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Supplementary Information

Knee kinematics:

A complete description of the kinematic data capture and resulting tibio-femoral movement patterns has been reported by Postolka and co-workers [1], but for completeness, a brief summary is provided here: A moving single-plane fluoroscope [2, 3] was used to capture radiographic images of the knee joint at 25Hz throughout multiple trials recording complete cycles each of level walking, downhill walking (10° declined slope), and stair descent (3 steps, each with 18cm height). After familiarisation trials, subjects performed level walking followed by downhill walking and then stair descent, with approximately 15 minutes resting time between activities. Five valid trials (with the knee remaining within the fluoroscopic field of view throughout the complete activity cycle) were used in this study. Accuracy of the reconstructed kinematics was previously assessed in our moving fluoroscope set-up, indicating 0.3°-1.0° error in rotational outputs, 0.2-0.6 mm for in-plane translations, and up to 7.1 mm for out-of-plane translations [4]. To reduce this out-of-plane error, the medio-lateral translation of the tibio-femoral segments was additionally visually checked and where appropriate re-fitted until a range of within ±3 mm was achieved (also reported for the natural knee [5]).

Three-dimensional models of each subject's knee that included structural and material property data of the femur, tibia, and fibula were recreated from computed tomography scans (Brilliance 64, Philips Healthcare, Netherlands and Siemens Somatom Definition AS, Siemens Healthcare GmbH, Germany). Bones were captured between 20cm proximal to 20cm distal of the knee with a resolution of 0.5x0.5 mm and a slice thickness of 1 mm. Using an intensity-based 2D/3D registration approach [4], the 3D models were then registered to the 2D fluoroscopic images to obtain accurate kinematics of the knee joint (Fig 1 in the manuscript).

The reconstructed kinematics indicated peak knee flexion angles during level walking, downhill walking, and stair descent of 62.8±5.5°, 70.1±4.7° and 95.0±5.7°, with corresponding maximum internal tibial rotation angles of 9.9±6.9°, 10.6±7.7°, and 12.3±7.6°, which generally occurred shortly before toe-off. The average range of abduction/adduction varied from 6.8° to 7.3° across the three studied activities. The average range of antero-posterior (AP) translation of the nearest contact points were comparable between activities, but considerably larger for the lateral (15.5±6.1mm) than the medial (10.3±3.2mm) condyle over the stance phase of level walking [1].

Sensitivity of the utilized multi-body modelling technique to uncertainty in kinematic inputs has been extensively assessed in our previous studies [6, 7]. In particular, maximum MCL elongation was changed by 0.4% due to a 1mm change in antero-posterior translation, 0.7% for 1° change in abduction-adduction, and 0.08% for 1° in internal-external tibial rotation. LCL exhibited the greatest sensitivity to out-of-plane translational error, yet its maximum elongation was changed by only 0.2% per 1mm change in mediolateral translation. Moreover,

when sensitivity to model parameters was considered, the maximum aMCL elongation was changed by 0.4% when its femoral attachment was moved 1mm in the anterior-posterior and proximal-distal directions. Sensitivity of collateral ligament elongation patterns to variation in tibial attachment position of the bundles was negligible.

Additional figures and tables:
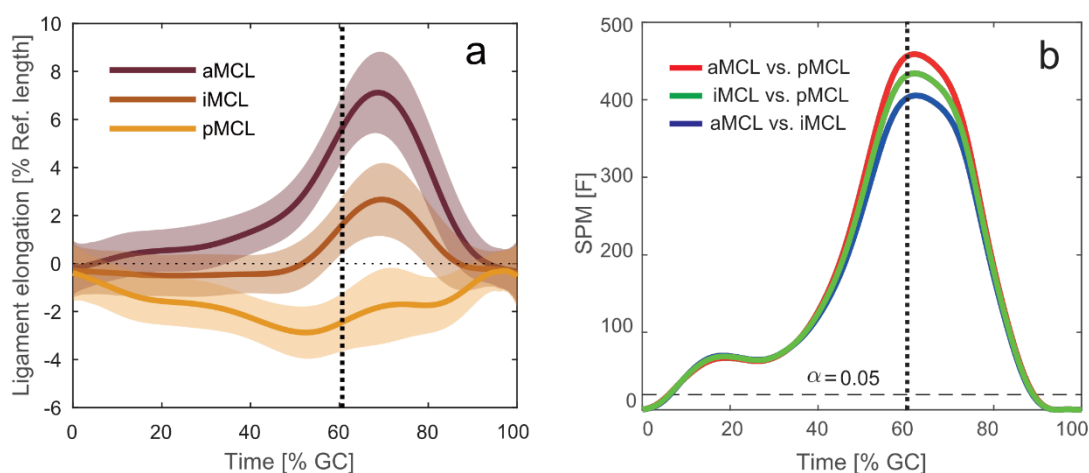
Fig s1. a) Elongation patterns of the three MCL bundles during downhill walking (solid lines represent inter-subject means while the shading represents ± 1 inter-subject standard deviation; horizontal dashed line represents the F-statistics corresponding to the significance level of $\alpha=0.05$). **b)** F-value for the SPM tests demonstrates that a regional dependency of the ligament elongation patterns exists for most of the activity cycle. The vertical dotted line represents the average toe-off time for the ten subjects.
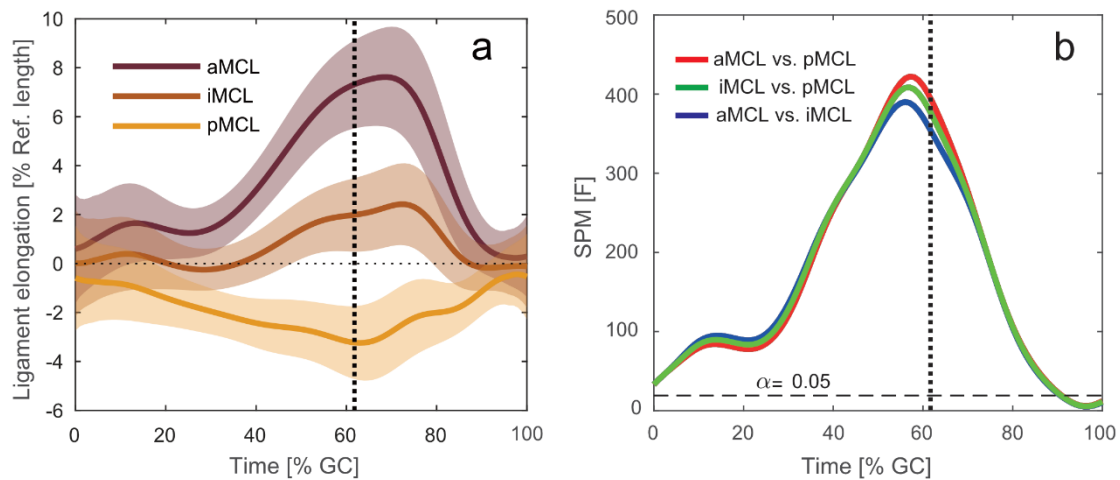
Fig s2. a) Elongation patterns of the three MCL bundles during stair descent (solid lines represent inter-subject means, while shading represents ± 1 inter-subject standard deviation; horizontal dashed line represents the F-statistics corresponding to the significance level of α=0.05). **b)** F-value for the SPM tests indicates a strong regional dependency of the ligament elongation patterns for most of the activity cycle. The vertical dotted line represents the average toe-off time for the ten subjects.

Table s1. F_{max} and F-statistics (in bracket) for the SPM tests performed to assess task-dependency of the ligament elongation patterns during stance and swing phases of the studied activities.

	Stance phase			Swing phase		
	LW vs. DW	*LW vs. SD*	*DW vs. SD*	*LW vs. DW*	*LW vs. SD*	*DW vs. SD*
aMCL	2.42 (10.10)	3.74 (11.40)	11.11 (8.08)[a]	0.13 (10.48)	36.36 (9.08)[b]	3.57 (10.10)
iMCL	0.87 (9.91)	4.47 (11.41)	3.96 (8.04)	0.10 (10.49)	14.06 (9.13)[c]	2.71 (10.10)
pMCL	6.22 (9.90)	6.76 (11.41)	2.17 (8.06)	0.13 (10.52)	4.78 (9.18)	1.74 (10.12)
LCL	4.76 (10.40)	1.25 (11.16)	1.28 (7.26)	9.56 (10.36)	1.20 (8.61)	3.80 (9.72)

Significant SPM; [a](between 45° and 46°), [b](between 61° and 70°), and [c](between 66° and 70°).

Matlab script for repeated measures ANOVA based on statistical parametric mapping (https://spm1d.org):

```
clear;
clc

% Load data:
dataset    = spm1d.data.uv1d.anova1rm.LigamentElongationData();
[Y,A,SUBJ] = deal(dataset.Y, dataset.A, dataset.SUBJ);% Y is the
ligament %elongation matrix, A and SUBJ are categorical variables
with the same length as Y %that represent the Bundle/activity and
subject number

% Conduct SPM analysis:
spm_bs    = spm1d.stats.anova1(Y, A);  %between-subjects model
```

```matlab
spm        = spm1d.stats.anova1rm(Y, A, SUBJ);  %within-subjects
model
spmi       = spm.inference(0.05); % For multiple tests, the
significance level %should be adjusted based on Bonferroni
correction
disp(spmi)



% Plot SPM graphs:
close all
spmi.plot();
spmi.plot_threshold_label();
spmi.plot_p_values();
hold on
plot(spm_bs.z, 'r')
```

References:

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5. Gray HA, Guan S, Thomeer LT, Schache AG, de Steiger R, Pandy MG. Three-dimensional motion of the knee-joint complex during normal walking revealed by mobile biplane x-ray imaging. J Orthop Res. 2019;37(3):615-30. doi:10.1002/jor.24226.

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