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Original Article

Effect of moxibustion on knee joint stiffness characteristics in recreational athletes pre- and post-fatigue

Yufeng Zhang [a], Zirong Bai [b], Zhiye Zhang [b], Peng Yuan [c], Yilin Xu [d], Zun Wang [b], David Sutton [e], Jun Ren [f], Eamonn Delahunt [g], [h], Dan Wang [c], *

[a] School of Exercise and Health, Shanghai University of Sport, Shanghai, 200438, China
[b] College of Acumox and Tuina, Health Preservation and Rehabilitation, Nanjing University of Chinese Medicine, Nanjing, 210046, China
[c] School of Elite Sport, Shanghai University of Sport, Shanghai, 200438, China
[d] Sports Biomechanics Laboratory, Jiangsu Research Institute of Sports Science, Nanjing, 320113, China
[e] Shanghai Elite Sport Training Center, Shanghai, 202150, China
[f] Shanghai Institute of Cardiovascular Diseases, Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai, 200032, China
[g] School of Public Health, Physiotherapy and Sports Science, University College Dublin, Dublin, D04 V1W8, Ireland
[h] Institute for Sport and Health, University College Dublin, Dublin, D04 V1W8, Ireland

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ABSTRACT

Objective: Joint stiffness results from the coupling of the nervous system and joint mechanics, and thus stiffness is a comprehensive representation of joint stability. It has been reported that moxibustion can alleviate general weakness and fatigue symptoms and subsequently may influence joint stiffness. This study investigated whether moxibustion could enhance knee joint stiffness in recreational athletes pre- and post-fatigue.
Methods: Eighteen participants were randomized into intervention (5 males: 20.6 ± 1.5 yr; 4 females: 20.8 ± 1.5 yr) and control groups (5 males: 19.4 ± 0.9 yr; 4 females: 20.5 ± 0.6 yr). The intervention group received indirect moxibustion applied to acupoints ST36 (bilateral) and CV4 for 30 min every other day for 4 consecutive weeks. The control group maintained regular exercise without moxibustion. Peak torque (PT) of right knee extensor, relaxed and contracted muscle stiffness (MS) of vastus lateralis, and knee extensor musculoarticular stiffness (MAS) was assessed with an isokinetic dynamometer (IsoMed 2000), myometer, and free oscillation technique, respectively. Measurements were taken at three time points: pre-intervention, post-intervention/pre-fatigue, and post-fatigue.
Results: MAS ($P = 0.006$) and PT ($P = 0.007$) in the intervention group increased more from pre-to post-intervention compared with the control group. Post-fatigue MAS ($P = 0.016$) and PT ($P = 0.031$) increased more in the intervention group than in the control group.
Conclusion: Moxibustion enhanced PT and knee MAS, suggesting that this intervention could be used in injury prevention and benefit fatigue resistance in young recreational athletes.

1. Introduction

An estimated 50%–66% of all sports-related injuries involve the lower extremities, with knee injuries accounting for 30%–45% of all such injuries.[1] Anterior cruciate ligament (ACL) injury is one of the most common knee joint injuries.[2] ACL injury results in the instability of the knee joint, which negatively impacts athletic performance.[2] Long-term injury to the meniscus and cartilage may also result from ACL injury.[2] Therefore, prevention of ACL injury is essential for knee joint health.

Stiffness is often defined as the relationship between an applied force and elastic deformation.[3] It plays an integral role in regulating and controlling human movement, and contributes to joint stability.[4–6] Effective joint stiffness involves the change in torque in various components like the joint capsule, ligaments, muscles, and skin, while muscle stiffness (MS) pertains to the relationship between force and deformation within a single muscle.[7] Stiffness serves as a comprehensive risk factor for injury prediction,

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* Corresponding author.
E-mail address: danwang_dana@163.com (D. Wang).

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surpassing metrics like strength.[8,9] Research suggests that excessive stiffness may be linked to bony injuries, while insufficient stiffness may contribute to soft tissue injuries.[10,11] Maintaining optimal muscle and joint stiffness is vital for providing adequate support and protection to the joints.[5]

Fatigue is common in sports and ordinary life. It can adversely affect various biomechanical and neuromuscular factors such as rate of force development, muscle activity, strength, proprioception, and kinaesthesia.[12,13] These symptoms can negatively influence athletic performance and increase the risk of musculoskeletal injury. In addition, fatigue of active stabilizing muscles and increased joint laxity have been proposed as potential mechanisms for ACL injury.[14] It has been proved that stiffness would be changed with fatigue in our previous studies.[15] Therefore, we speculated that delaying the onset of fatigue could reduce stiffness, thus reducing the occurrence of ACL injuries.

As a traditional Chinese medicine, moxibustion can affect blood flow and “qi” (energy) through the acupoints using a burning moxa stick.[16] Studies have shown that moxibustion has anti-fatigue properties.[16] Moxibustion could reduce the damage that high-intensity exercise causes to the liver, myocardium, skeletal muscle, and kidney cells[17] and improve glycogen levels in muscle and the liver, ensuring sugar is supplied to nerves, muscles, and other tissues.[18] In addition, moxibustion could enhance levels of central nervous system neurotransmitters such as dopamine (DA),[19] which delays the occurrence of central fatigue. Furthermore, it has been reported that moxibustion can relieve lower limb fatigue after exercise, as determined by biomechanical testing.[20] However, no research has investigated whether moxibustion intervention can effectively improve knee stiffness, including knee stiffness caused by fatigue.

Therefore, this study aimed to investigate whether moxibustion could improve knee joint stiffness characteristics in recreational athletes pre- and post-fatigue. We hypothesized that moxibustion would improve knee joint stiffness, and reduce knee stiffness decline caused by fatigue.

2. Methods

2.1. Participants

Eighteen participants were randomized into intervention and control groups (Table 1). The specific inclusion criteria were: (1) recreational athletes aged 18–30 who engage in regular (at least three times per week) field and court sports such as football, volleyball, and basketball. Body mass index (BMI) ≤ 25 [if BMI >25, body fat $\leq 25\%$ (male) or $\leq 35\%$ (female) (assessed via skinfold thickness) were deemed acceptable]; (2) no serious lower limb injury necessitating substantial medical intervention or resulting in severe functional impairment within the past 3 months (3) no serious diseases (e.g., cardiovascular, respiratory, metabolic, neurological, mental, and skeletal muscle diseases, hemophilia); that affect athletic performance; (4) no drug usage; (5) received no moxibustion treatment in the past 4 weeks; (6) no alcoholism. Participants were required to fill in a medical history questionnaire and the Physical Activity Readiness Questionnaire form during screening.[15] All participants were required to sign an informed consent form before participating in the study.

2.2. Study design

A single-blind randomized controlled trial was performed and the study proposal followed the CONSORT checklist. To minimize potential biases, the data analysis was conducted by a researcher who was blinded to the group allocation of the participants. Each participant was required to visit our laboratory and be familiar with the protocol before testing. The following evaluations were performed at three time points (pre-intervention, post-intervention/pre-fatigue, post-fatigue): 1) peak torque (PT) testing of the right knee extensor, 2) relaxed muscle stiffness (relaxed MS) of the right vastus lateralis (VL), 3) contracted muscle stiffness (contracted MS) of the right VL, and 4) musculoarticular stiffness (MAS) of the right knee extensor. Participants were requested to maintain a normal diet and refrain from exercise in the preceding 24 h before testing. In the intervention group, indirect moxibustion was applied to acupoints ST36 (bilateral) and CV4 for 30 min every other day for 4 consecutive weeks. The control group maintained regular exercise without moxibustion. A flowchart of the measurements is shown in Fig. 1.

2.3. Intervention

The acupoint ST36, located on the Stomach Meridian of Foot Yangming, is a vital acupoint known for enhancing overall physical strength and nourishing the spleen and stomach.[21] The acupoint CV4, belonging to the Conception Vessel, has long been used as a primary acupoint for strengthening and nourishing deficiencies, effectively treating various types of weakness and debilitation.[21] For moxibustion treatment targeting the musculoskeletal system, both the ST36 and CV4 acupoints were specifically chosen due to their commonly recognized benefits, such as reducing exercise fatigue and improving muscle strength.[16,17] One end of the moxa stick was ignited and fixed above the acupoints with custom-made devices (Fig. 2). Moxibustion was applied at a distance of 2–3 cm from the skin. The distance between the moxa stick and the participants' acupoint was adjusted according to the participants' tolerance level. Each acupoint was stimulated for 30 min to make the skin red without burning. The frequency of moxibustion was once every two days for four weeks according to previously established protocols.[21,22]

2.4. Measurements

2.4.1. Pre-intervention assessment

Relaxed MS: The relaxed MS of the VL was measured by a myometer (Myoton-3; Muomeetria, AS, Tallinn, Estonia) composed

Table 1
Demographic data of participants.

	Intervention group (n = 9)		Control group (n = 9)		*P*-value
	Male (n = 5)	Female (n = 4)	Male (n = 5)	Female (n = 4)	
Age (yr)	20.6 ± 1.5	20.8 ± 1.5	19.4 ± 0.9	20.5 ± 0.6	NS
Height (m)	1.71 ± 0.06	1.67 ± 0.04	1.82 ± 0.06	1.61 ± 0.03	NS
Weight (kg)	64.1 ± 10.4	58.0 ± 1.6	79.6 ± 16.0	50.5 ± 5.5	NS
BMI (kg/m2)	22.0 ± 2.9	20.9 ± 1.2	24.0 ± 4.1	19.4 ± 2.1	NS

BMI, body mass index. NS, nonsignificant where $P > 0.05$.

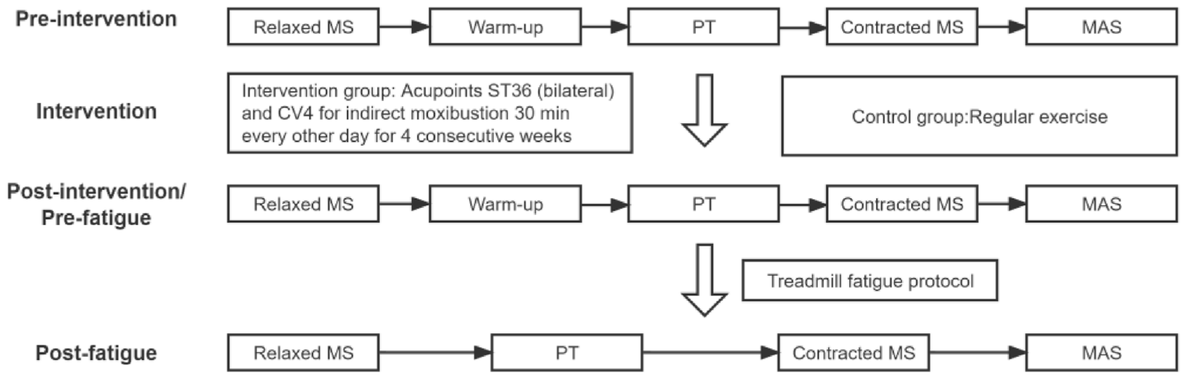

Fig. 1. Flowchart of the measurements. PT, peak torque; relaxed MS, relaxed muscle stiffness; contracted MS, contracted muscle stiffness; MAS, musculoarticular stiffness.

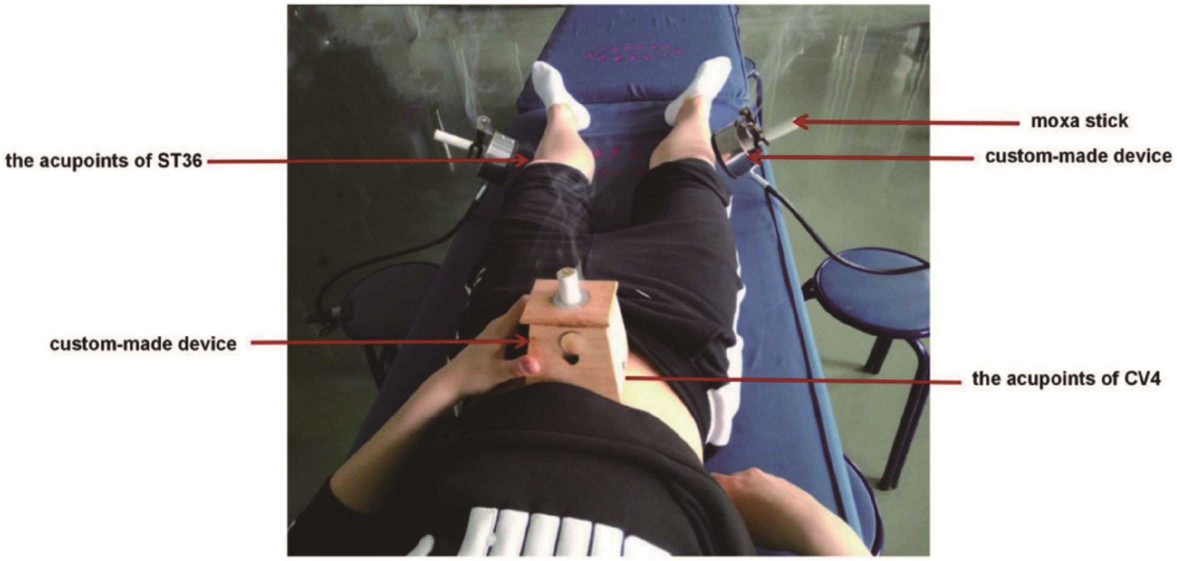

Fig. 2. Moxibustion on acupoints ST36 (bilateral) and CV4 with custom-made devices.

of a probe and an accelerometer, with a sampling frequency of 3200 HZ. We fixed the participants' pelvis with straps to prevent hip extension during the testing. The flexion angle of the hip joint was 105° (full extension of the hip joint is 180°), and the flexion angle of the knee joint was 150° (full extension of the knee joint is 180°). In addition, to reduce the compensatory contribution of the upper body, participants were asked to place their hands across their chest during the whole testing process. The probe was placed vertically on the muscle belly two-thirds of the way between the anterior superior iliac spine and the lateral midpoint of the patella. The probe was gently lowered onto the muscle belly of the VL and an automatic mechanical impact was delivered to the muscle (duration of 15 ms, force of 0.3–0.4 N, and local deformation of a few millimeters). The resultant damping free oscillation was recorded by the accelerometer inside the probe, and the real-time MS data was shown directly on the screen. Relaxed MS (no external load) was measured 5 times continuously and the average of the 5 measurements was used for analysis.

Warm-up: Participants cycled at 70–80 revolutions per minute (RPM) with a power output of 50 W for 4 min.

PT: Each participant was seated on the knee extensor dynamometer, and the lateral epicondyle of the femur was aligned with the axis of the dynamometer. The point of force transfer was at the crossbar directly in front of the lateral malleolus. PT of the right knee extensor was measured by an isokinetic dynamometer (IsoMed 2000; D. & R. Ferstl GmbH, Hemau, Germany). Participants sat in the same position as in the relaxed MS measurement. After being familiarised with the experimental procedure, they were instructed to produce a maximum voluntary isometric contraction (MVIC) of their knee extensors as quickly as possible for approximately 3 s. Strong verbal encouragement and visual target stimulation were used to ensure maximum contraction. Each participant was required to complete 3 MVIC trials, and the maximum value was used to determine the load used in contracted MS and MAS measurements. Force signals were multiplied by the individual lever arm length to produce torque values ($N \cdot m$).

Contracted MS: Contracted MS was measured at the same sitting position used in the relaxed MS measurement with an external load of 30% of the MVIC. The evaluation procedure was the same as for the relaxed MS measurement.

MAS: The MAS of the right knee joint was measured by the free oscillation technique, which is a comprehensive measurement incorporating the stiffness of the muscle-tendon unit, surrounding articular surfaces, ligaments, and skin.[15] The sitting position of the participants was the same as that of the relaxed MS measurement. They were required to support a load of 30% of the MVIC on the anteriorly distal portion of their lower leg. An external perturbation of 100–150 N was applied to the bar by the investigator and the ensuing oscillations were recorded by a uniaxial accelerometer inside the sensors (Delsys Inc, Boston, MA, USA) attached to the distal end of the lever arm of the dynamometer. Accelerometer data were sampled at 148 Hz and recorded on a personal computer with a 16-bit A/D converter. The signal was filtered by a Butterworth low-pass filter (third order) with a cut-off frequency of 4 Hz. Each participant completed 5 MAS trials with 1 min rest between trials. The average value of 3 successful trials was used for analysis.

2.4.2. Post-intervention/pre-fatigue assessment

Measurements (Relaxed MS, PT, contracted MS, and MAS) in the pre-intervention assessment period were repeated during the post-intervention/pre-fatigue assessment period just before the treadmill fatigue protocol.

2.4.3. Treadmill running fatigue protocol

The intervention and control groups completed a treadmill running fatigue protocol [23],[27] Participants were required to avoid strenuous exercise for at least 24 h and avoid drinking caffeinated or alcoholic beverages for at least 12 h before the testing. The treadmill running fatigue protocol was as follows: participants started running at 8 km/h with the treadmill at a constant grade of 1° inclination; the speed was increased by 2 km/h every 3 min. Participants were required to run until complete exhaustion [fatigued state, rate of perceived exertion (RPE) $\geq$ 17]. Verbal encouragement was provided toward the end of each run. A heart rate detector (Polar A300, Kempele, Finland) and RPE were used to record heart rate and subjective exertion, respectively, 30 s after each speed increase and immediately after the treadmill running fatigue protocol was completed.

2.4.4. Post-fatigue assessment

Measurements (relaxed MS, PT, contracted MS and MAS) in the pre-intervention assessment period were repeated during post-fatigue assessment period following the treadmill running fatigue protocol.

2.5. Statistical analysis

Statistical power was calculated as 0.996 using G-Power version 3.1.9.2, with a two-tailed α of 0.05, a total sample size of 18, 2 groups, 3 measurements, and an effect size of 0.95 based on data from this study. Specifically, the desired effect size was converted from $\eta^2{}_P$ of MAS. Two-way repeated measures ANOVA was used to investigate differences at three periods of the following four dependent variables: 1) PT, 2) relaxed MS, 3) contracted MS, and 4) MAS. The within-participant independent variable was time, and the between-participant independent variable was the intervention. Greenhouse-Geisser corrections were used when the assumption of sphericity (Mauchly's test) was violated. Levene's test was used to determine the homogeneity of variance. To reduce type II errors, effect sizes (η^2P) were calculated. Post-hoc tests were evaluated for statistical significance by using the Benjamini - Hochberg method[24] for false discovery rate (<5%). Data were reported as mean ± standard deviation (SD), and statistical analyses were conducted at a 95% level of significance ($P < 0.05$). All statistical analyses were performed using IBM SPSS Statistics 24.0.

3. Results

A total of 26 participants were screened and enrolled, but 8 participants were excluded due to the violation of eligibility criteria or withdrawal of consent. The remaining 18 participants were randomly assigned into one of two groups (intervention group: 9, control group: 9). During the study, no participant dropped out and failed to complete the analysis (Fig. 3). No side effects were reported by any participant.

Two-way repeated measures ANOVA found no significant difference in the interaction between group and time point in relaxed MS ($F = 0.546$, $P = 0.585$, $\eta^2{}_P = 0.033$) and contracted MS ($F = 0.964$, $P = 0.392$, $\eta^2{}_P = 0.057$). However , there was a significant difference in the interaction between the group and time point in PT ($F = 14.693$, $P < 0.001$, $\eta^2{}_P = 0.478$) and MAS ($F = 14.641$, $P < 0.001$, $\eta^2{}_P = 0.478$).

The post-hoc comparisons showed that PT ($F = 9.747$, $P = 0.007$, $\eta^2{}_P = 0.379$) and MAS ($F = 9.995$, $P = 0.006$, $\eta^2{}_P = 0.385$) increased more in the intervention group than the control group from pre-intervention to post-intervention/pre-fatigue. In addition, PT ($F = 5.622$, $P = 0.031$, $\eta^2{}_P = 0.260$) and MAS increased ($F = 7.265$, $P = 0.016$, $\eta^2{}_P = 0.312$) more in the intervention group from post-intervention/pre-fatigue to post-fatigue when compared to the control group (Fig. 4; Table 2).

4. Discussion

The primary findings of our study were that, when compared to the control group, the intervention group displayed increased PT and MAS from pre-intervention to post-intervention/pre-fatigue and increased PT from post-intervention/pre-fatigue to post-fatigue. However, there was no significant interaction of time and group for relaxed MS and contracted MS.

4.1. Changes from pre-intervention to post-intervention/pre-fatigue

PT increased in the intervention group compared with the control group from pre-intervention to post-intervention/pre-fatigue, which suggests moxibustion improves muscle strength. Similarly, Zhao et al. found that moxibustion on acupoints DU4 and ST36 increased grip strength in orienteering athletes.[25] Ju et al. also found that warm moxibustion combined with conventional quadriceps muscle strength training was more effective for improving muscle strength than simple quadriceps muscle strength training.[26] During PT measurement, participants were required to perform MVIC of the knee extensor as quickly as possible, an action that uses anaerobic metabolism. The energy used in the anaerobic exercise is from adenosine triphosphate-creatine phosphate (ATP-CP) decomposition and glycolysis.[27] Moxibustion can increase the body's ability to absorb nutrients, which boosts ATP, CP, and glycogen reserves in the body.[18,27,28] High-load exercise training often causes exercise-induced low blood testosterone, which is one of the main reasons for the decline in the body's exercise capacity and prolonged fatigue.[29] A related study showed that participants' PT of knee extensors improved after oral testosterone supplementation.[29] Therefore, moxibustion could increase PT through increasing blood testosterone concentration by regulating hypothalamus-pituitary-gonad axis dysfunction and imbalance during high-intensity exercise.[30]

MAS increased in the intervention group compared with the control group from pre-intervention to post-intervention/pre-fatigue. As summarized by Wang et al., volitional muscle contraction increases joint stiffness by more than four-fold, which indicates that muscle mass, muscle fiber type, cross-bridge mechanics, and other properties related to muscle force production explain MAS changes.[15,31] Therefore, increased PT in the intervention group could explain increased MAS.

4.2. Changes from post-intervention/pre-fatigue to post-fatigue

PT decreased significantly in the control group but not in the intervention group after the fatigue protocol. The decrease in the

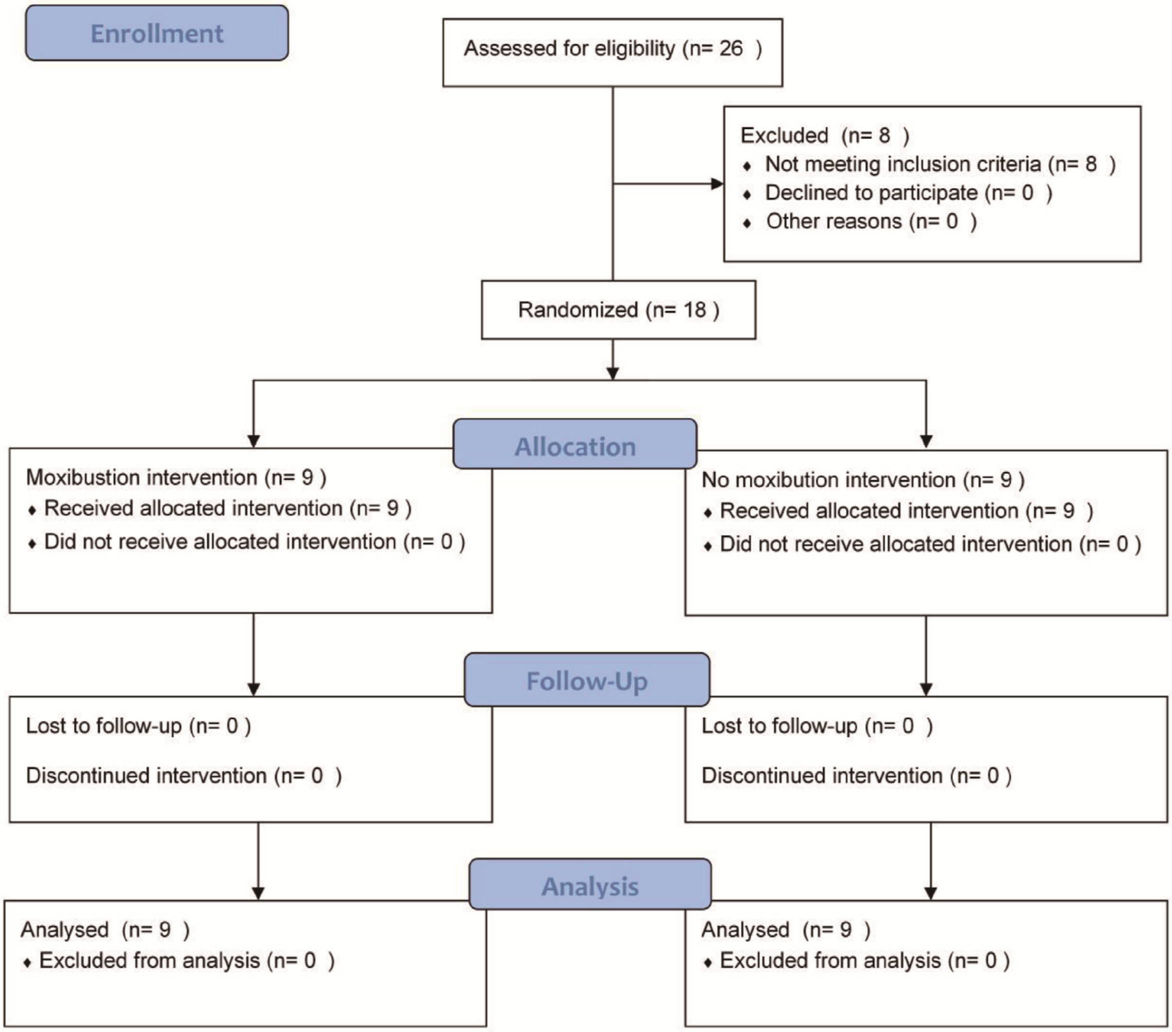

Fig. 3. Flow diagram of the recruitment and allocation of participants.

control group is consistent with previous findings.[14,15,32] First, fatigue leads to reduced coordination of extensors and flexors around the knee joint such as vastus lateralis and biceps femoris, which results in a reduction of output power because of changes in muscle coordination patterns.[33] Second, fatigue reduces the activity of Na^+-K^+-ATP enzymes and disturbs the concentration of Na^+ and K^+ inside and outside cells (i.e., the maintenance of Na^+ and K^+ gradients across sarcolemma and T tubular membranes), eventually leading to impairment of excitation contraction coupling.[33,34] In addition, fatigue can lead to an increase in metabolites such as adenosine monophosphate (AMP), inosine monophosphate (IMP), creatine, Mg^{2+}, H^+, and inorganic phosphate which inhibits normal physiological activities of cells and affects muscle contraction mechanisms.[33,35] Among these metabolites, H^+ and Pi play a major role in limiting the production of strength and energy in high-intensity exercise.[35] Accumulation of lactic acid in the blood and increased H^+ concentration reduce the activity of metabolic enzymes in the body (e.g., phosphofructokinase and glycogen phosphorylase), inhibit ATP produced by glycolysis, and compete with troponin for myofilament sliding, eventually reducing muscle contraction.[33,35] Inorganic phosphate affects Ca^{2+} release from the sarcoplasmic reticulum and accelerates detachment of myosin and actin,[35] reducing the amount of strong binding of cross-bridges.[35]

The results indicate that PT increased in the intervention group after fatigue when compared with the control group. Moxibustion can inhibit creatine kinase overflow in muscle cells, reduce urine nitrogen in serum, and reduce lactic acid content in the blood.[18] It can also increase the concentration and activity of antioxidant enzymes (e.g., glutathione peroxidase and superoxide dismutase) and increase the level of non-enzyme antioxidants such as vitamin C which reduce free radicals and prevent tissue injuries.[16–18,28,36] Moreover, moxibustion can increase energy supply for nerves, muscles, and other tissues during exercise.[18,28]

After fatigue, MAS increased in the intervention group compared with the control group, which suggests that moxibustion intervention effectively prevents a decline in MAS after fatigue. Due to the relationship between PT and MAS, the previously mentioned mechanisms for muscle contraction could also explain why MAS rises after the fatigue protocol. In addition, MAS is regulated by the central nervous system.[3] Consequently, central fatigue influences the level of MAS. DA, one of the monoamine neurotransmitters in the brain, is related to central fatigue.[37,38] Higher dopaminergic activity can improve athletic performance,[37,38] and increased central DA concentration induced by caffeine intake can suppress fatigue.[39] The smoke produced from moxibustion can also promote the release of DA in the brain.[19] Therefore, moxibustion could delay the occurrence of central fatigue by adjusting neurotransmitter levels in the brain, which may also be a reason for the rise of MAS in

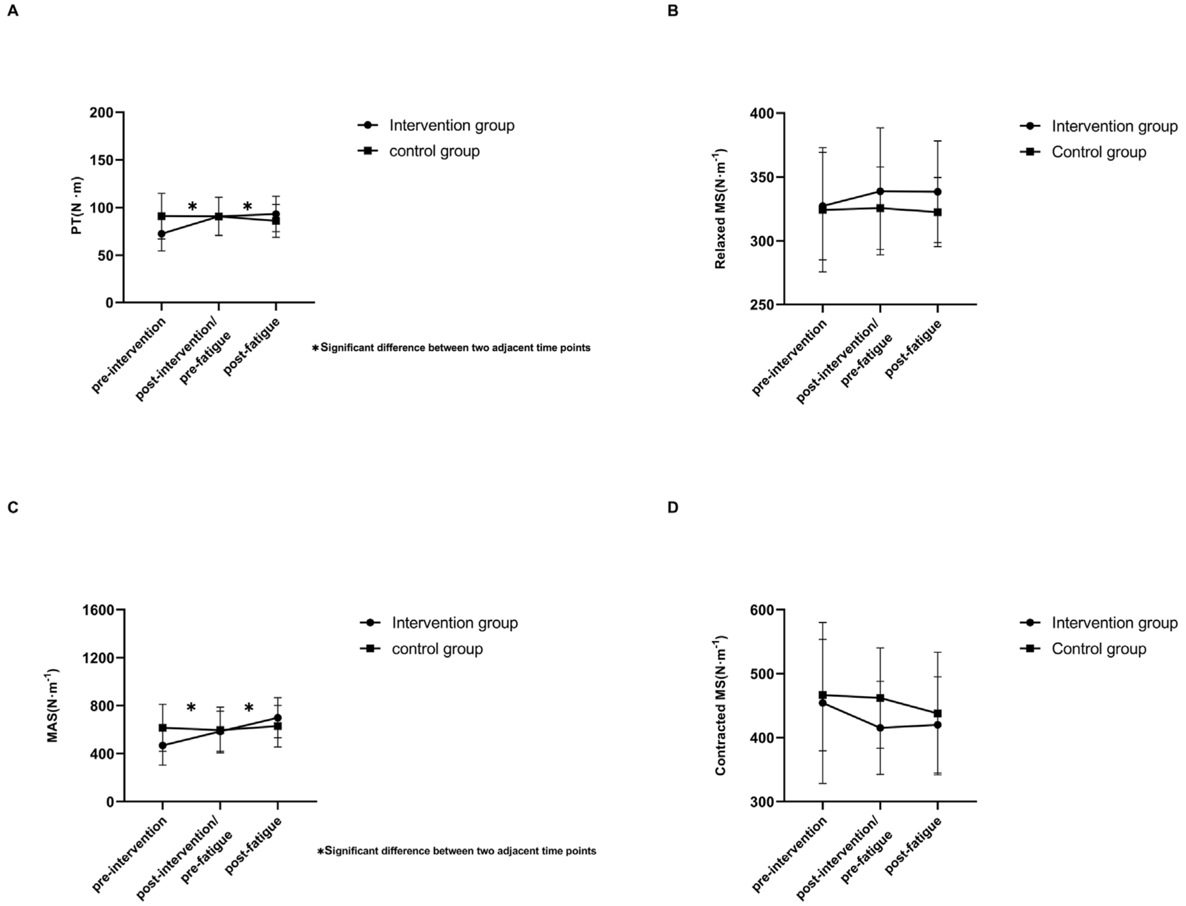

Fig. 4. Comparison of joint stiffness parameters for control and intervention groups at three periods (a) Peak torque (PT) in the intervention group increased from pre-intervention to post-intervention/pre-fatigue and from post-intervention/pre-fatigue to post-fatigue when compared to the control group. (b) There was no significant difference in relaxed muscle stiffness (MS) between the intervention group and control group at the three time points. (c) Musculoarticular stiffness (MAS) in the intervention group increased from pre-intervention to post-intervention/pre-fatigue and from post-intervention/pre-fatigue to post-fatigue when compared to the control group. (d) There was no significant difference in contracted MS between the intervention group and control group at the three periods.

Table 2
Parameters comparison between two groups at three time points ($\bar{x}$±s).

	Intervention group			Control group		
	pre-intervention	post-intervention/pre-fatigue	post-fatigue	pre-intervention	post-intervention/pre-fatigue	post-fatigue
PT (N · m)	72.5 ± 18.0	90.7 ± 20.1	93.3 ± 18.6	91.0 ± 24.0	90.7 ± 19.8	86.0 ± 17.2
Relaxed MS (N · m^{-1})	327.3 ± 42.0	338.8 ± 49.7	338.4 ± 39.7	324.3 ± 48.6	325.7 ± 32.2	322.6 ± 27.0
Contracted MS (N · m^{-1})	454.4 ± 125.8	415.4 ± 72.8	420.0 ± 75.0	466.7 ± 87.1	462.0 ± 78.3	437.9 ± 95.7
MAS (N · m^{-1})	468.8 ± 164.6	586.6 ± 167.9	699.7 ± 166.9	615.4 ± 196.6	597.1 ± 190.4	629.8 ± 172.7
RPE			19.0 ± 1.5			19.3 ± 0.7
HR			193.9 ± 9.9			191.0 ± 7.3

PT, peak torque; MS, muscle stiffness; MAS, musculoarticular stiffness; RPE, rate of perceived exertion; HR, heart rate.

the intervention group after fatigue.

4.3. Non-significant findings

There were no significant changes in relaxed and contracted MS between the intervention group and control group from pre-intervention to post-intervention/pre-fatigue and from post-intervention/pre-fatigue to post-fatigue. The quadriceps muscle is composed of four muscles: vastus medialis, vastus intermedius (VI), rectus femoris, and VL. However, we measured MS of VL as representative of MS of the quadriceps.[31] The contribution rate of VL to knee extension contraction is 20%–30%,[43] and thus the significant improvement of the PT in quadriceps could be attenuated in the MS of VL, resulting in nonsignificant changes in relaxed and contracted MS in the intervention group post-intervention and fatigue protocol.

4.4. Limitations

VI is the main extensor in the quadriceps. With increased torque of the knee extensor, the contribution rate of VI to knee extension contraction decreases, while the other three muscles' contribution increases,[40] although VI's contribution rate to the extensor torque is still above 40%.[40] However, we did not choose VI as the representative of the quadriceps due to its deep anatomical location. In the future, the development of equipment for the measurement of deep MS measurement may solve this problem.

The participants in our study were recreational athletes. Additional studies could be conducted on professional athletes to evaluate the application of these results for possible injury prevention. In addition, even though the power of the study has been calculated, the sample size was relatively small when the study was designed as a randomized controlled trial in human beings. Future research should expand the sample size to strengthen the validity of the data presented in this study.

In summary, moxibustion increased PT and MAS of the knee extensors pre- and post-fatigue but did not influence MS of VL significantly. Therefore, as an alternative intervention method, moxibustion might enhance muscle strength in the knee extensors, improve stiffness characteristics of the knee joint, and alleviate fatigue-induced stiffness decline.

Ethics approval and consent to participate

The study was approved by Shanghai University of Sports, China (approval number: 102772020RT101).

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Availability of data and materials

The data used to support the findings of this study are included within the article and available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no competing interests.

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