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ORIGINAL ARTICLE

Effects of a patellar strap on knee joint kinetics and kinematics during jump landings: an exploration using a statistical parametric mapping and Bayesian approach

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Abstract

Purpose The aim of the current research was to investigate the effects of a patellar tendon strap on knee joint kinetics and kinematics during a vertical jump task using a statistical parametric mapping (SPM) and Bayesian approach.

Methods Twenty-eight (14 male and 14 female) participants performed a vertical jump task under two conditions (patellar tendon strap/no-patellar tendon strap). Biomechanical data were captured using an eight-camera 3D motion capture system and force platform. Participants also subjectively rated the comfort/stability properties of the patellar tendon strap and their knee joint proprioception was examined with and without the strap using a weight bearing joint position sense test. Differences between patellar tendon strap/no-patellar tendon strap conditions were examined using SPM and Bayesian analyses and subjective ratings using Chi-squared tests.

Results The results showed that neither knee joint kinetics or kinematics were affected as a function of wearing the patellar tendon strap. The findings did show that the knee brace helped to significantly increase participants perceived knee stability, but there were no improvements in weight bearing knee proprioception.

Conclusions The current investigation indicates that the utilization of a patellar tendon strap akin to the device used in the current study does not appear to reduce the biomechanical parameters linked to the aetiology of knee pathologies, during vertical jump movements.

Keywords Biomechanics · Patellar tendon strap · Kinetics · Kinematics

Introduction

The physiological and psychological benefits of physical activity, sport and exercise are well-established [1]; and physical inactivity is recognised as one of the principal amendable risk factors linked to cardiovascular and other chronic pathologies such as type II diabetes mellitus, cancer, hypertension and depressive symptoms [2]. Therefore, several national/international initiatives have been introduced, seeking to encourage the adoption of a physically active lifestyle [3].

However, despite the incontrovertible health benefits that are mediated through regular physical activity, they are also known to be associated with a high incidence of musculoskeletal injury [4]. Injury is viewed as the only drawback of regular physical activity, but is unfortunately recognised as a common complaint associated with substantial issues [5]. The management/treatment of injuries associated with physical activity and sport is challenging for both patients and clinicians, and places significant economic stresses on the global healthcare system [6].

Importantly, Hootman et al. [7] observed in an examination of 15 different sports, that the lower extremities were the most common location for injury. Specifically, the knee has been shown to be the most commonly injured musculoskeletal site in athletes, accounting for 23.2–31% of all sports injuries [8] and as many as 60% of all sports-related

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surgeries [9]. Furthermore, a significant proportion of those partaking in physical activity and exercise will experience knee pain each year [10], with a significant proportion being associated with patellar tendinopathy and patellofemoral pain syndrome [11, 12].

Chronic patellar tendinopathy (often referred to as jumper's knee) is a musculoskeletal condition, responsible in both recreational and elite athletes, for as many as 25% of all soft tissue injuries [13]. Patellar tendinopathy is epitomized by localized pain and tenderness of the tendon itself at its proximal origin on the inferior pole of the patella [14]. This condition is mediated by activities that frequently and excessively load the patellar tendon, with failed reparative response due to insufficient rest between bouts of exercise/training [15]. It has, therefore, been recommended that treatment strategies for patellar tendinopathy concentrate on reducing the loading of the tendon [16]. Chronic tendinopathy is initiated 1–3 months after the commencement of pain symptoms [17], mediated by the absence of inflammatory cells within the tendon itself [16]. The pathological region at the inferior pole of the patellar is distinct, in that tendinopathy is associated with relative growth of the tendinous tissue, disorganisation of the collagen fibers, and a reduction in differentiation between adjoining collagen bundles [18]. Patellar tendinopathy is known to be both recurring and debilitating for those seeking to engage in physical activity, sport and exercise [12]. Cook et al. [19] revealed that > 33% of those experiencing patellar tendinopathy were unable to return to their habitual physical activity regime within 6 months. Even more concerning were the observations of Kettunen et al. [20] that 53% of athletes presenting with this pathology were forced to permanently withdraw from their chosen sport.

Similarly, patellofemoral pain, which typically manifests as retropatellar or diffuse peripatellar pain [21], is renowned as the most predominant orthopaedic condition in sports medicine [22]. The total occurrence of patellofemoral pain ranges from 8.8–17% [23]; although the incidence rate is considerably greater in active populations, with a recent observational analysis indicating that 25% of female and 18% of male athletes were affected [24]. Pain symptoms force 74% of patients to attenuate their engagement with sport/physical activity, and causes many athletes to permanently, and prematurely end their participation in sport [25]. Therefore, many patellofemoral pain patients develop associated psychological disorders including mental distress, pain-related fear, reduced self-efficacy and kinesiophobia [26, 27]. Patellofemoral pain is exasperated by athletic tasks/disciplines that frequently and excessively load the joint [21], and elevated patellofemoral joint stress [28], knee flexion, and knee adduction [29] are regarded as the biomechanical factors most strongly linked to the development of patellofemoral pain. Although treatment efficacy for patellofemoral pain is promising in the short term, the longer-term prognosis is poor, with between 71 and 91% of individuals facing ongoing symptoms up to 20 years following diagnosis [30]. Importantly, those who experience patellofemoral symptoms may later present with radiographic evidence of osteoarthritis at this joint [31].

Because both patellar tendinopathy and patellofemoral pain syndrome typically necessitate expensive long-term rehabilitation regimes [16, 32], prophylactic modalities are becoming increasingly important. The patellar tendon strap, a band worn just below the knee, in the soft tissue between the pole of the patella and tibial tubercule, is one of the most frequently adopted external devices for the treatment/circumvention of knee pathologies [33]. However, despite their frequent utilization, there has been relatively little research attention related to the efficacy of patellar tendon straps in reducing risk from chronic knee injuries.

Lavagnino et al. [14] examined the effects of a patellar tendon strap on localized strain at the proximal aspect of the patellar tendon typically affected by tendinopathy. They measured participants in a static position during weight bearing and non-weight bearing and quantified tendon strain using radiographic images. Their findings confirmed that localized strain was significantly decreased as a function of using the tendon strap, from which it was concluded that they may limit excessive patella tendon strain. Demirbüken et al. [33] examined the influence of a patellar tendon strap on weight-bearing asymmetry during squatting in those with and without knee osteoarthritis. The findings of this analysis showed that no statistical improvements were mediated as a function of the patellar tendon strap. Rosen et al., [34] examined the acute effects of a patellar tendon strap during single-limb landings in athletes with and without patellar tendinopathy. Patellar tendon straps reduced self-reported pain, produced less hip rotation, knee adduction, ankle inversion and decreased landing forces in those with patellar tendinopathy. Rosen et al. [35] similarly examined the influence of patellar tendon straps on quadriceps' muscle activity during drop-jump landings in male athletes with and without patellar tendinopathy. Their findings showed that in both tendinopathy and control groups, the patellar tendon strap reduced vastus lateralis pre-activation. Finally, both de Vries et al. [36] and de Vries et al. [37] who examined proprioception using a knee joint position sense test found that knee joint proprioception was enhanced in those with low proprioceptive acuity. To date, however, there has yet to be any published investigation of the biomechanical effects of patellar tendon straps on patellar tendon kinetics, patellofemoral stress or lower extremity kinematics linked to the aetiology of chronic knee pathologies.

Finally, whilst clinical musculoskeletal literature has made significant progress in identifying the risk factors related to the aetiology chronic knee pathologies and the effects of different conservative treatment modalities on these factors. These biomechanical parameters are habitually explored in scientific literature through the extraction of individual kinetic/kinematic values using a procedure called discrete point analysis [38]. Statistical parametric mapping (SPM) may, therefore, represent a more effective process for the analysis of time-based data, as it is able to explore an entire data series [39]. This removes potential bias in the extraction of individual discrete variables, and also reduces the likelihood of a type II error by eliminating requirement for multiple analyses [40]. Similarly, Bayesian analyses have also become considerably more prevalent and practicable in the last decade years [41]. Nonetheless, despite their prospective benefits [42] and the plethora of statistical publications supporting their adoption, their utilization in biomechanical analyses remains limited. To date there has yet to be any biomechanical investigation which has examined the effects of different patellar tendon straps on the biomechanical parameters linked to the aetiology of chronic knee pathologies using an SPM and Bayesian approach.

Therefore, the aim of the current investigation was to examine the influence of a patellar tendon strap on knee joint kinetics and kinematics during the vertical jump, using SPM and Bayesian analyses. An investigation of this nature may provide important clinical information to athletes and physical therapists regarding the prophylactic efficacy patellar tendon straps for the attenuation of biomechanical parameters linked to the aetiology of chronic knee pathologies.

Methods

Participants

Fourteen male (age = 27.71 ± 5.50 years, height = 1.77 ± 0.05 m, mass = 73.51 ± 5.69 kg) and fourteen female (age = 28.00 ± 4.96 years, height = 1.66 ± 0.04 m, mass = 64.43 ± 2.62 kg) were recruited to this study. Participants were excluded from the study if there was evidence knee pathology or there had been previous knee surgery. Written informed consent was provided and the procedure was approved by the University ethics committee (STEMH = 637).

Patella strap

A single patellar tendon strap was utilized in this investigation, (Bionix 1), which was worn on the dominant (right) limb in all participants. Participants performed their vertical jumps in the patellar tendon strap and no-patellar tendon strap conditions in a counterbalanced manner.

Procedure

Participants were required to complete five repetitions of a counter movement vertical jump in which they were required to use full arm swing and also to commence and land the jump on the force platform. The landing phase of the jump movement was quantified and was considered to have begun when > 20 N of vertical force was applied to the force platform and ended at point of maximum knee flexion [43].

Kinematics and ground reaction force (GRF) information were synchronously collected. Kinematic data were captured at 250 Hz via an eight camera motion analysis system (Qualisys Medical AB, Goteburg, Sweden) and kinetic data using a force platform (Kistler, Kistler Instruments Ltd., Alton, Hampshire) which operated at 1000 Hz. Dynamic calibration of the motion capture system was performed before each data collection session. To quantify lower extremity segments in six degrees of freedom, the calibrated anatomical systems technique was utilized [44]. To define the anatomical frames of the pelvis, thigh, shank and foot retroreflective markers (19 mm) were positioned onto the, iliac crest, anterior superior iliac spine (ASIS), and posterior super iliac spine (PSIS). In addition, further markers were placed unilaterally onto the, medial and lateral malleoli, greater trochanter, medial and lateral femoral epicondyles calcaneus, first metatarsal and fifth metatarsal heads of the affected limb. Carbon-fiber tracking clusters comprising of four non-linear retroreflective markers were positioned onto the thigh and shank segments. In addition to these the foot segments were tracked via the calcaneus, first metatarsal and fifth metatarsal, and the pelvic segment was tracked using the PSIS and ASIS markers. The hip joint centre was determined using a regression equation, which uses the positions of the ASIS markers and the centres' of the ankle and knee joints were delineated as the mid-point between the malleoli and femoral epicondyle markers. The test–retest reliability of this marker set has been confirmed through previous analyses [45].

Static calibration trials were obtained with the participant in the anatomical position in order for the positions of the anatomical markers to be referenced in relation to the tracking clusters/markers. A static trial was conducted with the participant in the anatomical position in order for the anatomical positions to be referenced in relation to the tracking markers, following which those not required for dynamic data were removed. The *Z* (transverse) axis was oriented vertically from the distal segment end to the proximal segment end. The *Y* (coronal) axis was oriented in the segment from posterior to anterior. Finally, the *X* (sagittal) axis orientation was determined using the right hand rule and was oriented from medial to lateral.

In addition to the biomechanical information, the effects of the patella strap on knee joint proprioception were also examined using a weight bearing joint position sense test. This was conducted, in accordance with the procedure of Drouin et al. [46], whereby participants were assessed on their ability to reproduce a target knee flexion angle of 30° whilst in single leg stance. To accomplish this, participants were asked to slowly squat to a knee flexion angle of 30°, which was verified using a handheld goniometer by the same researcher throughout data collection. Participants then held this position for 15 s during which time the knee criterion position was captured using the motion analysis system. Following this, participants were asked to return to a standing position and wait for 15 s, following which they reproduced the target angle as accurately as possible but without guidance via the goniometer. Again, this position was held for a period of 15 s and the replication trial was also collected using the motion analysis system. This above process conducted on three occasions in both the brace and no-brace conditions in a counterbalanced order and between each trial each participant walked for 20 ft to eliminate any proprioceptive memory of the previous trial. The absolute difference in degrees calculated between the criterion and replication trials was averaged over the three trials to provide an angular error value in both brace and no-brace conditions, which was extracted for statistical analysis.

Following completion of the biomechanical data collection, in accordance with Sinclair et al. [47], participants were asked to subjectively rate the patella strap in relation to performing the movements without the device in terms of stability and comfort. This was accomplished using three point scales that ranged from 1—more comfortable, 2—no-change and 3—less comfortable and 1—more stable, 2—no-change and 3—less stable.

Processing

Dynamic trials were processed using Qualisys Track Manager, and then exported as C3D files. Ground reaction force and marker data were filtered at 50 Hz and 15 Hz, respectively, using a low-pass Butterworth 4th order filter, and processed using Visual 3-D (C-Motion, Germantown, MD, USA). Internal moments were computed using Newton–Euler inverse-dynamics, allowing net knee joint moments to be calculated. Angular kinematics of the knee joint were calculated using an *XYZ* (sagittal, coronal and transverse) sequence of rotations.

Patellofemoral loading was quantified using a model adapted from van Eijden et al. [48], in accordance with the protocol of Wilson et al. [49] in that co-contraction of the knee flexor musculature was accounted for Hamstring and gastrocnemius forces were calculated in accordance with previously established procedures [50]. Hamstring and gastrocnemius forces were multiplied by their moment arms relative to the knee flexion angle [51], and then summed to generate a knee flexor moment. The knee flexor moment was added to the net knee extensor moment quantified using inverse dynamics and divided by the quadriceps moment arm [4], to obtain quadriceps force adjusted for co-contraction of the knee flexors. Patellofemoral force was then quantified in accordance with the protocol of van Eijden et al. [48].

Patellofemoral joint stress was quantified by dividing the patellofemoral force by the patellofemoral contact area. Patellofemoral contact areas were obtained in accordance with the sex specific data of Besier et al. [52]. Patellofemoral force (BW) and stress (KPa/BW) were normalized by dividing the net values by bodyweight.

In addition, Patellar tendon loading was quantified using a model similarly adapted from Janssen et al., [53]. Again, the derived knee flexor moment was added to the net knee extensor moment quantified using inverse dynamics, and then divided by the moment arm of the patellar tendon, generating the patellar tendon force. The tendon moment arm was using the data of Herzog and Read [54]. All patellar tendon forces were normalized by dividing the net values by bodyweight (BW). Patellar tendon forces (BW) were normalized by dividing the net values by bodyweight.

Following this, the three-dimensional knee joint kinematics, patellar tendon and patellofemoral kinetics were extracted during the entire landing phase and time normalized to 101 data points for each participant. In addition, because SPM utilizes time normalized data we also calculated the total patellofemoral/patellar tendon force impulse (BW·s) and patellofemoral stress impulse (KPa/BW·s) using a trapezoidal function during the landing phase. Finally, the patellofemoral and patellar tendon force instantaneous loading rates (BW/s) were also quantified maximum increase in vertical force between adjacent data points.

Statistical analyses

Differences in lower extremity kinetics and kinematics during the landing phase were examined using 1-dimensional SPM approach using MATLAB 2017a (MATLAB, MathWorks, Natick, USA), in accordance with [40], via the source code available at http://www.spm1d.org/. In agreement with Pataky et al. [55], SPM was implemented in a hierarchical manner, analogous to a 2 (Patellar strap) × 2 (Gender) mixed ANOVA, with post hoc analyses in the event of a significant interaction. The alpha (α) level for statistical significance for SPM was set at the 0.05 level. In addition to this, for patellofemoral/patellar tendon impulse and instantaneous load rates descriptive statistics of means and standard deviations (SD) were calculated for each condition/gender. Differences in patellofemoral/patellar tendon impulse instantaneous loading rates (i.e., parameters that could not be contrasted using SPM) were examined using Bayesian factors

(BF) to explore the extent to which the data supported the alternative (H_1) or null (H_0) hypotheses i.e., that there were or were no meaningful differences between patellar tendon strap and no-patellar tendon strap conditions for both males and females. Bayes factors were interpreted in accordance with the recommendations of Jeffreys, [56]. Finally, participants' subjective ratings of stability and comfort were examined using Chi-squared (χ^2) tests. Discrete statistical tests were conducted using SPSS v25.0 (SPSS, USA).

Results

Statistical parametric mapping

No significant differences in knee joint kinematics were observed (Fig. 1). However, for patellofemoral force there was a main effect of GENDER, which showed that females were associated with greater patellofemoral force during the early landing phase (Fig. 2).

Discrete parameters

For knee joint proprioception there was substantial evidence in support of H_0 for both males (BF = 0.25) and females (BF = 0.32). For patellofemoral instantaneous load rate there was again substantial evidence in support of H_0 for both males (BF = 0.28) and females (BF = 0.23). For the patellofemoral force integral there was substantial evidence for H_0 in males (BF = 0.20) and anecdotal evidence in females (BF = 0.61). For the patellofemoral stress integral there was substantial evidence for H_0 in males (BF = 0.20) and anecdotal evidence in females (BF = 0.78). For patellar tendon instantaneous load rate there was anecdotal evidence for H_0 in males (BF = 0.35) and substantial evidence in females (BF = 0.24). Finally, for the patellar tendon integral there was substantial evidence for H_0 in males (BF = 0.21) and anecdotal evidence in females (BF = 0.61) (Table 1).

Subjective ratings

In males, the subjective ratings of comfort indicated that, 3 participants rated that the tendon strap improved comfort, 10 no-change and 1 reduced comfort. The Chi-squared test was significant ($\chi^2 = 9.57$, $P < 0.05$) and significantly more participants found that the tendon strap has no effect on knee comfort. In females, the subjective ratings of comfort indicated that, seven participants rated that the tendon strap improved comfort, five no-change and two reduced comfort. The Chi-squared test was non-significant ($\chi^2 = 2.71$, $P > 0.05$).

In males, the subjective ratings of stability indicated that, 11 participants rated that the tendon strap improved perceived stability, three no-change and zero reduced stability. The Chi-squared test was significant ($\chi^2 = 13.86$, $P < 0.05$) and significantly more participants found that the tendon strap enhanced knee stability. In females, the subjective ratings of stability indicated that, nine participants rated that the tendon strap improved perceived stability, three no-change and two reduced stability. The Chi squared test was significant ($\chi^2 = 6.14$, $P < 0.05$) and significantly more participants found that the tendon strap enhanced knee stability.

Discussion

The aim of this investigation was to examine the influence of a patellar tendon strap on knee joint kinetics and kinematics during a vertical jump task, using SPM and Bayesian analyses. An investigation of this nature may provide important information regarding the effects of patellar tendon straps on the biomechanical parameters linked to the aetiology of chronic knee pathologies.

Importantly, the current investigation showed using both SPM and Bayesian analyses that neither patellofemoral of patellar tendon loading parameters were meaningfully influenced as a function of the patellar tendon strap. This finding opposes those of Lavagnino et al. [14], examined the effects of a patellar tendon strap on localized strain at the proximal aspect of the patellar tendon typically affected by tendinopathy. They measured participants in a static position at 60° of knee flexion rather than during a dynamic situation, which may explain the lack of agreement between the two investigations. This observation may be clinically meaningful as both chronic patellar tendinopathy and patellofemoral pain syndrome are mediated through excessive and frequent loading [15, 28]. Therefore, the findings from the current investigation indicate that patellar tendon straps may not be effective in attenuating the biomechanical parameters linked to chronic knee injuries.

However, the examination using SPM did show that during the early landing phase, females where associated with statistically larger patellofemoral joint forces than males. This observation concurs with those observed previously in different movements [57], in that females were associated with enhanced patellofemoral joint loading compared to age matched males. Importantly epidemiological analyses have shown that females are at increased risk from patellofemoral pain in relation to age-matched males [58]. Given the proposed association between knee joint loading and patellofemoral joint pathology [28], the current investigation appears to insight into the high incidence of patellofemoral pain in female athletes.

In addition, similar to the kinetic analyses, the current investigation showed that three-dimensional knee joint kinematics were not meaningfully influenced as a

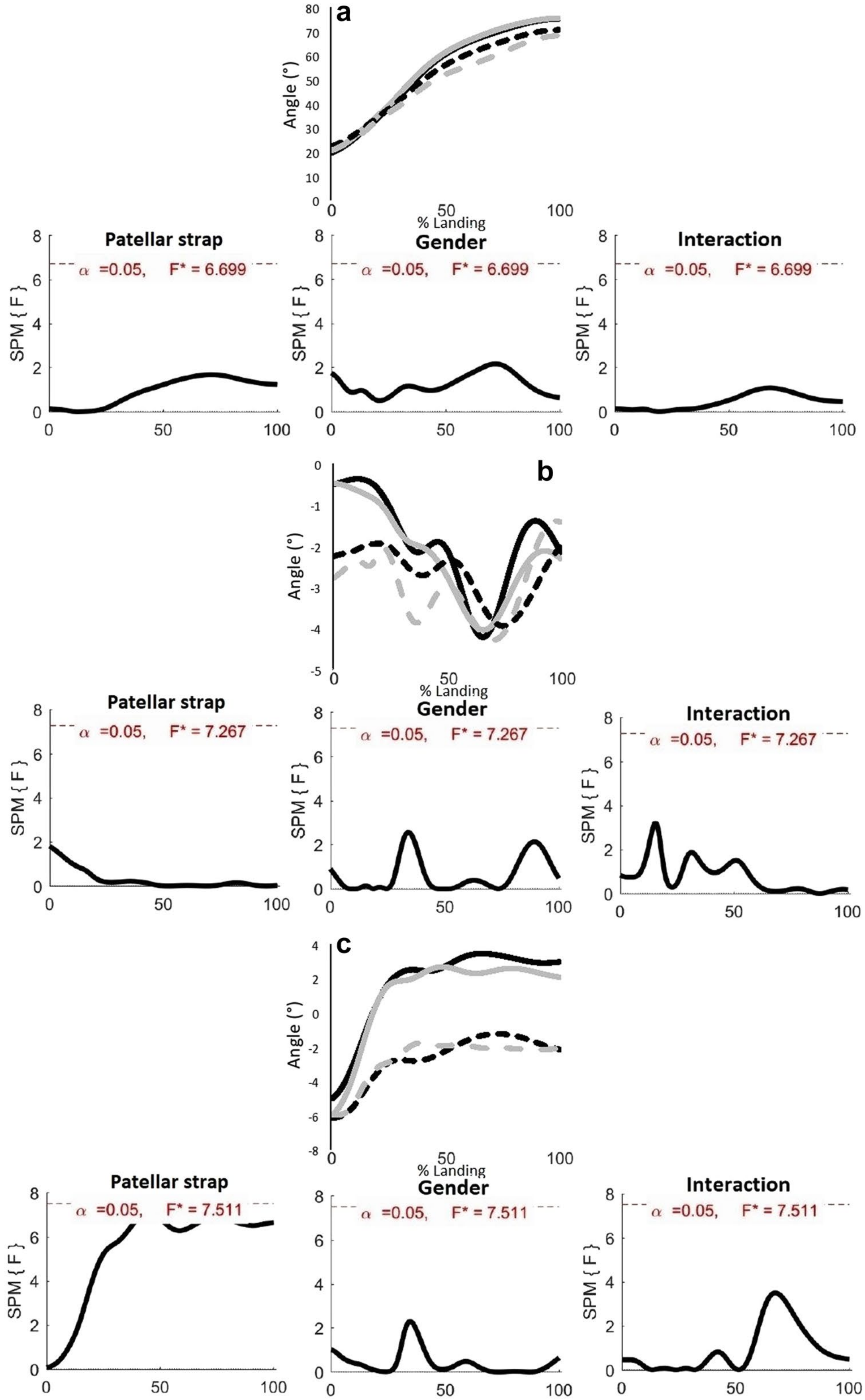

Fig. 1 Three-dimensional knee kinematics; **a** sagittal plane, **b** coronal plane and **c** transverse plane and associated SPM comparisons (black = male no patellar tendon strap, grey = male patellar tendon strap, black dash = female no patellar tendon strap, grey dash = female patellar tendon strap) (color figure online)

function of the patellar tendon strap. This observation, does not agree with those of Rosen et al., [34] who found a patellar tendon strap produced less hip rotation, knee adduction and ankle inversion in those with and without patellar tendinopathy. Athletes with patellar tendinopathy have been shown to exhibit decreased knee flexion angles during jumping activities (62). Similarly, those with patellofemoral pain have been shown to exhibit increased knee

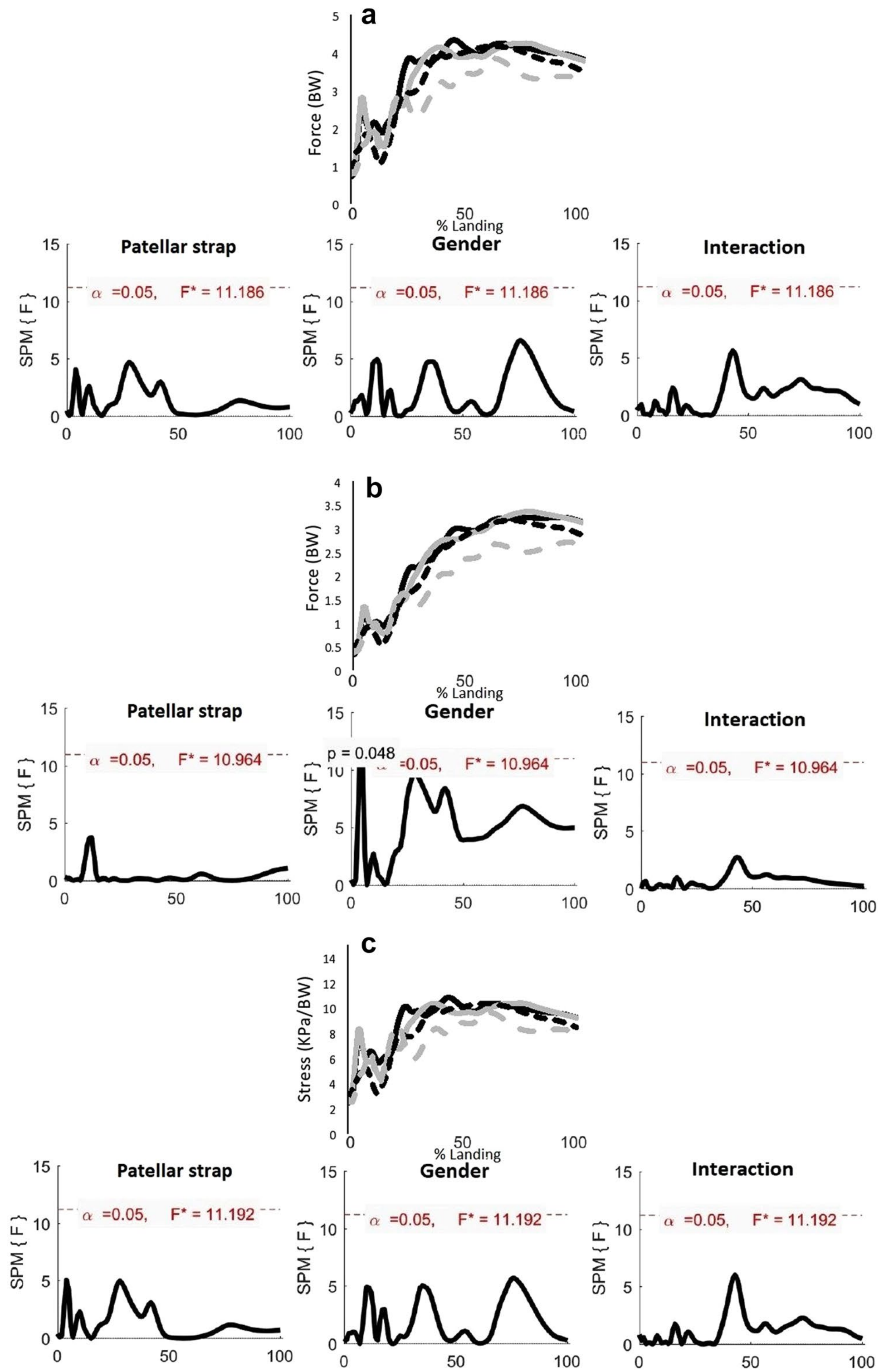

Fig. 2 Knee kinetics; **a** patellar tendon force, **b** patellofemoral force and **c** patellofemoral stress and associated SPM comparisons (black = male no patellar tendon strap, grey = male patellar tendon strap, black dash = female no patellar tendon strap, grey dash = female patellar tendon strap) (color figure online)

flexion, knee adduction and hip internal rotation in relation to non-pathological controls [29]. As such, the findings from the current investigation indicate that patellar tendon straps may not unequivocally reduce the three-dimensional kinematic parameters linked to the aetiology of chronic knee pathologies.

The current investigation also showed that knee joint proprioception was similarly not meaningfully affected by the patellar tendon strap. This observation opposes those of previous analyses indicating that patellar tendon straps improve knee proprioception. It is possible that the differences observed between analyses is due to the different approaches

Table 1 Discrete kinetic and proprioception parameters (mean and SD)

	Male				Female			
	Patellar strap		No-patellar strap		Patellar strap		No-patellar strap	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Proprioception error (°)	5.51	4.40	4.69	4.50	5.61	3.68	4.52	2.85
Patellofemoral instantaneous load rate (BW/s)	293.18	85.07	308.98	122.14	253.82	123.74	244.73	95.39
Patellofemoral force integral (BW s)	0.49	0.17	0.50	0.21	0.45	0.30	0.38	0.23
Patellofemoral stress integral (Kpa/BW)	1.64	0.55	1.66	0.63	1.63	0.84	1.38	0.59
Patellar tendon instantaneous load rate (BW/s)	572.03	163.09	606.97	244.24	487.69	230.48	473.49	192.41
Patellar tendon integral (BW s)	0.66	0.19	0.68	0.27	0.60	0.35	0.52	0.26

used to measure knee proprioception, as although de Vries et al. [36, 37] also utilized knee joint position sense analyses, this was not assessed during weight bearing. However, despite this the current study did reveal that perceived knee joint stability was significantly improved when using the tendon strap. This is an interesting observation taking into account the absence of meaningful alterations in knee joint kinetics, kinematics and proprioception, and thus it is not possible in the context of the current investigation to determine the clinical importance of improved perceived stability. Nonetheless, in future longitudinal analyses is it recommended that the clinical implications of perceived changes be examined further using patellar tendon straps.

A potential limitation to the current investigation is that patellofemoral and patellar tendon loading indices were obtained using a musculoskeletal modelling based approach. This was a necessary procedure due to the invasive nature of obtaining in vivo musculoskeletal kinetic measurements. Although this approach accounts for co-contraction of the knee flexor musculature, further work is still required to improve the efficacy of subject specific musculoskeletal models of the knee joint, making possible further developments in clinical biomechanical analyses. In addition, a further drawback to the current study is that it non-injured participants were examined, meaning that the findings are not generalizable to athletes with existing knee joint pathologies. Future, analyses should, therefore, seek to determine the clinical efficacy of patellar tendon straps as treatment modalities for athletes with existing knee injuries.

Conclusion

This study showed using SPM and Bayesian analyses that patellofemoral and patellar tendon kinetic parameters were not affected as a function of the patellar tendon strap. Similarly, three-dimensional knee joint kinematics were not meaningfully influenced as a function of the patellar strap. The findings did show, however, that the patellar strap helped to increase perceived knee stability. The current investigation, therefore, indicates that the utilization of a patellar tendon strap akin to the device used in the current study does not appear to reduce the biomechanical parameters linked to the aetiology of chronic knee pathologies, during vertical jump landing movements.

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Compliance with ethical standards

Conflict of interest The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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