

Please cite the Published Version

Roldán, E, Reeves, ND, Cooper, G and Andrews, K (2017) In vivo mechanical behaviour of the anterior cruciate ligament: A study of six daily and high impact activities. *Gait & Posture*, 58. pp. 201-207. ISSN 0966-6362

DOI: <https://doi.org/10.1016/j.gaitpost.2017.07.123>

Publisher: Elsevier

Version: Accepted Version

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***In vivo* mechanical behaviour of the anterior cruciate ligament: A study of six daily and high impact activities**

ABSTRACT

The anterior cruciate ligament (ACL) plays a key role in the stability of the knee joint restricting the rotation and anterior tibial translation. However, there is a lack of knowledge of the *in vivo* ACL mechanical behaviour during high impact manoeuvres. The motion of 12 young participants with healthy knees was captured while they performed the following activities: walking, running, cross-over cutting, sidestep cutting, jumping and jumping with one leg. The *in vivo* ACL length and strain were estimated using experimental kinematic data and three degree of freedom (DOF) knee model. The *in vivo* ACL tensile forces were determined with a well-established force/strain relationship obtained through ACL tensile tests. Statistical regression models between ACL length with respect to angles for each activity have been performed in order to better understand the ACL failure mechanisms. The maximum ACL tensile force was observed during jumping vertically at maximum effort with two legs (1.076 ± 0.113 N/BW). Surprisingly, the peak tensile ACL force for all subjects during crossover cutting (0.715 ± 0.2647 N/BW) was lower than during walking (0.774 ± 0.064 N/BW). Regression coefficients for crossover cutting indicated that excessive knee rotation and abduction angles contribute more significantly to the ACL elongation than in activities such as walking or running. These findings suggested that the ACL is subjected to multidirectional loading; further studies will be performed to investigate torsion, tensile and shear force on the ligament.

Key Terms: Anterior cruciate ligament (ACL); daily and high impact activities; *in vivo* kinematics; knee model; ACL tensile forces; ACL strain.

INTRODUCTION

The ACL is a key component in the stabilization of the knee joint, restricting the rotation and the anterior tibial motion and has one of the higher rates of injury of the human knee ligaments [1]. It is estimated that 78% of ACL injuries occur during noncontact manner, where sudden decelerations, landing from a jump, cross-over cutting or pivoting are present [2]. The majority of these injuries were recorded in young athletes [3]. Anatomical (femoral notch distance or laxity), hormonal and biomechanical factors have been researched to understand the important factors contributing to these higher rates of injury [4,5]; however the key factors contributing to ACL mechanical behaviour during high-risk activities and ACL rupture remain unclear.

Experiments using cadaveric knees [6] determining anthropometrical parameters (ACL insertion points locations or femoral notch distance) or understanding the behaviour under mechanical loading exhibited limitations reproducing the *in vivo* mechanical behaviour of the ACL. Video and motion capture techniques [7,8] were also used to understand ACL injury mechanisms through the study of joint kinematics but disregarded the ACL length, strain or force. Strain gauges have also been employed to assess the *in vitro* ACL strain [9] and *in vivo* ACL strain in cycling and squatting [10,11], but in the last case is invasive, requires surgery and the gauge is attached to only a small location of the ACL, not recording the strain across the whole ligament. Experiments with motion capture and biplanar fluoroscopy [12,13] and studies with motion capture and musculoskeletal simulations [14–16] determined ACL length and

strain during walking and jumping with both legs but activities with a high injury rates such as jumping with one leg or cutting have not been evaluated.

Due to the wide range of techniques used to determine *in vivo* ACL strain and force and the limitations associated to each technique, there are differences in the results obtained, and comparison between activities is complicated. During walking for example, there is large disparity with some studies reporting peak ACL forces of 0.2 N/BW [17] and others of more than 1 N/BW [18,19]. Therefore it is necessary to develop and implement a reliable and easy methodology to investigate different activities in one study to allow valid comparison. In this study, a musculoskeletal model with motion capture was used to determine the ACL length, strain and tensile force at all ranges of motion for six different activities: walking, running, sidestep cutting, crossover cutting, jumping with one leg and drop landing and jumping with maximum effort with two legs.

The aim of this study was to estimate *in vivo* ACL strain and tensile force through a motion capture in combination with a 3 DOF knee model while daily and high impact activities are performed, to better understand high-risk manoeuvres and compare the ACL behaviour between activities.

METHODS

Participants

Twelve young adults with no previous ACL injuries (7 men and 5 women; mean \pm SD age: 27.3 \pm 3.3years, height: 1.7 \pm 0.09 m and mass: 71.6 \pm 15.5 kg) were

recruited. Test subjects gave informed consent and the study was approved by the university ethics committee.

Protocol

A 10-camera motion capture system (Vicon 612 system, Oxford Metrics, UK) was used to collect kinematic data sampled at 100 Hz. A modification of the Cleveland Clinic marker set [20], consisted of 33 reflective markers placed on specific anatomical landmarks of the lower and upper body of the subjects, was utilized to collect the kinematic measurements.

All activities were performed over level ground and the participants were asked to perform them at their self-selected speed in order to not modify the correct execution of the task. Activities were: walking, running in a straight line, crossover cutting turning suddenly, sidestep cutting running straight then stepping at the right side with the right leg and keeping running straight, dropping from a 30cm height jumping box and perform a vertical maximum effort jump with both legs, and jumping horizontally three times with one leg. Tape was placed on the floor with the purpose of all subjects undertaking the activity in the same manner, moreover three force plates were used just for references to perform the tasks. The tape was used to indicate the starting and ending point of all the activities and the trajectories of the cutting tasks. Once the subjects were familiarised with the activities (performing them until their execution were correct) the kinematic data was collected for each participant over three successful repeated trials.

The data were processed in Vicon Nexus 1.8.5. software (Vicon Motion Systems Ltd, UK) and exported to OpenSim 3.3 (Sim TK, Stanford, CA) for the kinematics analysis.

ACL elongation and force estimation

The *in vivo* ACL length at each timepoint was determined by tracking the coordinates of the ACL insertion points from the processed kinematic data derived from motion capture and a musculoskeletal model scaled for each participant according to their anatomy. The developed model was based on the Gait 2392 model [21] implemented in OpenSim software. Our model consisted of 27 DOF of which 6 corresponded to the knee joints (3 DOF for each knee), 12 segments represented the bones and 92 musculotendon actuators. The anterior cruciate ligaments were modelled as an elastic passive soft tissue as previously described in Roldán et al. [22].

The strain at each instant was calculated from the ligament length change at each timepoint and the unloaded length. The *in vivo* unloaded length of the ACL for each participant was determined from a reference length (ACL length at knee full extension calculated for each subject) and reference strain (taken from literature [23]) following previous studies [22,23].

ACL tensile forces were calculated from a force/strain relationship reported in Blankevoort and Huiskes et al. [23] where the ligament was simulated by a non-linear spring, and its viscoelastic behaviour was mimicked with a damping element in parallel to the spring following the method implemented in previous studies [22]. Subsequently these estimated forces were normalized for Body Weight (BW) allowing comparison between subjects.

Notice that although the behaviour of both ACLs were collected, for the sake of simplicity only the results for the right knees are presented.

Similar approaches to determine the ACL elongation and tensile force were used in Debski, Darcy and Woo et al. [24] when testing cadaveric knees.

Statistical analysis

Multiple linear regressions were performed to estimate the relationship between right ACL length and right knee angles for each activity (considering positive sign knee flexion, external rotation and abduction angles). The coefficient of determination (R^2) decreases considerably when the sample size increases. In our case, the sample size for each activity was very large, reaching 3410 degrees of freedom for the two-legged jumping activity. Therefore, in order to verify the goodness of fit it was necessary not only consider the R^2 value, but also to have low standard error of the estimation, a highly significant F test with value of F above unity, highly significant regression coefficients (P value < 0.001) with short confidence intervals, non-collinearity between independent variables with a condition index below 15, and the normal distribution of the residuals. The Pearson coefficient signs indicated to us the behaviour of the ACL length when one of the knee angles varies. The sign of regression coefficients and Pearson coefficients could be different, since Pearson coefficients measure how an independent variable is related with the dependent variable and the regression coefficients indicates how all independent variables are related with the dependent variable.

RESULTS

All activities

The peak ACL force and strain, position of the maximum ACL length and elongation of the ACL for each activity are presented in table 1. The maximum ACL tensile force and elongation were seen during jumping with two legs.

Every subject exhibited a similar pattern of knee angles and ACL length performing the same activity, therefore changes in ACL length for both sexes

(considering sex as a categorical variable with 0 for men and 1 for women) can be predicted by changes in the three knee angles (quantitative variables) through multilinear regression models. The models performed in this study were highly significant with a high Fisher's F, highly significant regression coefficients (P value < 0.001) with short confidence intervals demonstrating the precision of the estimation, a low standard error of the estimation, non-collinearity of the independent variables and normal distribution of the residuals. The signs of Pearson correlation coefficients (table 2) indicated that the ACL length is longer in males, when the knee is extended, knee internal rotation increases and with more valgus angle.

Walking

Analysing the walking motion, the maximum ACL length was recorded at full knee extension (stage C in figure 1) at heel off. The ACL length minimum was at 50° of knee flexion (during the swing phase). A maximum was observed at maximum knee flexion (around 65°) due to a decrease of knee rotation angle.

The following function (1) predicts the ACL length for walking.

$$\text{ACL length (m)} = 0.03317 - 0.002415 * \text{Sex} - 0.00009 * \text{Knee flexion angle (}^\circ\text{)} - 0.000144 * \text{Knee external rotation angle (}^\circ\text{)} + 0.00006 * \text{Knee abduction angle (}^\circ\text{)} \quad (1)$$

Jumping with two legs

Five ACL length peaks were found in all participants. Figure 2 a) shows the stages for this activity, where A corresponds to the moment just before reaching the force plates where the knees are extended or almost extended, B is the maximum flexion of the knee after the drop where valgus and internal rotation angles increase, C is when the participant extended completely the knees during the jump (flight phase of

the jump), D is the moment where the subject reached the highest height jumping and E is the maximum flexion of the knee after the jump.

Regression coefficients were used to determine the relationship between gender, knee angles and ACL length at each stage for this activity:

$$\text{ACL length (m)} = 0.02932 - 0.000675 * \text{Sex} - 0.000036 * \text{Knee flexion angle (}^\circ\text{)} - 0.000168 * \text{Knee external rotation angle (}^\circ\text{)} - 0.000054 * \text{Knee abduction angle (}^\circ\text{)} \quad (2)$$

Jumping with one leg

A total of six ACL length peaks were found in all participants; the first (stage C), third (stage G) and fifth (stage J) peaks corresponded to the moment at toe off, the second (stage E), fourth (stage H) and sixth (stage K) were when the participants were jumping with their leg extended. Stages F and I corresponded with the moment when the participants were landing flexing their knee. Stage B was the moment when the participant flexed the knee to gain momentum for the jump and D was the moment when the participants were jumping with the knee slightly flexed. Figure 2 b) shows all these stages for the ACL length versus the knee angles.

The relationship between gender, knee angles and ACL length is shown in the equation (3):

$$\text{ACL length (m)} = 0.03149 - 0.00224 * \text{Sex} - 0.000087 * \text{Knee flexion angle (}^\circ\text{)} - 0.000159 * \text{Knee external rotation angle (}^\circ\text{)} - 0.00005 * \text{Knee abduction angle (}^\circ\text{)} \quad (3)$$

Running

Three ACL length peaks were found in all participants, the first peak corresponded to heel strike being the highest of the three peaks (stage A), the second peak is at toe off (stage B) and the third is at maximum knee flexion (stage C). This situation can be observed in figure 3 a).

In the regression model for running, the constant, sex and rotation angle were highly significant; contributing to the prediction of the dependent variable. However, the coefficients of knee flexion and abduction angles were not significant (P value 0.55 and 0.18 respectively), which means that these angles do not contribute significantly to the model. The predicted function for running was:

$$\text{ACL length (m)} = 0.02999 - 0.00306 * \text{Sex} - 0.0000019 * \text{Knee flexion angle (}^\circ\text{)} - 0.000084 * \text{Knee external rotation angle (}^\circ\text{)} - 0.000022 * \text{Knee abduction angle (}^\circ\text{)} \quad (4)$$

Sidestep cutting

Similar to the previous activity (running) three ACL length peaks were found in all our participants, the first peak corresponded to heel strike (stage A), the second peak was at toe off (stage B) and the third at maximum knee flexion (stage C). See figure 3 b).

As it was observed running, the constant, sex and rotation angle provided high information in predicting the dependent variable in the regression model, however the coefficients of knee flexion and abduction angles were not significant (P value 0.55 and 0.62 respectively), which means that these angles do not contribute significantly to the model. The function model obtained was:

$$\text{ACL length (m)} = 0.0282 - 0.001997 * \text{Sex} + 0.000002 * \text{Knee flexion angle (}^\circ\text{)} - 0.000086 * \text{Knee external rotation angle (}^\circ\text{)} - 0.0000096 * \text{Knee abduction angle (}^\circ\text{)} \quad (5)$$

Crossover cutting

As observed in the activities of “running” and “sidestep cutting” the pattern of the ACL length show three peaks for all participants in the same stages. The first peak corresponds to heel strike (stage A), the second peak is at toe off (stage B) and the third is at maximum knee flexion (stage C), as it can be observed in figure 3 c).

The function that predicted the ACL length for crossover was:

$$\text{ACL length (m)} = 0.02775 - 0.00239 * \text{Sex} + 0.000019 * \text{Knee flexion angle (}^\circ\text{)} - 0.000666 * \text{Knee external rotation angle (}^\circ\text{)} + 0.000058 * \text{Knee abduction angle (}^\circ\text{)} \quad (6)$$

DISCUSSION

The novelty of this study was the estimation of *in vivo* ACL length and tensile forces during different dynamic activities, which could provide an insight into high-risk activities for ACL injuries and rehabilitation protocols or provide mechanical specifications for ACL implants.

The peak ACL forces were previously reported to range between 0.2 N/BW and 1 N/BW during walking [16]. We observed a peak ACL tensile force of 0.774 ± 0.064 N/BW during walking corresponding to 28.5% of the reported failure strength of the ACL [25,26]. Therefore the value we report is in the same range as the ACL loads previously reported. We observed a peak strain during walking of 13%, which agrees with a previous study based on motion capture and biplanar fluoroscopic images the same activity [12].

The highest peak of ACL tensile force was recorded jumping with both legs (1.076 ± 0.113 N/BW) and their values were around 4 times higher than jumping with one leg (0.774 ± 0.064 N/BW); the knees were more flexed during the execution of one-legged jumping in all participants, decreasing the ACL elongation and consequently the estimated ACL tensile force.

From our study we observed that non-contact activities with high ACL injuries rate such as crossover cutting or sidestep cutting are surprisingly associated with lower tensile forces in the ACL than daily activities such as walking. This evidence indicates that the failure mechanisms for these activities are likely due to a combination of loads,

not just tensile loading. If we analyse the Pearson correlation coefficients for cutting, we observed more significant linear relationship between rotation and abduction angles with the ACL length than with the flexion angle, therefore knee rotation and abduction angles contribute greatly to the elongation of the ACL for these activities as it was predicted by Mc Lean et al. [27]. It is known that small knee flexion angles generate higher ACL tensile forces, the greater the valgus (abduction) angle the higher ACL loading is observed [14,28,29] and the greater rotation angle the ACL could be subjected to a higher torsional load. Therefore, this study demonstrates for the first time that to understand the mechanical behaviour of the ACL a study of its tensile, shear and torsional loading is needed. Future work should estimate shear and torsion loads for each activity studied in this paper.

There are two main limitations of this study. The first limitation is the assumption of having 3 DOF per knee instead of 6 DOF, in future studies a model including 6 DOF for each knee will be performed. The second limitation is that the ACL tensile forces were estimated from the ACL strain and this estimated by tracking the coordinates of the ACL insertion points and considering the ACL as a single straight bundle; however, it is known that the fibre bundles of the ACL are twisted and wrapped around the bones.

In conclusion, this study determined and compared *in vivo* ACL length and tensile forces during dynamic activities using a viscoelastic ligament model for a wide range of activities. The results of the study could inform research into ACL injury mechanisms or provide mechanical specifications for manufacturing improved ACL implants. It established highly significant regression models between ACL length with respect to knee angles for the different activities enabling prediction of the behaviour of the ligament according to given angles for each activity. Finally, the peak tensile ACL

force during cutting or running was lower than walking, suggesting that the failure mechanisms are due to multidirectional (including shear and torsion) loading instead of simply tensile loading in isolation.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest in this work.

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Table 1. Peak right ACL tensile forces, elongation, strain and position of the maximum right ACL length for each activity (mean±SD).

Activity	Max. Force (N)	Max. Force (N)/BW	Max. Elongation (mm)	Max. Strain	Max. ACL Length Position
Walking	513.068±8.337	0.774±0.064	6.15±0.28	0.132±0.00248	Heel off
Jumping with two legs	726.003±64.222	1.076±0.113	7.32±1.21	0.175±0.01284	Flight phase of the jump
Running	368.243±113.627	0.624±0.216	5.28±0.05	0.097±0.00249	Heel strike
Sidestep cutting	472.703±168.209	0.836±0.323	5.33±0.01	0.107±0.04027	Heel strike
Crossover cutting	425.869±147.602	0.715±0.265	5.36±0.15	0.107±0.03237	Heel strike
Jumping with one leg	149.352±55.474	0.243±0.092	3.86±0.17	0.047±0.01616	Flight phase of the jump

Table 2. Multiple linear regression parameters.

Activity	Fisher's F	Pearson Coefficients				Standard error of estimation	R ²
		Sex	Flexion	Ext. Rotation	Abduction		
Walking	615.27*	-0.446*	-0.526*	-0.483*	0.206*	0.002	0.614
Jumping with two legs	359.44*	-0.313*	-0.209*	-0.435*	0.06*	0.0028	0.297
Running	136.02*	-0.542*	-0.6*	-0.391*	0.137*	0.0025	0.355
Sidestep cutting	60.63*	-0.398*	-0.07*	-0.414*	0.083*	0.0029	0.243
Crossover cutting	91.43*	-0.469*	0.098*	-0.424*	0.221*	0.0027	0.307
Jumping with one leg	891.81*	-0.574*	-0.414*	-0.646*	0.146*	0.002	0.662

* Highly significant P value < 0.001

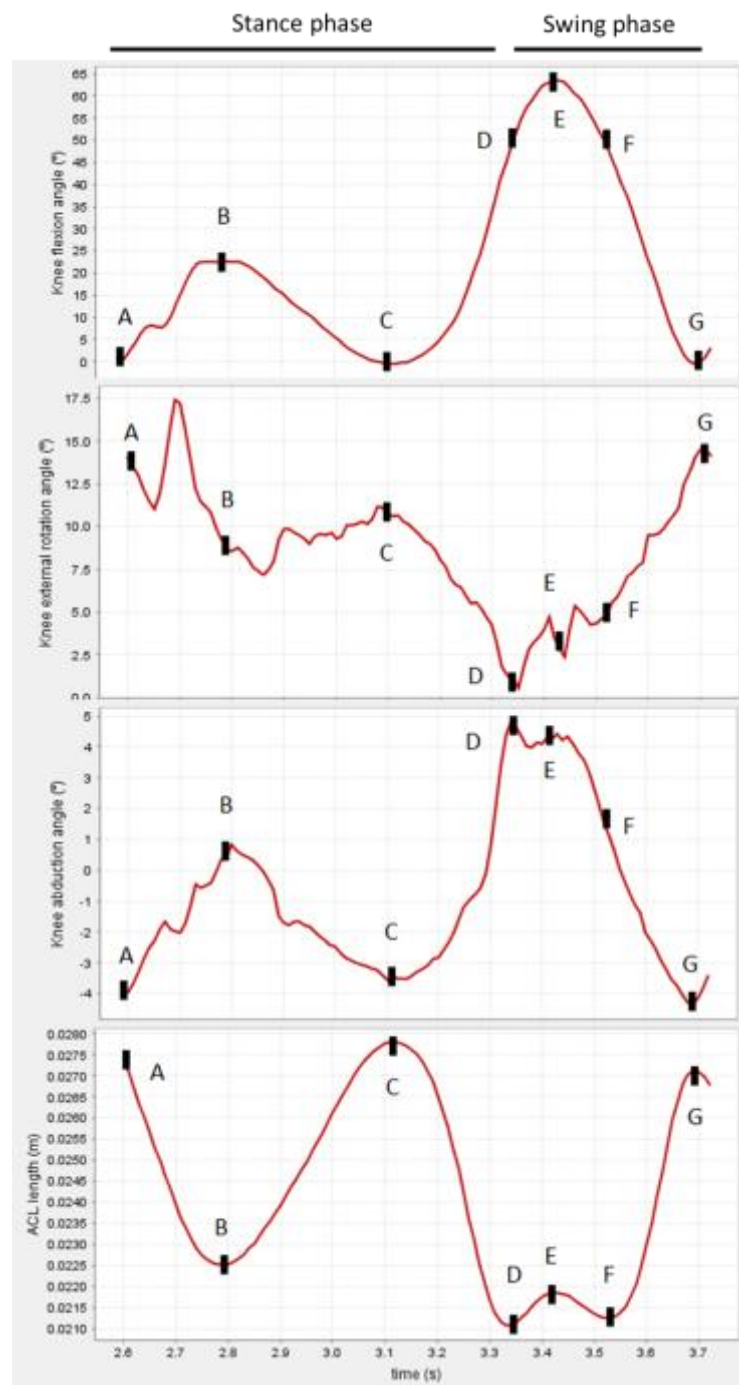


Figure 1. Example right knee angles (°) and right ACL length (m) from one participant during walking (Stages A and G correspond to heel strike, B to foot flat, C heel off, D toe off, and E and F midswing.)

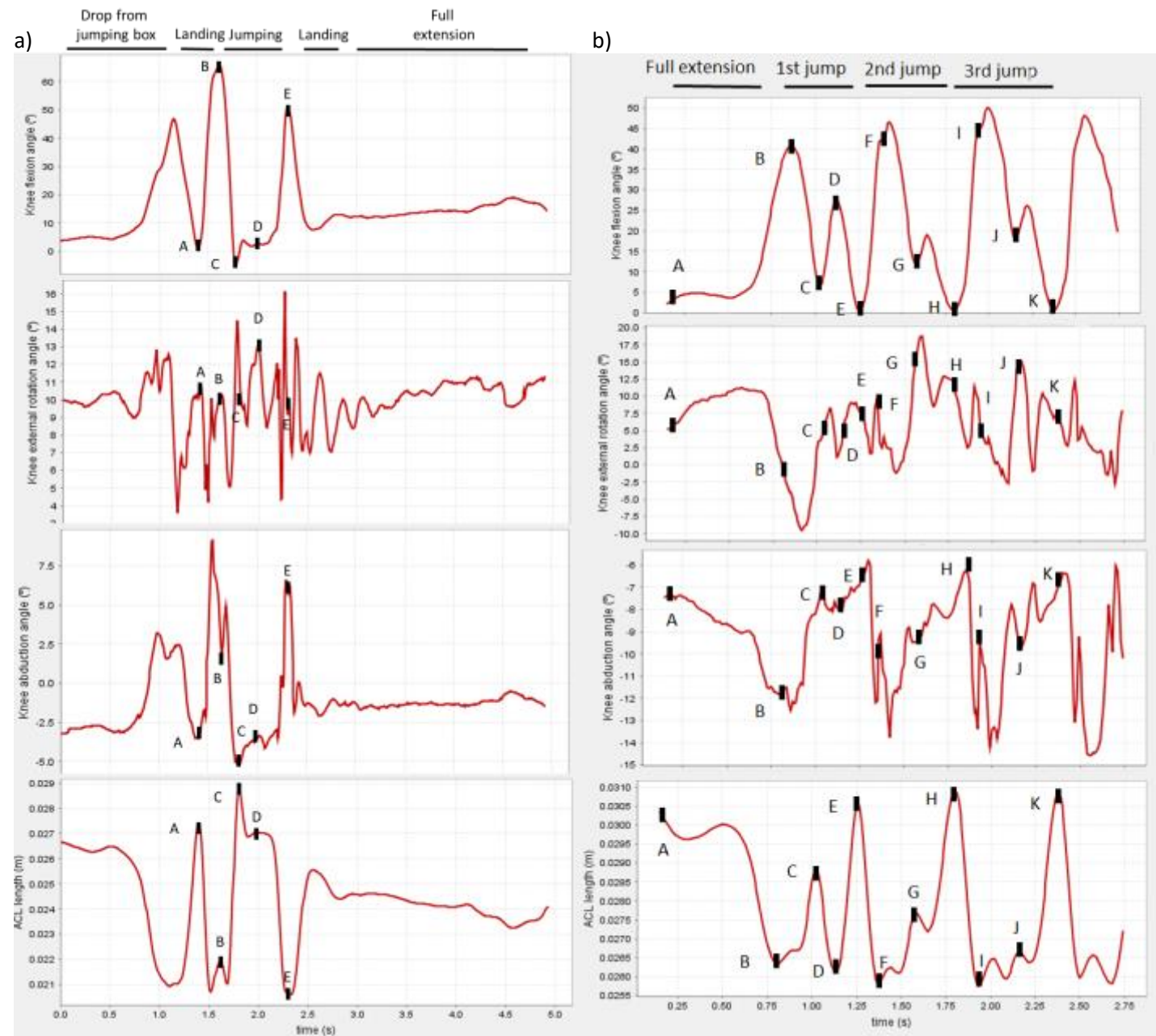


Figure 2. Example right knee angles (°) and right ACL length (m) from one participant a) jumping with two legs b) jumping three times with one leg.

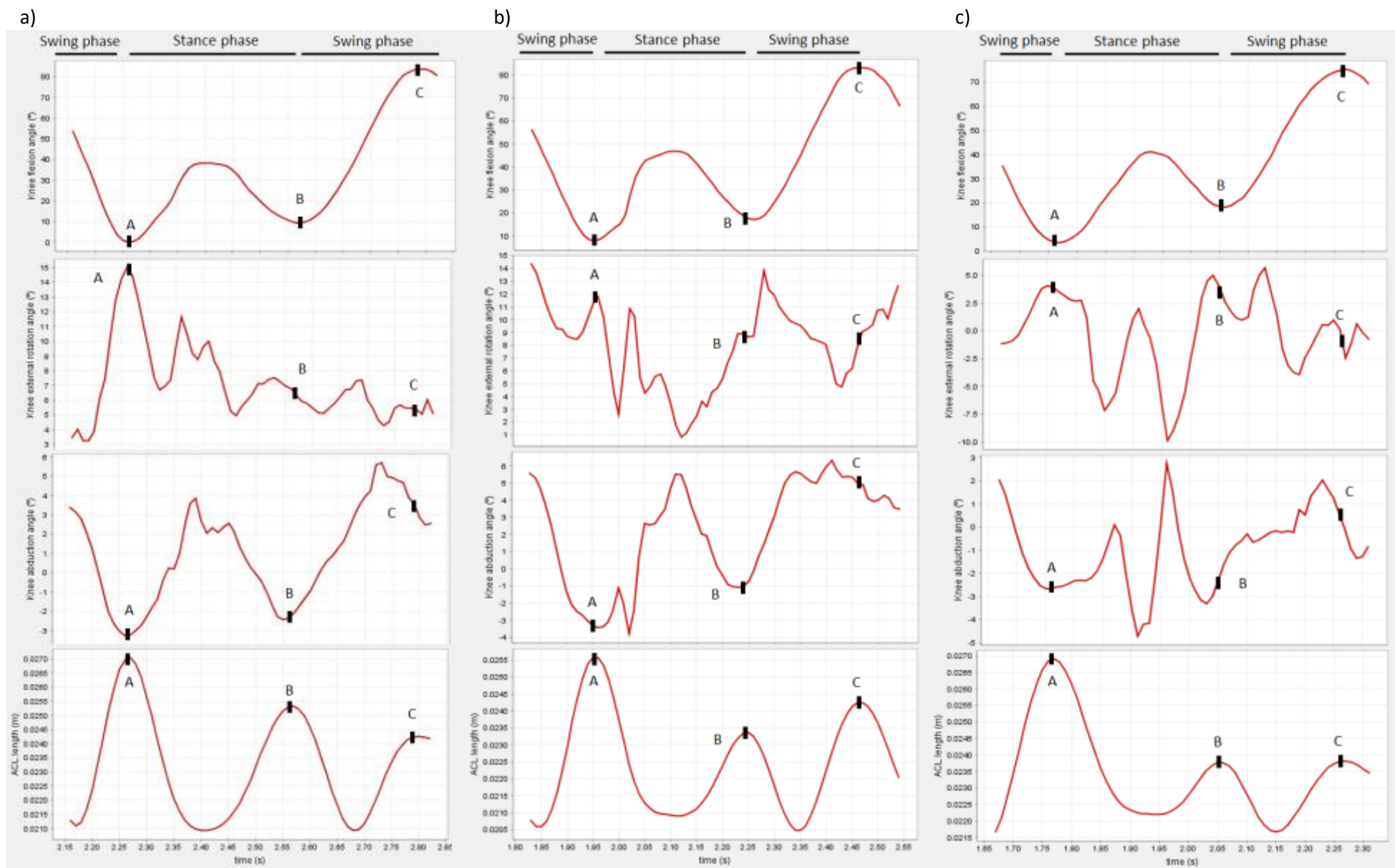


Figure 3. Right knee angles (°) and right ACL length (m) from one of our participants a) running, b) sidestep cutting, c) crossover cutting.