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Biomechanical assessment of Functional Landing Tests for ACL injury: gender differences

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Author: **Federico Adragna**

Student ID: 953098
Advisor: Prof.ssa Manuela Galli
Co-advisor: Prof.ssa Chiarella Sforza
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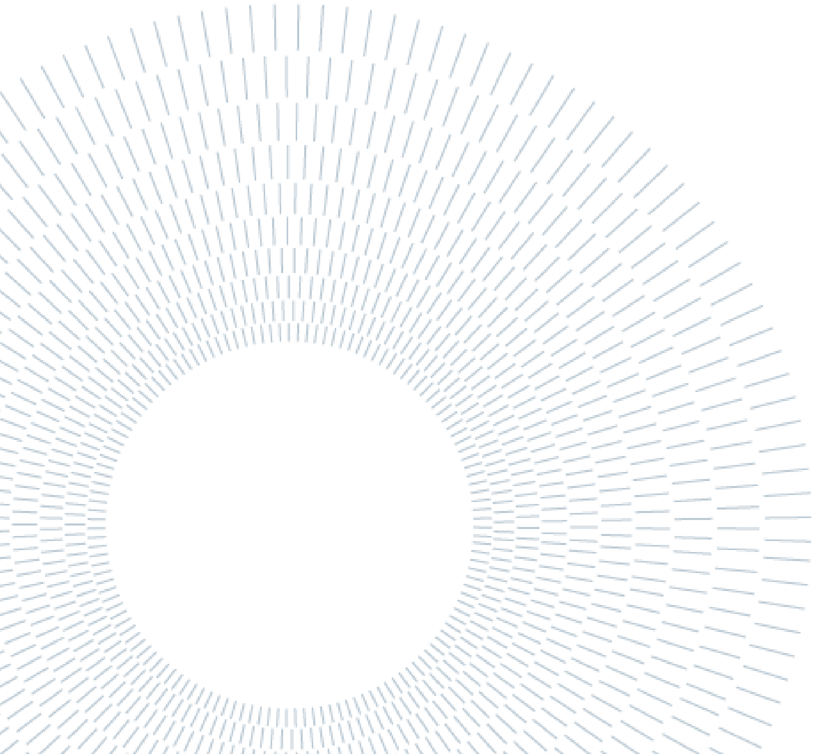


Abstract

Introduction. Non-contact injuries of the anterior cruciate ligament (ACL) are one of the most common injuries that can occur in team sports. Cutting, landing, and pivoting are typical maneuvers in which these injuries occur, inducing unusually high loads on the joint when executed with extended hip and knee and valgus knee. Women are demonstrated to have higher injury rates than men due to anatomical, neuromuscular and hormonal factors. Clinicians developed a series of “Functional Tests” to assess lower limb kinematic variables associated with ACL injuries, to be employed in injury prevention, rehabilitation and training programs. Landing tasks have been considered a great evaluation tool, but the lack of their standardization makes it difficult to compare results. The purpose of this study is to assess gender differences in lower limb kinematics when executing multi-planar landing tasks.

Materials and Methods. 20 males and 20 females (22.0 ± 2.1 years old) performed single-leg drop landing tests followed by a subsequent jump in one of four directions (vertical, lateral, forward, medial). Hip and knee joint angles were collected with a motion capture system and analyzed using Visual3D. The statistical analysis of the variables recorded at ground contact and in the following 100 ms was performed with the Two-Way ANOVA, with gender and test as factors.

Results. Women exhibited significantly less hip flexion and knee external rotation, and higher knee abduction at ground contact. Women had increased peak hip adduction and knee abduction, but less peak hip flexion and knee flexion than men. Between-test comparisons showed that the execution of the lateral second jump generated the highest peak hip adduction with respect to the other tests, while the medial jump the lowest. The central second jump induced higher peak hip adduction than the medial jump combined with the lowest peak knee flexion.

Discussion. The assessment of the proposed Functional Tests highlighted significant differences in male and female lower limb kinematics, showing how women executed a “stiffer” landing, with adducted hip and abducted knee, considered primary ACL injury risk factors. The between-tests comparison showed that the drop jump followed by the central jump induced a high hip adduction and the lowest knee flexion, this may imply a higher stress on the knee during the execution. The new elements introduced with these combined tasks may assist in developing a standardization of landing functional tests that clinicians can use in the evaluation of lower limbs biomechanics.

Keywords: **ACL**, **injury**, **landing**, **tests**, **gender**, **kinematics**.

Riassunto

Introduzione. Gli infortuni da non contatto al legamento crociato anteriore (LCA) sono tra i più comuni negli sport di squadra. Cambi di direzione e atterraggi sono tipici gesti che possono causare questi infortuni, in quanto sottopongono l'articolazione a sovraccarichi inusuali quando sono eseguiti con anca e ginocchio estesi e ginocchio valgo. È dimostrato come le donne abbiano tassi di infortunio più alti degli uomini a causa di fattori anatomici, neuromuscolari e ormonali. Professionisti del settore hanno sviluppato una serie di "Test Funzionali" per valutare variabili biomeccaniche degli arti inferiori associate ad infortuni del LCA, per poi utilizzarli in programmi di prevenzione, riabilitazione e allenamento. I test che comprendono atterraggi sono considerati un ottimo strumento di valutazione, ma la mancanza di una loro standardizzazione rende difficile il confronto tra i risultati. Lo scopo di questo studio è quello di valutare le differenze tra sessi nella cinematica degli arti inferiori durante l'esecuzione di atterraggi multi-planari.

Materiali e Metodi. 20 maschi e 20 femmine (età 22.0 ± 2.1 anni) hanno eseguito atterraggi monopodalici seguiti da un secondo salto in una di quattro direzioni (verticale, laterale, centrale e mediale). Tramite un sistema di analisi del movimento sono stati raccolti dati sulla cinematica di anca e ginocchio, poi analizzati in Visual3D. L'analisi statistica delle variabili al contatto con il terreno e nei successivi 100 ms è stata eseguita con un test ANOVA a due vie, con sesso e test come fattori.

Risultati. Le donne avevano significativamente meno flessione dell'anca e rotazione esterna del ginocchio, ma più abduzione del ginocchio al contatto con il terreno. Avevano inoltre valori di picco di adduzione dell'anca e abduzione del ginocchio maggiori, ma minore flessione di anca e ginocchio. I confronti tra test hanno mostrato che l'esecuzione del secondo salto laterale generava il picco maggiore di adduzione dell'anca, mentre il mediale il minore. Il secondo salto centrale induceva un picco più alto di adduzione dell'anca rispetto al mediale, unito al picco più basso di flessione del ginocchio.

Discussione. I test proposti hanno sottolineato differenze significative nella cinematica degli arti inferiori di uomini e donne, mostrando come le seconde eseguano atterraggi più "rigidi" con anca addotta e ginocchio valgo, considerati fattori di rischio per infortuni al LCA. Confronti tra test hanno mostrato che il salto centrale induceva elevata adduzione dell'anca e la minore flessione del ginocchio, ciò potrebbe indicare carichi più alti sul ginocchio. I nuovi elementi introdotti potrebbero aiutare nello sviluppo di una standardizzazione dei test con atterraggi, utilizzati da professionisti per la valutazione della biomeccanica degli arti inferiori.

Parole Chiave: **LCA**, **infortuni**, **atterraggi**, **test**, **sesso**, **cinematica.**

Contents

1 Introduction

1.1. General background

Sport practice has always been a constant in the everyday life of an individual and it is encouraged as part of a healthy lifestyle. In the last decade, sport participation increased in children of all ages in the U.S. (*State of Play 2021*, Aspen Institute, 2021), highlighting a positive trend of sports interest among young populations. However, more participation is linked with more injuries, and sports injury rates in youth and young adults are at the highest ever (Emery & Pasanen 2019). Injuries are detrimental not only to a professional athlete's career, but also to young people and those who participate in recreational activities. Joint injuries sustained in the early stages of an athlete's career may lead to increased risk of developing post-traumatic osteoarthritis (Richmond et al. 2013), weaker joint muscles and poor dynamic balance (Whittaker et al. 2018), conditions that will affect not only their possible future in sports but, more importantly, their everyday life. Participation in any physical activity must be balanced with the injury risk; then, there is the necessity of an evolution in sport practice, especially towards reducing or eliminating those issues that can compromise the athletic performance.

Non-contact injuries, which occur without physical contact with other players (Yu et al. 2007), are the most common injuries in sports, up to 78% of total injuries (Nyland et al. 1997, Kobayashi et al. 2016). Particularly frequent are non-contact lower limb injuries (Hootman et al. 2007), which generally involve the knee and ankle joints. (*Figure 1*).

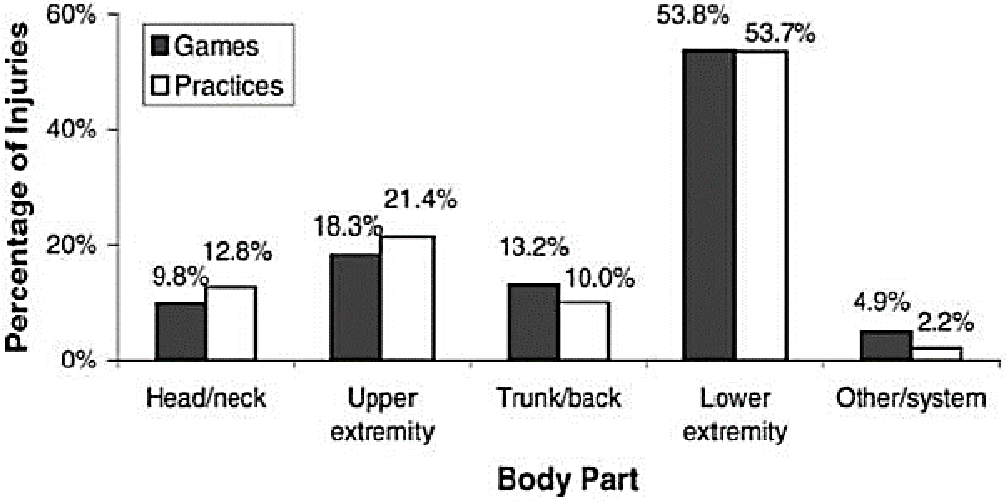


Figure 1: *Percentage of injuries per body part in sports (Hootman et al. 2007).*

Knee injuries, and more specifically, anterior cruciate ligament (ACL) tears, are recognized as one of the most common injuries in sports (Ferretti et al. 1992). It is estimated that up to 250 000 ACL injuries occur every year, reaching 2.8 to 3.2 occurrences every 10000 hours of play in women's collegiate basketball and soccer respectively (Smith et al. 2012). These kinds of injuries are particularly alarming not only for their immediate consequences, such as intense pain and the inability to participate in further activities, but also due to the following recovering process.

Typically, a complete rupture of the ligament requires the athletes to undergo a surgical procedure, with six to eight months to completely recover (Notarnicola et al. 2016). In most cases the knee joint is permanently damaged, leading to a considerable risk of relapse and the possibility of chronic limb instability even after the – supposed – complete healing of the joint (Brown et al. 2014). Moreover, the healing of such a wound is a considerably heavy burden also thinking about the financial aspect: the treatment of ACL injuries, in a year, is suspected to cost more than 3 billion dollars in the USA only (Hewett et al. 2016).

Most of non-contact injuries happen in those sports that involve sudden decelerations, landings, pivoting maneuvers and "*out of control*" play (Griffin et al. 2000). The great majority of team sports, such as football, basketball, and volleyball, all heavily involve the aforementioned risky motion patterns, thus leading to a great number of incidents. Landings (Boden et al. 2009) and changes of direction (CoD) (Alentorn-Geli et al. 2009) are widely recognized as the most dangerous movements, regarding ACL injury, that a player can perform during a match or training session. Their riskiness further increases when they are executed in unusual or unanticipated conditions. These movements involve instantly putting all the weight of the body on one or both limbs, leading to a sudden increase of the load applied to the knee. Thus, landings and CoDs can have catastrophic consequences on the knee if they are executed in a non-correct way, for instance when following an unprecedented event on the field or due to hazardous movements (Yeow et al 2011).

Despite improved knowledge on ACL injuries and injury prevention, the rate of injuries in sports, such as professional football and basketball, is not declining (Walden et al. 2015, Bullock et al. 2021). Thus, researchers continue to inquire on the possibility to predict – and prevent – ACL ruptures. Furthermore, some authors believe that "*the plethora of available tests may be burdensome to clinicians*" (Hewett et al. 2019), thus highlighting the necessity of simple and standardized tests that can be effectively employed.

It is finally clear and vital that sport scientists, physicians, physiotherapists and all those professionals that are involved in the training and care of athletes continue to work together to establish more impactful ways to prevent such dreadful events to happen.

1.2. Non-contact injuries

As previously stated, the most frequent injuries that can happen during a match or training session of team sports are non-contact injuries, especially the ones occurring to the joints of the lower limbs (Hootman et al. 2007). One of the most frequent sport gestures that lead to an injury is the single-leg landing (Ali et al. 2014), typical in football, basketball and volleyball. Commonly, the activity of landing on one leg after a leap or jump is linked with a sudden increase in the load that the joints are subjected to; sometimes, the impact is so powerful that the ligamentous structures can be permanently damaged, compromising the athlete's joint integrity (Ali et al. 2014). This can only increase the importance of correctly assessing the risks linked with such physical task.

Furthermore, there is a general agreement in literature that females are two to eight times more at risk, when compared to males, of sustaining a serious lower limb injury, in particular regarding the ACL (Griffin et al. 2000, Agel et al. 2005). In multidirectional women's sports, up to 70% of all ACL injuries occur via a non-contact mechanism (Taylor et al. 2016). It is conventionally accepted that there are a multitude of factors that cause this gender disparity, and they have been classified as anatomical, hormonal, and neuromuscular factors (Hewett et al. 2005). Women execute sports gestures with different movement patterns (Griffin et al. 2000), and their biomechanical characteristic may lead to a further increase in the injury rate.

1.3. Knee anatomy and injuries

1.3.1. Anatomy

The knee joint is composed of three articulations (*Figure* 2).

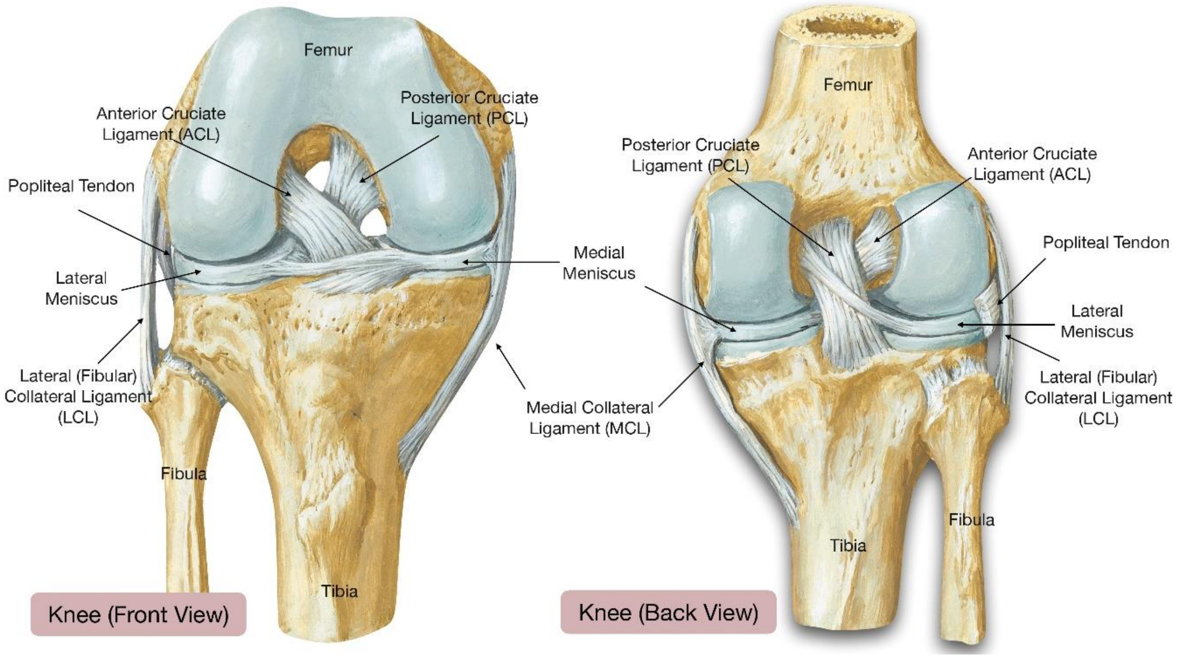


Figure 2: *Anatomy of the knee (The Knee World © 2020).*

Proximal tibiofibular joint

It is classified as a plane type synovial joint, and it connects the proximal epiphysis of the tibia and the fibula. It offers a very limited range of motion but plays a greater role in stability and weight bearing. During the dorsiflexion of the foot, a common movement that can happen during a landing, it acts as a shock absorber / damper.

Its relative stability is guaranteed by the capsule, the tibiofibular ligaments, and the lateral collateral ligament.

Patellofemoral joint

Classified as an angular ginglymus, it is the articulation between the posterior surface of the patella and the anterior surface of the distal femur. It allows the tendon of the quadriceps, which is a knee extensor, to be inserted directly over the knee, thus increasing the efficiency of the muscle. The patella is located within the quadriceps femoris tendon; it provides a fulcrum that increases the power of the knee extensors and provides stabilization.

Tibiofemoral joint

It is classified as a hinge type synovial joint, and as such it guarantees the knee to have only two degrees of freedom:

- Flexion – extension, in the sagittal plane.
- Intra – extra rotation, in the transversal plane.

The articular surface of the tibiofemoral joint is composed by the femoral condyles (medial and lateral), and the tibial condyles (again, medial and lateral), all of which are lined with hyaline cartilage. Between two condyles we find the menisci, which are defined as medial and lateral as well. These C-shaped fibrocartilage structures fulfill the task of dampening the load that is generated by the weight of the upper body by enlarging the surface area on which such pressure is applied.

The knee is stabilized by five principal ligaments, which are:

- Patellar ligament, it links the inferior aspect of the patella and the tibial tuberosity, its function is to secure the patella itself in its site.
- Collateral ligaments, medial and lateral. The former attaches to the medial condyle of the femur proximally and to the tibial one distally; the latter attaches to the lateral condyle of the femur proximally and to the head of the fibula laterally. Their function is to stabilize the joint on the frontal plane, avoiding excessive abduction and adduction of the knee. They also limit the range of rotation of the joint, allowing it to rotate only when it is in a flexed position.
- Cruciate ligaments, anterior (ACL) and posterior (PCL). The first one attaches to the internal surface of the lateral condyle of the femur and on the anterior intercondylar region of the tibia. The second one attaches to the internal surface of the medial femur condyle and to the posterior intercondylar region of the tibia. Their primary function is to stabilize the knee as well, especially in the anteroposterior direction, limiting the excessive anterior (ACL) and posterior (PCL) dislocation of the tibia.

Moreover, they contribute during the flexion and extension of the knee, helping the femoral condyles to correctly slide on the tibial surface. Both the ACL and the PCL are contained in the capsule.

1.3.2. ACL injuries

The ACL is the main ligamentous structure that avoids the anterior dislocation of the tibia. A healthy ACL can resist to uniaxial tensions of more than 2500 N, but physiological loads are usually well under this threshold (Brotzman & Wilk 2007). During the gait, forces applied to the ligament are in the order of 400 N, and during running they can go up to 1700 N (Brotzman & Wilk 2007). It is clear, then, that the uniaxial load on the ligament can surpass the rupture point only during unusual stress conditions, such as awkward landings and cutting maneuvers, situations that often happen during high intensity physical activity. Approximately 70% of ACL injuries occur via a non-contact way, such as when performing sudden decelerations, landings, pivoting maneuvers and “*out of control*” plays (Griffin et al. 2000), in which the body weight of the athletes themselves generates great forces, and thus excessive loads, on the ligament (Yu et al. 2007). Studies conducted on cadavers showed that the anterior shear force of the proximal end of the tibia was the primary determinant of strain in the ACL, while pure knee valgus and intra-extra rotation did not have significant effects on ACL loading (Berns et al 1992).

Nevertheless, when combined with anterior shear force of the tibia, both knee valgus and intra-extra rotation greatly increased the strain on the ligament (Berns et al. 1992, Markolf et al. 1995). Quadriceps muscles are the major contributor to the anterior shear force at the proximal end of the tibia through the patella tendon (Yu et al. 2007), and the applied quadriceps force causes the ACL to sustain high strains when knee flexion angle is less than 30° (Durselen et al. 1995). It is also known that increasing posterior ground reaction forces (GRFs) during athletic tasks increases ACL loading by inducing a higher quadriceps muscle contraption, due to the need of balancing a flexion moment relative to the knee (Yu et al. 2006). It can be then summarized that sagittal plane biomechanics are the major mechanism of ACL loading, although knee valgus-varus and internal-external rotation movements contribute as well. (Yu et al. 2007, *Figure 3*).

Figure 3: *Mechanism of ACL rupture following a single-leg landing during a professional volleyball match. In the last panel, excessive knee valgus can be seen.*

The extensive research of Pfeifer et al., in 2018, identified the major risk factors that can lead to ACL injuries in athletes. The authors stated that these factors can be categorized as extrinsic or intrinsic. Intrinsic factors are inherent to the physical characteristics and can be subdivided in either modifiable (e.g., muscular strength or flexibility) or non-modifiable (e.g., the anatomical structure of the athlete). On the other hand, extrinsic factors are outside of the control of the individual (such as weather conditions or playing surface). Given the nature of the non-modifiable factors, and the extrinsic ones, it is clear that the risk of an ACL injury will always exist, though it has been demonstrated that it can be contained through training programs and prevention protocols (Hewett et al. 2005).

Following a complete ACL rupture, all athletes who intend to return to high levels of sport activity must undergo surgical procedures to reconstruct the damaged ligament (Evans et al. 2014). A conservative therapy, preferred for older patients (Brotzman & Wilk 2007), leads in fact to further damage to the menisci and degenerative pathologies. There has been a drastic improvement in surgical procedures in the past decades: since the 1980s, arthroscopic procedures have allowed surgeons to reconstruct the ligaments without damaging the capsule and have dramatically reduced the time needed for the player to compete again (Chambat et al. 2013). The surgery is typically performed in the weeks following the injury, techniques that employ the use of autologous tendons are demonstrated to have a success rate of 97% (Notarnicola et al. 2016).

Given its highly debilitative impact, the rehabilitation process for this kind of injury often begins before the intervention and allows the athlete to perform some kind of physical activity after four to five months (Brotzman & Wilk 2007). However, this is permitted only without further complications, that sometimes follow the procedure, such as mobility loss in the knee region. Moreover, the return to sport is only allowed by physicians if the individual reaches specific healing targets such as the absence of pain and joint effusions, and if the ratio between quadriceps and hamstrings is > 70% (Brotzman & Wilk 2007).

Finally, it is of the utmost importance that the injured competitors follow specific post-rehabilitation protocols, aimed at preventing the occurrence of chronic complications such as osteoarthritis (Neuman et al. 2008).

1.4. Functional Tests

It is widespread in the scientific community the notion that some lower extremity movement patterns may influence the risk of sustaining ACL injuries. These patterns are usually decreased flexion in the sagittal plane of the knee, hip and trunk, knee valgus and intra or extra rotation of the leg (Padua et al. 2009, Taylor et al. 2016). As previously stated, the occurrence of a non-contact ACL injury is influenced by extrinsic and intrinsic factors. The latter are particularly important, because they are related to the single individual: some of them are non-modifiable (e.g., ligament laxity or anthropometric characteristics like height), but others – such as muscle strength and joint flexibility – can be improved following specific training programs (Pfeifer et al. 2018). Researchers have thus tried to design methods to evaluate knee performance with the aim of better assessing the influence of natural predisposition and training to lower limb biomechanics: a series of physical exercises, called "Functional Tests" (FTs), to simulate, in a controlled and safe environment, the stress put on the lower limbs during sports participation. The evaluation of specific biomechanical parameters recorded during FTs is often used to assess whether a previously injured individual could return to high intensity physical activity (Abrams et al. 2014). FTs that are commonly employed to assess ACL injury risk are squats (*Figure 4a*), CoDs (*Figure 4b*) and landings (*Figure 4c*) (Kivlan et al. 2012, Nedergaard et al. 2019, Collings et al. 2019).

Figure 4: *Execution of single-leg squat (a), change of direction (b), single-leg landing (c). Figure (a), (b) and (c) are adapted from, respectively, Fitarelli et al. (2020), Zago et al (2021) and Nedergaard et al. (2019).*

There is still much discussion around which task is best suited to assess injury prevention protocols. Squat tests involve the most controlled movements that can allow researchers to clearly identify dangerous movement patterns (Zeller et al. 2003), but some consider them to not correctly resemble real playing situations (Jones et al. 2014). CoDs are thought to be an effective screening test due to the high relevance of pivoting in injury situations (Nedergaard et al. 2019), but other authors acknowledge them as non-realistic due to the absence of a landing and the consequent loads on the knee. Finally, FTs that involve landings are considered as representative of real injury movement patterns, more so if combined with a side-cutting maneuver, as Krosshaug et al. suggested for future studies in 2016. During the last three decades, researchers have made great effort to assess the correct method to study the kinematics, kinetics and energy dissipation of the lower limbs during a landing task. Functional tests that involve landings are usually divided in two categories: double-leg and single-leg.

1.4.1. Double-leg landings

Typical double-leg landings evaluated by researchers are:

- Vertical Jump (VJ), during which the individual jumps upwards with maximum effort (Cruz et al. 2013).
- Forward Jump (FJ), in which the subject jumps forwards, often stopping at a predetermined distance (Cruz et al. 2013).
- Drop Landing (DL), during which the athlete steps off from a box or an elevated position and lands (Self & Paine 2001).
- Lateral Jump (LJ), in which the athlete jumps laterally, reaching a predetermined point (Taylor et al. 2016).
- Drop Jump (DJ), which is a DL immediately followed by a VJ (Hewett et al. 2005) or a FJ.

Figure 5: *Execution of a double-leg DJ. Adapted from Padua et al. (2009).*

As depicted above, many studies have been focused on double-leg landings to assess the intricacies of lower limb kinematics. Different landing techniques have been studied by Self & Paine (2001) to address the role of the ankle in ACL injury motion patterns. Hewett et al. (2005) employed double-leg DJs to explore whether increased knee valgus could effectively predict subsequent ACL injuries, allowing technicians to identify "at-risk" subjects before the injury occurs. The results confirmed that knee valgus could be considered a predictor of ACL injury risk. Similarly, Noyes et al. (2005) utilized a DJ protocol to assess if a neuromuscular training program could be effective in reducing knee valgus in female subjects, succeeding in their intents. Furthermore, some authors felt the need of starting to develop a clinical screening tool, the Landing Error Scoring System (LESS), to "*reliably identify subjects with potential high-risk biomechanics*" (Padua et al. 2009). The LESS was established by its creators to be a reliable tool when used to analyze results of DJs. Some studies proceeded further in this direction trying to establish normative values for knee valgus angle after a DJ and DL (Herrington & Munro 2010), to allow other authors to better compare their work results. Finally, other works involved DLs to establish the effect of fatigue and gender on landing biomechanics (Pappas et al. 2007, Wong et al. 2020).

Given the great relevance of double-leg landings in recent literature, it could be theorized that the scientific community has unanimously accepted these kinds of task as a suitable screening tool for ACL injuries. On the contrary, some authors discovered that a double-leg DJ is, in fact, a poor screening test for ACL injuries (Krosshaug et al. 2016). In their publication, the researchers evaluated more than 700 elite female athletes who competed in team sports that heavily involved landings and stated that there was no correlation between variables deriving from the double-leg DJ test and increased injury risk. However, functional tests such as the DJ and DL have been vastly used to better understand how the lower limb joints and muscles behave in a landing situation.

1.4.2. Single-leg landings

Thus far, most authors recognize the single-limb tasks as more predictive of real injury situations in disciplines like volleyball and basketball, because they highlight significantly greater knee valgus (Pappas et al. 2007) and less knee flexion (Yeow et al. 2010) compared to double-leg landings. Furthermore, over 70% of ACL injuries occur during unilateral foot contact (Boden et al. 2009), and literature suggests that landing on a single leg generates an increased load on all the lower limb joints, since they must bear the weight of the whole body (Taylor et al. 2016).

There is a great variety of single-leg landings that have been used to further increase researchers' knowledge about lower extremity biomechanics; most of these tasks are the same as those performed with two legs, such as the DJ (*Figure 6*) and DL, but single-leg landings have also been executed from increasing horizontal distances

(Ali et al. 2013) and vertical heights (Ali et al. 2014). Moreover, they have often been coupled with side cutting maneuvers to better represent the real field setting (Jones et al. 2014).

Figure 6: *Execution of a single-leg DJ.*

Single-leg landings have been at the center of many studies in the last two decades: Taylor et al. (2016) discussed the benefits of choosing single-leg maneuvers to correctly assess ACL injury risk factors, while also proposing to couple the tasks with lateral cuts to better represent the real playing situation. Other authors discussed the influence of hip strength on knee mechanics during single-leg DLs in females (Lawrence et al. 2008) and the effect of increasing starting heights (Ali et al. 2014) or leg dominance (Mokhtazardeh et al. 2017) when performing single-leg landings. Nagano et al. (2009) tried to deepen knee kinematics comprehension by using different kinds of landing task and succeeded in identifying single-leg plant-and-cutting as the task that poses the greatest risk to the knee, given the increased knee abduction and internal tibial rotation compared to double-leg landings. Researchers also tried to better evaluate lower limb kinetics (Ali et al. 2014) and energy dissipation at ground contact (Yeow et al. 2011). The study of these aspects allowed the authors to establish that an extended knee is more prone to injuries due to inefficient load dissipation through ligaments instead of muscular structures, and that single-leg landings pose a threat to the ACL due to the higher loads and increased frontal plane motions involved.

1.4.3. Landing variations

Although the simple DLs and DJs have been described as the most common, for both double and single-leg landings, there are several variations of FTs that can be used, with variations generally concerning: (1) the presence or not of a FJ after landing, and its distance; (2) the presence or not of a VJ immediately after landing; (3) the presence or absence of an immediate side cut (medial or lateral) after landing; (4) the use of a box to perform a DL and the starting height. These variations also interact with each other, resulting in an extremely high number of combinations that allow for great experimentation but also limit the capability of authors to compare results. With the purpose of better understanding all the intricacies related to the use of FTs in a clinical setting, many authors focused on evaluating and comparing different kinds of tasks and landing variations. Taylor et al. (2016) compared the results of double and single-leg FJs followed by a VJ and secondly of the same tasks but jumping in the lateral direction instead of forwards. The authors concluded that double-leg landings remain less adequate to represent lower limbs biomechanics in sports practice, even when combined with a following VJ, while the single-leg FJs combined with the VJ better mimic real-life situations and must thus be preferred in a laboratory setting. Similarly, Heebner et al. (2017) chose to focus on the differences regarding knee kinematics when performing single-leg and double-leg DLs, forward and finally stop jumps. The authors concluded that "*different landing tasks elicited different biomechanical responses*" and "*depending on the goals, using multiple assessment tasks should be considered*", suggesting that caution may be needed when comparing results of different studies.

An effort has also been made in terms of studying different variations of the same landing (such as different length or height of the jump) or whether the addition of a sequential movement (such as a cutting or forward / lateral jump) could better simulate the real injury situation. Investigations regarding drop landings from different heights highlighted that double-leg landings allow better shock absorption than single-leg ones, especially from a more elevated box (Yeow et al. 2010). Furthermore, it has been emphasized how landing from a higher starting point increases the impact load, inducing kinetic and kinematic changes that expose the ACL to a greater risk of injuries (Mokhtazardeh et al. 2017).

Inquiries about how a sequential task could influence the outcome of a landing showed that caution should be warranted when comparing different exercises (Cruz et al. 2013). However, it seems clear that athletes who poorly perform in a single-leg landing also do so in the case of pivoting and cutting maneuvers (Jones et al. 2014). Ultimately, recent research seems to show that neither single-leg DLs nor multi-planar side jumps can effectively mimic the high knee loads frequently seen in real injury situations, though authors acknowledge that performing more complex tasks (such as a landing followed by a cutting maneuver) more closely resembles what realistically happens on the playing field (Nedergaard et al. 2019).

As the scientific community deepened its understanding about the topic, more studies were conducted to explore how whole-body kinematics could influence knee behavior during landings. Many authors studied the role of ankle (Self & Paine 2001, Hargrave et al. 2003), hip (Lawrence et al. 2008), trunk (Blackburn et al. 2008) and arm (Chaoudari et al. 2005) kinematics in influencing the performance of the knee in terms of shock absorption, peak angle, and momentum. General consensus is that all of the aforementioned anatomical parts play a role in the final behavior of the knee, given that every limb is connected to the others forming a "*kinematic chain*" (Blackburn 2008). Nevertheless, most authors continue to focus their studies directly on the knee.

Ultimately, it can be stated that at present times there is no absolute agreement about which particular exercises can be utilized by scholars and technicians to better assist athletes in their training and prevention of new or recurring injuries (Heebner et al. 2017, Nedergaard et al. 2019).

1.4.4. Gender differences

Historically, one of the most discussed topics when approaching lower limbs biomechanics is the comparison between male and female athletes' motion patterns. This is due to the fact that female players have been linked with increased – from two to eight times – ACL injury rates (Griffin et al. 2000, Agel et al. 2005). This evident difference in injury rates has been linked to many factors. A few of them are anatomical, such as females presenting a narrower intercondylar notch (Pfeifer et al. 2018) and a smaller ACL volume (Charlton et al. 2002). Some of these factors are neuromuscular, for example reduced hamstrings strength. Finally, some authors believe hormonal factors (Slauterbeck & Hardy 2001) and being in the pre-ovulatory menstrual phase (Beynnon et al. 2006) may lead female athletes to a heightened risk of sustaining ACL injuries.

Functional Tests make no exception, and gender influence has been the primary objective of numerous studies. In current literature, comparisons between male and female counterparts when performing FTs, such as DLs and DJs, highlighted female subjects to have: (1) increased tibial rotation (Nagano et al. 2007), often coupled with a low hamstrings/quadriceps strength ratio (HQR) (Salci et al. 2004, Nagano et al. 2007); (2) a more “stiff” landing technique, which involves low knee flexion at ground contact and causes the ligamentous structures to bear the majority of the generated load (Decker et al. 2003, Schmitz et al. 2007); (3) low ankle plantarflexion angles, associated with high GRFs (Ali et al. 2013); (4) an increase in knee valgus, which is known to intensify the stress on the ACL (Russell et al. 2006, Pappas et al. 2007); (5) poor shank external rotators strength, which could be the cause of the increased tibial internal rotation previously seen (Kiriyama et al. 2009); (6) greater coronal plane excursions for the hip, knee and ankle (Ford et al. 2006). All these differences are commonly identified as high-risk factors for ACL injuries in women.

1.5. Gaps in the literature

Numerous authors acknowledged limitations that could have prevented their research from further progressing in the exploration of the topic of FTs. Some works concentrated exclusively on male (Yeow et al. 2011) or female (Cruz et al. 2013) athletes. Quite a few studies only employed double-leg landings (Hewett et al. 2005, Noyes et al. 2005), and some authors even deemed the tasks employed as too simple because the knee was not adequately stressed (Padua et al. 2009). Finally, some studies were limited to a too small pool of subjects (Salci et al. 2004, Ali et al. 2013).

Moreover, one main issue is the difficulty encountered when comparing the outcomes of different studies. This is primarily due to: (1) different landing techniques and exercises employed; (2) uncertainty around which height to select when performing DJs and which horizontal distance to pick when performing forward jumps. Although almost all studies on landings involved the use of similar exercises, as previously discussed, there are many variations that can be employed when performing analyses on the matter, and this leads to inconsistent results.

The lack of standardized tasks has made it difficult to understand how FTs can be employed to assist athletes. An effort must be made to develop a series of tests that can be used to improve training programs, rehabilitation protocols and screening procedures.

In recent years, researchers have inquired whether some of the most used tests could be actually inadequate to correctly assess injury risk and hazardous joint mechanics. Krosshaug et al. (2016) concluded that "*vertical DJs cannot be used as a screening test to predict ACL injuries in elite soccer and handball players*" due to an inability to associate the variables obtained from a DJ to increased injury risk; Collings et al. (2019) conducted a narrative review and discovered that "*a drop landing is unlikely to be a highly functional task that has high external validity in relation to common injury situations*", due to the absence of key factors like unplanned movements and external perturbations; finally, Kotsifaki et al. (2021) concluded that Single Leg Hop Distance (SLHD) is a "*poor measure of knee performance*", even though it is extensively used when evaluating an athlete's rehabilitation progress following ACL injuries.

Further research on the topic should focus on ultimately stating which tasks to employ to correctly assess lower limbs biomechanics, and perhaps should aim to introduce innovative exercises that improve the results of future studies. A standardized test should ideally: (1) use personalized horizontal jumping distances, based on the single athlete's capabilities; (2) involve the execution of complex movements, such as landing and cutting combinations, with the goal of exploring different motion patterns; (3) employ drop heights that allow to compare the performance of athletes having different heights. Currently, there is no set standard about this matter and various authors elected to use different elevations, for instance 20 cm (Kiriyama et al. 2009), 30 cm (Self & Paine 2001, Schmitz et al. 2007) 40 cm (Pappas et al. 2007), 60 cm (Ali et al. 2013), selected without a strict reasoning.

1.6. Purpose of this study

Following the discussed considerations, the present study has two main purposes. The first one is to introduce a new series of functional tests, designed starting from those used in previous studies but with three main distinctions: (1) the use of an adjustable starting height for the jumps, depending on the height of the single athlete; (2) the introduction of different forward jump distances, based on the maximum jump distance of every individual, with the purpose of correctly adjusting the test difficulty to each player’s performance; (3) the combination of DJs with vertical and forward jumps and cutting movements, in lateral and medial direction, which was suggested by previous authors (Nedergaard et al. 2019) but not often used in combination with DJs. Secondly, this study aims to further proceed in the exploration of the gender differences showed in FTs, comparing the performances of female and male subjects when executing the proposed tasks.

Lastly, even though not a direct goal of this study, my intention is to provide sport scientists, physicians, physiotherapists and every other professional working alongside athletes with simple, effective and standardized tasks which I believe could be used to improve injury prevention programs and rehabilitation protocols.

2 Materials and Methods

2.1. Subjects

Forty recreationally active athletes, 20 males and 20 females, were recruited for this observational study with a cross-sectional design. All of them attended the experimental sessions voluntarily. According to our inclusion criteria, the subjects were to be young (aged between 18 and 30 years), physically active, participating in sports at least two times a week, and healthy (no reports of a lower limb injury in the previous six months). Exclusion criteria included any history of knee surgery and diagnosis of a condition affecting balance.

Subjects' age ranged from 18 to 25 years, the body mass was 53.0 to 96.0 kg, and their height was 153.0 to 193.0 cm. More detailed information can be found in *Table 1*.

Table 1: *Anthropometric measures (mean ± SD) and subjects characteristics. Independent t-test was used to compare measures between groups. * denotes a significant difference, p < 0.05.*

Gender	**N**	**Age [years]**	**Height [cm]**	**Body Mass [kg]**	**Training Sessions Per Week**	**Dominant Lower Limb**	**Sport**
Female	20	21.9 ± 2.2	170.0 ± 7.7	63.8 ± 8.7	3.6 ± 1.0	Right: 17 Left: 3	Volleyball: 9 Soccer: 5 Gymnastics: 2 Long Jump: 1 Ultimate Frisbee: 1 Boxing: 1 Rugby: 1
Male	20	22.0 ± 2.1	179.0 ± 5.6	73.9 ± 9.0	3.4 ± 0.9	Right: 16 Left: 4	Soccer: 7 Volleyball: 4 Basketball 3 Beach Volley: 1 Karate: 1 Boxing: 1 Tennis: 1 Parkour: 1 Climbing: 1
Independent t-test (p-value)		0.883	< 0.001*	< 0.001*	0.628		
Total	40	22.0 ± 2.1	174.5 ± 8.0	68.9 ± 10.1	3.5 ± 1.0	Right: 33 Left: 7	

All participants were informed of the risks and benefits of the study prior to signing an informed consent form approved by the Ethical Committee. Benefits of this study included the delivery of a report containing data on their performance in the tasks, and the possibility to contribute to a study that can ultimately lead to more significant information about ACL injuries. Risks of the study were muscle soreness due to the number of eccentric muscle contractions during landing tasks and injuries to the lower limbs, similarly to the hazards that may be incurred in during any jump activity.

Research had been previously approved by the Ethical Committee of the University of Milan (Nr. 46/21) and it also conformed to the Declaration of Helsinki of 1964.

2.2. Instrumentation

2.2.1. Stereophotogrammetry system

The study was conducted in the Movement Analysis Laboratory of the University of Milan, equipped with a stereophotogrammetry system composed of 9 BTS-Smart E cameras (BTS S.p.A, Milan, Italy). The cameras (*Figure 7*) are composed by Charge Coupled Device (CCD) sensors, which react to infrared radiation (wavelength 700–1000 nm). A ring of LEDs is situated around the lens of every camera: the emitted infrared light is reflected on the surface of passive reflective markers, purposely placed on anatomical landmarks on the subject's body, and is captured again by the cameras.

Figure 7: *Acquisition camera.*

The system allows the 3D reconstruction of the position of every marker that moves in the *acquisition volume*. The cameras were positioned in the laboratory in order to guarantee that every marker was being recorded by at least two non-aligned cameras. The sampling frequency of the system was set to 60 Hz (one frame every 16.7 ms), which was acknowledged as appropriate considering the kind of movement recorded. Data acquisition is handled by a central computer through the dedicated software SmartCapture, which is integrated in the motion capture system itself. The spatial and temporal information obtained by the cameras is then processed to define marker trajectories and the consequent kinematics of the musculoskeletal system of the subjects.

The calibration of the system was executed prior to every recording session, following the instructions of the system's manufacturer. The calibration begins with a static phase, necessary to define the global reference system; a dynamic phase follows to define the acquisition volume in which the subject will execute the physical tasks. The static phase lasts approximately 10 seconds, and it is performed placing a Cartesian triad which defines X, Y and Z directions of the global reference system of the laboratory. The triad is equipped with markers, indicating the three directions, and was placed with the Y axis upwards (*Figure 8*) at the center of the acquisition volume.

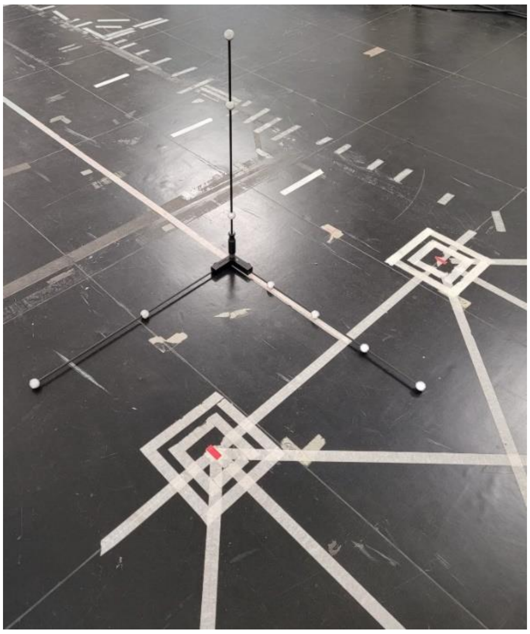


Figure 8: *Cartesian triad used in the static phase of the calibration process.*

The dynamic phase must be manually executed by an operator, who waves the *wand* (the Y axis detached from the triad) in every direction in the laboratory area where the tasks will be executed. This operation lasts approximately 120 seconds and allows the system to define the precise boundaries of the acquisition volume, which will be determined by crossing all 9 cameras' recordings to reconstruct the spatial movement of the markers with respect to the global reference system previously defined.

2.3. Experimental design

The study had the main goal of investigating lower limbs biomechanics during a series of landing FTs. In order to do so, subjects performed 4 variations of single-leg DJs in the Movement Analysis Laboratory and the collected data was subsequently analyzed. The proposed single-leg FTs included: (1) customized height of the DJ starting point proportional to the subject's height; (2) forward jump distance adjusted on the maximum single-leg horizontal forward jump of the individual; (3) the combination of a drop and a sequential jump in four directions.

2.3.1. Marker set

As previously discussed, kinematic data of the subjects were obtained through the optoelectronic motion capture system, which records the 3D instantaneous position of reflective markers (*Figure 9*).

Figure 9: *The three types of markers employed in the protocol.*

The markers were placed on the subject's body (skin-mounted markers), in proximity of specific anatomic landmarks meant to reduce the possible errors relative to the occurrence of skin motion artifacts (Fuller et al. 1997). Moreover, additional markers were mounted onto four rigid structures (*clusters*) attached to the thighs and shanks, following the indications of Manal et al. (2000).

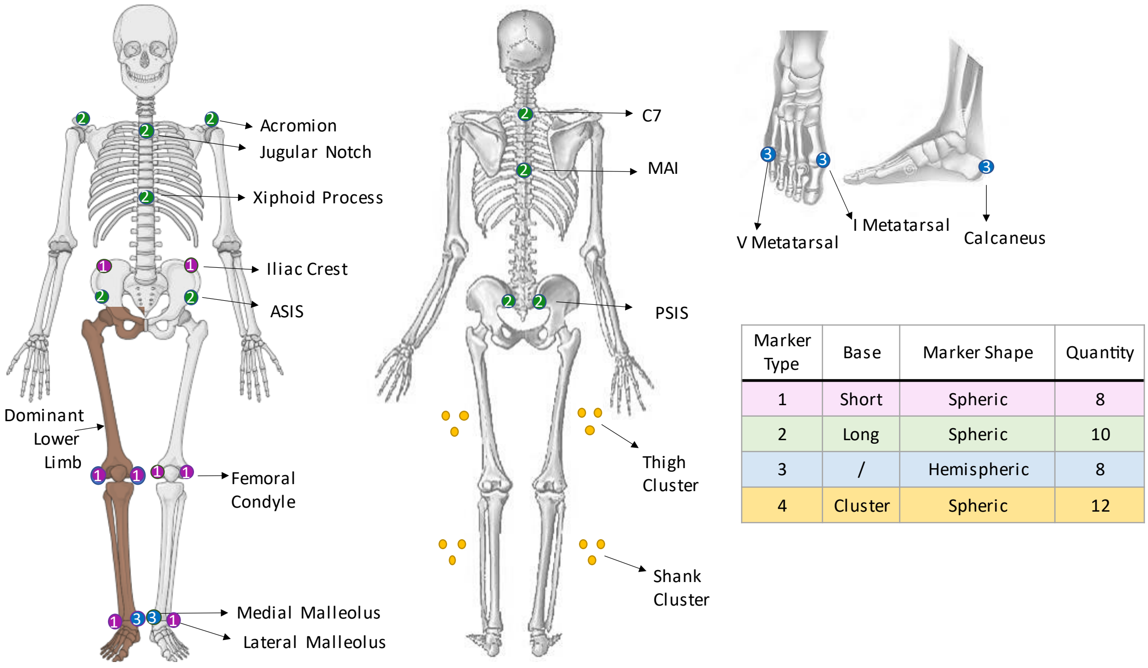
In this study, a marker set composed of 38 reflective markers was employed, of which 26 were positioned directly on the athlete's skin and 12 were organized in four T-shaped clusters of three markers each (*Figure 10*).

Figure 10: *Markers and marker clusters.*

Information about the complete marker set is indicated in *Table 2* and *Figure 11*.

Table 2: *List of all 38 markers and relative anatomic landmarks.*

Marker	**Marker type**	**Diameter [cm]**	**Anatomic landmark**
C7	Spheric	1.5	Seventh Cervical Vertebrae
RAC – LAC	Spheric	1.5	Right and Left Acromion
SJN	Spheric	1.5	Jugular Notch
PXI	Spheric	1.5	Xiphoid Process
MAI	Spheric	1.5	Midpoint between caudal extremities of Scapulae
RIPS – LIPS	Spheric	1.5	Right and Left Posterior Iliac Spines
RICR – LICR	Spheric	1.5	Right and Left Iliac Crests
RIAS – LIAS	Spheric	1.5	Right and Left Anterior Iliac Spines
RFLE – LFLE	Spheric	1.5	Right and Left Lateral Femoral Condyles
RFME – LFME	Spheric	1.5	Right and Left Medial Femoral Condyles
RFAL – LFAL	Spheric	1.5	Right and Left Lateral Malleolus
RTAL – LTAL	Hemispheric	1.0	Right and Left Medial Malleolus
RVMH – LVMH	Hemispheric	1.0	Right and Left Fifth Metatarsal
RFMH – LFMH	Hemispheric	1.0	Right and Left First Metatarsal
RCA – LCA	Hemispheric	1.0	Right and Left Calcaneus
RTH1, 2, 3	Cluster	1.5	Right Thigh
LTH1, 2 ,3	Cluster	1.5	Left Thigh
RSK1, 2, 3	Cluster	1.0	Right Shank
LSK1, 2, 3	Cluster	1.0	Left Shank



Marker Type	Base	Marker Shape	Quantity
1	Short	Spheric	8
2	Long	Spheric	10
3	/	Hemispheric	8
4	Cluster	Spheric	12

Figure 11: *Marker positions on the subject's body.*

The virtual representation of the subject is obtained through the construction of an anthropometric model (*Figure 12*) via the software SmartTracker (BTS S.p.A, Milan, Italy). This model only allows to correctly label markers and the relative anatomic landmarks. A second more complex model is created in the later stages of data elaboration. This second representation identifies the skeletal structure of the body, modelled as composed by rigid segments, each one defined by at least three non-aligned markers. The translation and rotation of every segment with respect to the contiguous ones define the kinematic of the body. These movements are permitted by the joints. Some segments, such as the foot and the trunk, contain more than one bone: this leads to errors when considering these structures as rigid. Nevertheless, even if it is a simplification of the real kinematics of the body, this approximation is generally accepted (Winter 2009).

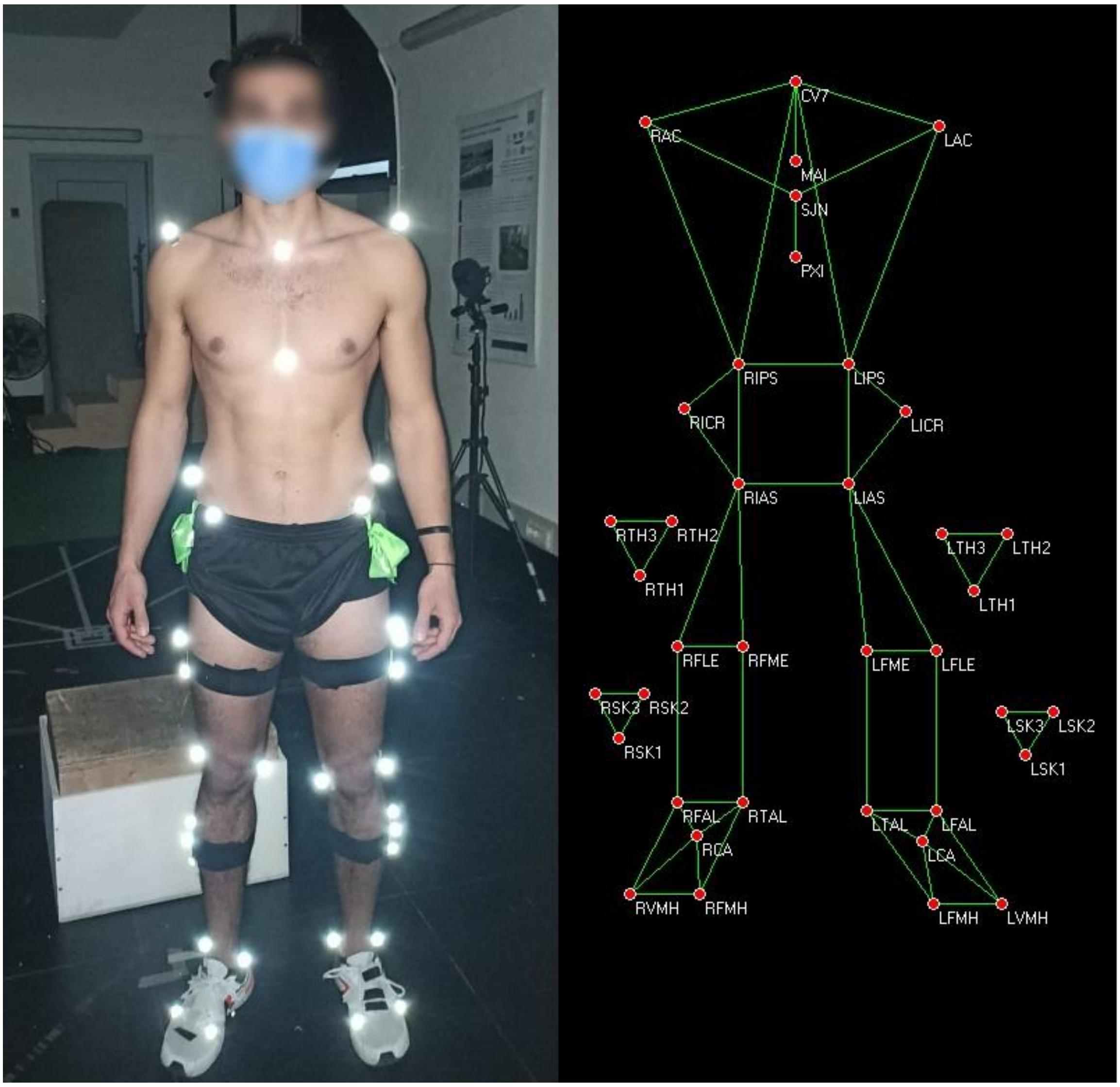


Figure 12: *Subject with markers positioned on his body and relative SmartTracker model.*

2.3.2. Study protocol

Every acquisition session began with the preparation of the marker set and the calibration of the optoelectronic system. Then, the subject was welcomed in the laboratory and was allowed to put on sports clothes. Every athlete was informed about the benefits and risks of the study and signed an informed consent form. Lastly, personal (age, date of birth, history of injuries, sport, number of trainings per week) and anthropometric (height, weight, dominant leg) data were collected. The dominant leg was defined as the limb one would use to kick a ball with maximum force.

Pre-test phase

Before the testing session, every athlete warmed up with 5 minutes of indoor cycling and simple jumping exercises, under the supervision of a sport scientist. Then, the operators positioned the markers following the discussed protocol. Another operator adjusted the height of the box to be used in the drop jumps: the platform consisted in a wooden cube, whose height was set as 20% of the subject's height, adjustable from 30 to 40 cm with resolution of 1 cm. After the warm-up, the athlete performed the maximum single-leg forward jump. Maximum forward jump distance was recorded after the athlete managed to jump – and maintain balance on one leg – to the same point in three consecutive trials. This had the purpose of defining the horizontal distance at which the box should have been positioned, with respect to the defined landing point at the center of the acquisition volume. Said distance was set to 60% of maximum jump distance. The average maximum jump distance was 163.9 ± 16.4 cm for females and 197.4 ± 17.1 cm for males. Finally, the pre-test phase concluded with the acquisition of the orthostatic trial, in which the subject stood still in anatomic position for 10 seconds. The trial was recorded through SmartCapture and it had the purpose of defining a static model of the single subject during the data elaboration phase.

Functional Tests

The core part of the trial consisted of four landing tasks: each exercise started with a drop landing (DL) in which the athlete jumped from the wooden box, landed on the dominant leg reaching a target on the ground, and immediately performed a second jump in one of four directions. The target was positioned at the previously discussed distance from the starting point. The landing variations (or conditions) are here presented:

- Drop Jump (DJ), in which the athlete performed the DL and immediately executed a vertical jump as high as possible on the dominant leg.

- Drop Jump Lateral (DJL), in which the athlete performed the DL and, immediately after landing, executed a maximal jump 45 degrees in the lateral direction with respect to the dominant limb.
- Drop Jump Central (DJC), in which the athlete jumped off the box as in the previous tasks and, after landing, immediately performed a maximal forward jump.
- Drop Jump Medial (DJM), in which the athlete jumped again off the box as in previous exercises and performed a maximal jump 45 degrees in the medial direction after landing, executing a cutting maneuver.

To ensure that athletes could correctly perform every task, visual indicators of jump targets and directions were applied on the ground (*Figure 13*) and operators closely followed the execution of each jump. The study protocol required the acquisition of three valid trials per condition. A trial was discarded when the subject: (1) failed to correctly reach the jumping target signaled on the ground; (2) failed to correctly jump in the indicated direction, for instance executing a jump in the lateral or medial direction but not following the 45° line; (3) excessively rotated the foot, in an attempt to anticipate the following lateral or medial jump. Ultimately, the tasks were executed in a random fashion, with the purpose of avoiding bias related to a predefined order in the execution of such conditions.

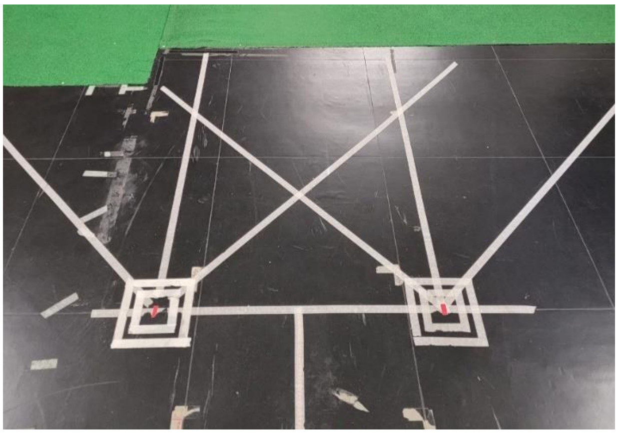


Figure 13: *Visual indicators representing the landing target and the lateral, central, and medial directions to follow when executing a jump.*

Post-test phase

After the completion of all landing tasks, the markers were removed from the body of the subject and the session was concluded.

2.4. Data analysis

2.4.1. Kinematic data elaboration

Data obtained from the trials were processed using the software SmartTracker, which was used to reconstruct the tridimensional trajectory of every marker during the tests. The first step of data elaboration (tracking phase) consisted in the assignment of a label to every marker: each label (as seen in *Table 2*) referred to a marker corresponding to a precise anatomical landmark. This was only done for the first frame of every task acquisition; then, the software is able to automatically assign the mentioned labels to the corresponding markers for the rest of the recording. The automated tracking of markers may generate errors due to the cameras briefly losing a marker or potential reflections that may disturb the cameras. Before proceeding, it was necessary to manually correct these imperfections. The final product of this first phase was a virtual 3D representation of the athlete executing each task, represented as a “stick-model” (*Figure 14*).

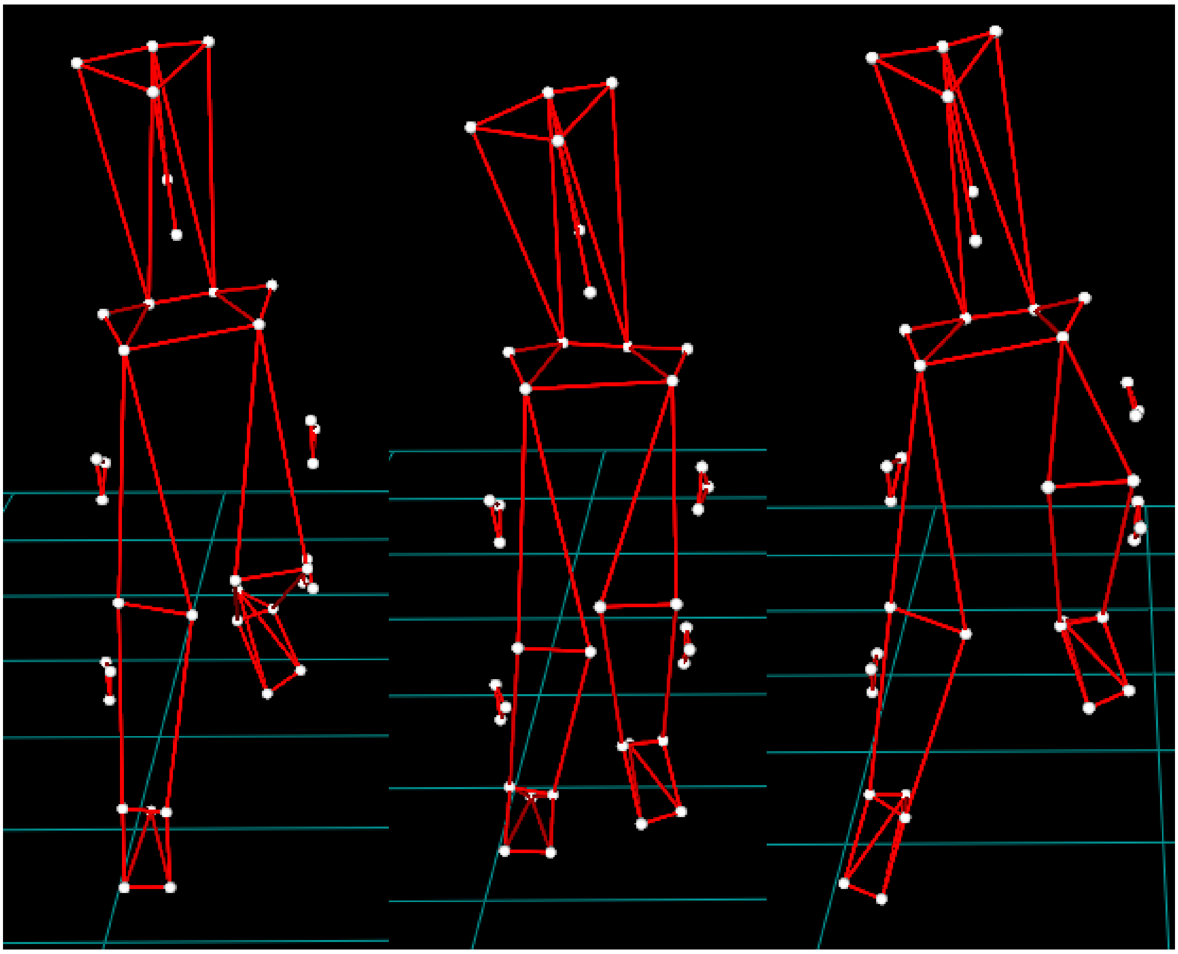


Figure 14: *SmartTracker virtual representation of a subject executing a DJM.*

After the completion of the tracking process, which was done for both static and dynamic data, the files were exported in the *.c3d* format in order to elaborate them further using the 3D biomechanics software Visual3D (C-Motion, Inc. Germantown, MD, USA), which allowed to extrapolate kinematic data.

The first step when using Visual3D is the creation of a reference model (*Figure 15*) for every subject, based on anthropometric data relative to the subject (height and weight) and the static acquisition file. The whole Visual3D reference model is created by defining a series of virtual rigid body segments, corresponding to the real body segments. In particular, it is necessary to define which markers identify proximal and distal extremities of each segment, in order to better assemble the model. Moreover, Visual3D allows to reconstruct internal anatomical landmarks, such as joint rotation centers, starting from the anthropometric data previously discussed and the position of the mentioned markers. This feature was used to virtually reconstruct the hip rotation center, fundamental to the model, in accordance with the results of the study by Bell et al. (1989). Finally, the model was applied to every dynamic file of the single subject, generated during the landing tasks (*Figure 16*).

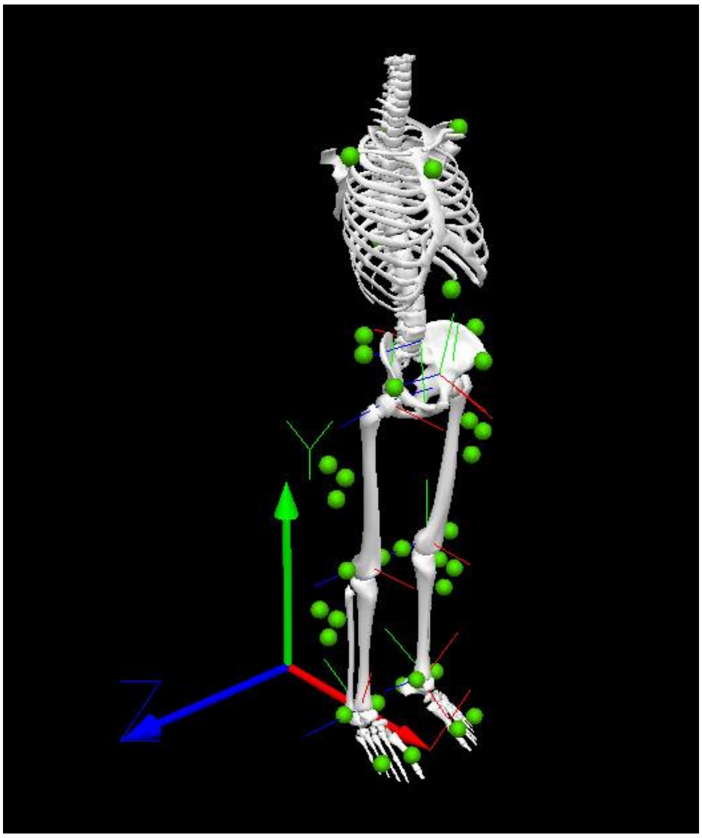


Figure 15: *Visual3D reference model of the lower limbs.*

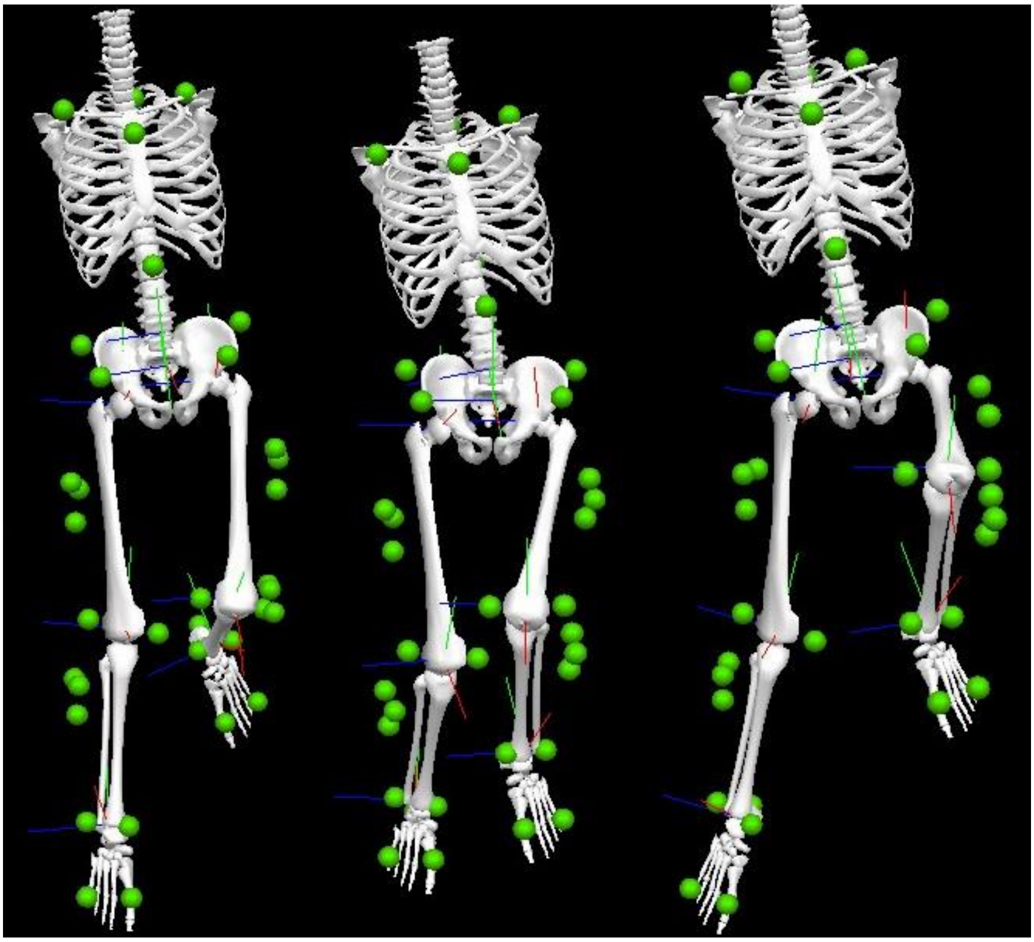


Figure 16: *Visual3D virtual representation of a subject executing a DJM.*

All kinematic calculations were performed using the *Computed_Model_Based_Data* menu in Visual3D, a tool that allows to automatically calculate joint angles as described in the dedicated paragraph. Kinematic variables – regarding the dominant limb – extrapolated from the acquisitions included: (1) hip and knee angles in all three planes at ground contact (defined as the frame in which the subject’s toe or heel first touched the floor) and (2) hip and knee peak angles in a time window of 100 ms after ground contact, which is recognized as one of the time frames when most ACL injuries occur (Krosshaug et al. 2007).

2.4.2. Joint angles

A joint angle represents the relative orientation between the local coordinate systems (LCSs) of the two connected segments. This orientation is defined as the roto-translation of the two reference systems with respect to their position and the rotation with respect to the global coordinate system (GCS) of the laboratory, previously defined during the static phase of the calibration process. The norm regarding the axes of the GCS that was followed during this study dictates that the X axis should be in the anterior-posterior direction, the Y axis in the vertical direction and the Z axis must be perpendicular to both X and Y, thus being in the medio-lateral direction. Regarding the LCS, the X axis was the one around which abduction (negative) and adduction (positive) movements occurred; the Y axis was the one around which intra (positive) and extra (negative) rotation occurred; finally, the Z axis was the one around which movements of flexion (positive) and extension (negative) occurred.

Knowing the translational vector and the rotational matrices of the LCSs belonging to both the proximal segment (${}^{g}\boldsymbol{o}_{p}$, ${}^{g}\boldsymbol{R}_{p}$) and the distal one (${}^{g}\boldsymbol{o}_{d}$, ${}^{g}\boldsymbol{R}_{d}$) with respect to the GCS, the kinematics of the whole joint can be expressed as

$$\boldsymbol{R}_j = {}^{g}\boldsymbol{R}_p^T \, {}^{g}\boldsymbol{R}_d$$

$$\boldsymbol{t}_j = {}^{g}\boldsymbol{R}_p^T \left({}^{g}\boldsymbol{o}_d - {}^{g}\boldsymbol{o}_p \right)$$

Where ${}^{g}\boldsymbol{R}_{p}^{T}$ is the transposed of the rotational matrix of the proximal segment's LCS with respect to the GCS (Cappozzo et al. 2005) (*Figure 17*). Based on this principle, the software Visual3D is able to calculate joint angles.

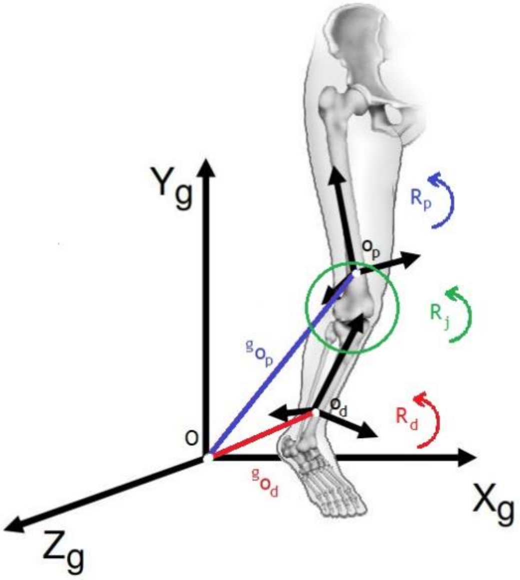


Figure 17: *Joint kinematics.*

2.4.3. Data post processing

Data obtained from the described process sometimes presented themselves as incomplete, due to the cameras losing a marker for a few frames or small issues occurred during the tracking phase. Moreover, signal noise tainted said initial data. In order to achieve better quality, an interpolation with a third-degree polynomial was performed, followed by a low pass Butterworth filter with a cutoff frequency of 6 Hz (as seen in Pappas et al. 2007). Both operations were conducted using the integrated tools in Visual3D. All the processing operations were included in a

Visual 3D *pipeline*. The utilization of this tool allows users to automatize calculations for every subject by generating an algorithm that includes all the steps previously described, from the reference model construction to the data polishing process. After its construction, the pipeline was saved and applied to every subject of the study.

Data analysis concluded with the export of kinematic parameters, in order to perform a statistical analysis.

2.5. Statistical analysis

Independent t-tests were performed to compare subjects' characteristics and anthropometrics between gender groups; significance was set at 5% ($p < 0.05$)

The following statistical analysis was carried out to compare the joint angles of male and female athletes at the initial contact (IC) and their peak values in the following 100 ms. This was done for all four tests (DJ, DJC, DJL, DJM).

The average values of the evaluated kinematic variables extracted from the three valid trials were calculated for each participant and used for the statistical analysis. The normality of all the resulting biomechanical parameters was evaluated with a Kolmogorov – Smirnov test. All data were normally distributed.

Each kinematic variable was evaluated with the Two-Way Analysis of Variance (ANOVA), in which factors were gender (male and female) and landing test (DJ, DJC, DJL, DJM). The significance level was set to $\alpha = 0.05$. If results were significant ($p < 0.05$) pairwise post-hoc comparisons, with Bonferroni corrections, were used to determine differences in the measured kinematic parameters between tests.

The effect size was evaluated using partial eta-squared, to identify the magnitude of the significant differences. The experimental effect was considered small for $\eta^2 = 0.01–0.06$, medium for $\eta^2 = 0.06–0.14$, and large for $\eta^2 > 0.14$ (Richardson 2010).

Finally, the post-hoc observed power was computed to evaluate the appropriateness of the chosen sample size.

All steps of the statistical analysis were performed using the software IBM SPSS Statistics v.27 (IBM Corporation, Armonk, NY, USA).

3 Results

An independent t-test was conducted to analyze anthropometric measures and subject characteristics (*Table 1*). Male subjects were significantly taller and had a higher body mass than female subjects (both p-values < 0.001), but no significant differences were found between genders when comparing age and training sessions per week.

Results of kinematic calculations and statistical analysis are reported in *Table 3*. It contains results relative to (1) the athletes' dominant limb kinematics at point of initial contact with the ground (IC) and (2) the peak values of the same angles in the following 100 ms. Both sections contain mean and standard deviation values of the joint angles, divided by gender, together with p-values of the ANOVA test and the effect size, expressed as partial η^2. It is important to note that *Table 3* contains the results of the gender comparison for joint angles in all four jumping tasks.

At IC, significant differences ($p < 0.05$) were found for hip flexion (HF IC), knee abduction (KAb IC) and knee internal rotation (KR IC) angles. Women showed significantly less hip flexion (mean difference of 2.6°, $p = 0.015$) when compared to males, with small effect size (partial $\eta^2 = 0.038$). Male athletes landed with an adducted knee, while women landed with a slightly abducted (valgus) knee (mean difference of 2.1°, $p < 0.001$), with medium effect size (partial $\eta^2 = 0.109$). Moreover, female athletes exhibited less knee external rotation when compared to male counterparts (mean difference of 2.1°, $p = 0.002$), with medium effect size (partial $\eta^2 = 0.063$). Results regarding hip adduction (HA IC), hip internal rotation (HR IC) and knee flexion (KF IC) did not highlight significant differences between male and female participants, with p-values respectively 0.983, 0.233, 0.995.

Peak values in the 100 ms following the IC had significant differences for hip adduction (HA P), hip flexion (HF P), knee abduction (KAb P) and knee flexion (KF P). Women landed with an adducted hip, while men landed with the hip in abducted configuration (mean difference of 2.3°, $p = 0.005$), with small effect size (partial $\eta^2 = 0.050$). Female athletes had, again, significantly less hip flexion than males (mean difference of 5.4°, $p < 0.001$), with medium effect size (partial $\eta^2 = 0.091$). When compared to male counterparts, females exhibited increased knee abduction (mean difference of 2.6°, $p < 0.001$) with medium effect size (partial $\eta^2 = 0.135$). Furthermore, men showed greater knee flexion than women (mean difference of 2.7°, $p < 0.001$), with medium effect size (partial $\eta^2 = 0.092$). Results regarding HR P and KR P were not significant (p-values respectively 0.998 and 0.141), thus there were no differences between males and females.

Table 3: *Kinematic variables (mean ± SD) and statistical results of the between-gender comparison for all tests. Significant values are marked with *.*

	Joint Angles [°]	Females	Males	p-value	Partial η^2
Initial contact					
	Hip Adduction	-13.0 ± 4.7	-13.0 ± 4.5	0.983	0.000
	Hip Internal Rotation	-6.3 ± 8.6	-7.9 ± 8.2	0.233	0.009
	Hip Flexion	37.2 ± 6.0	39.8 ± 7.4	0.015*	0.038
	Knee Abduction	0.6 ± 2.7	-1.5 ± 3.3	<0.001*	0.109
	Knee Internal Rotation	-0.9 ± 3.5	-3.0 ± 4.8	0.002*	0.063
	Knee Flexion	17.5 ± 3.4	17.5 ± 3.7	0.995	0.000
Peak					
	Hip Adduction	1.2 ± 5.5	-1.1 ± 5.8	0.005*	0.050
	Hip Internal Rotation	3.6 ± 7.3	3.6 ± 7.4	0.998	0.000
	Hip Flexion	47.9 ± 7.5	53.3 ± 10.0	<0.001*	0.091
	Knee Abduction	1.7 ± 3.1	-0.9 ± 3.7	<0.001*	0.135
	Knee Internal Rotation	4.7 ± 4.1	3.6 ± 5.1	0.141	0.014
	Knee Flexion	53.8 ± 4.3	56.5 ± 4.5	<0.001*	0.092

The influence of test types was significant only in HA P ($p < 0.001$), with a large effect size (partial $\eta^2 = 0.142$), and in KF P ($p = 0.020$) with a medium effect size (partial $\eta^2 = 0.063$). The results are reported in *Table 4*. The test type did not significantly influence the other variables ($p > 0.05$).

A *post-hoc* test, with Bonferroni type adjustment, was then executed to investigate the statistical significances among the four FTs with respect to the two mentioned variables. The analysis of HA P showed that the DJL induced the highest peak of adduction, while during the DJM the hip was more abducted than in other tests. In particular, during the execution of the DJL, peak hip adduction was significantly higher than in the DJ (mean difference of 3.1°, $p = 0.05$) and DJM (mean difference of 5.4°, $p < 0.001$). The HA P during the DJM resulted significantly lower than in DJC (mean difference of 4.4°, $p = 0.002$).

The knee was less flexed performing the DJC with respect to the other tests: the difference was significant only between DJC and DJM (mean difference of 2.7°, $p = 0.034$), but differences – although not significant – were found also between DJC and DJL ($p = 0.052$).

The post-hoc observed power of significant results for gender and between-tests differences ranges from 0.76 to 0.99.

Table 4: *Main results of HA P and KF P post-hoc analysis for comparison between tests after Bonferroni's correction. *Significant in Two-Way ANOVA ($p < 0.05$).*

Variable (deg)	**Test***	**Females**	**Males**	**Total**	**Comparison between tests (p-value)**			
					DJ	**DJC**	**DJL**	**DJM**
Peak Hip Adduction								
	DJ	0.8 ± 4.2	-2.2 ± 5.8	-0.7 ± 5.2			0.050	
	DJC	2.8 ± 5.0	-0.0 ± 4.9	1.4 ± 5.1				0.002
	DJL	3.9 ± 5.6	0.9 ± 5.7	2.4 ± 5.8	0.050			<0.001
	DJM	-2.7 ± 4.9	-3.2 ± 5.1	-3.0 ± 5.6		0.002	<0.001	
Peak Knee Flexion								
	DJ	54.6 ± 3.7	56.2 ± 4.2	55.4 ± 4.0				
	DJC	51.8 ± 5.1	54.9 ± 4.2	53.3 ± 4.9			0.052	0.034
	DJL	54.5 ± 3.9	57.3 ± 4.9	56.0 ± 4.6		0.052		
	DJM	54.4 ± 4.1	57.7 ± 4.2	56.0 ± 4.4		0.034		

Box plots showed in *Figure 18*, *Figure 19* and *Figure 20* allow to better represent the previously discussed results, while also providing insight about the athletes' performances in the four different tasks, which was not provided in *Table 3*. For clarity reasons, only box plots relative to significant – with respect to gender or test – results were included. Moreover, *Figure 20* contains kinematic results relative to peak hip adduction and knee flexion, which highlighted significant differences also relatively to the different tasks executed by the subjects, as previously discussed.

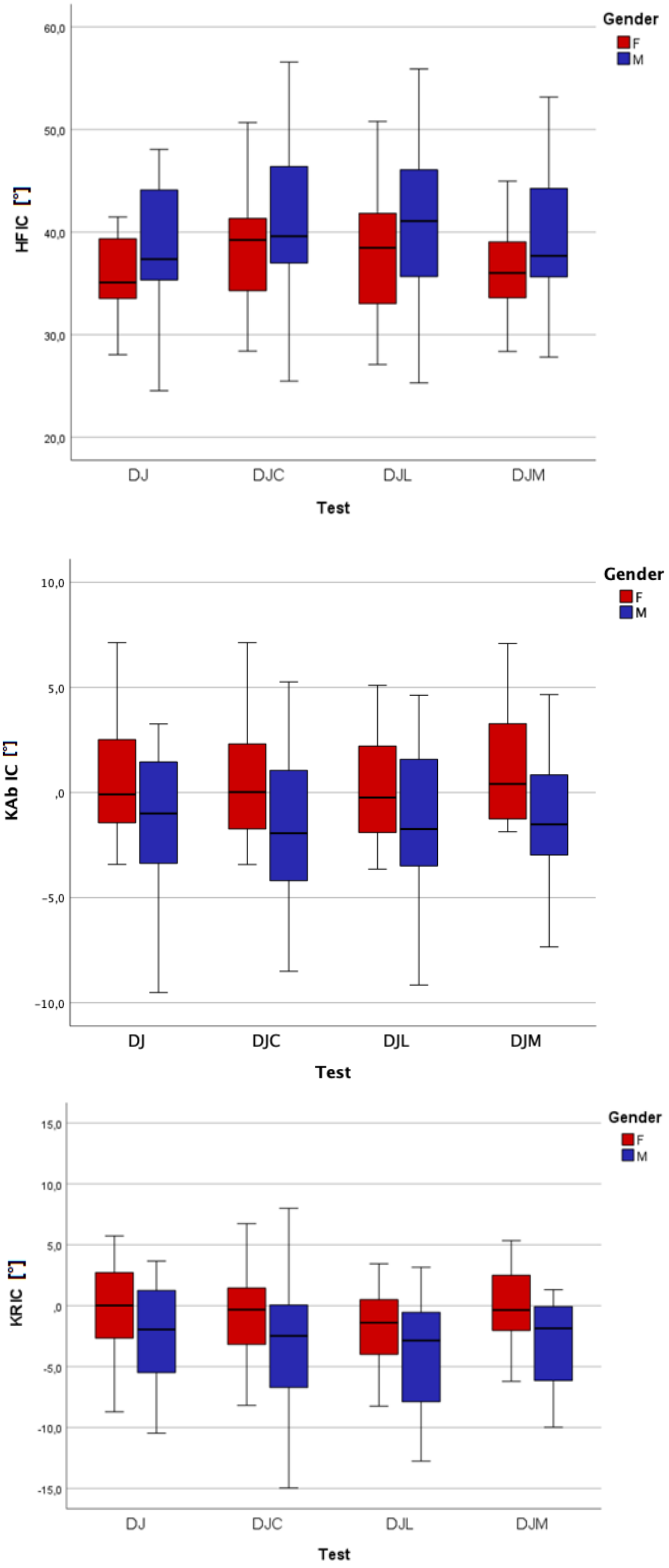


Figure 18: *Box plots showing HF (a), KAb (b) and KR (c) at IC when performing the four proposed tasks.*

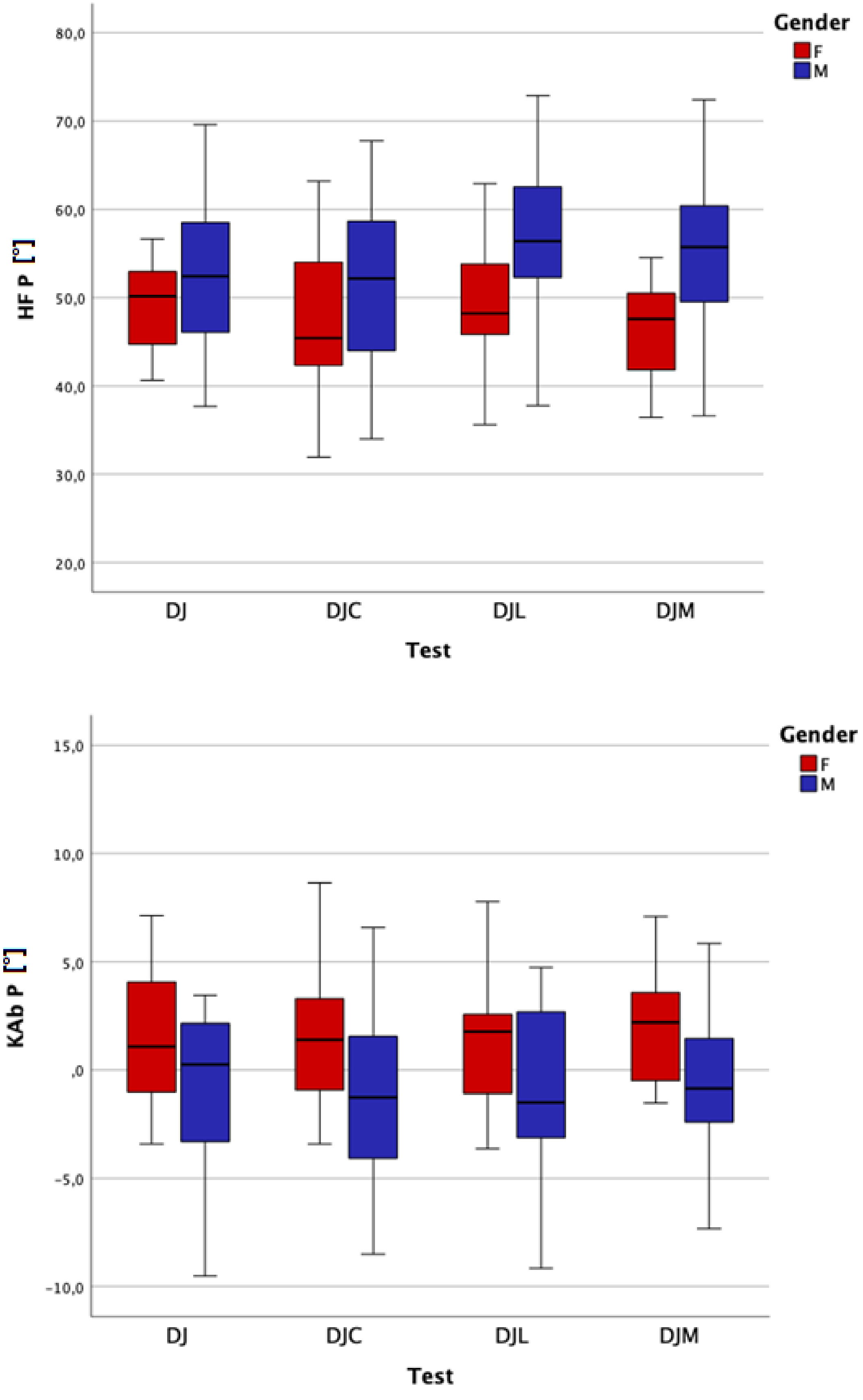
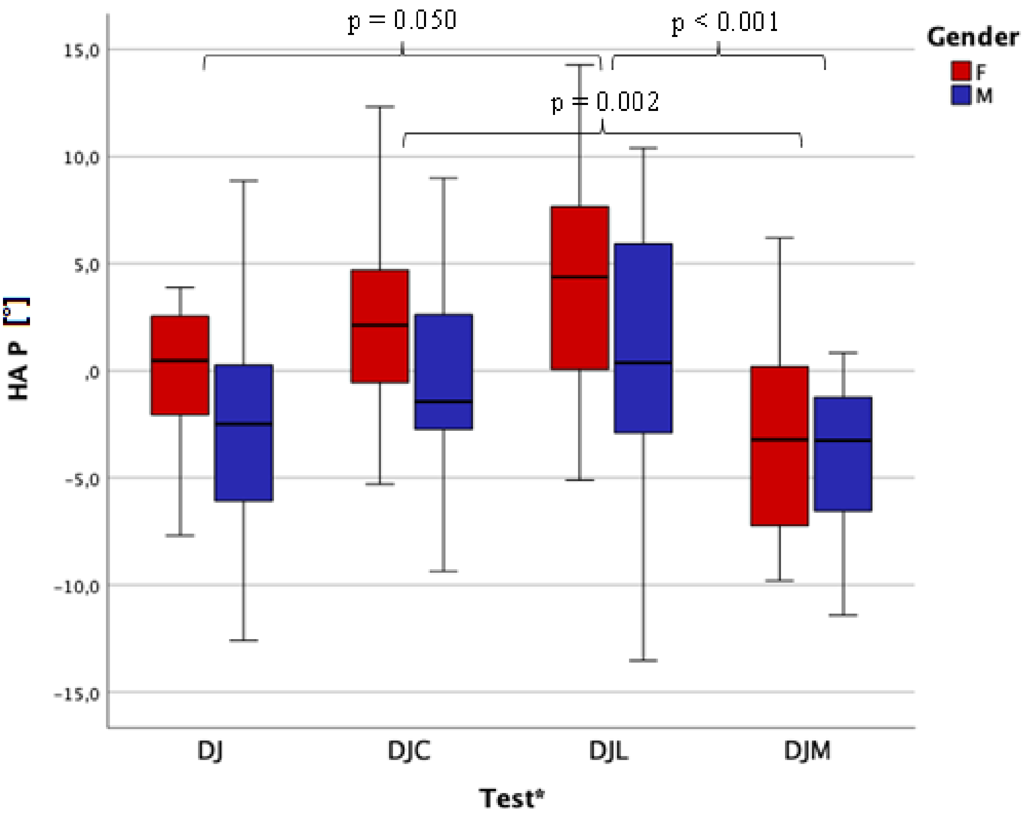


Figure 19: *Box plots showing peak HF (a) and KAb (b) in the next 100ms after IC when performing the four proposed tasks.*



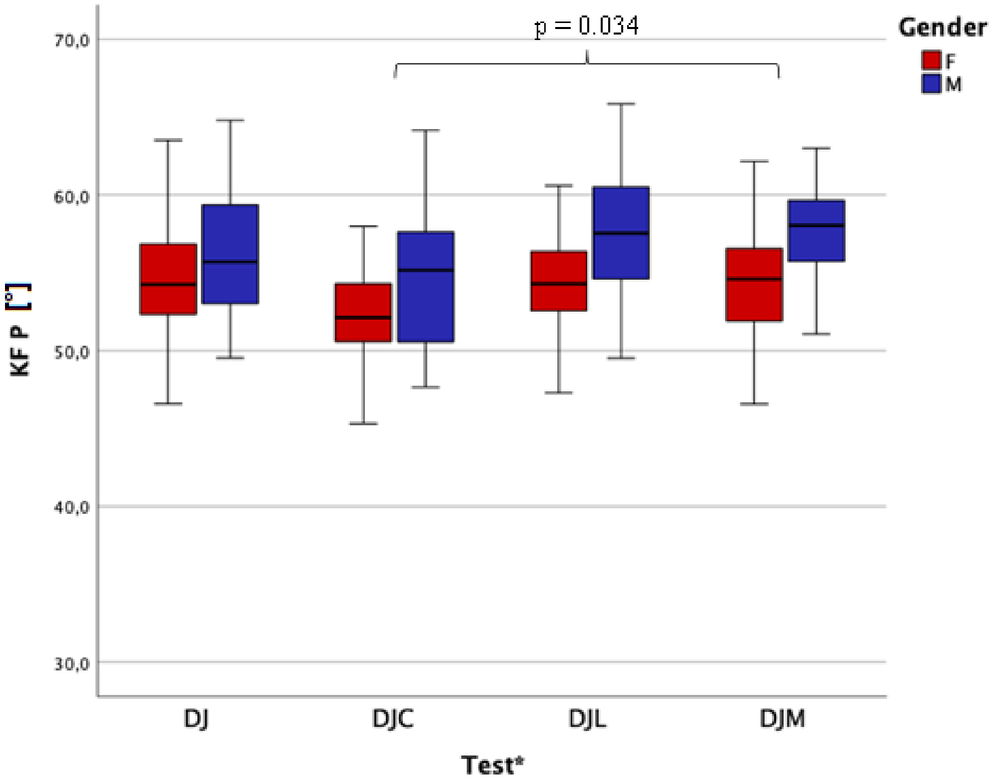


Figure 20: *Box plots showing peak HA (a) and KF (b) in the next 100ms after IC when performing the four proposed tasks. Brackets indicate significance and p-values of between-test comparisons after Bonferroni's correction.*

4 Discussion

Statistical analysis between groups showed height and body mass to be significantly different between male and female subjects. Age and number of training sessions per week were not significant, thus they do not constitute confounding factors when discussing the obtained results.

The main findings of this study concern gender differences in lower limb kinematics when performing four landing tasks in a controlled environment. 20 male and 20 female athletes performed a drop landing followed by a subsequent jump in four different directions (vertical, forward, lateral and medial). The results of post-hoc observed power analysis confirmed that the sample size was appropriate to analyze the variables chosen to achieve the aims of this study. Moreover, the selected sample size resulted comparable to those used in similar studies meant to analyze gender differences in landing FTs. For instance, 32 subjects (16 males and 16 females) were involved in the study by Pappas et al. (2007), 25 subjects (14 males and 11 females) were studied by Hovey et al. (2019), and finally 37 subjects (18 males and 19 females) were investigated by Nagano et al. (2007).

The results of this study highlighted different dominant limb biomechanics when comparing results of males and females. Non-contact ACL injuries are one of the most common injuries in sports (Ferretti et al. 1992), and female athletes are linked to increased ACL injury risk when compared to male counterparts (Arendt et al. 1995). This is due to biomechanical, neuromuscular, and hormonal factors: in this study, only biomechanical factors were considered.

More precisely, women exhibited significantly less hip flexion and knee external rotation, but higher knee abduction at ground contact when compared to men. Furthermore, female athletes showed significantly higher hip adduction and knee abduction, but lower peak hip and knee flexion in the 100 ms that followed ground contact, as the time window within the ACL injuries commonly occur (Krosshaug et al. 2007). While many authors have already investigated landing kinematics at ground contact (Decker et al. 2003, Fagenbaum et al. 2003, Russell et al. 2006), Sigurðsson et al. (2018) found that peak ground reaction forces – and consequently peak joint stress – happen within 100 ms from IC.

As previously stated, female athletes tend to perform differently when compared to male counterparts when executing functional tests (especially due to intrinsic factors) such as landing tasks. However, there is no set standard about what landing variations should be executed, making the comparison of results of different studies difficult. Nevertheless, many findings of this study were aligned with those found in previous literature.

At IC, women exhibited significantly less hip flexion than men. This is believed to increase risk of ACL injury in athletes because an extended lower limb may lead to inefficient load dissipation through ligament structures (Padua et al. 2009). This result is consistent with findings by Chappell et al. (2007), who evaluated lower limb kinematics in double-leg stop jumps. In addition, female subjects showed a less externally rotated and a more abducted knee when compared to males. Both frontal and transversal plane kinematics are recognized as key factors in knee injuries, as stated by Taylor et al. in 2016: excessive knee valgus and leg rotation increase strains on ligamentous structure and may lead to joint damage. Our findings concerning the angles at IC were even consistent with those of Russell et al. 2006: the authors investigated single-leg drop landings – although from a fixed height of 60 cm – and discovered women landed with a slightly valgus knee, while men tended to land with the knee in a varus position.

The study protocol yielded significant results also when analyzing lower limb kinematics in the 100 ms following IC. Women exhibited significantly higher hip adduction than men, who were found to have an abducted hip instead, something that did not happen at IC. This is commonly recognized as a risk factor for ACL injury, as excessive hip adduction can cause the knee to enter a valgus position (Powers 2010). These findings relate to those of Hovey et al. (2019), who investigated single-leg DLs and DJs. Female athletes showed again lower peak hip flexion than male counterparts, consistently with results of Schmitz et al. (2007), who inquired single-leg drop landings but did not focus on a specific time frame. Furthermore, women were found to have a significantly more abducted knee, while men exhibited a varus knee. Female athletes are recognized to have increased knee valgus at landing, and this may enhance the risk of ACL injury (Hewett et al. 2005). These findings are in agreement with those of Ford et al. (2006) who discovered female subjects to show higher knee abduction than males when performing single-leg drop landings. The authors evaluated a time window of 500 ms after IC, very similar to the one considered in this study, and obtained similar results. Lastly, women did not exhibit significant differences in knee rotation at peak when compared to men but showed a lower peak of knee flexion during the first 100 ms of the landing phase. Altered sagittal plane knee kinematics is believed to be a major risk factor for ACL injuries in female athletes (Griffin et al. 2000, Yu et al. 2007) due to the increased loads on the ligamentous structures when the knee itself is in an extended position. These findings are consistent with those of Lephart et al. (2002) and Schmitz et al. (2007), who both investigated gender differences in single-leg drop landings and found females, in average, had decreased maximal knee flexion after landing (4.6°) with respect to males, results comparable to those of the present study, that show a mean difference of 2.7°, but in the 100 ms following ground contact.

This study highlighted significant differences between male and female athletes when investigating knee external rotation at IC. Other studies found similar results when investigating single-leg landings, but only at peak values (Nagano et al. 2007, Kiriyama et al 2009). This could be due to the protocol employed, as in both previous studies men and women jumped from the same height. It is possible that not adjusting the task to the single athlete’s capability tainted final results. Moreover, previous works highlighted significant differences when comparing hip adduction and internal rotation angles of male and female participants at IC (Lephart et al. 2002, Chappell et al 2007). This study, however, did not show any significant difference with respect to the mentioned variables at the time of ground contact.

As previously discussed, when comparing results of different landing tasks, this study highlighted significant differences only in HA P and KF P. In particular, the hip was more adducted during the execution of the DJC and DJL, while it was abducted in the DJ and DJM. This could be associated to the motion pattern of the single task: the DJM required a second jump in the medial direction that made necessary an increase in hip abduction; conversely, in the DJL the subject jumped in the lateral direction thus executing a movement that demands hip adduction. Nevertheless, women exhibited a more adducted hip in all four tasks when compared to men, a result consistent with those of Hovey et al. (2019), who stated altered frontal plane motion patterns may lead females to sustain more injuries. Knee flexion was the lowest in the DJC with respect to other tasks, especially in females, although this difference is significant only when comparing the DJC with the DJM. Nevertheless, differences between DJC and DJL results are very close to the significance threshold. The execution of a DJC involved higher hip adduction and lower knee flexion than other tasks, thus it can be theorized that this particular task was the most demanding for the knee, due to the fact that it was executed in a particularly “stiff” manner. Women showed an even stiffer landing strategy when compared to men, highlighting once again one of the reasons of higher knee injury rates in female athletes.

In conclusion, the present study provided new elements that may be of assistance when evaluating lower limbs biomechanics to assess ACL injury risk in athletes. It is the first study in which subjects performed DJ tests with sequential jumps in four directions, starting from personalized heights and forward jump distances. The four proposed landing tasks were designed to evaluate different knee motion patterns that are usually associated with ACL injuries and allowed to represent typical sport gestures – such as single-leg landings and cutting maneuvers – that are commonly linked with increased non-contact ACL injury risk in team sports. Moreover, although different tasks elicited different results, male and female subjects consistently showed different biomechanical behaviors when executing the proposed tasks, with female athletes performing riskier – in terms of ACL injury – motion patterns. Furthermore, the participants of this study were recreationally active athletes: while enrolling top-tier athletes is certainly useful to better comprehend the biomechanics at the highest levels, most injuries occur in less competitive environments as recreationally athletes constitute the majority of players. Evaluating landing performances in subjects who are believed to engage in less competitive activities could help designing specific training programs tailored to their skill level. Lastly, it can be stated that results of this study are mainly aligned with those of similar previous works, but they certainly provide new elements that may require further exploration and discussion.

4.1. Limitations and future developments

This study produced relevant results, but it still had some inevitable limitations.

Firstly, only kinematic variables were investigated. Excessive joint load is the primary cause of knee injury and, although joint angles play a central role in determining how the stress is applied, an analysis of the forces applied to the ligamentous structures would have been beneficial to the goals of the presented work. Thus, the use of a force platform to study landing kinetics would have greatly enhanced the results of the investigation.

Secondly, as previously discussed, there are many ACL injury risk factors other than biomechanical ones. This study only focused on the latter, but a comprehensive evaluation of, for instance, ovarian and hypophyseal hormonal levels in female athletes may offer more information about the causes underlying gender differences in landing tasks.

Moreover, this investigation enrolled athletes who compete in different disciplines, with different backgrounds and skill levels. This variety is common in similar studies, since male and female athletes, even when competing in the same sport, are often believed to possess different physical capabilities. As far as training levels are concerned, no gender-related differences were observed. In general, this may indicate that males and females were experiencing a similar weekly load at the moment of the tests. Moreover, the majority of subjects (60% of females and 75% of males) competed in the same three sports (football, volleyball, boxing). Nevertheless, a less heterogeneous subject pool may have provided different results, although this remains only a theory.

Lastly, it should be stated that landing tasks executed in a safe and controlled environment (e.g., the laboratory) often cannot resemble real life playing situations. In realistic play settings, athletes are subjected to many different stimuli, and often find themselves reacting to inputs in an unanticipated manner. This may lead to incorrect motion patterns and injuries, thus making the playing field inherently more dangerous and unpredictable than the laboratory, where athletes execute pre-programmed movements.

Following these limitations, future developments of the presented work should include: (1) force platforms, in order to assess lower limb kinetics as previously discussed; (2) an analysis of additional risk factors, together with biomechanical ones; (3) a more homogeneous subject sample, consisting in athletes with as much as possible similar skill level; (4) the possibility to conduct experimentations directly on the playing field, although this remains difficult to implement.

Furthermore, future studies should focus their attention also on ankle kinematics and kinetics since they are believed to play a fundamental role in defining the landing technique of an individual. Lastly, it would be interesting to compare the performances of athletes who already suffered ACL injuries to those of healthy subjects. Assessing whether players can return to the field is central in their rehabilitation, and it is one of the current uses of FTs in clinical practice: analyzing previously injured subjects’ results in the discussed landing tasks could improve the ability of clinicians to decide if an athlete’s recovering is complete.

5 Conclusions

In order to further expand knowledge about landing functional tests, 40 healthy subjects – 20 males and 20 females – were recruited to perform four variations of a drop jump task in a controlled environment. The study had the goal of highlighting gender differences in the execution of a drop landing followed by a subsequent jump in one of four directions (vertical, lateral, forward, medial). The protocol was developed to improve those of previous studies, mainly adjusting the task difficulty to the athletes' anthropometric measures and real jumping capabilities. Moreover, the study focused on multi-planar jumps. These improvements were made in an attempt to introduce standardized tests that clinicians could employ in the future.

An analysis of lower limb kinematics showed that women exhibited less hip flexion and knee external rotation, but more knee abduction at ground contact with respect to men. Similar results were found in the 100 ms following ground contact, in which female athletes had higher peak hip adduction and knee abduction, but lower peak hip and knee flexion than male counterparts. All these altered motion patterns are commonly associated with an increased risk of sustaining ACL injuries and are usually found in female subjects, thus contributing to the higher non-contact ACL injury rates in women. The statistical analysis of the results confirmed them to be of great significance. Moreover, the proposed tasks involved knee loading patterns that may have been fundamental in better highlighting said results.

Furthermore, between-test comparisons highlighted significant differences in peak hip adduction and knee flexion in the execution of different tasks. The subjects showed increased hip adduction when executing the DJL and DJC, while showing decreased knee flexion in the DJC. These findings allow to theorize that the DJC may have been a particularly demanding task for the knee, as both these kinematic results are linked with increased knee injury rates, especially in females.

The results of the presented work are in agreement with those found in previous literature. Elements introduced in this study, such as personalized starting heights and forward jump distances, were beneficial to the execution of the landing tasks and played a crucial role in obtaining significant results. Nevertheless, further research is required to recommend whether these adjustments could be used to develop standardized tests.

In conclusion, there is a strong need in the scientific community for a standardization in the execution and use of functional tests to evaluate lower limbs biomechanics. The proposed tests and the results of this study may be of assistance in developing new or improved tasks to be used by clinicians in injury prevention and recovery, and in designing new training programs for athletes.

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List of Figures

List of Tables

List of acronyms

ACL: Anterior Cruciate Ligament

CCD: Charge Coupled Device

CoD: Changes of Direction

DJ: Drop Jump

DJC: Drop Jump Central

DJL: Drop Jump Lateral

DJM: Drop Jump Medial

DL: Drop Landing

FJ: Forward Jump

FT: Functional Test

GCS: Global Coordinate System

GRF: Ground Reaction Force

HA IC: Hip Adduction at Initial Contact

HA P: Peak Hip Adduction

HF IC: Hip Flexion at Initial Contact

HF P: Peak Hip Flexion

HQR: Hamstrings / Quadriceps strength Ratio

HR IC: Hip internal Rotation at Initial Contact

HR P: Peak Hip internal Rotation

IC: Initial Contact

KA IC: Knee Adduction at Initial Contact

KA P: Peak Knee Adduction

KF IC: Knee Flexion at Initial Contact

KF P: Peak Knee Flexion

KR IC: Knee internal Rotation at Initial Contact

KR P: Peak Knee internal Rotation

LCS: Local Coordinate System

LESS: Landing Error Scoring System

LJ: Lateral Jump

PCL: Posterior Cruciate Ligament

SLHD: Single Leg Hop Distance

VJ: Vertical Jump