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Human Kinetics
ORIGINAL INVESTIGATION

Rugby Players Exhibit Stiffer Biceps Femoris, Lower Biceps Femoris Fascicle Length to Knee Extensors, and Knee Flexors to Extensors Muscle Volume Ratios Than Active Controls

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Purpose: This study aimed to determine if hamstring-strain-injury risk factors related to muscle structure and morphology differed between rugby union players and controls. ***Methods:*** The biceps femoris long head (BFlh) fascicle length and passive muscle stiffness and relative and absolute muscle volume of knee flexors (KF) and extensors (KE) were measured in 21 male subelite rugby players and 21 male physically active nonathletes. ***Results:*** BFlh fascicle length was significantly longer (mean difference [MD] = 1.6 [1.7] cm) and BFlh passive muscle stiffness was significantly higher in rugby players (MD = 7.8 [14.8] kPa). The absolute BFlh (MD = 71.9 [73.3] cm^3), KF (MD = 332.3 [337.2] cm^3), and KE (MD = 956.3 [557.4] cm^3) muscle volumes were also significantly higher in rugby players. There were no significant differences in the relative BFlh and KF muscle volumes. The relative KE muscle volumes were significantly higher in rugby players (MD = 2.3 [3.7] cm^3/kg). However, the percentage BFlh fascicle length:KE (MD = –0.1% [0.1%]), BFlh/KE (MD = –0.9% [1.9%]), and KF:KE (MD = –4.9% [5.9%]) muscle volume ratios were significantly lower in the rugby players. BFlh muscle volume significantly correlated with BFlh fascicle length (r = .59, r^2 = .35) and passive muscle stiffness (r = .46, r^2 = .21). ***Conclusion:*** Future prospective studies should examine whether there are threshold values in BFlh passive muscle stiffness and BFlh fascicle length:KE, BFlh:KE, and KF:KE muscle volume ratios for predicting hamstring strain injuries.

Keywords: muscle architecture, muscle stiffness, hamstring injuries

Hamstring strain injuries (HSIs) are common in team sports and cause the greatest time lost to playing and training in rugby union.[1,2] The effect of HSI on player availability is further compounded by the high proportion of recurrent injuries,[3] suggesting that this injury is difficult to rehabilitate effectively.[4] Therefore, scientists have focused on identifying risk factors for HSIs over the last 2 decades to develop optimal hamstring injury prevention strategies.

The biceps femoris long head (BFlh) is the most susceptible to injury among the hamstrings muscle group, accounting for more than 80% of all HSIs.[5] The late swing phase of running is the most vulnerable time for hamstring injuries.[6,7] During this phase of running, the hamstrings behave as an antagonist to the quadriceps femoris and contract eccentrically to control the quadriceps femoris muscle during tibial deceleration.[8] At this moment, the biceps femoris is exposed to the highest stretch, reaching about 110% of its length.[9] HSIs generally occur when the muscle fascicles cannot resist this excessive tensile force.[10] Despite the multifactorial nature of his,[11] muscle imbalance, particularly insufficient hamstring strength in comparison with the quadriceps, has been strongly suggested as a risk factor for HSIs.[12] Muscle structural and morphological risk factors for the HSIs have also been proposed in recent years,[13–15] including shorter BFlh fascicle length,[14] and increased passive hamstring stiffness.[15] Whether the nature of rugby union match play or training leads to alterations in these risk factors and a potential increase in the risk of HSI incidence and recurrence is unclear. In addition to comparing the BFlh fascicle length and passive muscle stiffness between rugby players and controls, this study aimed to compare BFlh fascicle length to knee extensors (KE), knee flexors (KF) to KE muscle volume ratios between the groups. Strength imbalances between KF and KE, favoring KE, are risk factors for HSIs.[12] When considering that muscle volume is a strong predictor of strength and power (r^2 = .8–.9),[16] examining these ratios can be an indicator of possible strength imbalances favoring the knee extensors and can be an indicator of increased tensile force on the biceps femoris fascicles due to higher quadriceps strengths,[12] or shorter BFlh fascicle lengths.[14]

Therefore, the primary aim of this study was to compare BFlh fascicle length, passive stiffness, and thigh muscles' morphology in

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male rugby union players and healthy active controls. The secondary aim was to assess relationships between the BFlh muscle volume, fascicle length, and passive muscle stiffness. We hypothesized that rugby players would have (1) greater muscle volume due to long-term training and competition, (2) longer BFlh fascicle length due to the potentially larger muscle size, (3) higher BFlh passive stiffness due to the potentially higher muscle volume, and (4) lower BFlh fascicle length/KE, BFlh/KE and KF/KE muscle volume ratios due to potentially higher KE activities compared with healthy controls.

Methods

Subjects

Male participants were recruited via email and verbal advertisements among the rugby teams in Tokyo and the university population of Tokyo Metropolitan University. Inclusion criteria for the rugby group were (1) being male, healthy, and actively playing rugby in the Japanese university division 1 rugby union competition, (2) absence of an acute lower extremity injury, (3) absence of a known hamstring injury and traumatic knee injury history such as anterior cruciate ligament injury, and (4) being between 18 and 35 years old. Inclusion criteria for the control group were: (1) being male and healthy; (2) not having a totally sedentary lifestyle to minimize the adverse effects of a sedentary lifestyle on our measures; (3) not performing any regular strength, power sports, and any sports discipline-specific training; (4) absence of an acute lower extremity injury; (5) absence of a known hamstring injury and traumatic knee injury history such as anterior cruciate ligament injury; and (6) being between 18 and 35 years old. Training regimens, training and injury histories of the rugby group were recorded via a written questionnaire. The physical activity status of the control group was measured using the International Physical Activity Questionnaire short form.[17] The dominant thigh was determined as the preferred kicking leg. The Tokyo Metropolitan University, Arakawa campus ethics committee provided ethical approval (code: 20067) according to the Declaration of Helsinki.[18] Written informed consent, health screening questionnaires, and a standard magnetic resonance imaging (MRI) questionnaire were read and signed by the participants before study enrollment.

Design

A cross-sectional study design was used in this study to compare those habituated to rugby participation and the active control group. A priori sample size calculation was calculated for KF to KE muscle size ratio,[19] using G*Power software,[20] for 0.8 effect size, 80% statistical power, and .05 alpha level in a total of 42 participants were equally divided for both groups (21 rugby group and 21 for the control group; 1:1 allocation ratio). Additionally, the required sample size of reliability assessments for the BFlh fascicle length and stiffness measurements of this study were calculated by referring to the intraclass correlation coefficient (ICC) values of previous reliability studies investigating ultrasound-measured BFlh fascicle length and ultrasound-based shear wave elastography measured stiffness.[21,22] The lowest ICC value for the BFlh fascicle length and stiffness measurements in these studies was .81. Therefore, the required sample size for this reliability was calculated based on a .8 ICC value, which is lower than the lowest reliability value among the mentioned biceps femoris fascicle length and stiffness reliability assessment scores by the previous studies.[21,22] In light of guidelines,[23] for ICC reliability studies, the required sample size was estimated as 7 for 2 measurements, .8 ICC value, and 80% statistical power.

Methodology

Both groups underwent BFlh fascicle length measurements via B-mode ultrasonography, the BFlh passive stiffness assessments via ultrasound-based shear wave elastography, and thigh muscles' volume measurements via MRI of both legs. Additionally, measures of BFlh fascicle length and passive stiffness measurements were repeated twice on different days in 7 participants' to calculate the reliability of the measurements. The same researcher (Furuta), who was experienced in musculoskeletal ultrasound imaging, performed the scanning during the all fascicle length and passive muscle stiffness measurements alongside another experienced researcher (Yagiz).[24] The MRI scanning was performed by Kuruma an experienced researcher in MRI measures for musculoskeletal assessments. The first author (Yagiz) completed the fascicle length digitizations, and boundary tracings of the muscles for calculating the BFlh fascicle lengths, and BFlh, KE, and KF muscle volumes.

BFlh Fascicle Length

A linear array transducer of a 2-dimensional B-mode ultrasound (i18LX5 [46-mm width], Aplio i800, Canon Medical Systems) was used to assess the BFlh fascicle length by following the methodology of a recent study.[24] Participants laid down in the prone position on a standard medical bed without performing any voluntary muscle contraction. An ultrasound image of the BFlh fascicles along the BFlh muscle orientation was taken from the 50% distance between the trochanter major and popliteal crease (Figure 1). The fascicle length was calculated via the manual linear extrapolation method in light of a recent study,[24] by using the ImageJ software (National Institutes of Health).

BFlh Passive Muscle Stiffness

The BFlh muscle stiffness was measured by using the same ultrasound machine and transducer as for the BFlh fascicle length measurement by using the validated procedure for passive stiffness of individual muscles via shear wave elastography function of the ultrasound machine.[25] Precisely the same procedure for the BFlh fascicle length was followed during the BFlh muscle stiffness measurements with an addition that a 10-mm elastogram was taken from the central region of the BFlh muscle belly to calculate the passive muscle stiffness of the BFlh (Figure 2).

Muscle Volume Measurements

The MRI scans and further muscle volume calculations were completed using a 3-Tesla (3T) MRI imaging system with air coil technology (SIGNA Premier 3.0T, GE Healthcare). Participants assumed a supine position with neutral hips and extended knees in the magnet bore with a strap on both limbs to prevent unwanted movements. T-1 weighted contiguous axial MRI scans were recorded starting from the iliac crest and finishing at the tibial condyles (field of view 420 mm × 420 mm, slice thickness: 10 mm, interslice thickness: 0 mm). The KF and KE muscle boundaries were manually traced using OsiriX MD software (version 13.0.1, Pixmeo SARL). All the visible cross-sectional areas of the KE and KF muscles were outlined in each axial MRI image (Figure 3). To calculating the absolute BFlh, KE (rectus femoris, vastus intermedius, vastus medialis, and vastus lateralis), and KF (the biceps

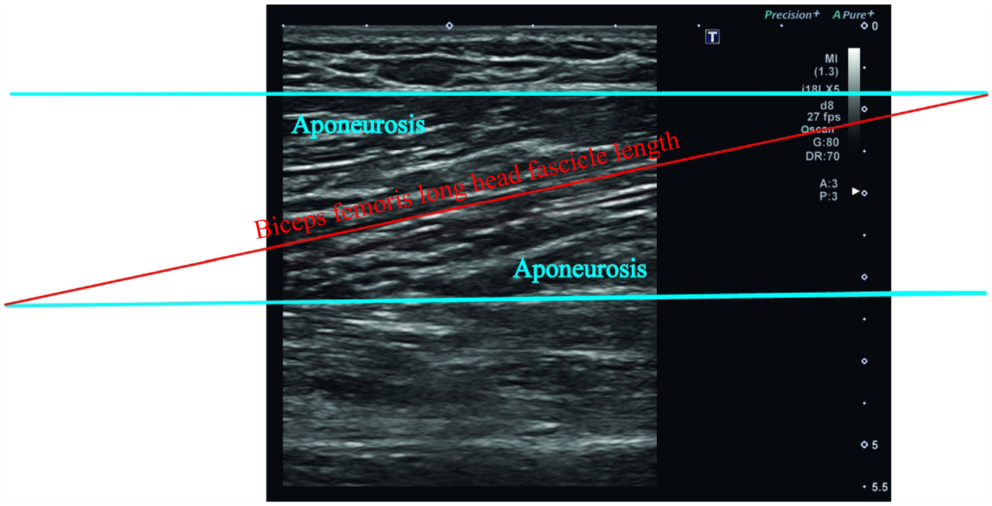

Figure 1 — Example of biceps femoris long head fascicle length measurements via B-mode ultrasound. Fascicle length was calculated based on the manual linear extrapolation method by following the methodology of a recent study.[24]

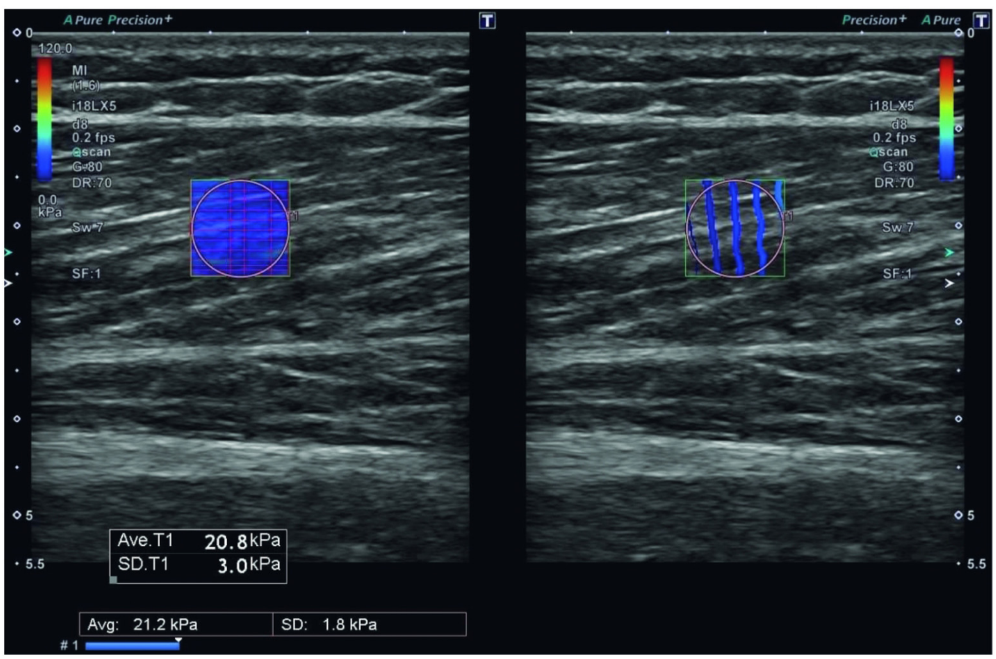

Figure 2 — Example of a biceps femoris long head passive muscle stiffness measurement via ultrasound-based shear-wave elastography. A 10-mm elastogram was taken from the central region of the biceps femoris longhead muscle belly.

femoris short and long heads, gracilis, sartorius, semimembranosus, and semitendinosus) muscle volumes, their cross-sectional areas were summed and multiplied by the slice thickness.[26] Relative muscle volumes were calculated by dividing the absolute muscle volume values by the individual participants' body mass (in cubic centimeter per kilogram).

Statistical Analyses

The SPSS software (version 27) was used for statistical analyses of this study. The BFlh fascicle length, passive muscle stiffness, absolute and relative muscle volume of the BFlh, and overall KE and KF values of both legs were compared using the 1-way analysis of variance between the groups. However, due to little differences between the values of the legs and the dominant leg's higher tendency to the occurrence of HSIs,[27] this article presented only the results for the dominant legs to improve the clarity of the text. The homogeneity of variances was tested via Levene statistics. The reliability of the BFlh fascicle length and passive muscle stiffness measurements were assessed via ICC analysis based on 2-way random absolute agreement. An ICC value lower than .5, .5 to .75, .75 to .9, and over .9 were accepted as poor, moderate, high, and very high reliabilities, respectively. In addition, linear regression analyses were performed to find out relationships between the BFlh muscle volume, fascicle length, and passive muscle stiffness. The correlation coefficient values $R \leq .3$, $R = .3$

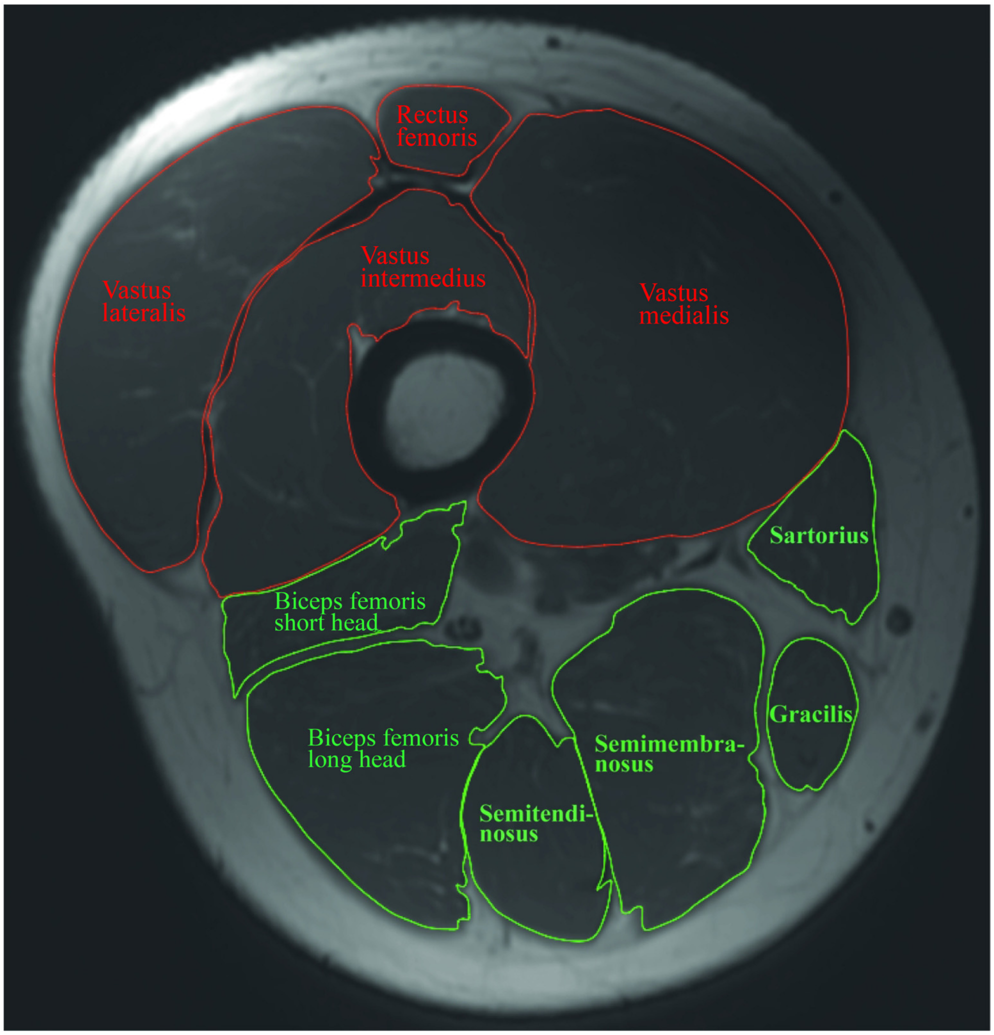

Figure 3 — An example cross-sectional MRI image of the right thigh to show knee flexor and extensor muscles' cross-sectional areas for future use in muscle volume calculations. MRI indicates magnetic resonance imaging.

to .5, $R = .5$ to .7, $R = .7$ to .9, and $R \geq .9$ were considered very weak, weak, moderate, strong, and very strong. The alpha level for the statistical significance was set at .05 for all the statistical analyses of this study.

Results

Initially, 47 male participants were recruited for the study. However, 4 participants did not meet the inclusion criteria of this study on the prescreening forms, and one participant did not attend all the measurements. Therefore, 5 participants were excluded from the study, and 42 participants, 21 for the rugby (age: 20.4 [1.2] y; height:173 [4.6] cm; body mass: 87.4 [10.9] kg) and 21 for the control (age: 22.1 [1.1] y; height: 170.5 [6.1] cm; body mass: 63.8 [7.8] kg) groups completed the study. Rugby players exhibited 9.1 (3.4) years of rugby training. The players performed 18.4 (3.9) hours for 5.6 (1.1) sessions of rugby training per week. The weekly rugby training consisted of 38% resistance training at the gym, 33% rugby-specific training, and 29% agility and sprint and endurance training. Additionally, the rugby group had 1.3 (0.7) rugby matches per week. On the other hand, the control group was performing 0.8 (1.3) vigorous-intensity, 2.4 (3.2) moderate-intensity, and 5.3 (6.2) low-intensity nonsport-specific exercise such as running and walking. By recruiting this control group, we aimed to minimize the potential adverse effects of sedentary lifestyles and minimize any sport-specific alterations in the outcome measures.

Seven participants underwent intratester and interday reliability assessments across 2 separate sessions on different days. As a result, the BFlh fascicle length (ICC = .97; 95% confidence interval, .78–.99), and passive muscle stiffness (ICC = .91; 95% confidence interval, .42–.98) measurements were graded as very highly reliable.

Statistical analyses showed that rugby players exhibited significantly longer BFlh fascicles, higher BFlh passive muscle stiffness, and higher BFlh absolute muscle volume (Table 1). However, there were no differences in the relative muscle volume values between the groups (Table 1). The fascicle lengths of 6 participants (3 from the rugby and 3 from the control group) were not calculated due to unclear fascicular paths in the images.

Based on the absolute muscle volume comparisons between the groups, muscle volumes of the knee extensors and flexors were significantly higher in the rugby players than in the controls (Table 2). However, the rugby players only demonstrated higher relative muscle volumes of the knee extensors than the controls

(Table 2). The groups had no significant differences in relative KF' muscle volumes (Table 2).

The percentage of BFlh/KE, BFlh/KF, and KF/KE muscle volume ratios were compared between the groups. In consequence, the rugby group displayed significantly lower percentage BFlh/KE and KF/KE muscle volumes (Table 3). However, there were no significant differences in BFlh/KF muscle volume ratios between the groups (Table 3).

The BFlh muscle volume exhibited moderate positive correlations with the BFlh fascicle length value (Table 4). The BFlh muscle volume also illustrated weak positive correlations with the BFlh passive muscle stiffness (Table 4).

Discussion

To the authors' knowledge, this is the first study to compare BFlh fascicle length; passive muscle stiffness; and the BFlh, KE, and KF muscle volumes between rugby players and healthy, physically active controls to assess the potential long-term adaptations of these structures to rugby-specific training and match play. The main

Table 1 Biceps Femoris Long Head's Fascicle Length, Passive Muscle Stiffness, and Absolute and Relative Muscle Volume Results of the Groups, Mean (SD)

	Rugby	Control	Difference	*P* for homogeneity of variances	*P* for mean difference
Fascicle length, cm	10.7 (1.2)**	9.1 (1.1)	1.6 (1.7)	.912	<.001
Passive muscle stiffness, kPa	24.7 (10.5)*	16.9 (7.6)	7.8 (14.8)	.094	.009
Absolute muscle volume, cm^3	288 (41.2)**	216.2 (45.3)	71.9 (73.3)	.611	<.001
Relative muscle volume, cm^3/kg	3.3 (0.4)	3.4 (0.7)	−0.1 (0.8)	.049	.573

*Significantly higher than the other group ($P < .05$). **Significantly higher than the other group ($P < .001$).

Table 2 Absolute and Relative Total Muscle Volumes of the Knee Extensors and Knee Flexors Based on the Groups, Mean (SD)

	Rugby	Control	Difference	*P* for homogeneity of variances	*P* for mean difference
Absolute knee extensors, cm^3	3017.4 (429.3)**	2061.1 (310.4)	956.3 (557.4)	.136	<.001
Relative knee extensors, cm^3/kg	34.6 (2.8)*	32.3 (3.5)	2.3 (3.7)	.464	.027
Absolute knee flexors, cm^3	1374.2 (205.6)**	1041.9 (192.8)	332.3 (337.2)	.874	<.001
Relative knee flexors, cm^3/kg	15.7 (1.3)	16.3 (2.5)	−0.6 (3.1)	.032	.32

*Significantly higher than the other group ($P < .05$). **Significantly higher than the other group ($P < .001$).

Table 3 KF to KE Percentage Muscle Volume Ratios, BFlh to KE Percentage Muscle Volume Ratios, and BFlh Fascicle Length to KE Percentage Muscle Volume Ratios of the Groups, Mean (SD)

	Rugby	Control	Difference	*P* for homogeneity of variances	*P* for mean difference
KF:KE%	45.7 (4.1)	50.6 (4.78)*	−4.9 (5.9)	.185	.001
BFlh:KE%	9.6 (0.9)	10.5 (1.45)*	−0.9 (1.9)	.044	.019
BFlh:KF%	21 (1.8)	20.8 (1.9)	0.3 (2.9)	.603	.637
BFlh fascicle length:KE%	0.4 (0.6)	0.5 (0.1)*	−0.1 (0.1)	.346	.004

Abbreviations: BFlh, biceps femoris long head; KE, knee extensors; KF, knee flexors.
*Significantly higher than the other group ($P < .05$).

Table 4 Correlations Between the BFlh Morphological Variables

	BFlh muscle volume**	BFlh passive muscle stiffness*
BFlh fascicle length	$r = .59$, $r^2 = .35$, $P < .001$	$r = .34$, $r^2 = .12$, $P = .021$
	BFlh muscle volume*	**BFlh fascicle length***
BFlh passive muscle stiffness	$r = .46$, $r^2 = .21$, $P = .002$	$r = .34$, $r^2 = .12$, $P = .042$

Abbreviation: BFlh, biceps femoris long head.
*Significantly correlated ($P < .05$). **Significantly correlated ($P < .001$).

findings of the study showed that long-term exposure to rugby-specific training and competition led to greater absolute muscle volume of the KE and KF, an increase in BFlh stiffness and BFlh fascicle length. Notably, KE muscle volume relative to body mass was also greater in rugby players, which contributed to lower KF to KE ratios and lower ratios for BFlh fascicle length/KE muscle volume and BFlh to KE muscle volume.

Our study has revealed that playing rugby led to significantly longer BFlh fascicles (mean difference [MD] = 1.6 [1.7] cm). However, playing rugby leads to significantly lower percentage BFlh fascicle length/KE muscle volume ratios (MD = –0.1% [0.1%]), lower KF to KE percentage ratios (MD = –4.9% [5.9%]), lower BFlh to KE percentage muscle volume ratios compared with controls (MD = –0.9% [1.9%]). Likewise, rugby players showed significantly higher KE muscle volume values relative to body mass (MD = 2.3 [3.7] cm^3/kg) than the controls. However, they did not show differences in KF muscle volume values relative to body mass compared with controls (MD = –0.6 [3.1] cm^3/kg). Another important finding from this study was that long-term rugby-specific training and match play increased the BFlh passive muscle stiffness (MD = 7.8 [14.8] kPa).

In their prospective study, Timmins et al highlighted BFlh fascicle length as a risk factor for HSIs.[14] However, one of the limitations of this study was not assessing the architectural characteristics of the hamstrings relative to knee extensors. The knee extensors contribute to the increased tensile force during the eccentric action of the hamstrings by behaving as an antagonist. Indeed, our study found significantly lower percentage ratios of BFlh fascicle length/KE muscle volume for the rugby group compared to control (MD = –0.1% [0.1%]). These results may indicate that rugby-specific adaptations in the BFlh fascicle length/KE muscle volume can theoretically increase the tensile force in the series of sarcomeres in the BFlh fascicle length and might increase risk of HSIs. When considering that KE muscle volume is one of the strongest predictors of concentric muscle power,[16] this alteration can increase the tensile force on the BFlh fascicles during the late swing phase of running due to the antagonist behavior of the KE and might increase the vulnerability of the muscle. Unfortunately, this argument requires further investigation due to the lack of prospective and retrospective studies addressing the predictive abilities of architectural and morphological parameters of the hamstrings relative to KE for the HSI. Therefore, future prospective studies should examine whether there are threshold values in the BFlh fascicle length/KE muscle volume, BFlh/KE and KF/KE muscle volume ratios for predicting HSIs. Additionally, retrospective studies should compare BFlh fascicle length/KE muscle volume, BFlh/KE and KF/KE muscle volume ratios of rugby players prone to hamstring injuries with injury-free rugby players.

Additionally, this study has shown that long-term rugby-specific training and match play led to higher BFlh passive muscle stiffness (MD = 7.8 [14.8] kPa). This observation might further compound HSI risk, as evidence has suggested that increased hamstring stiffness is a risk factor for HSIs.[15] However, much of the previous research on passive muscle stiffness have adopted techniques which included the measurement of the whole muscle–tendon complex of the agonist muscles. These techniques are limited by not being capable of evaluating the stiffness of the individual muscles.[28] However, the use of shear wave elastography in this study has allowed an accurate assessment of passive stiffness.[25] Our findings revealed that long-term rugby participation leads to increases in passive muscle stiffness of the BFlh. However, previous research,[15] showing that increased hamstring stiffness is a risk factor for HSIs, employed less valid assessments free oscillation technique to assess the passive stiffness of the muscle and tendon of all KF, rather than measuring the passive stiffness of the individual muscles. Therefore, new prospective studies are needed to assess the predictive ability of the BFlh passive muscle stiffness and determine if there is a predictive threshold value for the HSIs using the updated technology,[25] namely shear wave elastography.

Our study employed the "gold standard" measurement of MRI for the muscle volume assessments.[25] However, no "gold standard" measurement method of the BFlh fascicle length exists in the literature.[29] Additionally, the effect size of exercise can vary between the ultrasound assessment methods, such as the trigonometric equation method potentially leads to overestimated BFlh fascicle length,[21] and size of exercise effect on the BFlh fascicle length compared with manual linear extrapolation, panoramic ultrasound scanning,[30] or diffusion-tensor MRI.[31] Despite this study using the manual linear extrapolation method, which does not significantly overestimate the BFlh fascicle length,[21] and effects of exercise on the BFlh fascicle length,[30] using this method is still a limitation of this study due to lacking a "gold standard" method for the BFlh fascicle length assessments.[21] This study measured the muscle volume of the major KF naturally located at the thigh. However, lateral and medial gastrocnemius, popliteus, and soleus muscles also contribute to knee flexion, despite not being their primary activity. Thus, not measuring the volume of these muscles can be another confounding factor of this study. Initially, the body mass and age were significantly different between the groups. This study recruited 7 participants for the intrarater interday reliability assessments of the BFlh fascicle length and passive muscle stiffness measurements. However, not performing the reliability assessment on a larger sample with an additional interrater reliability assessment could be a limitation of this study. Despite this study mainly comparing ratios of the KF relative to extensors of individuals, significant body mass differences between the groups can be another confounding factor of this study. Lastly, this study recruited subelite players from the top Japanese university rugby league (division 1) with 9.1 (3.4) years of rugby-specific training history. Still, this study could not recruit players competing at the top-level international leagues, which may be another limitation.

Practical Applications

Habitual participation in rugby union training and competition results in an alteration in structure and morphology of the thigh musculature, which may increase the risk of HSI. These alterations include an increase in KE muscle volume relative to the KF, and an increase in bicep femoris passive muscle stiffness. Coaches and athletes are advised to maintain balanced developments between the KF and extensors by adjusting rugby training regimens. More focus on the development of muscle hypertrophy, strength qualities, and mobility in the hamstrings muscle group as part of a prehabilitation routine is recommended. For example, eccentric hamstring training (eg, eccentric hip extension, Nordic hamstring, and Russian belt exercises) can be performed for the purpose.

Conclusions

This study reveals that long-term rugby-specific training and match play may lead to imbalanced developments between the knee flexor (KF) and extensors favoring the knee extensor (KE) muscles.

Specifically considering hamstring strain injury (HSI) mechanism, increased biceps femoris long head (BFlh) passive muscle stiffness, and smaller BFlh fascicle length/KE ratios might increase the risk of HSIs. To support this argument, evidence should be gathered from future prospective studies examining whether there are threshold values in the BFlh passive muscle stiffness, BFlh fascicle length/KE, BFlh/KE and KF/KE muscle volume ratios for predicting HSIs.

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