Relationships among hamstring muscle optimal length and hamstring flexibility and strength

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Abstract

Background: Hamstring muscle strain injury (hamstring injury) due to excessive muscle strain is one of the most common injuries in sports. The relationships among hamstring muscle optimal lengths and hamstring flexibility and strength were unknown, which limited our understanding of risk factors for hamstring injury. This study was aimed at examining the relationships among hamstring muscle optimal length and flexibility and strength.

Methods: Hamstring flexibility and isokinetic strength data and three-dimensional kinematic data for hamstring isokinetic tests were collected for 11 male and 10 female recreational athletes. The maximal hamstring muscle forces, optimal lengths, and muscle lengths in standing were determined for each participant.

Results: Hamstring muscle optimal lengths were significantly correlated to hamstring flexibility score and gender, but not to hamstring strength. The greater the flexibility score, the longer the hamstring muscle optimal length. With the same flexibility score, females tend to have shorter hamstring optimal muscle lengths compared to males. Hamstring flexibility score and hamstring strength were not correlated. Hamstring muscle optimal lengths were longer than but not significantly correlated to corresponding hamstring muscle lengths in standing.

Conclusion: Hamstring flexibility may affect hamstring muscle maximum strain in movements. With similar hamstring flexibility, hamstring muscle maximal strain in a given movement may be different between genders. Hamstring muscle lengths in standing should not be used as an approximation of their optimal lengths in calculation of hamstring muscle strain in musculoskeletal system modeling.

Keywords: Injury risk factor; Muscle biomechanics; Muscle length–tension relationship; Muscle optimal length; Muscle strain; Muscle strain injury

1. Introduction

Hamstring muscle strain injury (hamstring injury) is one of the most common injuries in track and field, soccer, Australian football, rugby, and American football involving high-speed running, jumping, and kicking, accounting for up to 29% of all injuries in these sports. Although most hamstring injuries do not require surgical treatment, athletes typically need 2 to 8 weeks to recover from the injuries and get back to their preinjury level of activity, which results in substantial time and financial losses. Athletes who sustained hamstring injuries have a high reinjury rate of 12%–31%. Reinjured hamstrings take an even longer time to recover. Repeated hamstring injury may result in longer rehabilitations, chronic pain, disability, and even the end of an athletic career. Because of the significant financial and time loss and significant consequences of hamstring injuries, intensive efforts have been made to prevent hamstring injuries and improve rehabilitation in the past 3 decades. A recent extensive review of literature with detailed injury rates, however, revealed that injury and reinjury rates remained unchanged, which indicate a need for further studies on hamstring injury prevention and rehabilitation.

To effectively prevent and rehabilitate hamstring injury, identifying risk factors for the injury is critical. Flexibility and strength are 2 proposed risk factors for hamstring injury. However, the results of clinical studies on the effects of hamstring flexibility and strength on the risk of hamstring injury are
inconsistent. Several studies showed that the risk of hamstring injury negatively correlated to hamstring flexibility,\textsuperscript{15–17} whereas other studies showed no correlation.\textsuperscript{18–20} In addition, studies showed that the risk of hamstring injury negatively correlated to the ratio of hamstring to quadriceps muscle strength,\textsuperscript{19,21,22} whereas other studies showed no correlation.\textsuperscript{16,23,24} 

Several studies using animal models demonstrated that a muscle strain injury occurs when the muscle is stretched or during an eccentric contraction, and active muscle strain reaches a certain magnitude regardless of muscle force and strain rate.\textsuperscript{25–30} These results suggest that the direct cause of muscle strain injury is muscle strain instead of muscle force and strain rate. Like other materials, muscle strain is defined as the ratio of muscle length deformation to muscle resting length, which itself is defined as the maximum muscle length at which the parallel elements are not generating force.\textsuperscript{31} Muscle resting length can be approximated as the muscle optimal length, which is defined as the muscle length at which the force generated by muscle contractile elements is maximal.\textsuperscript{32,33} The greater the hamstrings optimal lengths, the lower the maximal hamstrings muscle strains in a given athletic task with similar range of lower extremity motion.

Hamstring flexibility and strength should be correlated to hamstring optimal lengths if they are risk factors for hamstring injury. However, the relationships of hamstring muscle optimal lengths with hamstring flexibility and strength are still unknown. An in vivo study that investigated the optimal knee flexion angle at which isokinetic knee flexion moment was maximal showed that legs recovered from hamstring injury had a greater optimal knee flexion angle in comparison to legs without the injury for the same athletes.\textsuperscript{34} This result indicates that legs with hamstring injury may have shorter muscle optimal length in comparison to legs without injury. Alonso et al.\textsuperscript{35} reported that the mean optimal knee flexion angles were 75° for legs with tight hamstring muscle and 65° for legs with more flexible hamstrings muscles. Other studies showed that 6 to 8 weeks of stretching training improved hamstring flexibility and decreased optimal knee flexion angle by 4° to 10°.\textsuperscript{36,37} These results indicate that hamstring muscle optimal lengths may be correlated to hamstring flexibility. However, the relationships of hamstring muscle optimal lengths with flexibility have not been established. Our literature review also did not reveal any association between hamstring strength and muscle optimal lengths. Furthermore, several studies indicated that muscle flexibility and strength were correlated,\textsuperscript{38–40} whereas our literature review revealed that these indications have not been confirmed. In addition, hamstring muscle lengths in standing were used as an approximation of hamstring muscle optimal lengths to estimate hamstring muscle strains in athletic tasks.\textsuperscript{31–42} Obtaining hamstring muscle length in standing is easier than obtaining hamstring muscle optimal lengths. However, the relationships of hamstring muscle lengths in standing with their optimal lengths are still unknown.

The purpose of this study was to determine the relationships among hamstring muscle optimal length, flexibility, and strength, and the relationship between hamstring muscle optimal length and hamstring muscle length in standing. We hypothesized that hamstring muscle optimal length would be positively correlated to hamstring flexibility and strength. We also hypothesized that hamstring strength and flexibility would be significantly correlated. In addition, we hypothesized that hamstring muscle optimal length would be significantly different from but significantly correlated to hamstring muscle length in standing.

2. Materials and methods

2.1. Participants

Twenty-one college students (11 males and 10 females) regularly participating in exercise and sport activities volunteered to participate in this study and all participants gave written consent. The means of ages, standing heights, and body masses were 24.7 ± 2.9 years, 174.0 ± 3.1 cm, and 65.6 ± 5.9 kg, respectively, for male participants; and 23.6 ± 0.9 years, 163.8 ± 3.8 cm, and 53.5 ± 4.4 kg, respectively, for female participants. All participants had no history of hamstring injury or other lower extremity injuries that prevented them from performing the tasks in this study. The use of human subjects was approved by the Institutional Review Board of Beijing Sport University.

2.2. Protocol

Each participant had a 5–10 min warm-up including jogging and stretching, then underwent a passive straight leg raise (PSLR) test\textsuperscript{43} (Fig. 1) to evaluate hamstring flexibility and an isokinetic strength test to determine hamstring muscle optimal length for each leg. Each participant had 3 PSLR trials for each leg. The body position in maximum hip flexion angle in each PSLR trial was recorded. In the hamstring isokinetic strength test, retroreflective markers were placed bilaterally at the anterior superior iliac spine (ASIS), the top of the crista iliaca, the greater trochanter, the lateral and medial femur condyles, the lateral and medial malleolus, the tibial tuberosity, and the center of the second and third metatarsals. An additional marker was placed on the L4-L5 interface. The participant performed a calibration trial in a standing position, then the marker on

![Fig. 1. Passive straight leg raise test and hip flexion angle.](image-url)
L4-L5 was removed. The participant was then seated on the IsoMed2000 strength-testing system (D&R Ferstl GmbH, Hemau, Germany) with a hip flexion of 90°. The thigh and the lower leg of the test leg were secured on the seat and the dynamometer arm, respectively, of the strength-testing machine, in such a way that only knee flexion/extension movements were allowed and the knee flexion/extension axis was aligned with the rotation axis of the dynamometer. The rotation speed and range of the dynamometer arm movement were set at 10°/s and 110°, respectively, with the dynamometer arm positioned at leg fully extended as 0°. The participant had 3 isokinetic knee flexion trials with maximum effort for each leg with a 90 s rest between trials. Three-dimensional (3D) trajectories of reflective markers and knee flexion torques were recorded for each trial.

2.3. Data collection

The body position with maximal hip flexion angle in the PSLR test was recorded using a high-definition digital camera with its optical axis perpendicular to the sagittal plane of the participant. 3D trajectories of reflective markers were recorded using a videographic system with 10 video cameras (Oqus 400; Qualisys, Gothenburg, Sweden) at a sample rate of 100 frames per second and Qualisys Track Manager software. The knee flexion torque data measured by the dynamometer in the strength-testing system were collected using a MegaWin 2.4 system (Mega Electronics Ltd., Kuopio, Finland) at a sample rate of 100 samples per channel per second. The videographic and dynamometer data collections were time synchronized by the Qualisys Track Manager computer program package.

2.4. Data reduction

Digital photos of the maximal hip flexion angle in the PSLR test were digitized using the Shixun Motion Analysis System Version 4.0 (Beijing Sport University, Beijing, China). The hip flexion angle in each PSLR trial was reduced as the angle between the vector from the hip joint center to the knee joint center and the vector from the acromion process to the hip joint center (Fig. 1). The average of the maximal hip flexion angles from 3 PSLR trials was used as the hamstring flexibility score for each leg.

The raw 3D trajectories of all reflective markers in each hamstrings isokinetic strength-testing trial were filtered through a low-pass digital filter at a cut-off frequency of 10 Hz. The 3D local coordinates of the L4-L5 marker in a pelvis reference frame were estimated using the 3D coordinates of markers on the ASIS and the right top of the crista iliaca in the standing calibration trial. The 3D trajectories of the L4-L5 marker in the laboratory reference frame were then estimated from its 3D local coordinates and the 3D trajectories of the markers on the ASIS and the right top of the crista iliaca in each strength-testing trial.

The moment generated by hamstrings muscles (\( M_{\text{Ham}} \)) at a given time point during strength testing was reduced as the difference between the moment measured by the strength-testing system and the moment of the gravitational force on shank and foot relative to the knee joint center. The moment due to gravitational force was calculated from the mass of shank and foot and the locations of the center of mass of shank plus foot and knee joint center. The strength-testing trial that had the maximal peak moment was selected for further data reduction and used in data analysis.

Muscle length of a given hamstring muscle in a selected strength-testing trial for each leg was determined as the distance between origin and insertion of the muscle and normalized to femur length, defined as the distance between hip and knee joint centers. The 3D trajectories of the origins and insertions of each hamstring muscle in the laboratory reference frame were calculated from the location and orientation of the corresponding segment reference frames and the 3D local coordinates of the origins and insertions in the corresponding segment reference frames (Table 1). The detailed calculations can be found elsewhere.

The total hamstring force (\( F_{\text{Ham}} \)) at a given time during the strength testing was calculated as

\[
F_{\text{Ham}} = \frac{M_{\text{Ham}}}{P_{\text{BL}}R_{\text{BL}} + P_{\text{BS}}R_{\text{BS}} + P_{\text{SM}}R_{\text{SM}} + P_{\text{ST}}R_{\text{ST}}}
\]

where \( P_{\text{BL}}, P_{\text{BS}}, P_{\text{SM}}, \) and \( P_{\text{ST}} \) were relative physiological cross-sectional areas of biceps long head (28.54%), biceps short head (12.88%), semimembranosus (46.46%), and semitendinosus (12.12%), respectively, whereas \( R_{\text{BL}}, R_{\text{BS}}, R_{\text{SM}}, \) and \( R_{\text{ST}} \) were the moment arms of these 4 muscles relative to the knee joint center, respectively. The moment arm of each hamstring muscle was calculated as the distance between the knee joint center and the action line of the corresponding muscle in the sagittal plane of the shank. The maximal \( F_{\text{Ham}} \) was identified for each leg and used as the measure of hamstring strength of the given leg. The force of each hamstring muscle was calculated as the product of \( F_{\text{Ham}} \) and corresponding physiological cross-sectional area. Muscle optimal length of a hamstring muscle was identified as the muscle length corresponding to the calculated peak muscle force of the given hamstring muscle. Muscle lengths in standing position were also reduced from the standing calibration trial.

2.5. Data analysis

To test our first hypothesis, linear regression analysis with a dummy variable was performed to determine the relationships of optimal lengths with flexibility score and strength for each of the biceps long head, semimembranosus, and semitendinosus muscles. The full regression model was

\[
y = a_0 + a_1x_1 + a_2x_2 + a_3\beta + e
\]

where \( y \) was muscle optimal length, \( x_1 \) was hamstring flexibility score, \( x_2 \) was hamstring strength, \( \beta \) was the dummy variable representing gender (\( \beta = 0 \) for male, \( \beta = 1 \) for female), \( a_0 \) to \( a_3 \) were regression coefficients, and \( e \) was the residual. The best regression equation was determined through a backward selection procedure. A regression coefficient was kept in the regression equation if (1) the contribution of the corresponding term to the regression measured by partial \( R^2 \) was greater than 0.03...
and statistically significant and (2) the overall regression was statistically significant.

Linear regression analysis with a dummy variable was also performed to test our second hypothesis about the relationship between hamstring strength and flexibility score. The full regression model was

\[ y = a_0 + a_1x + a_2\beta + e \]

where \( y \) and \( x \) were hamstring strength and flexibility score, respectively, and \( \beta \) was the dummy variable representing gender (\( \beta = 0 \) for male, \( \beta = 1 \) for female). The best regression equation was determined using the same procedure and criteria as used for data analysis testing the first hypothesis.

Finally, paired \( t \) test and linear regression analysis with a dummy variable were performed to test our third hypothesis by determining the relationship of optimal length and the length in standing for each hamstring muscle. The full regression model was similar to that used in testing the second hypothesis with \( y \) as muscle optimal length and \( x \) as the corresponding muscle length in standing. All data analyses were performed using SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as the type I error rate less than or equal to 0.05.

3. Results

Length–tension relationships of the hamstring muscles were significantly affected by the flexibility score (Fig. 2). The best regression equation for the optimal muscle length \( (y) \) of the long head of the biceps as a function of flexibility score \( (x) \) and gender \( (\beta) \) was

\[ y = 0.9382 + 0.0015x - 0.0336\beta \quad (R^2 = 0.535, \ p = 0.001) \]

with the fractional contributions of flexibility score and gender to the overall regression as 0.3457 \( (p < 0.001) \) and 0.1893 \( (p < 0.001) \), respectively (Fig. 3A). The best regression

| Muscle          | Attachment | Reference frame | Side | Coordinates (%) | Reference length  |
|-----------------|------------|-----------------|------|-----------------|-------------------|
| Biceps long head| Origin     | Pelvis          | Right| -31.97 -67.07 -26.86 | Pelvis width      |
|                 | Insertion  | Tibia           | Right| -2.69 -11.32 10.30 | Tibia length      |
| Biceps short head| Origin    | Femur           | Right| 1.48 -50.49 6.40  | Femur length      |
|                 | Insertion  | Tibia           | Right| -2.69 -11.32 10.30 | Tibia length      |
| Semimembranosus | Origin     | Pelvis          | Right| -27.36 -64.22 -20.89 | Pelvis width      |
|                 | Insertion  | Tibia           | Right| -5.80 -19.16 6.05  | Tibia length      |
| Semitendinosus  | Origin     | Pelvis          | Right| -28.27 -70.29 24.15 | Pelvis width      |
|                 | Insertion  | Tibia           | Right| -2.00 -16.41 3.38  | Tibia length      |

Note: Pelvis width is the distance between the left and right anterior superior iliac spines; tibia length is the distance between the knee and ankle joint centers; femur length is the distance between the hip and knee joint centers.

Fig. 2. Hamstring muscle length–force relationships of 2 participants with different flexibility: (A) biceps long head; (B) semimembranosus; (C) semitendinosus. Muscle length was normalized as a fraction of femur length (FL). Muscle force was normalized as a fraction of body weight (BW).
equation for the optimal muscle length \( (y) \) of the semimembranosus as a function of the flexibility score \( (x) \) and gender \( (\beta) \) was

\[
y = 0.9747 + 0.0012x - 0.0178\beta \quad (R^2 = 0.350, p = 0.001) \quad (5)
\]

with the fractional contributions of flexibility score and gender to the overall regression as 0.2991 \((p < 0.001)\) and 0.0509 \((p = 0.079)\), respectively (Fig. 3B). The best regression equation for the optimal muscle length \((y)\) of the semitendinosus as a function of the flexibility score \((x)\) and gender \((\beta)\) was

\[
y = 1.003 + 0.0011x - 0.0161\beta \quad (R^2 = 0.316, p = 0.001) \quad (6)
\]

with the fractional contributions of flexibility score and gender to the overall regression being 0.2512 \((p < 0.001)\) and 0.0658 \((p = 0.056)\), respectively (Fig. 3C). Hamstring strength had no significant contribution to either of above regressions (partial \(R^2 = 0.012\), \(p = 0.505\) for the long head of the biceps; partial \(R^2 = 0.001\), \(p = 0.942\) for the semimembranosus; and partial \(R^2 = 0.001\), \(p = 0.901\) for the semitendinosus).

Hamstring flexibility score was not significantly correlated to hamstring strength \((R^2 = 0.006, p = 0.622)\). Hamstring muscle optimal lengths were significantly greater than corresponding muscle lengths in standing \((p < 0.001)\) (Table 2).

Hamstring muscle optimal lengths were not significantly correlated to corresponding muscle lengths in standing \((R^2 = 0.074, p = 0.082\) for biceps long head; \(R^2 = 0.024, p = 0.326\) for semimembranosus; and \(R^2 = 0.036, p = 0.232\) for semitendinosus).

4. Discussion

The results of this study partially support our first hypothesis that hamstring muscle optimal lengths would be positively correlated to hamstring flexibility and strength. The results showed that hamstring muscle optimal lengths were significantly correlated to hamstring flexibility score but not to hamstring strength, which partially support our first hypothesis. The best regression equations showed that the greater the flexibility score, the longer the hamstring muscle optimal length. These results confirmed the results of previous studies that the optimal

| Muscle          | Normalized muscle length | In standing position |
|-----------------|--------------------------|----------------------|
| Biceps long head|                          |                      |
| Male            | 1.09 ± 0.05              | 0.96 ± 0.02          |
| Female          | 1.07 ± 0.05              | 0.96 ± 0.02          |
| Semimembranosus |                          |                      |
| Male            | 1.09 ± 0.04              | 1.00 ± 0.01          |
| Female          | 1.09 ± 0.04              | 1.01 ± 0.02          |
| Semitendinosus  |                          |                      |
| Male            | 1.11 ± 0.04              | 0.97 ± 0.02          |
| Female          | 1.11 ± 0.04              | 0.96 ± 0.02          |

Note: Muscle optimal lengths and lengths in standing position were normalized to femur length; that is, the distance from hip joint center to knee joint center.
knee flexion angle for maximal knee flexion moment decreased as hamstring flexibility score increased, which indicate that hamstring muscle optimal lengths may be affected by hamstring flexibility.

The results of this study support flexibility as a risk factor for hamstring injury. Previous studies showed that muscle strain injury was only related to active muscle strain, not muscle force. Muscle strain is defined as the ratio of muscle length deformation to muscle optimal length, demonstrating that with the same muscle length deformity, the shorter the muscle optimal length, the greater the muscle strain. We showed that the better the hamstring flexibility, the longer the hamstring muscle optimal length. These results combined together suggest that in a given movement, athletes with good hamstring flexibility may have lower maximal hamstring muscle strains, and they imply that athletes with good hamstring flexibility may have lower risk for hamstring injury compared to athletes with poor hamstring flexibility.

The variation in hamstring muscle optimal lengths cannot be completely explained by the hamstring flexibility measure in this study. Hamstring flexibility was represented by hip joint range of motion with a straight knee in this study. Hip joint range of motion is affected not only by hamstring flexibility but also by hip joint capsule and ligament tightness. This may explain the relatively low contribution of hamstring flexibility score to regressions of hamstring muscle optimal lengths in this study.

Hamstring muscle optimal lengths were not only correlated to flexibility but also to gender. The results of this study showed that with the same flexibility score, the hamstring muscle optimal lengths of females were shorter in comparison to males. Our literature review failed to find an explanation for this gender difference. These results, nevertheless, indicate that with the same flexibility, females may have greater maximum hamstring muscle strain and thus higher risk for hamstring injury in comparison to males. This indication, however, is inconsistent with what clinical studies showed. Data from the National Collegiate Athletic Association Injury Surveillance System showed that men have significantly higher rates of hamstring injury than women in soccer, baseball, softball, and indoor track. Opal et al. showed that high school boys were at a greater risk of hamstring injury than high school girls in track and field and that there was no difference in the risk of sustaining hamstring injury between male and female collegiate athletes. One possible explanation of this discrepancy is that males might have larger range of motion compared to females when performing the same tasks. Future studies are needed to confirm the results of this study and previous clinical studies.

Although the results of this study showed that hamstring muscle optimal lengths and strength were not correlated, this does not necessarily mean that hamstring strength is not a risk factor for hamstring injury. Flexibility can be categorized as static or dynamic. Static flexibility represents the tolerance to stretch and is measured as range of joint motion, whereas dynamic flexibility represents the resistance to stretch and is measured as muscle stiffness. Biomechanical models of muscle force demonstrated that muscle strength is a determinant of muscle stiffness. In a given position, the greater the muscle strength, the greater the muscle stiffness. Increasing muscle strength may increase muscle resistance to elongation to prevent muscle from being overstretched and cause a strain injury. This may be particularly important for prevention of hamstring injury in those athletic tasks, such as kicking, in which hamstrings were substantially stretched in follow-up movements.

The results of this study showed that hamstring flexibility and strength were not significantly correlated. These results do not support our second hypothesis, indicating that differences in hamstring flexibility do not explain differences in hamstring strength across individuals. These results were inconsistent with the finding of previous studies, which demonstrated relationships among muscle strength, stiffness, and flexibility, indicating that muscle flexibility and strength were negatively correlated. This discrepancy can be explained by the effect of muscle flexibility on the muscle length–tension relationship found in this study. The results of this study showed that hamstring muscle length–tension relationships of participants who had better flexibility were shifted toward the direction of increased muscle length. With shifted length–tension relationships, a person with good flexibility would have lower muscle force compared to a person with poor flexibility when the muscle length was shorter than the optimal length (Fig. 2). The strength-testing positions described in previous studies were likely to be set in such a way that muscle lengths were shorter than optimal lengths for most of the subjects. Therefore, resulting in a negative correlation between muscle flexibility and strength. The hamstring strength in this study was measured as the maximal hamstring force in the length–tension relationship rather than joint torque or muscle force at a given lower extremity position. As the results of this study showed, this maximal concentric contraction force is not affected by the flexibility.

The results of this study showed that hamstring muscle optimal lengths were significantly longer than but not significantly correlated to corresponding hamstring muscle lengths in standing. These results partially support our third hypothesis, indicating that even if normalized to a body dimension, hamstring muscle length in a given position cannot be used as an approximation of hamstring muscle optimal length. Furthermore, optimal length is an important muscle parameter in muscle biomechanical models for estimating muscle force. A recent study demonstrated that estimated muscle forces are sensitive to optimal lengths. Using previously published muscle optimal lengths in muscle biomechanical models may result in significant errors in estimated muscle forces even if these muscle optimal lengths are normalized to a body dimension. This is because of a large between-individual variation in muscle optimal lengths owing to a large between-individual variation in flexibility, as the results of this study showed. Muscle optimal lengths may need to be individualized for accurate estimates of muscle force.

The relationships between hamstring flexibility and optimal lengths found in this study are limited as cross-sectional relationships. These cross-sectional relationships only suggest that individuals with good hamstring flexibility may have longer...
hamstring muscle optimal lengths compared to those with poor flexibility, which does not necessarily mean that improving hamstring flexibility would result in an increase in hamstring optimal muscle lengths and thus a reduction of the maximal hamstring muscle strains in athletic tasks for a given individual. Studies have demonstrated that the cross-sectional difference in muscle optimal length was due to a difference in a number of sarcomeres in series in the muscle.\textsuperscript{55–59} Whether the number of sarcomeres in series in muscles can be increased through flexibility training is still unknown. Although studies also showed that flexibility training could decrease optimal knee flexion angle,\textsuperscript{36,37} whether flexibility training actually increased hamstring muscle optimal lengths is still unknown. Future longitudinal studies are needed to confirm that a cause-and-effect relationship exists between hamstring muscle optimal lengths and hamstring flexibility.

The calculated hamstring muscle forces in this study might contain errors when muscle lengths are short. These errors would mostly likely be due to errors in calculated muscle moment arms. We calculated the moment arm of each hamstring muscle as the distance between the knee joint rotation center and the action line of the corresponding muscle. The knee joint rotation center was defined as a fixed point at the middle of the line connecting the medial and lateral femoral condyles. The actual knee rotation center moves distally toward the tibial plateau as the knee flexion angle is greater than 30°.\textsuperscript{60} The errors in the location of actual knee joint rotation center might have resulted in underestimated hamstring muscle moment arms and underestimated hamstring muscle forces when the knee flexion angle was greater than 30°. This issue may explain the inconsistency in the muscle length–tension relationships between this study and previous reports. Although these errors in calculated hamstring muscle forces do not seem to affect the results of this study, hamstring muscle moment arms may be calculated using other techniques, such as ultrasound, to avoid errors in calculating hamstring muscle forces. Furthermore, future studies are needed to confirm and explain the gender difference in the relationship between hamstring flexibility and muscle optimal length found in this study. In addition, future studies are needed to determine whether muscle flexibility affects the maximal muscle strain in \textit{in vivo} movements.

\textbf{5. Conclusion}

Hamstring muscle optimal lengths are positively correlated to hamstring flexibility across individuals. With the same flexibility score, females have shorter hamstring muscle optimal lengths compared with males. Hamstring muscle optimal lengths are not correlated to hamstring strength. Hamstring flexibility and strength are not correlated across individuals. Hamstring muscle optimal lengths are longer than but not correlated to the hamstring muscle lengths in standing.

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\textbf{Authors’ contributions}

XW carried out the experiments, performed the data processing and statistical analysis, and drafted the manuscript; BY and HL conceived of the study, and participated in its design and coordination and helped to draft the manuscript; FQ and WEG helped to draft the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

\textbf{Competing interests}

The authors declare that they have no competing interests.

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