WHAT CAN WE LEARN FROM *IN VIVO* BIOMECHANICAL INVESTIGATIONS OF LOWER EXTREMITY?

Mario Lamontagne[1-2], D.L. Benoit[3], D.K. Ramsey[4], A. Caraffa[5-6], G. Cerulli[5-6]

1- School of Human Kinetics and 2- Dept. of Mechanical Engineering, University of Ottawa, Ottawa, Canada; 3- Dept. of Mechanical Engineering, University of Delaware, Newark-DE, USA; 4- Dept. of Physical Therapy, University of Delaware, Newark-DE, USA; 5- Dept. of Orthopaedic Surgery, University Hospital of Perugia, Perugia, Italy; 6- Let People Move, Biomechanics Laboratory, Perugia, Italy

In vivo biomechanical investigations of human movement are needed to better understand function and injury mechanism of the musculoskeletal system and to validate models or methods that otherwise could not be validated. In this report, we showcase two biomechanical approaches that use *in vivo* experiments to directly measure skin movement artefacts and the role of hamstring neuromuscular control in protecting the anterior cruciate ligament (ACL). The study on skin movement artefacts revealed that surface markers provided kinematics which can present repeatable patterns within a participant for various movements. However these repeatable patterns must not be misinterpreted as accurately representing skeletal kinematics, at least beyond the sagittal plane of movement. In the second investigation the neuromuscular control of the hamstring and gastrocnemius muscles showed a protective mechanism to prevent excessive ACL elongation, whereas the quadriceps muscles resisted against the collapsing of the knee joint after foot impact with the ground. This paper highlights the value of in vivo experimentation in contributing to our understanding of biomechanical functions or processes.

KEY WORDS: In vivo measurements, kinematics, ACL strain, skin markers

INTRODUCTION: Quantitative biomechanical analysis is important for gaining a systematic understanding of normal and pathological joint motion during human performance. Biomechanical analysis of human motion often comprises of measuring kinematics, kinetics and neuro-muscular activity of a series of joints. Accurate and precise measurements of the kinematics and kinetics of joints are needed to understand the function or malfunction of the musculoskeletal system during human movement performance (Cerulli *et al.*, 2003; DeFrate *et al.*, 2004; Fleming & Beynnon, 2004; Komistek *et al.*, 2005; Li *et al.*, 2005; Ravary *et al.*, 2004). Yet knowledge about joint kinematics and kinetics are limited by the accuracy of the measurement system used (Ramsey & Wretenberg, 1999).

Usually joint kinematics are obtained by attaching reflective markers to the segment skin. Based upon rigid body mechanics, three-dimensional kinematics assumes that markers placed on the skin represent the position of bony landmarks for the segment. However skin markers move in relation to the bony landmarks resulting in relative errors (Ishii *et al.*, 1997). Consequently considerable questions remain concerning the accuracy of joint kinematics (Reinschmidt *et al.*, 1997a).

Reinschmidt *et al.*, (1997b) have reported segmental errors due to skin movement artefact of around 5°. *In vivo* measurements of skeletal motion by using markers fixed on bone pins represents an accurate but ethically questionable technique (Lafortune *et al.*, 1992; Ramsey *et al.*, 2001; Ramsey *et al.*, 2003) which represents the most accurate method for determining bone movements (Cappozzo, 1991). Since *in vivo* and invasive methods for joint kinematics are not suitable for routine analyses, surface marker optimisation methods have been proposed to correct skin movement artefacts through the application of clusters of markers (Andriacchi *et al.*, 1998; Cheze *et al.*, 1995; Lucchetti *et al.*, 1998). Some methods reported reduced kinematics errors between 25 and 33%, however, no studies have validated their techniques with *in vivo* kinematics data of healthy subjects during physiological motions.

From a clinical perspective investigations of ligament and tendon loading are essential to gain insight into injury mechanism (Zavatsky & Wright, 2001) and may lead to the prevention of injuries. Several *in vivo* strain studies (Arms *et al.*, 1984; Beynnon & Fleming, 1998; Beynnon *et al.*, 1997a; Beynnon *et al.*, 1997b; Cerulli et al., 2003; Fleming *et al.*, 1999; Fleming *et al.*, 2001; Li *et al.*, 2004; Yoo *et al.*, 2005; Zavatsky & Wright, 2001) have provided insight into the biomechanical function of ligaments but have offered limited information for activities involving neuromuscular control. Only few investigations have investigated the *in vivo* ACL mechanical behaviour during dynamic motions such as the jump, quick stop, and cut (Benoit *et al.*, 2000; Beynnon & Fleming, 1998; Cerulli et al., 2003; Fleming et al., 1999) . However, the direct relationship between ACL elongation and neuromuscular control of the knee joint flexor and extensor muscles has not been well investigated and remains unclear.

In this report, we present two biomechanical approaches to address (1) skin movement artefacts during motion analysis and (2) neuromuscular control of the hamstrings to protect the ACL through *in vivo* experimentation using direct measurement methods. The first objective was to quantify the error caused by skin movement artefact when reporting the kinematics of the tibio-femoral joint during movements that incorporate sagittal and non-sagittal plane rotations. The second objective consisted of investigating the relationship between in-vivo ACL strain and neuromuscular control during jumping, stopping, and cutting movements. Finally, this paper will reinforce the value of *in vivo* direct experimental data in biomechanical research.

METHODS: The methodology has been divided to represent the two investigations. The first study consists of quantifying the error caused by skin movement artefact when reporting the kinematics of the tibio-femoral joint during walking and cutting (Benoit et al, 2005). The second consisted of investigating the preventive role of the hamstrings in relation with knee flexion angle and *in vivo* ACL elongation during jumping, stopping and cutting motions.

Skin movement artefacts

Participants: Eight healthy male participants with no history of knee injury or prior surgical treatment of the lower limbs were selected by an orthopaedic surgeon to participate in the study. All participants were informed of the risks involved with all procedures and all foreseeable complications. A consent form was accepted and signed by all participants and the study was conducted with the approval of the Ethics Committee of the Karolinska Hospital, Stockholm Sweden.

Surgical Procedure: A complete description of the surgical technique, as well as associated limitations and methodological concerns can be found in Ramsey et al., (2003). In short, stainless steel Apex self-drilling/self-tapping pins (Stryker Howmedica AB Sweden, 3.0 mm diameter, #5038–2-110) were inserted into the distal femur and proximal tibia of the right leg. Following surgery participants were then transported by wheelchair to the motion analysis laboratory (Astrid Lindgren Hospital-Stockholm, Sweden) for data collection. The pins remained inserted for the duration of the test. Upon completion of the experiments (approximately 2 hours), participants returned to the operating room to have pins removed.

Motion recordings: Triads consisting of four non-collinear 7 mm reflective markers (pin-markers) were affixed to the bone pins. Additional clusters comprised of four 10mm surface markers (skin-markers) were affixed onto the lateral and frontal aspects of both the right thigh and shank. Skin markers were spaced 10-15cm from adjacent markers within their respective cluster and their arrangement was chosen to ensure they remained non-coplanar in at least two camera views throughout the range of motion. Other reflective markers were also placed to define the segmental anatomical coordinate system and they were recorded and removed prior to the movement trials. Motion recordings and the force platform signal were synchronously collected.

Bone pin- and skin-marker trajectories were simultaneously tracked within a $0.8m^3$ measurement volume (1.1m × 0.8m × 0.9m) using four infrared cameras (ProReflex, Qualisys AB, Sweden), sampling at a frequency of 120 Hz. Markers coordinates were

transformed using the direct linear transform (DLT) and the raw 3D coordinates exported and saved to a local computer for later analysis.
Participants walked along a 12m walkway at a self selected pace. Five successful walking and cutting trials were recorded for each participant. Prior to performing the lateral cutting manoeuvre, participants jumped for maximal horizontal distance. Their longest measurement was recorded and marked on the floor to determine the proper takeoff distance to the force platform. From an initial standing position the participant pushed off using the left leg and, upon landing onto their right foot, immediately pushing off the platform, cutting to the left at an angle of approximately 45°. Each movement task was followed by a standing reference trial. The orientation of the target clusters from the first reference trial was matched against the second to verify the pins did not bend and the triad did not rotate during testing.
Kinematic analysis: Custom-made software (Matlab, Mathworks, USA) was developed and validated to process the 3D kinematic information derived from the bone pins and surface markers respectively (Lafontaine *et al.*, 2003). The kinematic patterns were described using the terminology and the ordered operations of the Joint Coordinate System (JCS) (Grood & Suntay, 1983). Kinematic data (movement of the tibia relative to the femur) for both the pin and skin markers were computed and low-passed filtered at 12Hz using a 20th order FIR digital filter (Matlab) and normalised to 100% stance phase (foot-strike to toe-off). Pre-foot-strike was expressed as a function of the normalised stance phase and ranges from -10% (or the longest duration of pre-foot strike for that given participant) to 0% (foot strike).
Statistical analysis: Three points of interest during the stance phase of the walking and cutting cycle were chosen for statistical analysis: heel strike (HS); mid-stance point (corresponding with maximum knee flexion angle during the first 60% of stance) (MS); and toe-off (TO). The kinematic data derived from the bone-pins was considered the 'Gold Standard' of measurement. Paired, two-tailed Student's T-tests were used to determine if skin derived kinematics at the three time-points differed from those derived from the bone-pins.

In-vivo ACL strain and neuromuscular response

Participants: Three healthy males from the Medical School at the University of Perugia (mean: age; 25yrs; height: 167cm; weight: 71.5kg) with no previous knee joint injuries were volunteered for the study. The participant was introduced to the surgical procedure and the laboratory testing protocol. After giving informed consent and prior the Differential Variable Reluctance Transducer (DVRT) implantation, the participants were instructed and practiced the requested movements (Jump, Stop and Cut). The participant was instructed to hop and reach the target as quickly as possible, landing with the instrumented leg and stopping in the landing position, without touching the right foot to the ground for at least two seconds. At all times the participant was asked to maintain partial knee flexion in the instrumented leg (at least 10°) to prevent impingement of the DVRT on the condylar notch. The task was repeated until the participant was able to perform it comfortably without going into full extension. The other movements were also trained during the pre-trial session.
Surgical Procedure: Later during the day, the DVRT was implanted on the antero-medial band of the intact ACL. Due to the fact that muscular contraction affects ACL strain, it is necessary that the participant be conscious during the surgical procedures. All procedures are therefore performed under local intra-articular anaesthetic. The DVRT is inserted via arthroscopic knee portals: the antero-medial portal is used for the arthroscopic optic device and the antero-lateral portal is used to insert the DVRT. The DVRT is then inserted through a 10mm sleeve and trocar inserted through the antero-medial portal. The trocar is inserted into the joint space and pressed against the antero-medial bundle of the ACL. The trocar is then removed and the insertion tool inserted into the sleeve. The DVRT is thus introduced into the joint space and aligned with the ligament fibres. The barbed ends of the DVRT are then inserted into the ligament bundle and fixed in place. The sutures holding the DVRT onto the tool are then released and then removed, thus leaving the DVRT implanted into the ligament. The surgical instruments were removed and the wounds were closed around the exiting instrument wire and removal sutures. The wounds were then covered with sterile bandages and the limb wrapped in a sterile elastic bandage. The participant was then transported to

the biomechanics laboratory for data collection. The zero strain position of the ACL was determined using the technique previously described by Fleming (Fleming *et al.*, 1994). Four high-speed digital video cameras (JVC GR-DVL9600) connected a PC computer equipped with the SIMI* Motion system (SIMI* Reality Motion Systems GmbH) were positioned on the same side of the participants' instrumented leg to record all trials. The cameras recorded at a speed of 50 Hz and were zoomed to include only the instrumented leg in the field of view. The calibrated volume was approximately 1.5 m x 1.0 m x 0.75 m. The entire collection window was 8 seconds at 1000Hz for the electromyography, force plate, and DVRT signals and at 50 Hz for the kinematics data. A total of three trials per movements were collected. The zero-strain test was then repeated to ensure proper operation of the DVRT.

Data Processing: The rectified EMG signals recorded during the three motions were synchronised to match the time of DVRT, ground reaction force, and kinematics data. The rectified EMG signals were normalised by peak amplitude for the dynamic contractions of the three manoeuvres using the stopping motion EMG data as normalisation basis. All data were processed using SIMI Motion system. Manoeuvres were analyzed five frames before heel strike until the participant moved outside of the force plate. The data from all three trials was ensemble averaged over the cycle and the data reported corresponds to the average over the three trials.

RESULTS:

Skin movement artefacts

Of the eight participants, two were excluded due to technical problems. No participants reported significant pain or discomfort during the experiments and all reported being able to move their knee freely despite pin implantation. Absolute error between the skin-marker and pin-marker kinematics at heel strike, mid-stance and toe-off during the walking and cutting motions are reported in Table 1. A significant difference in reporting skin-marker derived kinematics with respect to actual tibio-femoral kinematics is evidenced at heel strike, mid-stance and toe-off for both walking and cutting rotations and translations. In the stance phase of walking the average rotational absolute error ranged from 2.1° to 4.4° while translational errors ranged from 3.3 to 13.2mm. In the cutting movement the range of absolute errors and maximum absolute error were higher for both rotations (3.6° to 14.4°) and translations (6.6 to 24.1 mm) respectively.

While the absolute error is the absolute difference between the skin-marker and pin-marker derived kinematics, the average standard error of the estimate (*S*) describes the error associated with predicting pin-marker based tibio-femoral kinematics from skin-marker derived kinematics. The average *S* for walking and cutting movements is found in table 2.

Table 1. Absolute error values of skin-marker derived kinematics at three time points during walking and cutting of knee rotations and translations: flexion extension (Flex/ext), adduction-abduction (Add/abd), internal-external rotation (Int/ext); medio-lateral.

		Rotations (degrees +/- StDev			Translations (mm +/- StDev)		
		Flex/ext	Add/abd	Int/ext	Med/lat	Ant/post	Dist/comp
Walk	Foot-strike	2.9 (2.6)	2.6 (2.8)	2.7 (2.0)	5.1 (2.6)	7.5 (4.4)	5.0 (2.9)
	Mid-stance	2.4 (2.0)	3.1 (3.3)	2.3 (1.0)	5.5 (3.2)	6.1 (5.4)	3.3 (2.4)
	Toe-off	2.8 (2.4)	4.4 (3.3)	2.1 (2.1)	8.3 (5.5)	13.2 (5.0)	5.0 (2.5)
Cut	Foot-strike	4.5 (3.3)	7.5 (6.6)	7.7 (8.9)	12.4 (13.8)	6.6 (7.7)	9.0 (8.4)
	Mid-stance	3.6 (2.2)	14.4 (12.3)	4.0 (2.9)	16.0 (13.3)	24.1 (19.8)	8.9 (6.9)
	Toe-off	4.1 (2.7)	6.7 (4.1)	7.8 (8.6)	10.1 (11.1)	10.8 (10.8)	9.1 (9.2)

These error values were higher in the cutting movement for all measured rotations and translations. This data was calculated by comparing the pin- and skin-marker data across all participant s for each trial and at every time point, with the average calculated across time points (n=110 walking, n=105 cutting due to a shorter pre-foot-strike phase).

Table 2. Average standard error of the estimate (S) describing the error associated with predicting tibio-femoral kinematics from skin-marker. Average calculated for each data point of the stance phase (average of 110 data points) based on the estimated prediction of all walking (n=25) and cutting (n=28) trials.

	Rotations (degrees)			Translations (mm)		
	Flex/ext	Add/abd	Int/ext	Med/lat	Ant/post	Dist/comp
Walk	2.5	3.6	2.9	6.0	6.8	2.8
Cut	5.7	5.7	5.1	13.7	16.3	12.2

In-vivo ACL strain and neuromuscular response

For the purpose of conciseness only the stopping motion is presented in this abstract but the complete report will be presented during the symposium. As shown in the figure 1, Knee angle, *in vivo* ACL elongation, vertical ground reaction force and EMG of the semitendinosis, and both Gastrocnemius are depicted. During the stopping task, the maximum peak elongation of the ACL coincided with the maximum knee angle and the maximum vertical ground reaction force occurred later in the cycle. The maximum normalised EMG of the semitendinosis and both gastrocnemius took place before the maximum ACL elongation. On the other hand, the quadriceps muscle reached their maximum after the peak GRF.

DISCUSSION: The purpose of the first investigation was to quantify the error caused by skin movement artefact when reporting the kinematics of the tibio-femoral joint during movements that incorporate sagittal and non-sagittal plane rotations. We found within participant data to be repeatable when using either the skin or surface mounted markers for both the walk and cut. This was encouraging however the error associated with skin movement artefact differed widely across participants. Unfortunately, skin movement of the thigh and shank may be large enough to mask the actual movements of the underlying bones, thus making reporting of knee joint kinematics potentially uncertain.

The data from this study suggests that the use of skin-markers to describe knee joint motion must be presented with an envelope of accuracy that describes the artefact imparted by skin movement of the markers. Benoit et al (2005) have proposed using the average standard error of the estimate values of table 2 for reporting skin-marker derived knee joint kinematics. This estimate of the error associated with predicting tibio-femoral kinematics from skin-markers would allow for the reporting of kinematics within a 65% confidence interval (for 95% confidence interval use 1.96 x S). This provides a tool for ensuring that differences reported between groups are not within the range of skin movement error but instead represent true group differences.

In the second investigation the participant's neuromuscular strategy anticipated the landing impact for all motions (albeit to a lesser degree during the cut) by contracting the hamstrings and gastrocnemius muscles with high intensity, whereas the quadriceps muscles contracted right at or after foot contact with the ground. This illustrates that the hamstring and gastrocnemius muscles may act to protect the ACL from elongation during landing from jumping and cutting, while the quadriceps muscles played their anti-gravitational role to avoid collapsing of the knee when the foot impacts the ground. The instrumented portion of the ligament in this study also corresponds to the portion of the ligament that may first be damaged during ACL injuries (Zavatsky & Wright, 2001). The fact that the strain increased in the stressful movement in this portion of the ACL indicated that the movement might cause an increased load across the ligament fibers.

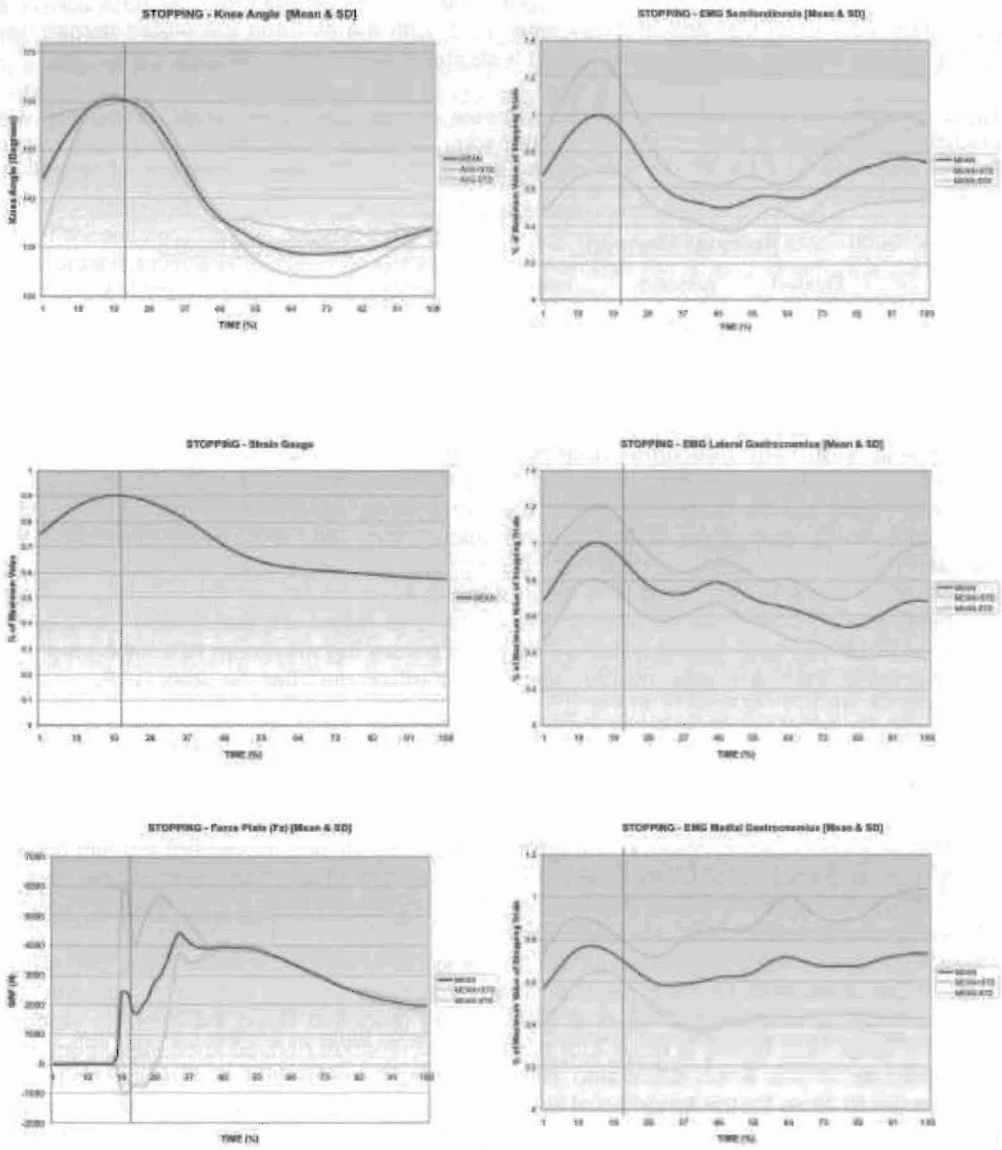

Figure 1. Knee angle, ACL *in vivo* elongation, ground reaction forces with the peak normalised EMG of the semitendinosus, and both Gastrocnemius. Note the vertical line indicates the maximum ligament strain.

CONCLUSION: The first study exposes the limitations of evaluating knee joint kinematics using surface markers. The absolute errors presented in this study offer a guideline to which conclusions may be drawn from 3D knee joint kinematics under similar testing conditions. We reported a minimum assessment of skin movement artefact and potential guidelines when discussing findings. A very important observation from this study is that the surface marker derived from kinematics can present repeatable profiles within a participant for various movements. However these repeatable patterns must not be misinterpreted as

accurately representing skeletal kinematics, at least beyond the sagittal plane of movement where the error is small relative to the total movement.
The second investigation has confirmed that the stopping, jumping and cutting manoeuvres generate a relatively high level of ACL elongation that initiates at or just before foot contact when the leg is most extended. The anticipatory contraction of the hamstring and gastrocnemius muscles may play an important role in protecting excessive ACL elongation whereas the quadriceps muscle prevent the collapsing of the knee joint after the foot impact with the ground.
This article has demonstrated the benefits of using in vivo experimentation by reporting on two experimentations that yielded useful information which could not have been found without direct measurement.

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