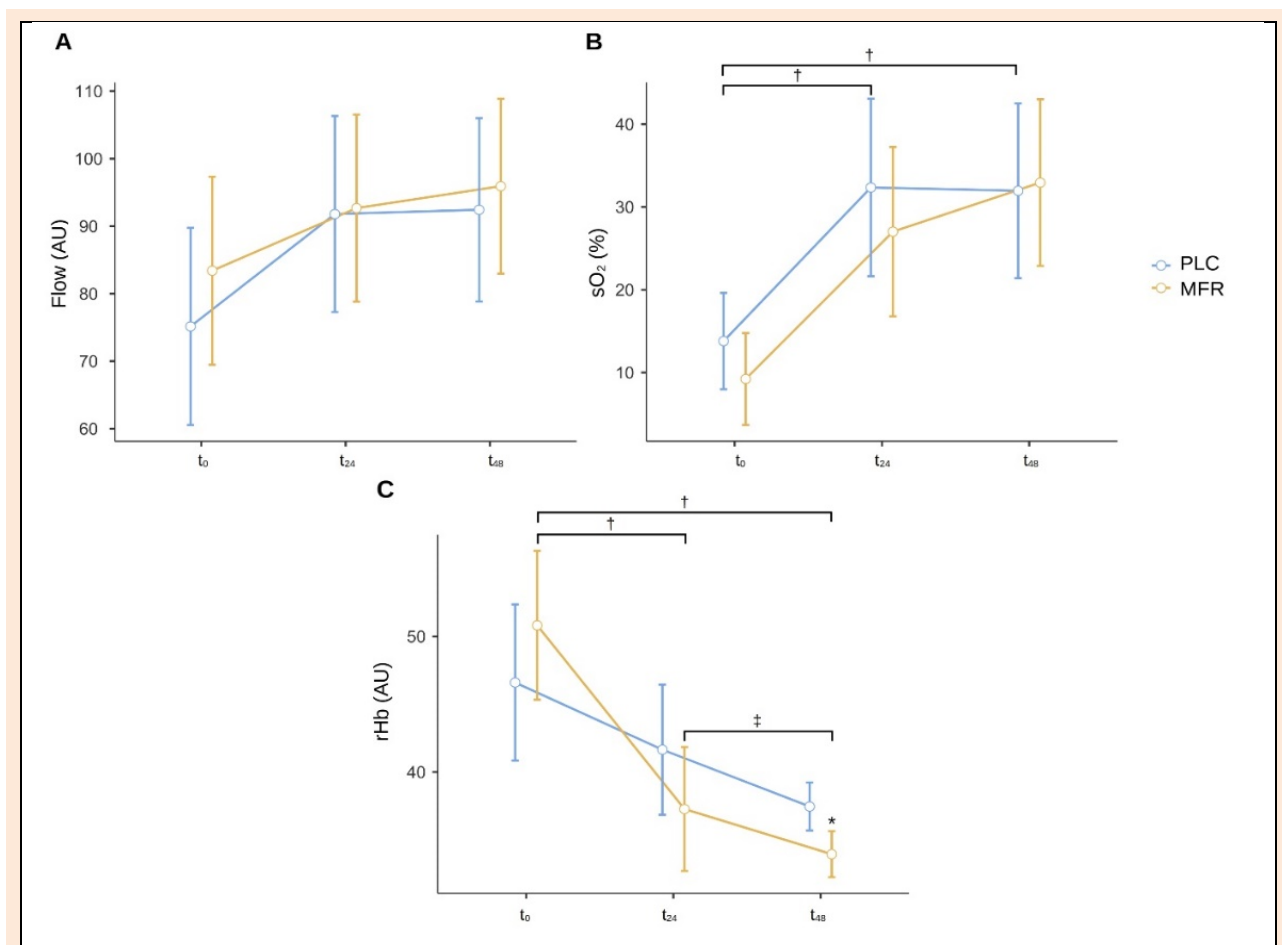


Figure 4. Microcirculation parameters. A Blood flow. B Oxygen saturation. C Relative haemoglobin. AU, arbitrary units; sO₂, oxygen saturation; rHb relative haemoglobin; t₀, baseline; t₂₄, 24 h after exercise; t₄₈, 48 h after exercise. † Significant time differences to t₀ at p < .05 level. ‡ Significant time differences to t₁ at p < 0.05 level. * Significant group differences at p < .05 level. Error bars show the 95% confidence interval.

Table 4. Descriptive statistics of pain parameters.

	Group	Time	Mean ± SD	95% Confidence Interval	
				Lower	Upper
Palpation Pain PLC n=10 MFR n = 11 (VAS mm)	PLC	t ₀	21.5 ± 12.9	12.3	30.7
	MFR	t ₀	15.5 ± 18.1	3.30	27.6
	PLC	t ₂₄	32.7 ± 16.2	21.1	44.2
	MFR	t ₂₄	27.6 ± 20.9	13.6	41.7
	PLC	t ₄₈	32.0 ± 12.7	23.0	41.1
	MFR	t ₄₈	25.0 ± 19.6	11.8	38.2
Pressure Pain Threshold PLC n=10 MFR n = 11 (N/mm)	PLC	t ₀	81.8 ± 18.6	68.4	95.1
	MFR	t ₀	84.9 ± 18.2	72.6	97.1
	PLC	t ₂₄	67.6 ± 22.6	51.5	83.8
	MFR	t ₂₄	68.1 ± 23.2	52.6	83.7
	PLC	t ₄₈ *	63.6 ± 11.3	55.5	71.7
	MFR	t ₄₈	82.6 ± 11.5	74.9	90.4

PLC, placebo group; MFR, myofascial release group; VAS, visual analogue scale; SD, standard deviation; t₀, baseline; t₂₄, 24 h after exercise; t₄₈, 48 h after exercise. * Significant group differences at p < .05 level.

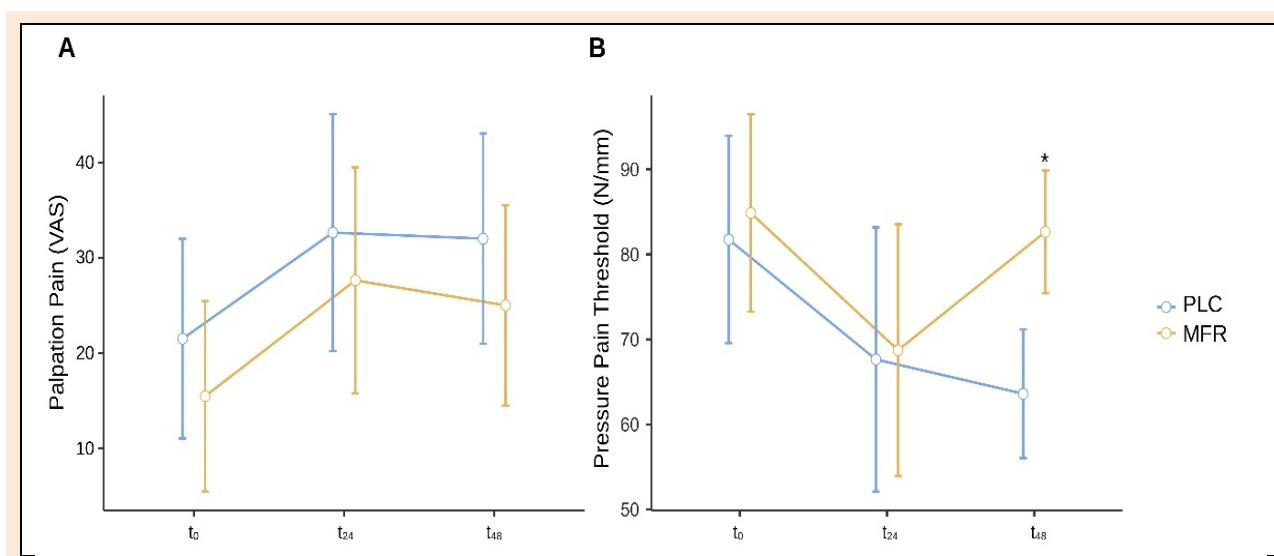


Figure 5. Pain parameters. A Palpation pain. B Pressure pain threshold. PLC, placebo group; MFR, myofascial release group; VAS, visual analogue scale; t₀, baseline; t₂₄, 24 h after exercise; t₄₈, 48 h after exercise; * Significant group differences at p < .05 level. Error bars show the 95% confidence interval.

Mixed-repeated-measures ANOVA revealed a significant interaction for the parametric outcome of PPT between time and group ($F(2, 38) = 3.49$, $p = 0.041$, partial $\eta^2 = 0.155$). According to the Tukey-HSD, PPT increased significantly in the MFR group versus the placebo group 48 h after eccentric exercise (20.69 N/mm, $p = 0.002$; Table 4; Figure 5B).

Linear relationship between ECT, ES and MFR

The R^2 for the overall linear model between ECT as well as ES stiffness and group membership was .542 (adjusted $R^2 = .491$), indicative for a high goodness-of-fit. ECT stiffness and group membership significantly predicted ES stiffness ($F(1,18) = 9.91$, $p = 0.006$). The regression equation was $ES\ stiffness = 2.279 + 0.587 \cdot ECT\ stiffness - 0.480 \cdot group\ membership$, where each m/s of ECT stiffness increases ES stiffness by 0.587 m/s, while group membership of MFR treatment decreases ES stiffness by 0.480 m/s (Figure 3C).

Discussion

The aim of this work was to investigate the effects of submaximal eccentric exercise on ECT and ES with regard to stiffening as well as microcirculation. In addition, it was investigated whether MFR has an influence on these outcomes and if there is a relationship between stiffening of the ECT and ES along with treatment conditions. The study follows on from a previous one that examined swelling and stiffening of the ECT after maximally strenuous eccentric exercise during DOMS (Brandl et al., 2023c). However, as severe post-competition DOMS rarely occurs in trained athletes, submaximal loads were investigated instead to focus practically on recovery treatments in a more realistic athlete environment (Barbas et al., 2011; Mohr et al., 2016; Mizumura and Taguchi, 2024).

ES stiffness was increased one and two days after eccentric exercise in both groups (MFR and placebo), whereas ECT stiffness remained unchanged. This is in

contrast to the results of Wilke et al. (2022), who found no changes in the stiffness of the biceps femoris muscle after maximal eccentric exercise, but an increase in stiffness of the ECT. Brandl et al. (2023b) and Tenberg et al. (2022) found ECT swelling after maximal eccentric exercise, which could trigger significant DOMS. It was assumed that the edema caused by fascial micro-injuries leads to stiffening of the ECT and not the muscle. However, Fu et al. (2024) also observed a presumably water content-dependent muscle stiffening in addition to ECT stiffening, but only found correlations between ECT and DOMS. Therefore, regarding the muscle, the results of previous studies are contradictory, as both a decrease and an increase in stiffness after eccentric exercise were found (Andonian et al., 2016; Wilke et al., 2022; Ličen and Kozinc, 2022; Fu et al., 2024).

The results of the present work suggest different mechanisms between submaximal and maximal eccentric exercise with and without the development of DOMS. Previous work has shown that injuries in the ECT increase intramuscular pressure due to edema (Schaser et al., 1999). However, this was observed in a model with severe soft tissue impacts and the extent to which this occurs after micro-injuries, as would be expected with submaximal eccentric exercise, needs further clarification.

MFR reduced ES stiffness by 14% one day after eccentric exercise (Figure 3A). MFR was found to have an effect on muscle contraction dynamics in a previous study (Brandl et al., 2022a), probably due to changes in neuromuscular control. It was hypothesized that adhesions caused altered muscle spindles and/or fluid changes in hyaluronan and the ability to lubricate the TLF, which could explain the influence of the intervention on the stiffness of the muscle (Stecco et al., 2016; Lohr and Medina-Portes, 2021; Brandl et al., 2022a). ECT stiffness does not appear to be affected by this mechanism. However, all measurements showed wide confidence intervals, indicating large inter-individual differences, whereby suspected MFR effects could not show significant effects, indicating a potentially higher complexity than could be determined by punctual ultrasound elastography measurements. While blood flow and oxygenation have been studied during and immediately after eccentric exercise, studies with longer observation periods, e.g. over several days, are rare. Ahmadi et al., (2008) monitored muscle oxygenation after eccentric exercise using near-infrared spectroscopy and found an almost similar increase in sO_2 of about 20% one day after eccentric exercise. This phenomenon was accompanied by a significant decrease in rHb and unchanged blood flow. They hypothesized that the oxygen consumption of the muscle may have decreased after eccentric exercise. However, in the present study, microcirculation was measured in the ECT rather than the muscle. Therefore, it can only be speculated what the 10% lower rHb level of the MFR group two days after eccentric exercise has for a beneficial effect. In a previous study, this was an indication of faster venous-capillary blood outflow (Brandl et al., 2022b).

Participants performed approximately 70% of repetitions of eccentric exercise of the trunk than in a previous

study in which significant DOMS was subsequently induced, indicating that the load in this study was rather submaximal. It was therefore expected that palpation pain would not change over time and between groups (MFR or placebo group), as this phenomenon was observed more in the setting of severe DOMS (Lau et al., 2015; Wilke et al., 2022; Tenberg et al., 2022; Brandl et al., 2023c; 2023b). The results were consistent with this assumption. Participants treated with MFR had a 30% higher PPT two days after eccentric exercise than the placebo group. This is in line with other studies investigating the beneficial effects of manual therapy techniques for the treatment after eccentric exercises (Nahon et al., 2021). However, study results are generally controversial and appear to be highly dependent on the setting, technique, targeted tissue of intervention and timing of application (Cheung et al., 2003; Nahon et al., 2021). Therefore, future work focusing on these modalities is needed for further clarification.

ECT stiffness and group membership predicted substantial ES stiffness. That the TLF is presumably capable of altering ES neuromuscular activity has already been mentioned. Given the controversial results of other studies (Wilke et al., 2022; Fu et al., 2024), which instead found an increase in ECT stiffness, one could hypothesize that the muscle-fascia relationship is more complex, recursively interdependent and probably related to the additional occurrence of DOMS.

Limitations

The study had some limitations. The therapist could not be blinded to sham or MFR treatment. Nevertheless, the participants and statisticians remained blinded to these modalities. According to the Credibility and Expectancy Questionnaire, both the MFR and the placebo group showed no significant differences in credibility and expectancy, indicating that the blinding was successful. Recent research suggests that the International Physical Activity Questionnaire may misrepresent seating-related items, whereas it has good reliability for other aspects. We used the questionnaire as an inclusion criterion (more than 4 hours of strenuous exercise per week). Therefore, the sitting-related items seemed to be rather irrelevant in this case. However, to mitigate a possible bias of the questionnaire, future studies should complement the assessment of physical activity with smart trackers that collect information on sedentary behavior.

The O2C laser and white light spectroscope measures tissue depth up to 8 mm, depending on the fiber optic probe used. However, it cannot be assumed that this device derives the microcirculation solely from the ECT. Participants with dermis and subcutaneous adipose tissue greater than 7 mm were excluded to minimize the influence of increased adipose tissue. Given the dense optical properties of TLF, it is likely that most of the microcirculation data originated from fascial rather than muscle tissue. Nevertheless, it is plausible that part of the blood flow originates from muscle tissue, which is why the term "myofascial" microcirculation was used throughout this study without distinguishing between TLF and muscle.

Conclusion

Submaximal eccentric exercise of the trunk significantly increases muscle stiffness of the ES 24 and 48 hours later, which is associated with increased oxygen saturation and decreased relative haemoglobin. MFR treatment could decrease relative haemoglobin 48 hours after eccentric exercise, which could hypothetically be due to increased blood flow or capillary venous outflow. However, this is highly speculative and the changes in microcirculation need to be clarified in further work. The MFR treatment had an additional effect on PPT 24 hours after eccentric exercise, suggesting a positive effect on eccentric exercise recovery. In terms of stiffness, the results were controversial to the current literature and merit further investigation. The results as a whole need to be confirmed by future work and the full understanding of the mechanisms behind the observations needs to be further addressed.

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Key points

- Submaximal eccentric exercise of the trunk increases muscle stiffness and oxygen saturation, while relative haemoglobin decreases.
- Manual myofascial release treatment decreases pressure pain threshold after submaximal exhausting exercise and potentially increases microcirculatory blood flow.
- Myofascial release treatment could have a positive effect on post-competition recovery.

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