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Knee Flexion Excursion Mediates the Association Between Quadriceps Stiffness and Knee Loads During the Mid-Stance Phase of Gait

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ABSTRACT

Lower strength and higher passive stiffness of the quadriceps have been associated with a higher risk of knee osteoarthritis/pain in aging populations. It is proposed that altered quadriceps properties would cause excessive knee loads, thus contributing to knee mechanical damages, but the relevant evidence is limited. This study aimed to explore relationships amongst quadriceps properties, knee flexions, and knee moments during gait. This study included 87 community-dwelling elders (65.9 ± 4.1 years; 57.5% females). Quadriceps strength was assessed using a Cybex dynamometer, and the passive stiffness of three superficial quadriceps heads was evaluated using shear-wave ultrasound elastography. Gait analysis was conducted to compute the knee adduction moment (KAM), knee flexion moment (KFM), KAM index, and knee flexion excursion (KFE) during the mid-stance phase. Associations amongst quadriceps properties, KFE, and knee moments were examined by partial correlations. Mediation analysis was used to explore the mediating role of KFE in associations between quadriceps properties and knee moments. Greater quadriceps strength was associated with a higher KAM ($r = 0.304$; $p = 0.006$). Greater stiffness of the rectus femoris (RF) was indirectly associated with a higher KAM index (mediation-effect [95% CI]: 0.084 [0.011, 0.191], $p = 0.017$) but a lower KFM (mediation-effect [95% CI]: −0.139 [−0.270, −0.041], $p = 0.004$), via a smaller KFE. Stronger quadriceps are correlated with a higher frontal knee moment. Stiffer RF is indirectly associated with a higher frontal knee load sharing through reduced sagittal knee motions, which could be a potential mechanism of stiffer RF for knee osteoarthritis.

1 | Introduction

Age-related modulations in quadriceps muscle include decreased strength and increased passive stiffness [1–3]. Lower quadriceps strength is linked to the occurrence of radiographic [4, 5] and symptomatic [6–8] knee osteoarthritis (OA). Furthermore, heightened passive stiffness of the quadriceps is associated with pain in individuals with symptomatic knee OA [9]. Increased passive stiffness in skeletal muscle results in a steeper length-tension curve (indicating decreased compliance) during joint movement [10]. Additionally, higher quadriceps stiffness has been associated with a diminished ability of the quadriceps to produce force [11]. A recent cohort study found that greater quadriceps stiffness was correlated with an increased risk of developing clinical knee OA in 12 and 24 months [6, 8]. It is hypothesized that the quadriceps generate internal forces to counteract external knee loads, thereby safeguarding the knee joint against mechanical damage [12, 13]. Nevertheless, connections between quadriceps characteristics and knee biomechanics remain inadequately defined. This knowledge would be advantageous for gaining a deeper comprehension of how quadriceps mechanical properties might influence knee OA or related symptoms in the aging population.

The knee adduction moment (KAM) is an indirect indicator of medial knee joint loading [14–16], with a higher KAM correlating with an increased risk of knee pain/OA [17, 18]. While stronger quadriceps are suggested to offer mechanical safeguarding for the knee joint, a notable correlation between quadriceps strength and KAM remains unestablished in patients with knee OA [19–21]. Furthermore, exercise-induced gains in quadriceps strength have not shown associations with changes in KAM among knee OA patients [22]. Interestingly, a positive relationship between quadriceps strength and KAM has been observed in healthy adults [23], challenging the proposed role of quadriceps strength in knee health [12, 13]. This discrepancy could be attributed to the fact that while the KAM is a widely recognized mechanical risk factor for medial knee OA progression, it is not the sole contributor to medial knee loading. According to mechanical principles, the KAM and KFM are interrelated, and both significantly contribute to the medial contact force during gait [24]. Evidence suggests that both the KAM and KFM are associated with cartilage changes over a 5-year period in knee OA [25]. The KAM is higher in more severe OA, and the KFM has a greater influence early in the disease process [26]. A recent cohort study revealed that lower KFM correlated with a heightened risk of chronic knee pain in the elderly [27]. Furthermore, a decline in KFM coupled with a relative increase in the KAM has been linked to knee OA progression, leading to the proposal of the KAM index as a more sensitive marker than KAM alone for reflecting medial knee joint loading [28]. Therefore, a more comprehensive understanding is required regarding the associations between quadriceps properties and external knee moments.

Changes in quadriceps properties could indirectly influence knee loads through their connections with sagittal knee movements. Research indicates that diminished quadriceps strength correlates with reduced knee flexion angle during the mid-stance phase of gait [29]. Moreover, increased passive stiffness of the quadriceps, characterized by decreased compliance, might also contribute to diminished sagittal knee movements, such as knee flexion excursion (KFE) during the mid-stance phase of gait [10]. Given that reduced sagittal knee motions have been linked to decreased knee flexion moments [30–32], it is plausible that quadriceps strength and stiffness are indirectly associated with external knee moments via their correlations with KFE.

This study aimed to investigate the associations of quadriceps properties with knee loads, via the potential mediation of knee flexion excursion during gait in healthy elders. We aimed to (i) examine relationships between quadriceps properties and knee moments; (ii) delineate relationships between quadriceps properties and KFE; and (iii) explore the potential mediation role of KFE in relationships between quadriceps properties and knee moments. We hypothesized that (i) lower quadriceps strength and greater quadriceps stiffness would be associated with higher KAM and KAM index, but a lower KFM; (ii) lower quadriceps strength and greater quadriceps stiffness would be related to smaller KFE; and (iii) the associations of quadriceps properties with dynamic knee moments would be mediated by KFE.

2 | Methods

2.1 | Study Design

This was a cross-sectional study with a mediation analysis.

2.2 | Participants

Participants were recruited from local communities in Hong Kong using convenience sampling. Inclusion criteria comprised individuals who were aged between 60 and 80 years and capable of walking 10 meters unaided. Exclusion criteria included individuals with any knee pain, knee or hip joint arthroplasty, recent knee injury or surgery within the past year, knee arthritis (e.g., osteoarthritis or rheumatoid arthritis), or neurological conditions (e.g., stroke or Parkinson's disease). During the screening visit, the candidate participants were asked whether they were experiencing or diagnosed with the above-mentioned conditions. Knee pain (on both knees) was rated on the numeric rating scale (NRS). A physical therapist with 8 years post-qualification experience (XH) screened the knee muscle contracture by performing passive knee range of motion tests. If the answer for any item was positive, any knee pain (NRS ≥ 1/10) was reported on either knee, and/or knee muscle contracture was suspected, he/she would not be eligible to participate in this study.

Data on age, sex, body mass, height, and body mass index (BMI) were recorded for all eligible participants. Participants were classified into two groups based on their exercise habits per week, as per the guidelines provided by the U.S. Department of Health and Human Services [33], distinguishing between sedentary and active lifestyles. The study adhered to the guidelines outlined in the Declaration of Helsinki and received approval from the human subjects' ethics sub-committee of the institution overseeing the research (ID no.: HSEARS20180110001). Before data collection, all participants provided written informed consent.

2.3 | Quadriceps Strength

The isokinetic peak torque of the quadriceps was evaluated using a Cybex isokinetic dynamometer (Cybex Co., Ronkonkoma, NY, USA) at a consistent angular velocity of 60°/s, following a previously established protocol [6, 8, 34]. The leg tested was chosen randomly. Participants were seated and instructed to perform concentric knee movements from maximum flexion to maximum extension with maximal effort, repeating this action five times. Before the test, participants underwent a warm-up trial at 50% of their maximal voluntary effort, and verbal encouragement was provided during the assessments. The peak torque values from the middle three extension trials were averaged and normalized to body mass (Nm/kg) [6, 8, 34]. Test-retest reliability from our lab was 0.90 [6].

2.4 | Quadriceps Stiffness

Shear-wave ultrasound elastography (SWUE) measures the speed of shear waves in skeletal muscles, providing a valid and reliable method to evaluate muscle stiffness [35]. Muscle shear modulus, which serves as an indicator of muscle stiffness [36], was assessed in the three superficial quadriceps muscle heads (rectus femoris [RF], vastus lateralis [VL], and vastus medialis [VM]) using a SWUE system integrated with an Aixplorer ultrasound scanner (Aixplorer Version 4.2; Supersonic Imagine, Aix-en-Provence, France) and a linear ultrasound probe (4–15 MHz, Super Liner 15–4; Supersonic Imagine), following a previously established protocol [6, 8, 37].

Participants assumed a supine position with their knees flexed at 60° and their hips neutrally positioned. This measurement position was chosen to ensure that muscle fibers were beyond the slack position [37]. Measurement sites were identified: 1/2 between the anterior superior iliac spine (ASIS) and the superior border of the patella for RF; 1/3 distally between the ASIS and the lateral border of the patella for the VL; and 1/5 distally between the ASIS and the medial border of the patella for the VM [37]. The transducer was oriented perpendicularly and aligned with the shortening direction of the lateral component of the RF fascicles and the muscle fiber directions of the VL and VM [6, 8, 37].

We applied ultrasound gel to the skin and ensured minimal pressure from the ultrasound probe to avoid compressing the underlying muscle. An 11-second video (1 sample per second with a spatial resolution of 1 × 1 mm) was recorded while the tested muscle was at rest. Each video was divided into 11 frames, with the middle five frames averaged for subsequent analysis. The region of interest was delineated based on the muscle thickness beneath the marked area. MATLAB (MathWorks Inc, Natick, MA, USA) was utilized to compute the shear modulus values (in kPa) for each muscle head. We calculated the sum of the shear moduli of the RF, VL, and VM to reflect the overall superficial quadriceps stiffness. All measurements were performed by a single examiner (LZP). Intra-rater reliabilities were determined to be 0.94, 0.89, and 0.94 for the RF, VL, and VM, respectively [6].

2.5 | Gait Analysis

Gait analysis was conducted in a motion and gait laboratory equipped with an eight-camera (MX T40) Motion Analysis System (Vicon, Oxford, UK) and two floor-mounted force plates (Advanced Mechanical Technology Inc., Watertown, MA, USA). Data were captured at sampling rates of 1,000 Hz for kinetic data and 100 Hz for kinematic data. The standard Plug-In-Gait marker set was employed on the pelvic, hip, knee, and ankle joints of both lower limbs [27, 38]. Marker trajectory data underwent digital filtering using a Woltring quintic spline filter with a low-pass cut-off frequency of 10 Hz [39]. Each participant walked barefoot without any assistance along a 10-meter walkway at a self-selected comfortable pace. Following three practice trials, data collection commenced. Five successful trials, characterized by clean foot strikes on the force plates for each leg, were recorded and saved for subsequent offline analysis.

We utilized Nexus software (Version 2.5, Vicon) to analyze knee joint kinetic and kinematic data. Heel contact and toe-off events were identified using a vertical ground reaction force threshold of 20 N [40]. Knee biomechanical outcomes were computed using a custom MATLAB program (MathWorks Inc, Natick, MA, USA). Peak KAM and KFM were determined as the maximum values during the mid-stance phase of the gait cycle along the frontal and sagittal planes, respectively. To determine the KAM index, we began by computing the total joint moment (TJM). This was achieved by calculating the square root of the sum of the squared values of the KFM, KAM, and knee rotation moment for each frame within the mid-stance phase. The peak TJM was identified as the highest TJM value observed during this phase. Subsequently, the KAM index was calculated as the percentage (%) of squared corresponding KAM over the squared peak TJM [28]. The KFE was identified as the angle of knee excursion from the knee flexion at initial heel contact (KFA_hc) to the peak knee flexion (KFA_peak) during the mid-stance phase. The mid-stance phase corresponds to approximately 17–50% of the stance phase and marks the time when the knee first reaches peak KAM, KFM, TJM, and KFA [28, 41]. Each participant's gait parameters were averaged over five successful trials. Knee kinetic parameters were normalized to body mass [27, 38]. Additionally, walking speed was recorded as the average speed across five successful trials.

2.6 | Statistical Analysis

Partial correlations were employed to investigate the associations: (i) between quadriceps properties and knee moments; (ii) between quadriceps properties and KFE; and (iii) between KFE and external knee moments, adjusting by age, sex, BMI (or body height), activity level, and walking speed. The body height was entered for models including quadriceps strength or knee loads, which had been normalized by body mass [6, 27, 34, 38]. For each model, the partial correlation coefficient (r) and corresponding p value were reported.

If significant associations between quadriceps properties and KFE, as well as between KFE and knee moments, were identified in the preceding analyses, mediation analyses would be conducted to explore the potential mediating role of KFE in relationships between quadriceps properties and knee moments, adjusting by age, sex, body height, activity level, and walking speed. The R package "Mediation" was used for the mediation analyses, using R statistical software (Version 4.1.2). The indirect ($a*b$), direct (c'), and total effects ($c = a*b + c'$) with their 95% confidence intervals (95% CI) and corresponding p values were computed for each mediation model, using the bootstrapping method (5,000 replications) [42]. Each mediation model was assessed using previously established criteria: (i) No effect (non-mediation): neither $a*b$ nor c' is statistically significant; (ii) Direct-only (non-mediation): c' is significant, but $a*b$ is not; (iii) Indirect-only (mediation): $a*b$ is significant, while c' is not; (iv) Partial mediation: both $a*b$ and c' are significant [8, 42]. For the partial mediation, the proportion mediated was computed by dividing the indirect effect by the total effect ($100\% * a*b/(a*b + c')$). [43]

The KFE was calculated based on KFA_hc and KFA_peak. Since quadriceps properties may influence KFE through their effects on KFA_hc and/or KFA_peak, we performed secondary analyses to explore the relationships between KFE and these two variables, using partial correlations controlling for age, sex, body height, activity level, and walking speed. This allowed us to better understand how KFE was determined by KFA_hc and KFA_peak within this cohort. Additionally, we investigated the associations between quadriceps properties and both KFA_hc and KFA_peak to assess whether quadriceps properties exert distinct effects on knee flexion angle at these two specific gait events. Evidence showed that increased KFA_hc [44] and decreased KFA_peak [45, 46] are related to OA severities. These analyses would provide further insight into the mechanisms by which quadriceps properties may influence knee kinematics during gait.

The study size was reached based on the availability of eligible participants during the study period. The significance level was preset at $p < 0.05$.

3 | Results

We initially screened 182 community-dwelling older adults, with 87 eligible participants (65.9 ± 4.1 years) included in this study. Table 1 presents the demographic and descriptive data of the participants.

TABLE 1 | Demographic and descriptive data.

	***n* = 87**
Demographics	
Age (years)	65.9 ± 4.1
Sex	
n (%) of females	50 (57.5%)
Body mass (kg)	59.6 ± 10.6
Body height (m)	1.60 ± 0.08
BMI (kg/m^2)	23.3 ± 3.6
Activity level	
n (%) of sedentary	36 (41.4%)
Walking speed (m/s)	1.20 ± 0.19
Quadriceps properties	
Quadriceps strength (Nm/kg)	1.14 ± 0.32
Total quadriceps shear moduli (kPa)	23.9 ± 4.9
RF shear modulus (kPa)	12.8 ± 3.6
VL shear modulus (kPa)	6.3 ± 2.0
VM shear modulus (kPa)	4.9 ± 1.0
Knee biomechanics	
KAM (Nm/kg)	0.60 ± 0.18
KFM (Nm/kg)	0.31 ± 0.25
KAM index (%)	43.8 ± 17.3
KFE (degree)	7.7 ± 3.6
KFA_hc (degree)	8.1 ± 5.8
KFA_peak (degree)	15.8 ± 7.7

Note: Values are mean ± SD, unless other indicates.
Abbreviations: KAM, knee adduction moment; KFA_hc, knee flexion angle at initial heel contact; KFA_peak, peak knee flexion angle; KFE, knee flexion excursion during mid-stance phase; KFM, knee flexion moment; n, numbers; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; TJM, total joint moment.

3.1 | Quadriceps Properties and Knee Moments

The greater passive stiffness of the RF was significantly associated with a higher KAM index (partial $r = 0.252$, $p = 0.022$; Figure 1A) and a lower KFM (partial $r = -0.220$, $p = 0.047$; Figure 1B), but not with KAM (Figure 1C). Greater passive stiffness of the total quadriceps also showed a significant association with a higher KAM index (partial $r = 0.227$, $p = 0.040$).

Greater quadriceps strength was significantly correlated with higher KAM (partial $r = 0.304$, $p = 0.006$; Figure 1F), but not with KFM (Figure 1D) or KAM index (Figure 1E) (Table 2).

3.2 | Quadriceps Properties and Knee Flexion Excursion During the Mid-Stance Phase of Gait

We found greater RF stiffness was significantly associated with the smaller KFE (partial $r = -0.231$, $p = 0.037$; Figure 2) during the mid-stance phase of gait. While KFE was not significantly related to quadriceps strength or the stiffness of other quadriceps muscle heads (Table 2).

3.3 | Knee Flexion Excursion and Knee Loads

The larger KFE was significantly associated with a lower KAM index (partial $r = -0.309$, $p = 0.005$; Figure 3A), a higher KFM (partial $r = 0.484$, $p < 0.001$; Figure 3B), but not with KAM (Figure 3C).

3.4 | Mediation Analysis

Based on the findings from the previous steps, we examined two models regarding the potential mediation role of KFE in the relationships between RF stiffness and knee moments (KAM index and KFM). We found that the greater RF stiffness indirectly associated with a higher KAM index ($a*b$ [95%CI]: 0.084 [0.011, 0.191], $p = 0.017$; c' [95%CI]: 0.201 [−0.019, 0.410], $p = 0.078$; Figure 4A) and with a lower KFM ($a*b$ [95%CI]: −0.139 [−0.270, −0.041], $p = 0.004$; c' [95%CI]: −0.120 [−0.320, 0.081], $p = 0.233$; Figure 4B), via a smaller KFE. While the direct paths were insignificant in either model.

3.5 | Secondary Analysis

The lower KFE was significantly associated with the lower KFA_peak (partial $r = 0.678$, $p < 0.001$) and the lower KFA_hc (partial $r = 0.301$, $p = 0.006$) (Figure S1). The greater RF stiffness was significantly associated with smaller KFA_peak (partial $r = -0.256$, $p = 0.020$) during the mid-stance phase of gait, but not with KFA_hc (Figure S2).

4 | Discussion

This study aimed to investigate associations amongst quadriceps properties, knee kinematics, and knee kinetics during gait.

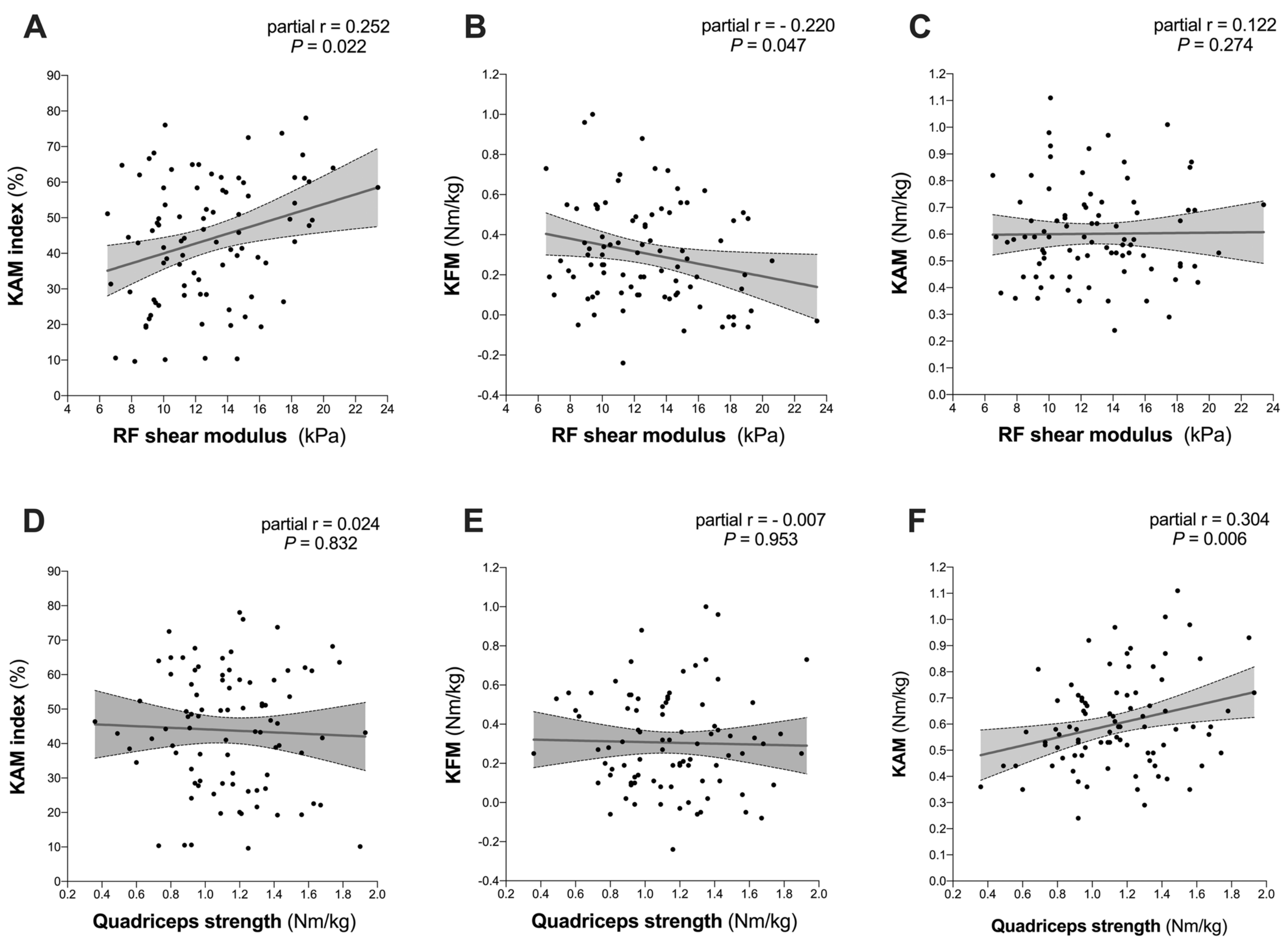

FIGURE 1 | Scatter plots between quadriceps properties and external knee moments: (A) RF stiffness and KAM index; (B) RF stiffness and KFM; (C) RF stiffness and KAM; (D) quadriceps strength and KAM index; (E) quadriceps strength and KFM; (F) quadriceps strength and KAM. KAM, knee adduction moment; KFM, knee flexion moment; RF, rectus femoris.

We found that greater passive stiffness of the bi-articular head RF, was indirectly associated with a higher KAM index but with a lower KFM, mediated by a smaller KFE during the mid-stance phase of gait. We also observed a significant relationship between the greater quadriceps strength and higher KAM.

This study represents the first attempt to investigate the relationship between passive properties of the quadriceps and external knee moments. We found the higher quadriceps stiffness, especially the bi-articular quadriceps head RF, was related to a higher KAM index. Furthermore, mediation analyses revealed that higher RF stiffness indirectly correlates with a higher KAM index and a lower KFM, via a smaller KFE. This implies that increased passive stiffness of the bi-articular RF, characterized by reduced compliance [10], may function as a rigid biomaterial limiting knee flexion during the mid-stance phase of gait. This speculation was further supported by the results of our secondary analyses showing that the greater RF stiffness was significantly associated with reduced KFA_peak (but not with KFA_hc), and the reduced KFA_peak is likely the more significant determinator of the lower KFE during the mid-stance phase of gait. Restricted sagittal knee motions coincide with a decrease in KFM, responding to knee joint flexion [45, 46], and may cause an increase in frontal knee load sharing. Given that a higher KAM index and a lower KFM have been related to the onset and progression of knee pain/OA [27, 28], this mechanical mechanism could potentially contribute to the development of knee symptoms/OA in the elderly [6, 8, 9]. Future studies employing prospective mediation designs are anticipated to validate this proposed mechanical pathway. Our mediation analysis revealed that the KFE is the indirect-only mediator in the relationship between RF stiffness and both the KAM index and KFM. This finding aligns with our proposed theoretical framework, suggesting that increased RF stiffness may impact knee moments exclusively through its influence on knee flexion during the mid-stance phase of gait [42]. Consequently, this underscores the importance of focusing on knee flexion during the mid-stance phase as a critical target outcome in intervention studies designed to reduce RF stiffness and alleviate knee loading.

The relationship between quadriceps stiffness and knee biomechanics was specifically pertained to the bi-articular RF. This aligns with a recent study showing that among the Vasti, the activation of bi-articular RF muscle has the greatest impact on knee contact force during walking [47]. During routine weight-bearing activities, eccentric contraction of the quadriceps is crucial for controlling dynamic knee motion and potentially reducing load on the knee joint during the mid-stance phase

TABLE 2 | Associations between quadriceps properties and knee biomechanics during gait.

	KAM		KFM		KAM index		KFE		KFA_hc		KFA_peak	
	r	*p*[a]	*r*	*p*[a]	*r*	*p*[a]	*r*	*p*	*r*	*p*	*r*	*p*
Quadriceps strength	**0.304**	**0.006**	– 0.007	0.953	0.024	0.832	– 0.076	0.499[a]	0.143	0.200[a]	0.077	0.493[a]
Total quadriceps stiffness	0.151	0.177	– 0.179	0.109	**0.227**	**0.040**	– 0.158	0.157[b]	– 0.151	0.175[b]	– 0.187	0.093[b]
RF stiffness	0.122	0.274	**– 0.220**	**0.047**	**0.252**	**0.022**	**–0.231**	**0.037**[b]	– 0.198	0.074[b]	**– 0.256**	**0.020**[b]
VL stiffness	0.109	0.328	– 0.001	0.990	0.058	0.604	0.078	0.484[b]	– 0.020	0.861[b]	0.020	0.860[b]
VM stiffness	0.083	0.459	– 0.084	0.455	0.094	0.400	– 0.098	0.379[b]	0.008	0.942[b]	– 0.038	0.738[b]

Abbreviations: KAM, knee adduction moment; KFA_hc, knee flexion angle at initial heel contact; KFA_peak, peak knee flexion angle; KFE, knee flexion excursion during mid-stance phase; KFM, knee flexion moment; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis.

[a]Adjusted by age, sex, body height, activity level, and walking speed.

[b]Adjusted by age, sex, body mass index, activity level, and walking speed. Significant association in **Bold**.

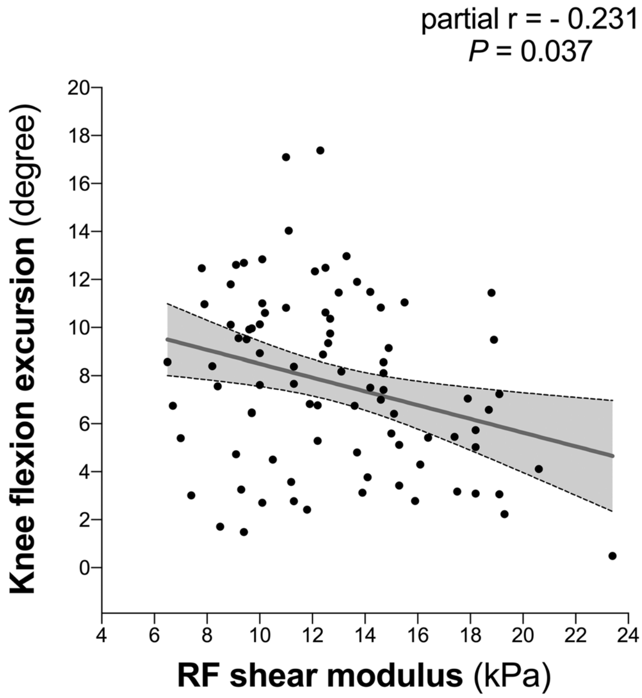

FIGURE 2 | Scatter plot between RF stiffness and knee flexion excursion during the mid-stance phase of gait. RF, rectus femoris.

[48]. In this process, the RF undergoes more dynamic stretching compared to other mono-articular quadriceps heads (e.g., VL and VM) [11]. Notably, our findings suggest that increased RF stiffness may cause a reduced KFE, primarily through a decrease in KFA_peak during the mid-stance phase, but rather than through changes in KFA_hc. This is particularly significant given that prior research has identified increased KFA_hc [44] and decreased KFA_peak [45, 46] as risk factors for knee OA. Therefore, interventions aimed at reducing RF tightness and enhancing knee flexion during the mid-stance phase could be beneficial for mitigating impact-related knee loading, potentially lowering the risk of knee OA in the aging population. Additionally, age-related increases in quadriceps stiffness are more pronounced in the RF [2]. This evidence suggests that heightened passive stiffness of the RF may require special attention among the elderly to enhance gait performance and promote better knee health.

We observed a positive relationship between quadriceps strength and KAM. It was consistent with the report from a previous study that stronger quadriceps was related to a higher KAM in healthy young women [23]. Notably, a recent study demonstrated that increased passive stiffness of the RF was associated with knee pain in individuals with knee OA, irrespective of quadriceps strength [9]. Additionally, findings from a prior clinical trial indicated that quadriceps strengthening was beneficial for alleviating knee symptoms but did not significantly impact external knee moments [22]. Therefore, greater quadriceps strength may generate more internal force for accommodating higher external knee loads (e.g., KAM) in healthy people [23]. In addition, its protection on knee health might be through other pathways, such as joint alignments. For instance, quadriceps strength has been associated with tibiofemoral and patellofemoral joint alignments [49, 50]. Future studies are warranted to investigate these hypotheses further.

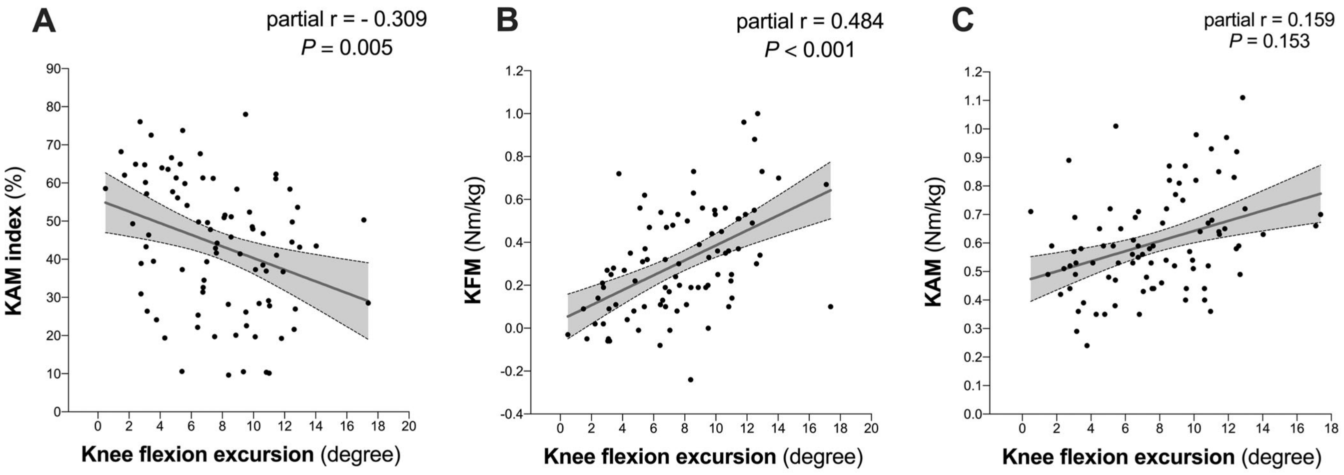

FIGURE 3 | Scatter plots between knee flexion excursion and external knee moments during the mid-stance phase of gait: (A) knee flexion excursion and KAM index; (B) knee flexion excursion and KFM; (C) knee flexion excursion and KAM. KAM, knee adduction moment; KFM, knee flexion moment.

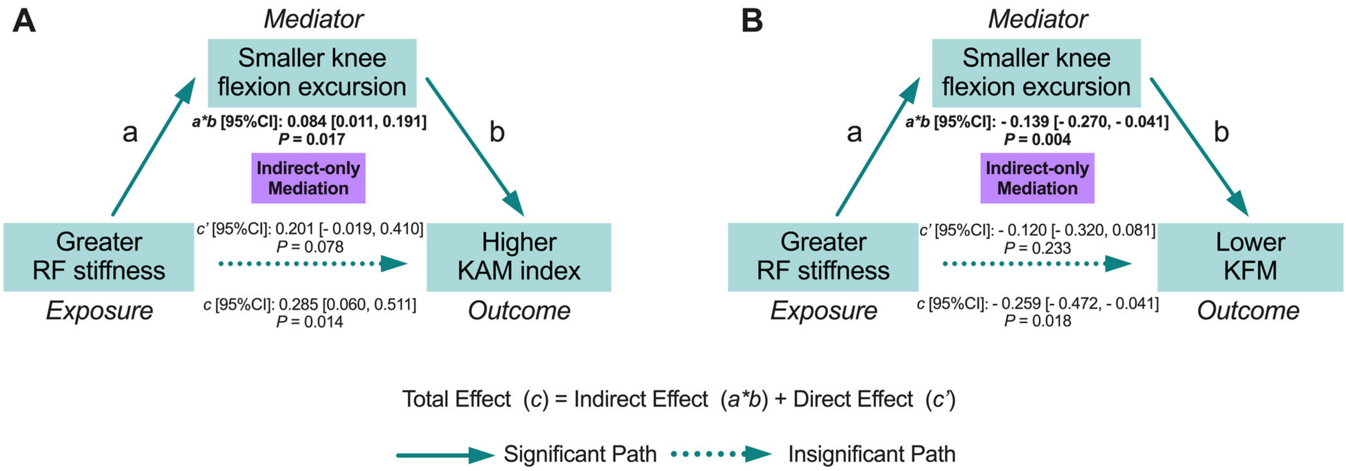

FIGURE 4 | Models of the potential mediating role of knee flexion excursion on associations between RF stiffness and external knee moments (A: KAM index; B: KFM) during the mid-stance phase of gait. CI, confidence interval; KAM, knee adduction moment; KFM, knee flexion moment; RF, rectus femoris.

4.1 | Limitations

This study has several limitations. We evaluated the stiffness of the three superficial heads of the quadriceps but not that of the vastus intermedius, as it is located deep below the superficial heads and cannot be reliably probed by SWUE [6, 8, 37]. Our findings indicate that it is the passive stiffness of the bi-articular head RF, rather than the mono-articular heads (VL or VM), that is associated with knee biomechanics. Therefore, it is less likely that the passive property of the deep mono-articular head vastus intermedius significantly affects knee biomechanics. Another potential limitation of our study is the use of the "Mediation" package in R for conducting mediation analyses. While this approach allowed us to assess mediation effects by controlling for potential covariates such as demographics, activity level, and walking speed, utilizing more sophisticated statistical methods could offer a more nuanced evaluation of the individual contributions of potential confounders to both the indirect and direct pathways within the mediation models. Such an approach would clarify the influence of these factors and strengthen the interpretation of the mediation results. Furthermore, we acknowledge that a cross-sectional study cannot establish cause-effect relationships. Building on the findings from this study and related evidence, we propose potential mechanical pathways regarding how altered quadriceps stiffness may contribute to the development of knee OA/pain in the elderly, which requires future studies with prospective mediation designs for examination.

5 | Conclusions

The study suggests that stronger quadriceps is related to higher knee adduction moment. Greater bi-articular rectus femoris stiffness indirectly correlates with a lower sagittal knee moment and a higher proportion of frontal knee moment, mediated by smaller sagittal knee motions during the mid-stance phase of gait.

Author Contributions

Siu Ngor Fu: writing – review and editing, supervision, resources, methodology, formal analysis, validation, funding acquisition,

conceptualization. **Changhai Ding:** writing – review and editing, formal analysis, validation, methodology. zongpan li: writing – review and editing – original draft, investigation, formal analysis, validation, methodology, data curation, conceptualization. **Aaron Kam-Lun Leung:** writing – review and editing, resources, formal analysis, validation, methodology. **Xiuping Huang:** writing – review, formal analysis, validation, methodology, data curation. **Chen Huang:** writing – review, software, formal analysis, validation, methodology, data curation. **Shan Su:** writing – review, validation, data curation. **Raymond C.K. Chung:** writing – review, formal analysis, validation, methodology, data curation. All authors approved the final version to be submitted.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.