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INFLUENCE OF FATIGUE ON MUSCLE ACTIVATION PATTERNS DURING CHANGES OF DIRECTION IN ÉLITE FEMALE SOCCER PLAYERS

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LIST OF SYMBOLS AND ABBREVIATIONS USED

CoD: Change of Direction

BMI: Body Mass Index

RoM: Range of Motion

ACL: Anterior Cruciate Ligament

PCL: Posterior Cruciate Ligament

CoM: Centre of mass

EMG: Electromyography

ENG: Electroneurography

sEMG: Surface Electromyography

GRF: Ground Reaction Force

GRS: Global Reference System

VAM: Maximum Aerobic Velocity

$[La^-]_b$: Lactate concentration

HR: Heart Rate

RPE: Rate of Perceived Exertion

SD: Standard Deviation

SPM: Statistical Parametrical Mapping

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ABSTRACT

Introduction: anterior cruciate ligament (ACL) injury is a significant problem in young female athletes, especially in sports that involve abrupt *sidecutting* manoeuvres and unexpected changes of directions. Non-contact injury is the most common mechanism of ACL wounds and it is determined by multifactorial aspects (intrinsic and extrinsic factors). A preventive neuromuscular and proprioceptive training programme is necessary to reduce the high incidence of this damage. It is essential to assess how fatigue changes some kinematic and kinetic parameters, while performing changes of direction. Due to fatigue, also muscle control, muscle reaction patterns, coordination and control of body may change, significantly.

Purpose of the study: the aim of this study is to analyse the electromyographic signal that describe the behaviour of the dominant lower limb during changes of direction. The test is performed by a pool of female subjects, during a fatigue protocol that includes continuous changes of direction.

Materials and methods: eighteen female subjects practicing high level soccer were evaluated (age range: 20-31 years). After a brief warm-up, composed by light running, exercises for joint mobility and stretching, lactate sampling was performed in order to monitor the level of fatigue. The functional test was based on the detection of electromyographic activity of the dominant lower limb during a shuttle run, at a set speed (70% of the maximum aerobic speed). Heart rate was also detected during the test. Finally, after a questionnaire on the subjective perception of effort, blood lactate sampling was repeated after 1, 3 and 5 minutes from the end of the exercise. The muscles we analysed are rectus femoris, vastus medialis, vastus lateralis, biceps femoris, semitendinosus, tibialis anterior, gastrocnemius medialis and gastrocnemius lateralis. Before being analysed, the electromyographic signal was filtered and rectified. Our aim was to assess fatigue influence on the signal intensity, the time of muscle activation and on the EMG frequency content.

Results: it was found that the increase in exercise intensity, in terms of repetition of the gesture, seems to not determine a univocal alteration in muscle activation patterns: some athletes, due to fatigue, increased the activation of some muscle groups, while other decrease it. Among participants there is a reduction in the frequency content of the signal, but there is not a common trend of a delay or an anticipation in the muscle onset times. Our statistical analysis claims that the behaviour of adaptation to a fatigue state is highly subject-dependent.

Discussion: the different adaptation to the exercise load confirms that we cannot assume fatigue as the only factor responsible for ACL tears. A reduction in muscle activity may be responsible for an overload of ligaments and other structures that compose the tibiofemoral joint; there are other external loads on the tibia that contribute to the generation of a risky condition for the leg.

Spectral changes of the electromyographic signal are determined by the decrease of the speed of propagation of the action potential, connected to the production of acid catabolites (effects of metabolism). During fatigue, there is also the accumulation of lactate, the decrease of the pH value and the exhaustion of substrates (phosphocreatine and ATP). There are also some changes that occur at the level of the central nervous system in the activation of the motor unit. Our outcomes are in line with these assumptions; the more the athletes were fatigued, the more they reduced their spike frequency.

The results confirm the strong specificity of the muscle patterns; therefore, it must be considered by acting on an individual level in the prevention programs.

SOMMARIO

Introduzione: la lesione del legamento crociato anteriore (ACL) è un problema significativo nelle giovani atlete, in particolare negli sport che comportano brusche manovre di spostamento laterale e inaspettati cambi di direzione. La lesione senza contatto con l’avversario è il meccanismo più comune dei danni dell’ACL ed è determinata da aspetti multifattoriali (fattori intrinseci ed estrinseci). Un programma di allenamento neuromuscolare e propriocettivo preventivo è necessario per ridurre l'elevata incidenza di questo danno. È essenziale valutare in che modo, mentre si eseguono cambi di direzione, la fatica modifica alcuni parametri cinematici e cinetici. A causa dell'affaticamento, anche il controllo muscolare, i modelli di reazione muscolare, la coordinazione e il controllo del corpo possono cambiare in modo significativo.

Scopo dello studio: lo scopo di questo studio è di analizzare il segnale elettromiografico che descrive il comportamento dell'arto inferiore dominante durante i cambi di direzione. Il test viene eseguito da un gruppo di soggetti femminili, durante un protocollo di fatica che include continui cambi di direzione.

Materiali e metodi: sono state valutate 18 calciatrici che praticano attività sportiva ad alto livello (fascia di età: 20-31 anni). Dopo un breve riscaldamento, composto da corsa leggera, esercizi per la mobilità articolare e lo stretching, è stato eseguito un campionamento del lattato per monitorare il livello di affaticamento. Il test funzionale si è basato sul rilevamento dell'attività elettromiografica dell'arto inferiore dominante durante una corsa shuttle, a una velocità impostata (70% della velocità aerobica massima). Durante il test è stata registrata la frequenza cardiaca. Infine, dopo un questionario sulla percezione soggettiva dello sforzo, il prelievo del lattato è stato ripetuto dopo 1, 3 e 5 minuti dalla fine dell'esercizio. I muscoli che abbiamo analizzato sono retto femorale, vasto mediale, vasto laterale, bicipite femorale, semitendinoso, tibiale anteriore, gastrocnemio mediale e gastrocnemio laterale. Prima di essere analizzato, il segnale elettromiografico è stato filtrato e rettificato. Il nostro obiettivo

era verificare l'influenza della fatica sull'intensità del segnale, sul tempo di attivazione muscolare e sul contenuto in frequenza.

Risultati: si è riscontrato che l'aumento dell'intensità dell'esercizio, in termini di ripetizione del gesto, non sembra determinare un'evidente alterazione dei pattern di attivazione muscolare; alcune atlete, a causa della fatica, aumentano l'intensità di attivazione di alcuni gruppi muscolari, mentre altre la diminuiscono. Tra i soggetti c'è una riduzione del contenuto in frequenza del segnale, ma non una tendenza di un ritardo o di un'anticipazione dei tempi di attivazione muscolare. La nostra analisi statistica afferma che il comportamento dell'adattamento a uno stato di affaticamento è fortemente dipendente dal soggetto.

Discussione: il diverso adattamento allo sforzo conferma che non possiamo assumere la fatica come unico fattore responsabile delle lesioni dell’ACL. Una riduzione dell'attività muscolare può essere responsabile di un sovraccarico dei legamenti e delle altre strutture che compongono l'articolazione tibiofemorale; ci sono altri carichi esterni sulla tibia che contribuiscono alla generazione di una condizione rischiosa per la gamba.

I cambiamenti dello spettro del segnale elettromiografico sono determinati dalla diminuzione della velocità di propagazione del potenziale d'azione, collegata alla produzione di cataboliti acidi (effetti del metabolismo). Durante l'affaticamento, si verificano anche l'accumulo di lattato, la diminuzione del valore del pH e l'esaurimento dei substrati (fosfocreatina e ATP). Ci sono anche alcuni cambiamenti che si verificano a livello del sistema nervoso centrale nell'attivazione dell'unità motoria. I nostri risultati sono in linea con questi presupposti; più le atlete erano affaticate, più riducevano la frequenza di sparo.

I risultati confermano la forte specificità dei modelli muscolari che, pertanto, deve essere considerata agendo a livello individuale nei programmi di prevenzione.

1. INTRODUCTION

Injuries significantly impair both individual and team performance in sport, thus their prevention must be a priority (Ekstrand et al., 2011). Anterior cruciate ligament (ACL) injuries continue to rise among athletes (Agel et al., 2016) and current prevention programs typically involve a combination of plyometrics, strength training, agility and balance exercises (Anderson et al., 2016; Gagnier et al., 2013). The aim is to create stable motor output by the athlete, even under sport-specific fatigued conditions in a complex athletic environment (Dingenen & Gokeler, 2017; Swanik, 2015).

Fatigue is deemed to have an important (but still not completely understood) role in the pathway to ACL injury. A fatigue state decreases the synergy of body segments activation during movement, owing to coordinative changes, to a reduction in degrees of freedom or to a loss of efficiency (Pol et al., 2019). Training to resist fatigue is an underestimated aspect of prevention protocols; currently, the most used measure of fatigue is incremental weakness related to playing time (Luig et al., 2017). However, injury surveillance data have not shown a consistent relationship between injury and fatigue as a result of playing time (Walden et al., 2011).

An important perspective to include in an injury prevention model is the fact that an imbalance between stress and recovery can generate several physical (e.g., increased fatigue level, decreased performance) and psychological (e.g., increased anxiety, emotional lability) responses (Ivarsson et al. 2019). Athletes can answer in two ways: they can adjust their activities (i.e., increasing recovery and decreasing training load) or ignore the physical and psychological reactions (i.e., increasing training effort and neglecting recovery). The second approach is generally associated with adverse outcomes and with an increased likelihood of becoming injured (Ivarsson et al. 2019; Williams and Andersen 1998).

Laboratory studies have shown conflicting results concerning the effect of fatigue on lower limb biomechanics, during athletic tasks (Barber-Westin and Noyes 2017;

Santamaria and Webster 2010); a reason could be the fact that these studies do not reflect the complexity of physical and psychological fatigue that occurs during an actual game (Windt and Gabbett 2017). In real conditions, environmental external factors and the contact with other players increase this complicacy. Moreover, fatigue can arise both for an acute workload and for lack of sleep; suboptimal recovery makes the athlete more fatigued and vulnerable to injury (Ivarsson et al., 2014, 2019; Johnson, 2011). Another cause of the onset of neuromuscular fatigue results from the accumulation of playing time (Gabbett, 2004; Wright et al., 2017), being the player potentially more vulnerable as the game progresses.

1.1. Non-contact injuries

Acute knee injuries represent a serious problem in ball and rackets sports, since they involve abrupt changes of direction (CoDs), such as landing, turning, and *sidecutting* manoeuvres (Beynnon et al., 2014; Faude et al., 2006; Myklebust et al., 1997; Pasanen et al., 2017). Most of these damages occur in non-contact conditions and under the influence of neuromuscular factors; they alter the biomechanical loading of the knee (Hewett et al., 2005a) and affect the magnitude and timing of muscle force production to stabilize this joint (Hewett et al., 2005b; Zebis et al., 2009).

Non-contact ACL injuries are particularly frequent and harmful (Smith et al. 2012): more than 200 000 ACL injuries are estimated to occur annually in the United States (Marshall et al., 2007; Prodromos et al., 2007). Such injuries are further defined as being accompanied by complaints lasting more than 4 weeks and by the absence from sport for 4 or more months. They are often associated with serious damages to the visceral system or to the musculoskeletal system, such as bone fractures or dislocations of the joint (Chomiak et al., 2000). Surgical reconstruction of the ACL is designed to restore the normal anatomy and biomechanics of the knee joint, to allow individuals to return to the sport activity, as soon as possible (Eckenrode et al., 2017). In their study on ACL-reconstructed patient, Gokeler et al., (2010) reported that it may require more time than 6 months after the reconstruction, to reprogram the neuromuscular system. In addition to the long recovery times necessary after these damages, there is a high risk of relapses in the long term and a chronic instability of the limb (Brown et al., 2014).

1.2. ACL injuries

Figure 1-ACL in normal condition and in a rupture state.

ACL ruptures (Figure 1) are among the most debilitating injuries in landing-, pivoting- and jumping- based sports (Ardern et al., 2011; Fortington et al., 2016). At least two-thirds of these injuries are sustained during non-contact circumstances and they commonly occur in athletes younger than 25 years (Kirkendall et al., 2002). The aetiology of ACL tears is multifactorial and it results from the interaction of several modifiable and non-modifiable risk factors (Hewett et al., 2010) (Table 1).

One potentially modifiable factor is neuromuscular fatigue, that leads to acute reductions in muscle strength, altered patterns of muscle activation, changes in hip and knee kinematics and increased ground reaction forces (Barber-Westin & Noyes, 2017; Benjaminse et al., 2019). All these conditions are hypothesised to increase the likelihood of non-contact ACL injury, during landing or sidestep.

Very few prospective studies have quantified fatigue in a meaningful way and there are no data to indicate that fatigue is, indeed, a risk factor for ACL injury (Barber-Westin and Noyes 2017; Benjaminse et al. 2019). Furthermore, none of the neuromuscular training interventions have proved to be successful in reduction of ACL injury rates (Webster and Hewett 2018).

Modifiable factors	
Biomechanical	Knee abduction Anterior tibial shear Lateral trunk motion Tibial rotation Dynamic foot pronation Fatigue resistance Ground reaction forces
Neuromuscular	Relative hamstring recruitment Hip abduction strength Trunk proprioception
Non-modifiable factors	
Anatomical	BMI Femoral notch index (ACL size) Knee recurvatum General joint laxity Family history
Developmental and hormonal	Sex, female Pubertal and post-pubertal maturation status Preovulatory menstrual status ACL tensile strength Neuromuscular shunt

Table 1-Modifiable and non-modifiable risk factors for ACL injury.

It is important to study how fatigue can alter certain physiological and biomechanical parameters of the knee joint, especially during the repetition of CoDs. The purpose is to obtain some specific prevention programs for non-contact injuries, in particular for those affecting the ligaments of the lower limb.

Even though musculoskeletal injuries are sport-specific and not sex-specific, there is a large difference in the total number and in the incidence of serious ACL wounds among men and women, especially in jumping and pivoting sports (Arendt et al., 1999).

1.2.1. Surgical treatment and consequences

After ACL rupture, athletes undergo a significant and aggressive period of rehabilitation and a reconstructive intervention using auto/allografts to stabilize the knee (Kaeding et al.,2015; Gottlob et al., 1999). The ACL is associated with poor healing capacity and the risk of a second injury is as high as 30% in the ipsilateral knee (Di Stasi et al., 2013). This wound frequently leads to chronic knee pain and makes athletes more susceptible to early-onset degenerative knee osteoarthritis (Oiestad et al., 2010); these conditions lead to a decline in athletic participation, but also to an enduring disability in later life (Ajuied et al. 2014).

Surgical interventions in this area have experienced important progress. Since the 1980s, thanks to arthroscopic techniques, intra-articular reconstructions avoid the need of arthrotomies; the time required for rehabilitation before returning to sport is reduced. The operation is carried out within 30 days from the injury, using autogenous tendons of the semitendinosus and gracilis muscles for the reconstruction. This type of intervention has a success rate of 97% (Bisciotti et al. 2016).

Since ACL rupture is highly debilitating, the rehabilitation process often begins before the operation itself; it allows a gradual return to sporting activity not earlier than 4-5 months after the intervention (Brotzman 2007). This return is subjected to complications; the most common is the loss of mobility of the district and, consequently, the achievement of specific objectives. For example, strength ratio between hamstrings and quadriceps ≥ 70% (Brotzman 2007). Moreover, quadriceps strength is greater than 85% compared to the contralateral, while hamstring strength is equal to 100% of the contralateral.

Even after the return to sport, it is also important to follow post-rehabilitation work protocols, to prevent later complications, such as arthro-fibrosis and osteoarthritis (Neuman et al. 2008).

1.2.2. Changes of Directions

Changes of directions are very frequent in team sports, in which a rapid adaptability is required in response to the race context. In a football match, for example, are registered until 700 changes of directions (Bloomfield et al., 2007), to dodge an opponent or an obstacle.

These movements can be classified in planned, when the athlete knows exactly when and with which modality the action is performed, or unplanned, when the decision to carry out a CoD is taken only a few moments before its realization. In this second case, the subject does not know the direction and the intensity in advance; unscheduled CoDs occur more frequently during sports, because of many external factors and as opponents' actions or game dynamics are not easy to foresee. In unexpected CoDs, muscle activity increases by 10-20% compared to those planned, while the load on the ligament structures of the lower limb is twice as high (Besier et al., 2003).

Furthermore, CoDs can be performed in two ways: sidestep cut (Figure 2, left) and crossover cut (Figure 2, right). The first involves a movement in which the free leg proceeds in the opposite direction with respect to the leg supporting the body weight. Contrarily, in the crossover cut the free leg swings towards the supporting limb, passing in front of the latter before touching down the ground (Schot et al., 1995). The effects of these actions on the joint can be also influenced by trajectory angles, that are a basis for classification; up to now, 45°, 90° and 180° CoDs have been explored.

Figure 2-CoD performed with sidestep cut technique (left) and with crossover cut technique (right).

This motor task involves an increasing muscle activity and an overloading of ligaments that stabilize the joint; there is a large risk of incurring injuries, like ankle sprains and ACL ruptures. It has been demonstrated that most non-contact injuries during CoDs occur in the initial phase of the contact and in the phase of weight acceptance (Brown, Brughelli, and Hume 2014).

An increase in the subject's height, in the Body Mass Index (BMI) or in the percentage of fat mass lead to a greater risk of non-contact injuries (Alentorn-Geli et al., 2009; Chaouachi et al., 2012; Kobayashi et al., 2016). The probability of ACL injury is increased by a reduction in flexion (Alentorn-Geli et al. 2009) and by an excessive rotation and abduction movements of the knee (Besier et al., 2003; David et al, 2007), which make the joint unstable and potentially vulnerable.

The authors attributed these results to the small amount of time to make appropriate postural adjustments before performance of the task such as the position of the foot on the ground relative to the body centre of mass (Besier et al., 2001).

1.2.3. Anatomy of the knee

The knee articular district is composed of a total of 3 different joints: Proximal Tibiofibular Joint, Femororotulea joint, Femorotibial joint. The main characteristics of these anatomical parts and their main functions in the stability of the knee are described below.

Proximal Tibiofibular Joint

It is classified as arthrodia, in which the proximal diaphyses of fibula and tibia are in contact. Therefore, it is characterized by scarce mobility and it is defined as a "compensation structure", acting like shock absorber. This function is very useful, especially when the anterior articular surface of the astragalus, during massive dorsal flexion of the foot, penetrates the anterior tibial-peroneal articular surface, widening it. This kind of dorsal flexion easily occurs during a CoD, when the passive stabilization of this joint is guaranteed by the ligaments of the head of the fibula.

Femororotulea joint

This angular ginglymus articulates the posterior face of the patella with patellar face of the femur (femoral trochlea). This joint contributes to the movement of flexion-extension of the knee.

Femorotibial joint

It is a double condyl-arthrosis, that functionally behaves like an angular ginglymus. Therefore, it guarantees knee movements in two different planes: flexion-extension (frontal plane) and rotation with flexed knee (transversal plane). The articular surface is composed of the convex faces of the femoral condyles (medial and lateral) and from the glenoid cavities of the tibial condyles (medial and lateral).

In Table 2 are reported the ranges of motion (RoM) of the knee.

The articular surfaces of the knee joint are covered with hyaline cartilage and, between them, there are two meniscuses interposed; they are opposed C-shaped connective structures, consisting of fibrocartilage, fixed to the joint capsule through the meniscofemoral ligaments. The function of these soft tissues is to amortize the load generated between the joint surfaces, by increasing the area of contact, with consequent distribution of the force on a larger surface and a pressure reduction.

Movement	Joint	Range of Motion (°)
Flexion	Femorotibial	140
	Femororotulea	
Extension	Femorotibial	0
	Femororotulea	
Internal rotation	Femorotibial	20-30
External rotation	Femorotibial	40-50

Table 2-Range of Motion (ROM) of the knee.

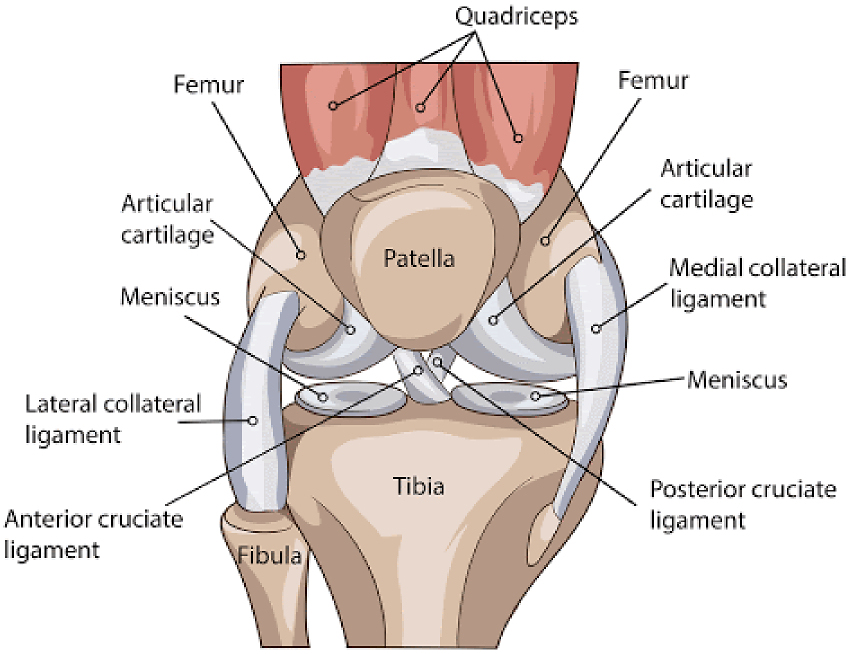


Figure 3-Anatomy of the knee.

The main ligaments that stabilize the knee joint are (Figure 3):

- *Patellar ligament*, that joins the inferior apex of the patella and the tibial tuberosity. It has the function of fixing the patella in its seat.
- *Collateral ligaments (medial and lateral),* that have lateral insertion on the lateral epicondyle of the femur and on the head of the fibula. Their function is to stabilize the knee joint on the frontal plane, limiting its extension and rotation. The rotation, in fact, is only admitted when the knee is flexed,

because when the joint is extended the collateral ligaments are in maximum tension, preventing lateral movements.

- *Anterior Cruciate Ligament (ACL),* it fits on the internal surface of the lateral condyle of the femur and on the anterior intercondylar area of the tibia.
- *Posterior Cruciate Ligament (PCL),* has insertion on the internal surface of the medial femoral condyle and on the posterior intercondylar area of the tibia.

In particular, the cruciate ligaments, during the flexion-extension movements, contribute to guide the slip of the femoral condyles on the tibial surface. For this reason, both PCL and ACL have an intracapsular course, but they insert outside the joint capsule.

Function of ACL

Thanks to its functional properties and complex anatomy, the main function of the ACL is to improve the passive stability of the knee joint in all three planes (Markolf et al. 1995), especially during dynamic activities. ACL limits the anterior translation of the tibia in the sagittal plane (Fleming et al. 2001; Markolf et al. 1995), the rotation of the knee in the transversal plane (Dürselen et al., 1995; Fleming et al., 2001; Markolf et al., 1995) and the abduction or adduction movement in the frontal plane (Markolf et al. 1995).

Performing tensile testing, Woo et al. (1991, 1999) reported the ultimate failure load of the native femur-ACL-tibia complex in younger cadaveric specimens (2160±157 N), and the mean ACL stiffness (242±28 N/mm); these values decrease with age and with the axis of loading. The forces applied on the ACL are usually below this threshold; in a normal gait cycle the ligament is subjected to a force of 400 N, which can reach a value of 1700 N during acceleration and deceleration in straight run (Brotzman 2007). The load easily exceeds the ACL breaking point during unusual combinations of stress for the knee joint, that occur in high intensity sport activities.

1.2.4. Involved muscle

In order to allow the performance of important tasks, such as walking, running, kicking and jumping, there are groups of muscles that flex, extent and stabilize the knee joint. The muscles include the quadriceps, hamstrings (Figure 4) and the muscles of the lower leg part (Figure 5).

The muscles of quadriceps femoris group (vastus lateralis, vastus medialis, vastus intermedius, and rectus femoris) are the extensors of the knee joint. These large muscles originate in the ilium and femur and insert on the tibia.

The hamstring muscles (biceps femoris, semitendinosus and semimembranosus) are the flexors of the knee. They extend across the posterior surface of the thigh from the ischium of the pelvis to the tibia of the lower leg. These muscles provide the dynamic knee joint stability.

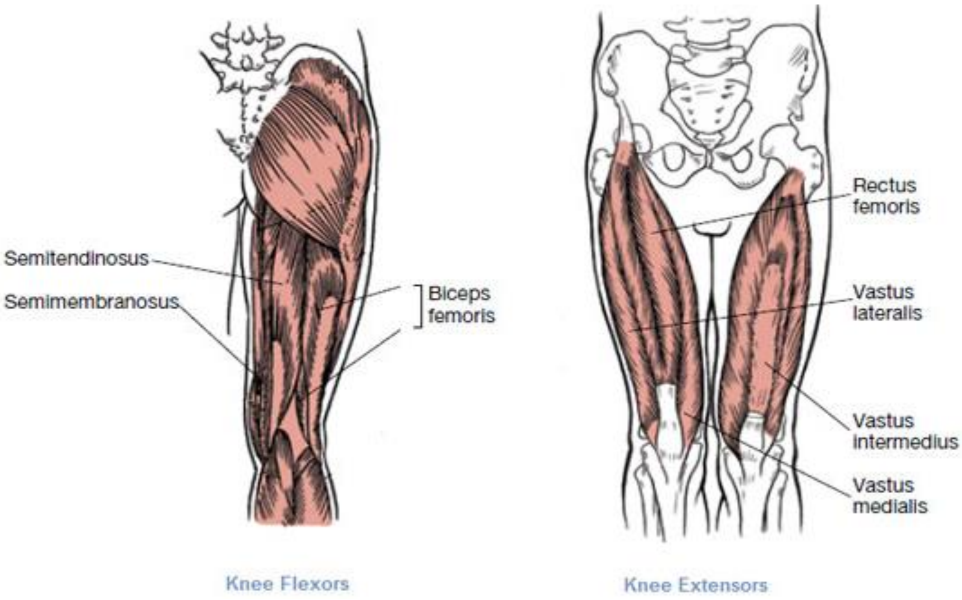


Figure 4-Flexor and extensor muscles of the knee.

In the lower leg region, there are the soleus and the gastrocnemius, that form the posterior muscular wall of the knee. They act as flexors of the knee and plantar flexors of the foot. The gastrocnemius is a biarticular muscle, that extends from the distal end of the femur and, through the calcaneal (Achilles) tendon, inserts into the calcaneus of the heel. The soleus is a monoarticular muscle, that together with the gastrocnemius form the triceps surae.

Some other muscles help to stabilize femur, tibia, and thigh muscles during the flexion of the knee, which requires some slight rotation of the tibia, provided by the contraction of the popliteus muscle.

Figure 5-Muscles of the lower leg region.

In the sagittal plane, the resulting anterior forward pull on the ACL decreases with increased knee flexion (Fleming et al. 2001; Markolf et al. 1995), while active quadriceps contraction is a major contributor to this anterior shear force (Draganich & Vahey, 1990; Dürselen et al., 1995; More et al., 1993; Renström et al., 1986). Due to the angular attachment of the patella ligament to the tibia, the contribution of quadriceps is more pronounced at more extended knee joint angles, between 0°–35° of flexion (Beynnon et al. 1995; Dürselen, Claes, and Kiefer 1995; Fleming et al. 2001); hence, this promotes an increased risk of ACL injury during forceful landing or sidecutting.

Co-activated muscles (Figure 6) help to protect the knee during dynamic tasks (Beynnon & Fleming, 1998; Li et al., 1999; Withrow et al., 2006, 2008). Antagonist–agonist relationships are crucial: anatomical and computer modelling studies have demonstrated that the hamstrings play an important role as ACL synergists; the hamstring recruitment reduces ACL loads from quadriceps and provide knee resistance to anterior tibial translation (Draganich & Vahey, 1990; Li et al., 1999; MacWilliams et al., 1999; Pandy & Shelburne, 1997; Renström et al., 1986).

Colby et al. (2000) reported that in young women, during sidecutting manoeuvres, hamstring muscles activation is submaximal during and after initial contact. A low level of hamstring activity and low angle of knee flexion at foot strike, coupled with unopposed forces of the quadriceps, could produce significant anterior displacement of the tibia (Chappell et al., 2007). One potential explanation could be that reduced magnitude of the hamstrings-quadriceps co-contraction would favour a more explosive jumping movement.

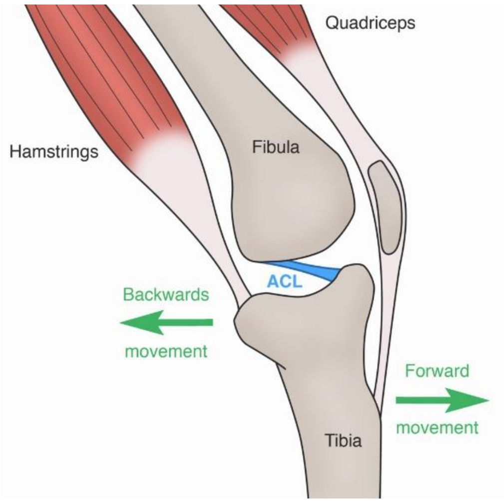


Figure 6-Hamstring and quadriceps contribution on ACL.

1.3. What is fatigue?

Fatigue can be defined as the decrease in the pre-match/baseline psychological and physiological function of the athlete (Place et al. 2008). A complex relationship between psychological and physical factors may increase the risk of injury, since they determine the way in which the player moves. For example, emotional stress can affect perceptual abilities, such as vision and reaction time (Andersen & Williams, 1997, 1999) and decreased alertness can reduce attention and decision making.

Due to fatigue, the athlete may be unable to respond fast enough to the somatosensory information and to the biomechanical demands of a rapidly changing physical environment (Swanik 2015). External physical loads can be perceived differently by each individual athlete and it is a poor predictor of fatigue (Windt and Gabbett 2017).

Despite a significant amount of research, in fact, little is known about the impact of fatigue on human performance and injury risk (Enoka and Duchateau 2016); this parameter is virtually unmeasurable, mainly because of the fragmentary interpretations of fatigue.

1.3.1. Task specificity

Fatigue is a transient exercise-induced reduction in the ability to produce force or power, whether or not the task can be sustained (Enoka and Duchateau 2008). A critical feature of this definition is the distinction between muscle fatigue and the ability to continue the task. Accordingly, muscle fatigue is not the point of task failure or the moment when the muscles become exhausted. Fatigue corresponds in a decline in performance when a task involves the sustenance of a maximal contraction. However, in most sporting activities tasks involve submaximal contractions and the onset of fatigue may not limit the ability to perform the task.

It is common to distinguish between central fatigue and peripheral fatigue (Gandevia 1992, 2001). In the first case, there is an exercise-induced reduction in the level of voluntary muscle activation, connected to both spinal and supraspinal factors (e.g., inhibition of spinal excitability). By contrast, peripheral fatigue leads to a reduction in the force-generating capacity of the muscle; it occurs at the level of neuromuscular junctions (McLean and Samorezov 2009) and is due to a reduction in the release of calcium ions from the sarcoplasmic reticulum (Cheng et al., 2018).

1.3.2. Muscle fatigue

Muscle fatigue can refer to a motor deficit or to a decline in mental function; it describes the gradual decrease in the force capacity of muscles or the end of a sustained activity. This kind of fatigue can be measured as a reduction in muscle force, as a change in electromyographic activity or as an exhaustion of contractile function in isometric conditions.

Muscle fatigue can be caused by many different mechanisms, ranging from the accumulation of metabolites within muscle fibres, to the generation of an inadequate motor command in the motor cortex (Enoka & Duchateau, 2008). There is not a global mechanism responsible for muscle fatigue, but it exists specificity about the task being performed, the age, the sex and the activation of the nervous system. For example, Baudry et al. (2007) found that old adults are more fatigable than young adults.

Usually, women are able to sustain a contraction for a longer duration than men, especially at lower contraction intensities (Clark et al., 2005; Hicks et al., 2001; Hunter et al., 2006), but not for maximal contractions (Baudry et al. 2007); the most common explanation for this sex difference is the greater muscle mass activated by men and a lesser reliance on glycolytic metabolism by women. Finally, the specificity of the impairments, during fatiguing contractions, also concerns an inadequate reduction in the activation of muscles by the nervous system, with a few electrical stimuli delivered to the motor nerve (Merton 1954).

In normal conditions, during landing, the more joints are flexed, the more the energy is absorbed and the less the impact is transferred to the knee. Fatigue produces a significant reduction of EMG amplitudes (Melnyk et al., 2007) and causes less energy absorption by the muscles (Mair et al., 1996). Consequently, energy is absorbed by other structures, such as ligaments. McLean et al. (2007) concluded that fatigue-induced modifications in the control of lower limbs may increase the risk of non-contact ACL injury.

Nyland et al. (1997) demonstrated that, during crossover cutting, in female athlete, quadriceps and hamstring eccentric work-induced fatigue produces earlier gastrocnemius and soleus activation, but a delay in the activity of femoral muscles. The authors concluded that posterior lower leg musculature acts as synergistic and compensatory dynamic knee stabilizer during the stressful functional pivot shift phase of the crossover cut.

1.3.3. Impact of fatigue on decision making

Fatigue is a disabling symptom, modulated not only by alterations in central and peripheral mechanisms, but also by subjective psychological factors, which influence the ability of an athlete to react to external stimuli.

Unanticipated single-leg tasks are functionally demanding and, thus, high-risk movements; one leg must adapt to the deceleration of the centre of mass (CoM) over a short time period (Orishimo and Kremenic 2006). The impact of fatigue on decision making may present the worst scenario for high-risk dynamic landing strategies in terms of load on the knee (Besier et al., 2001; Borotikar et al., 2008; McLean & Samorezov, 2009). Especially in sidecutting manoeuvres (Herman & Barth, 2016; Swanik et al., 2007), there is a perpetuating substantial degradation and overload of central control mechanisms. Additionally, Olsen et al., (2004) reported that ACL injuries occurred when team handball players were out of balance, or some form of perturbation (such as the contact with another player) altered the player's coordination. Thus, it appears that fatigue may contribute to other risk factors, but it is not an isolated risk element for ACL injury.

Therefore, there is the need to include training of cognitive processing speed (e.g., reaction time), as this appears to be a modifiable characteristic in athletes (Wilkerson et al., 2017).

1.3.4. Effect of fatigue on the biomechanics of the changes of direction

Neuromuscular fatigue leads to transient reductions in muscle strength and deleterious changes in lower limb kinematics and kinetics during potentially hazardous tasks, such as cutting or landing. Despite this, recent analyses demonstrate no relationship between player workload in training or competition and the occurrence of ACL injury (Barber-Westin and Noyes 2017; Benjaminse et al. 2019).

The most common ‘measure’ of fatigue in sport is the incremental workload related to playing time (Benjaminse et al. 2019). It is generally agreed that athletes who spend more time training or competing experience greater workloads and more fatigue, than those who spend less time in these activities.

By this logic, injuries would occur at a greater frequency later in the game or season. However, a recent meta-analysis (Doyle et al., 2018) reported no difference in this injury occurrence during the first or second halves of games or of a season.

Previous studies

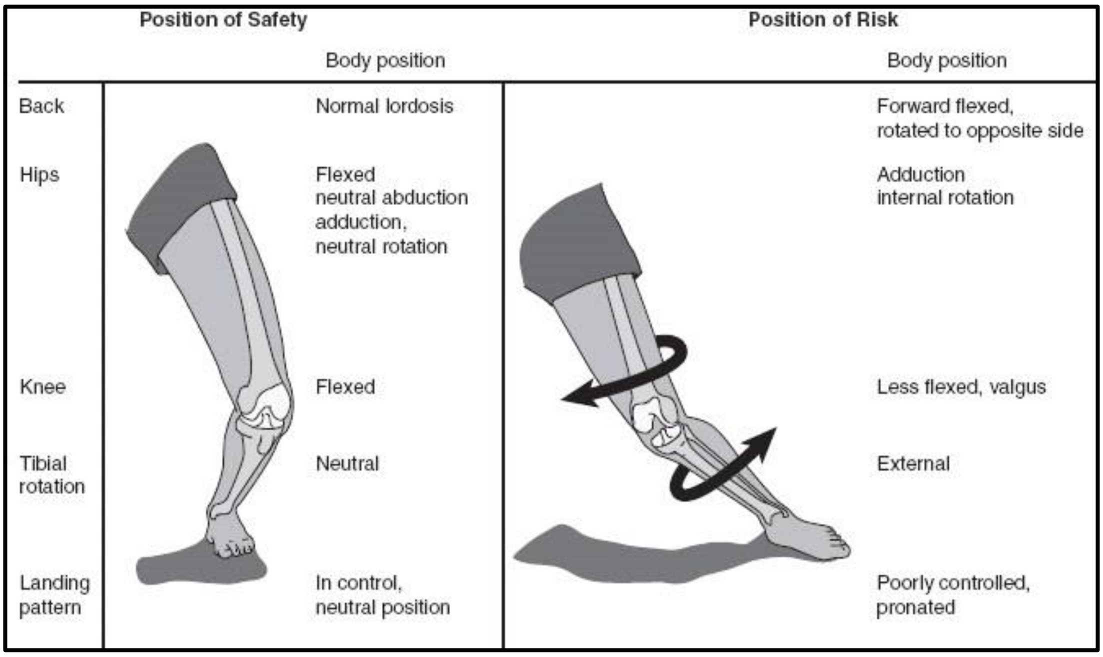
A number of studies have examined the acute effects that fatigue has on lower limb kinematics and kinetics (Barber-Westin and Noyes 2017); three-dimensional motion analysis and force platform systems assessed male and female athletes, landing from a jump or performing a cutting manoeuvre.

Consistent heterogeneity exists in how different authors have stimulated and measured the parameter of fatigue; some applied peripheral fatigue protocols on specific lower limb muscles, whereas others employed more general fatigue protocols (Barber-Westin and Noyes 2017; Benjaminse et al. 2019). All the authors agree to consider that the capacity to tolerate a given workload can vary significantly among athletes and that it is difficult to determine when participants are actually fatigued.

The main finding of these studies was that fatigue had no significant impact on most of the kinetic and kinematic variables. However, weakness did induce a change in movement in the sagittal plane, especially in the initial contact, when the landing strategy becomes stiffer.

Researchers have shown that movement mechanics change unfavourably during unanticipated execution of a task compared with anticipated ones (Besier et al., 2001; Collins et al., 2016; McLean & Samorezov, 2009; Weinhandl et al., 2013). Potentially, the firsts are more similar to a real game, in which the environment constantly changes and the athlete has only few milliseconds to act a strategy. In these situations, the athlete easily reaches a position of risk (Figure 7).

Indeed, evidence suggests that excessive dynamic knee valgus (Hewett et al., 2005; Kristianslund et al., 2014), poor trunk control (i.e. lateral flexion) (Donnelly et al., 2012; Hewett et al., 2005; Kristianslund et al., 2014), excessive dorsiflexion (Donnelly et al., 2012), and higher ground reaction forces (Hewett et al., 2005) may all elevate the risk for ACL tears. This theoretical framework may be intuitive, but it is equally defensible to suggest that fatigued athletes would, actually, move more slowly and produce less ground reaction forces than non-fatigued athletes. Such a strategy would be expected to reduce knee joint torques, to confer a protective effect. Up to now, there is little consensus on which argument is correct.



	Position of Safety	Position of Risk
	Body position	Body position
Back	Normal lordosis	Forward flexed, rotated to opposite side
Hips	Flexed neutral abduction adduction, neutral rotation	Adduction internal rotation
Knee	Flexed	Less flexed, valgus
Tibial rotation	Neutral	External
Landing pattern	In control, neutral position	Poorly controlled, pronated

Figure 7-Risky biomechanics of the CoD.

Several investigations have attempted to map the surface EMG (sEMG) activity of lower limb muscles, before and after fatigue (Orishimo & Kremenic, 2006; Pappas et al., 2007; Zebis et al., 2009). Even though sEMG is not a measure of fatigue, it is hypothesised that central fatigue leads to a reduction in voluntary drive (Taylor et al., 2006), reflected by a decrease in the sEMG amplitude. After the application of fatigue protocols during dynamic knee loading, reduced hamstring relative to quadriceps activation may be associated to lower knee flexion moments, greater knee valgus moments and increased ground reaction forces (Laughlin et al., 2011; Leppänen et al., 2017); this pattern of muscle activation is related to an increased risk of future ACL injury (Mette K. Zebis et al. 2009).

These results should be interpreted with caution, because of some limitations. First of all, the sEMG amplitude is influenced by the degree to which motor unit firing is synchronous (Yao, et al., 2000) and that increases during fatigue. Another limitation is the susceptibility to cross-talk from neighbouring muscles, which makes a reliable discrimination between closely muscles very difficult (Bourne et al. 2018). Unsurprisingly, sEMG studies have generated inconsistent results (Barber-Westin and Noyes 2017). Zebis and collegues (2011) reported a reduction in hamstring sEMG amplitude, following a simulated soccer match; Pappas et al., (2007) reported no change in this parameter following a fatiguing jumping protocol.

Even though fatigue protocols are based on the reach of a maximal fatigue level, Borotikar et al. (2008) showed that biomechanical adaptations (e.g., increase in initial contact hip extension angle and peak knee abduction angle) are seen already at the 50% level of fatigue.

In conclusion, healthy athletes deal well with induced fatigue. An explanation could be that the testing protocols used in the laboratory are too ‘simple’; thus, it is possible to counteract the effect of fatigue as the athlete can exclusively focus on task execution, with no other environmental distractions.

1.4. Electromyography

The term electromyography refers to a functional diagnostic technique, that records the electrical signal of a muscle as a voltage difference (Bracale et al., 2002), with the aim of evaluating the functioning of the muscle, during its contraction activity (electrical biopotential). The EMG signal is extremely complex and depends on several factors, such as the anatomical and physiological properties of the muscles, the state of the peripheral nervous system, the thickness of the subcutaneous fatty panel and the characteristics of the interface with the device. In the last 25 years, collection, recording and processing techniques of this kind of signal have considerably developed in diagnostic, therapeutic and rehabilitation areas, but also in sports (about biomechanics, movement analysis, training, muscle performance, fatigue, etc.).

1.4.1. History of electromyography

The first signs of a relationship between electrical activity and muscle contraction were found in the works of Redi, in 1666, but only two centuries later, in 1844, Matteucci gave explanation to this phenomenon. Even though the first recording of an electromyographic signal was in 1849 (by the Frenchman Emil du Bois-Raymond), the term electromyography was, actually, introduced with Marey, in 1890. The first effective analyses of the electromyographic signal date back to 1912, when Piper, a professor of physiology at the University of Berlin, noticed a decrease in surface muscle signals during static contractions. For the first time, in 1922, Gasser and Erianger (who were awarded the Nobel prize for their interpretations of muscles electrical activity, in 1944), were able to view the EMG signal on an oscillographic screen, thanks to the use of a cathode ray tube. The following year, Cobb and Forbes observed that the increase in signal amplitude was a parameter of interest, due to muscle fatigue during static contractions.

The most significant signal detection improvements were between the 1930s and 1950s, thanks to the developments in electronics (Cifrek et al., 2009); increasingly reliable tools allowed neurologists, kinesiologists and orthopedists to make a wider and more frequent use of electromyography. In the 1960s, there are the first clinical use of surface electromyography for the treatment of pathologies, thanks to electrically stable and low-noisy needles and skin electrodes (of silver or silver chloride). For example, the study on the morphology of the so called "motor unit action potentials" was developed for a diagnosis of neuromuscular pathology, while other studies are related to walking and to global muscle activity, in general.

1.4.2. Electromyographic techniques

The electromyographic analysis can be divided into needle electromyography (also called electroneurography, ENG) or surface electromyography (sEMG).

In ENG, a subcutaneous needle is placed in direct contact with the muscle of interest, recording electrical intramuscular activity; with this technique, used especially in the diagnostic field, it is possible to analyse only a single motor unit. On the contrary, sEMG highlights a group of motor units (nerves) and their conduction speed; the signal is collected through surface electrodes, easily applied with double-sided adhesive pads to the skin and placed with a precise arrangement in correspondence of the muscles of interest. These electrodes have poor selectivity and poor precision, especially when the muscle is small or very deep; moreover, in the use of these electrodes, a very frequent problem is the so called “cross-talk”, the disturbance created by the activity of other near muscles (Winter 2009).

1.4.3. sEMG and fatigue

The use of the sEMG had a considerable diffusion in the last thirty years, especially in the sports field, to quantify the timing of muscle activation during physical activities and to evaluate fatigue, in a non-invasive way. This measurement can provide quantitative and qualitative information on muscles coordination and coactivation, as well as on their contribution to the development of strength, precision and joint stability, for a given movement. According to Cifrek et al., (2009), the most representative parameters of fatigue are the increasing amplitude of the signal during the fatigue process and the displacement of the median frequency towards lower frequencies (Figure 8).

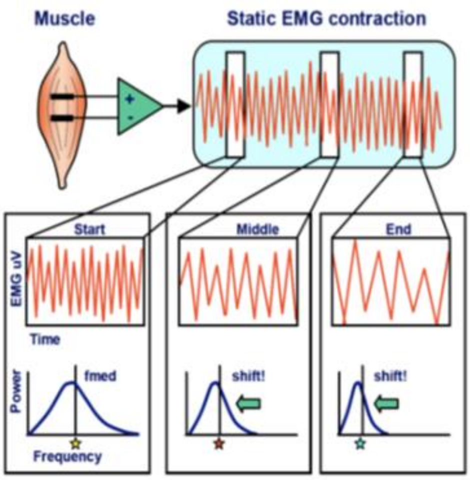


Figure 8-Shift of the median frequency of the EMG signal towards low frequencies as the subject's fatigue increases during a static contraction

1.5. ACL injuries in women soccer players

Female athletes appear to be at a particular high risk of sustaining non-contact ACL injury (Lind et al., 2009; Renstrom et al., 2008). Across sports, the overall-incidence of a first-time non-contact ACL rupture in female high school and college athletes have been reported to be as high as 0.112 per 1000 athlete exposures compared with 0.063 per 1000 athlete exposures in males (Beynnon et al. 2014); ACL injury incidence rates for women seems to peak during adolescence (age 14–18 years) with 227.6 (per 100,000 person years) compared to 113.2 (per 100,000 person years) for the following age group (age 19–25 years) (Sanders et al. 2016).

In order to design effective injury prevention programs, influential risk factors must be identified (van Mechelen et al., 1992); among them, anatomical and biomechanical factors, such as external loadings, neuromuscular control and specific muscle activation patterns. There are gender-specific differences in muscle activation and coordination in sidecutting manoeuvres; in young female, in fact, there is an elevated quadriceps activity (vastus lateralis) and a reduced medial hamstring activity, prior to landing (Bencke et al., 2018). For this reason, these athletes are more subjected to ACL non-contact injury, compared to the male counterpart, at a rate between two and seven times greater (De Loës et al., 2000).

To prevent this injury, it is important to optimize prophylactic training regimes targeting this specific age- and sex- group. To design prevention programs, we need to know the internal anatomy of the knee joint, the movements in all three planes and which muscles, as antagonists or synergists, are activated to support the mechanical load (van Mechelen et al., 1992). Three-dimensional kinematic and kinetic analyses (Chappell et al., 2002; Decker et al., 2003; Ford & Hewett, 2003; Lephart et al., 2002; Malinzak et al., 2001; Lin et al., 2012; Sinclair et al., 2012) have shown that, during deceleration or landing manoeuvres, females tend to exhibit:

- reduced hip, knee and ankle flexion angles;
- enhanced knee valgus angles;
- larger ground reaction forces (GRF);
- greater tibia anterior shear forces;
- larger knee extension and valgus moments;
- greater hip internal rotation;
- hip adduction and knee rotation.

Only few studies, adding electromyography, have investigated how specific patterns of muscle activation may influence the injury risk. Even less is known about the effect of specific training intervention on adapting muscle activation patterns.

1.5.1. H/Q ratio

Electromyography analyses have shown that females exhibit quadriceps dominance during the landing phase of a sidecutting manoeuvre and they take longer to generate maximum hamstring torque than their male counterparts (Nagano et al., 2011). This has been confirmed through the hamstring/quadriceps ratio (H/Q ratio), whose value is lower for female athletes compared to males, especially in the pubertal age (Ahmad et al. 2006). Women develop less strength, with a more favourable development in the quadriceps compared to the hamstrings.

1.5.2. Soleus/gastrocnemius ratio

In their study, Mokhtarzadeh. et al. (2013) quantified also the soleus and the gastrocnemius forces, whose kinetics are typically ignored. In fact, since the lines of action of these muscles are close to the long axis of the tibia, they are supposed to have a limited influence on the ligaments of the knee. Previous studies, instead, underlined how the soleus protects the ACL during landing manoeuvres by exerting a posterior force on the tibia and that gastrocnemius act as an ACL antagonist. The soleus/gastrocnemius ratio is statistically larger in male athletes, indicating a more favourable ratio in terms of protection from ACL injuries during high-intensity athletic movements.

1.5.3. Risk factors for ACL injuries in women

Risk factors influencing the onset of non-contact ACL injuries can be extrinsic (outside the body) or intrinsic (within the body) factors. According to the guidelines established by the Hunt Valley meeting (Griffin et al. 2006), they are divided into environmental, anatomical, hormonal, neuromuscular and biomechanical factors.

Environmental factors

For example, they include weather conditions, the playing surface and the friction coefficient. A dry surface raises friction and torsional resistance, improving the performance, but also increasing the risk for ACL injuries (Douglas & Martin, 1975; Heidt et al., 1996);

Anatomical factors

Among them, there are high BMI, joint laxity, Q-angle, ACL size and strength, pelvis and trunk positions. In this case, preventive mechanisms are limited, since anatomy is difficult to modify. For example, in female, higher values of the Q-angle (Figure 9) place the knee at a higher risk to static and dynamic valgus stresses (Buchanan et al., 2003) and determine a greater pelvic width to femoral length ratios.

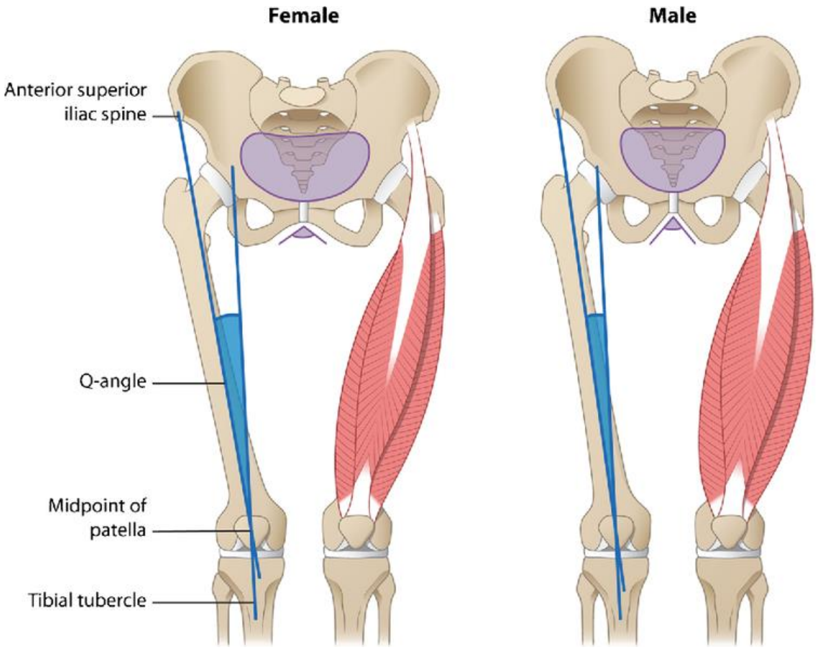


Figure 9-Q-angle different amplitude in women (left) and men (right).

Hormonal factors

It was demonstrated that the use of the oral contraceptive decreases the laxity of the ligament (Martineau et al., 2004). Moreover, there is a greater predisposition to the ACL injury during the pre-ovulatory phase (Hewett et al., 2007), concerning the phase of the menstrual.

Neuromuscular factors

These aspects concern the unconscious activation of muscles and dynamic restraints surrounding a joint, in response to sensory stimuli. Some forms of perturbation alter the coordination or the intended movement at the time of injury (Olsen et al., 2004); it is the case of the contact with another player, in which the neuromuscular system determines the biomechanics of playing actions. Unconscious muscle activation is crucial during many actions in sport; differences in neuromuscular control may explain the increased ACL injury risk shown by female soccer players. This cohort of subjects perform an increased adduction and internal rotation of the femur, reduced hip and knee flexion angles, increased dynamic knee valgus and increased quadriceps activity (Olsen et al. 2004).

ACL is an important site of proprioceptive feed-back in the control of muscles around the knee joint. We can define the hamstrings as receptor-dependent muscles, activated by ACL receptors, when the ligament is placed under stress. This behaviour suggests that decreased proprioception could have an impact on knee stability, justifying the inclusion of proprioception training in preventive and rehabilitation programs (Solomonow et al. 1987).

Biomechanical factors

Biomechanics of playing actions is necessary to understand the pathomechanics of ACL injuries and offer effective prevention programs.

In the sagittal plane, a decrease in knee flexion produces an increase in the angle of insertion of the hamstrings (ACL elevation angle); changes in its orientation influence the load on the ligament and, consequently, its ability to sustain elastic deformation without injury (Blackburn and Padua 2008). The structural properties of the ACL are maximized under tensile (longitudinal) loading conditions and minimized under non-axial (shear) loading conditions (Woo et al., 1991).

Yu et al., (2006) found that female athletes exhibit an increased hamstring activation before landing but a trend of decreased hamstring activation after landing; this excessive quadriceps activity may increase ACL loading, since muscle activation is a major determinant of muscle contraction force (Kaufman et al., 1991).

1.6. Objectives of this study

Female athletes have a risk of non-contact ACL injury from 6 to 8 times higher than men (Arendt & Dick, 1995), mainly because of morphological, anatomical and neuromuscular differences (Wilk et al., 1999).

In women the volume of the femoral head, in which the ACL is inserted, is significantly lower than that of men; this volume is related to the size of the ligament and to the differences in weight and height. These characteristics determine the weakness of the ligament for female athletes who, therefore, have a much higher probability of rupture (Charlton et al., 2002), due to this intrinsic ligamentous laxity.

The reference study (Zago et al. 2019) was conducted on a sample of male subjects who underwent a shuttle test on the distance of 5 meters, with CoD at 180 °; the technique used was the lateral cutting, performed at high intensity level. This analysis confirmed the effect of fatigue on the biomechanics of the joints: knee flexion is significantly reduced at the end of the exercise and it is inversely proportional to different physiological parameters; moreover, the times of foot-ground contact decrease significantly during the changes of direction, although not directly influenced by physiological variables.

The hypothesis behind our investigation is to verify if, in young female athletes, the onset of fatigue causes a change in the repetition of the motor gestures and in the pattern of muscle activation in high stress conditions; moreover, it is important to understand if fatigue can determine a reduction in the frequency content of the electromyographic signal and if the onset time of muscle activation changes during the test. What we expect from our study is that fatigued muscles absorb less energy before failure than non-fatigued ones (Mair et al. 1996) and that a fatigued status leads to reductions in muscle strength and, consequently, to deleterious alterations in joints kinematics and kinetics. All these things are supposed to increase strain on the ACL during potentially hazardous movements, such as cutting or landing (Benjaminse et al., 2008; Kernozek et al., 2008; Wojtys et al., 2016).

This study is meant to be a support for athletic trainers: it is important to arise the awareness in preventive programs and to make objective decisions on the resumption of post-injury sports activity; the creation of an athlete biomechanical profile would be helpful in ACL neuromuscular prevention training programs.

1.6.1. Limitations

Different fatigue protocols do not produce uniform alterations in lower limb biomechanical factors, that heighten the risk of non-contact ACL injuries. In addition, women exhibit different fatigue levels than men, also in relation to the specific task performed and to the muscle groups involved (Hunter 2009).

Some studies do not include a direct measure of muscle fatigue to indicate when the protocol should end; it is assumed that a fatigued state is reached especially with protocols based on repetitions of certain tasks (Cortes et al., 2014; Pappas et al., 2007; Sanna & O'Connor, 2008; Yamada et al., 2012; Beynnon et al., 2005; McLean & Samorezov, 2009). However, using time alone as an indicator of fatigue is problematic, because athletes have intrinsic sets of parameters that involve complex central and metabolic factors. The introduction of a uniform specific fatigue indicator (e.g., such as direct measurements of the heart rate and muscle power) would be more effective.

The large variation in findings indicates the need for continued research in this area and the refinement of fatigue protocols and of methods of analysis. Improved future studies may better determine which variables are the most relevant to the non-contact ACL injury.

Given the complex nature of fatigue (Taylor, Todd, and Gandevia 2006) and the methodological concerns associated with sEMG (Bourne et al. 2018; Yao, Fuglevand, and Enoka 2000), researchers should avoid making conclusions about the fatigability of muscles based on this measure alone.

Future works (Bourne et al. 2018; Yao, Fuglevand, and Enoka 2000), employing direct measures of voluntary activation (i.e. twitch interpolation), are needed to understand the impact of fatigue on the magnitude of lower limb muscle activity. Assessment techniques with high spatial resolution, such as functional magnetic resonance imaging (Bourne et al. 2018), may also be used to characterise the effects of fatigue on the 'patterns' of lower limb activation during high-risk activities.

2. MATERIALS AND METHODS

2.1. Study design and participants

This cross-sectional observational case series study involved 18 female subjects (20-31 years old, BMI 18.4 – 25.1 kg/m^2) on a voluntary basis. All the participants were young women, who played at high level of Italian Female Football League ("Serie A, B, C"). They were healthy and in good fitness conditions, in possession of a valid medical certificate and they did not experience any severe (> 28 days of absence from activity) or moderate (between 8 and 28 days) orthopaedic or muscle-tendon injury in the previous six months (Le Gall et al., 2006). Before taking part in the test, the football players signed an informed written consent, in which the aims of the study were described, together with the concerns about risks and benefits. All the details concerning anthropometric characteristics and physiological parameters of the participants are reported in Table 3.

In order to limit the variability factors in the calculation of physiological parameters, as precautions, the athletes were asked not to eat or drink anything different from water at least in the 2 hours before the test, as well as for the assumption of caffeine or nicotine. In the end, the last physical effort should have been more than 24 hours before the trial.

The research was conducted in two different periods: after the end of the 2018/2019 football season and during the first months of the 2019/2020 football season; participants practised at least three weekly training session and the match on Sunday (Le Gall et al., 2006). The project was approved by the Ethics Committee of the University of Milan (Nr. protocol: 1/2016) and it met the current ethical standards in sports and exercise research (Declaration of Helsinki, 1964).

The data acquisition phase was carried out in collaboration with Francesca Salaorni and Claudia Brunetti. In their works, they deal with the kinematics and the kinetics of the gesture, respectively, analysing how they change with fatigue.

Subject	Age	Role	Dominant Foot	Weight [kg]	High [cm]	BMI [kg/m²]	70% VAM [m/s]	Category
1	20	W	Left	47	156	19.3	2.5	C
2	22	S	Right	56	169	19.6	2.6	B
3	23	W	Left	56	167	20.0	2.7	B
4	21	GK	Right	60	163	22.6	2.3	B
5	31	M	Right	58	165	21.3	2.9	B
6	30	W	Right	47	154	19.8	2.6	C
7	20	M	Right	54	158	21.6	2.6	C
8	20	M	Right	55	160	21.4	2.6	B
9	23	W	Right	57	173	19.0	2.9	C
10	29	GK	Right	61	166	22.2	3.3	C
11	28	W	Left	52	156	21.3	3.6	C
12	26	W	Left	56	166	20.3	2.5	B
13	21	W	Right	58	160	22.7	2.5	C
14	21	GK	Right	64	168	22.7	2.3	B
15	27	W	Right	55	173	18.4	2.3	B
16	25	D	Left	77	175	25.1	2.6	B
17	27	D	Right	64	170	22.1	2.7	A
18	21	W	Left	53	160	22.7	2.6	A
Mean	24			57	164	21.2	2.7	
SD	4			7	6	1.7	0.3	

Table 3-Anthropometric characteristics and physiological parameters of each participant (BMI: Body Mass Index; VAM: Maximal Aerobic Speed; S: striker; M: midfielder; D: defender; W: wing; GK: goalkeeper).

2.1.1. Procedures

The trial consisted in a 5-metres shuttle run test; it was conducted at a speed calculated as 70% of the maximal aerobic speed (VAM) of the subject, as reported by Zago and collaborators (Zago et al. 2019). In order to analyse the kinematics of the subjects, 37 reflective markers were placed on specific anatomical landmarks of the athlete; thanks to an optoelectronic system, the positions of these markers were recorded, with a sampling frequency of 100 Hz. For the kinetic analysis, it was used a force platform system, to measure the ground reaction forces (GRFs) exchanged with the ground during the cutting manoeuvre. Finally, to evaluate the muscle activity during the movement, 8 electromyographic probes were positioned on the participant’s dominant leg, that is the one used to execute the CoD.

Marker set

Figure 10-Orthostatic position of the subject.

The software used to analyse all the data was Visual 3D (version 6.03.06, C-Motion, Inc. Germantown, MD, USA); this software supports almost any marker set, including 6 Degree of Freedom, Helen Hayes/Newington (e.g." Conventional Gait Model"), CODA, and many laboratory in-house systems. Tracking markers are placed on the segments in correspondence to relevant body landmarks that move minimally with skin movement. According to the anthropometric model (Figure 11), the markers were positioned in the following anatomical landmarks on the athlete (Figure 10): seventh cervical vertebra, bilaterally on acromion, olecranon, styloid process of radium, superior-anterior iliac spine, posterior iliac crest, lateral and medial femoral epicondyle, lateral and medial malleolus, calcaneus and foot (corresponding to the 1st and 5th metatarsus).

Furthermore, on the thigh and the tibia they were applied clusters of markers (Manal et al., 2000; McClay & Manal, 1999), made with 3D printing, where markers were located at a fixed distance; these rigid devices were necessary in the case some markers put on the leg were lost during the exercise, to allow a reconstruction of the model of the leg, during further analysis.

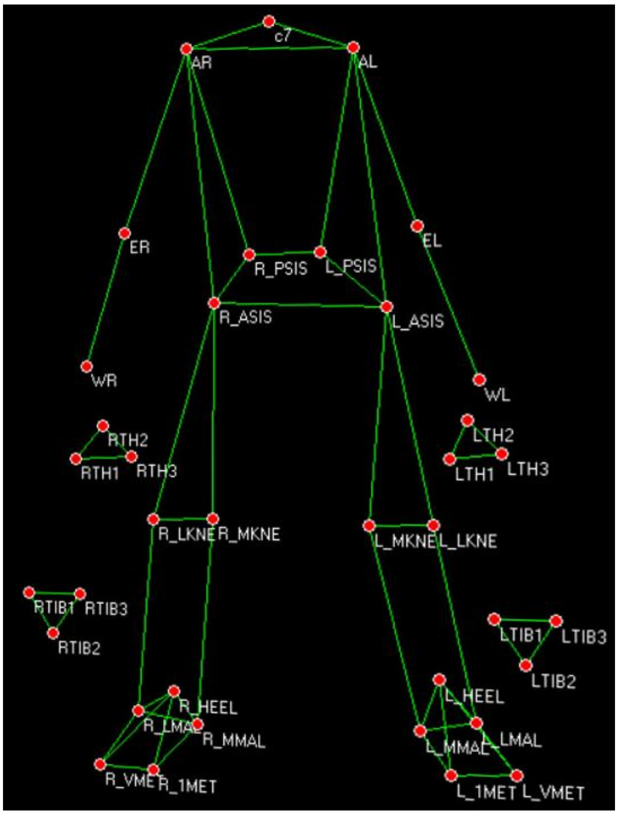


Figure 11-Anthropometric model.

All the correspondences between marker (or cluster) positions and anatomical landmarks are reported in Table 4.

Markers	**Anatomical landmark**
C7	Cervical vertebra
AR-AL	Acromion (right and left)
ER-EL	Radial epicondyle (right and left)
WR-WL	Styloid process of ulna (right and left)
R_ASIS-L_ASIS	Anterior-superior iliac spine (right and left)
R_PSIS-L_PSIS	Posterior-superior iliac spine (right and left)
R_LKNE-L_LKNE	Lateral femoral epicondyle (right and left)
R_MKNE-L_MKNE	Medial femoral epicondyle (right and left)
R_LMAL-L_LMAL	Lateral malleolus (right and left)
R_MMAL-L_MMAL	Medial malleolus (right and left)
R_HEEL-L_HEEL	Heel (right and left)
R_1MET-L_1MET	1st Metatarsus (right and left)
R_5MET-L_5MET	5th Metatarsus (right and left)
Clusters	
RTH1-RTH2-RTH3	Right thigh
LTH1-LTH2-LTH3	Left thigh
RTIB1-RTIB2-RTIB3	Right tibia
LTIB1-LTIB2-LTIB3	Left tibia

Table 4-Correspondence between marker and cluster positions and anatomical landmarks.

The anthropometric model was composed of rigid segments, which represented the skeletal structure of the athlete. Even though the assumption of bones as rigid segment is theoretically incorrect, it is commonly adopted in the analysis of movement; this hypothesis greatly simplifies the mathematics of the problem (Winter 2009). The relative position in time between two adjacent segments easily defines the joint kinematics.

EMG probes

In this study, on each athlete were positioned 8 sensors, to record simultaneously the activity of the lower limb muscles during the dynamic activity of the shuttle.

The muscles of interest are both extensors and flexors of the knee and ankle joints: rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), biceps femoris (BF), semitendinosus (ST), tibialis anterior (TA), gastrocnemius medialis (GM), gastrocnemius lateralis (GL).

As it is illustrated in Figure 12, all the participants, in a lying position, were asked to flex or extend the knee or ankle joint, in order to find the correct position and orientation of the probes; these positions corresponded to significant anatomical points. The details for this procedure, conducted according to the SENIAM guidelines (http://www.seniam.org/) are reported in Table 5. All the details on the used EMG system will be provided later.

Muscle	Type	Location of electrodes	Orientation of electrodes
Rectus femoris	Knee extensor	At 50% on the line from the anterior-superior iliac spine superior to the superior part of the patella.	In the same direction of the line from the anterior-superior iliac spine superior to the superior part of the patella.
Vastus medialis	Knee extensor	At 80% on the line between the anterior iliac spine superior and the joint space in front of the anterior border of the medial ligament.	Perpendicular to the line between the anterior iliac spine superior and the joint space in front of the anterior border of the medial ligament.
Vastus lateralis	Knee extensor	At 2/3 on the line from the anterior spina iliac superior to the lateral side of the patella.	In the direction of the muscle fibres.
Biceps femoris	Knee flexor	At 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia.	In the same direction of the line between the ischial tuberosity and the lateral epicondyle of the tibia.

Semitendinosus	Knee flexor	At 50% on the line between the ischial tuberosity and the medial epicondyle of the tibia.	In the same direction of the line between the ischial tuberosity and the medial epicondyle of the tibia.
Tibialis anterior	Ankle flexor	At 1/3 on the line between the tip of the fibula and the tip of the medial malleolus.	In the same direction of the line between the tip of the fibula and the tip of the medial malleolus.
Gastrocnemius medialis	Ankle extensor	On the most prominent bulge of the muscle.	In the direction of the leg.
Gastrocnemius lateralis	Ankle extensor	At 1/3 of the line between the head of the fibula and the heel.	In the same direction of the line between the head of the fibula and the heel.

Table 5-SENIAM guidelines for EMG probes placement.

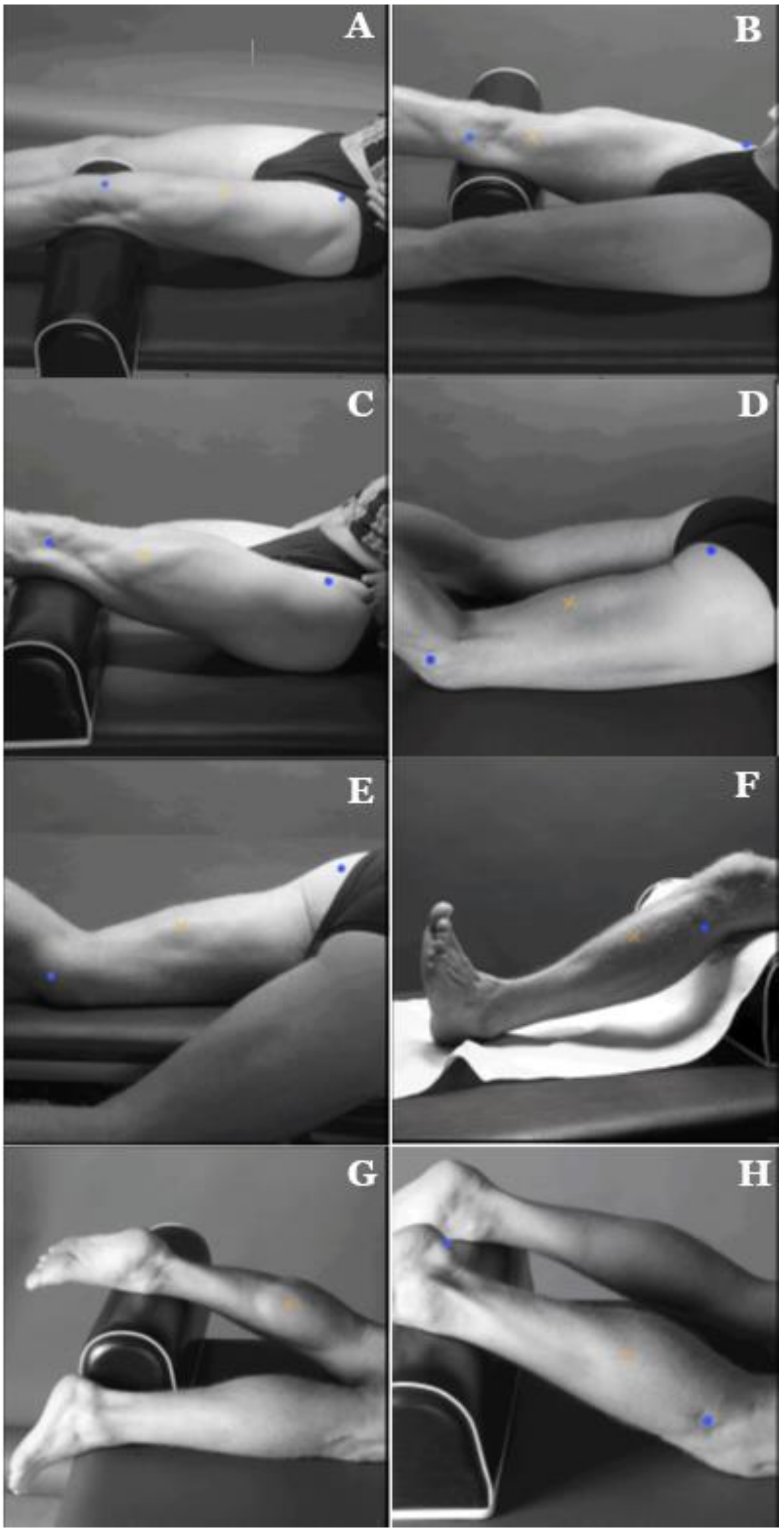


Figure 12-Positions of EMG probes on rectus femoris (A), vastus medialis (B), vastus lateralis (C), biceps femoris (D), semitendinosus (E), tibialis anterior (F), gastrocnemius medialis (G), gastrocnemius lateralis (H).

All these preparing procedures and the shuttle run test took place at the Laboratory "Luigi Divieti" of Politecnico di Milano. A mat of synthetic grass was positioned on the floor of the laboratory, in order to simulate an outdoor soccer field (Figure 13).

Figure 13-Experimental setting in the Laboratory "Luigi Divieti" of Politecnico di Milano.

The experimental protocol is made up of three parts, carried out on the same day:

1. Pre-test;
2. Shuttle run (fatigue) test;
3. Post-test.

Before starting the actual test, in the pre-test phase, the subject was submitted to a blood sample, taken from the ear lobe, in order to measure the lactate concentration ($[La^-]_b$) in rest conditions. This procedure was carried out by a medical personnel present during the surveys.

This phase was followed by a warm-up of about 5 minutes conducted by a professional physical trainer, during which each athlete, made light run and exercises, for joint mobility and stretching.

Figure 14-CoD at 180° during the shuttle run test.

The main trial of the test consisted of a run on the distance of 5 metres (Figure 14), with 180° CoDs, in which the racing speed was set at 70% of VAM (Zago et al. 2019). Each subject was guided by an acoustic signal provided by a metronome, in order to maintain the velocity and the frequency as much as possible consistent with this set. Heart rate and electromyography activity were monitored during the test, thanks to a heart rate monitor.

After the shuttle, participants were asked to express their perception of the effort (RPE), using the Borg 1-10 scale (Borg, 1982), given by the clinical staff. Furthermore, a blood sample was taken to measure how the concentration of lactate changed after 1, 3 and 5 minutes after the exercise. To monitor the status of fatigue, we considered the peak of blood lactate reached within 5 minutes after the end of the shuttle.

In parallel with the monitoring of physiological parameters, thanks to the optoelectronic system, it was acquired also the kinematics and kinetics of the subject.

All the parameters that characterized the physical trial of each participant are reported in Table 6.

Subject	Number of Cods	Time [s]	RPE	Basal $[La^-]_b$ [mmol/L]	$[La^-]_b$ peak [mmol/L]	HRpeak (bpm)	%HRmax
1	70	291	6	1.0	6.8	178	91.8%
2	29	125	6	-	11.8	179	92.9%
3	42	166	7	0.8	14.4	188	98.0%
4	70	305	7	1.0	6.7	184	95.2%
5	17	67	8	1.4	9.6	173	92.9%
6	29	117	6	1.2	10.3	184	98.4%
7	41	163	8	0.9	11.2	187	96.4%
8	30	129	9	1.5	15.3	173	89.2%
9	34	135	7	1.0	9.3	178	92.8%
10	38	165	7	0.8	11.9	177	94.3%
11	26	109	3	1.1	13.3	174	92.4%
12	28	122	5	1.0	11.2	173	91.1%
13	32	136	5	1.2	6.9	192	99.3%
14	55	244	4	1.0	-	182	94.2%
15	70	303	6	0.9	9.3	-	-
16	20	82	6	1.4	15.8	186	97.6%
17	21	85	7	0.8	9.9	185	97.8%
18	26	107	5	1.2	11.4	188	97.3%
Mean	38	158	6	1.1	11.1	181	94.8%
SD	17	76	1	0.2	2.6	6	2.9%

Table 6-Parameters of the shuttle test of each participant (RPE: Rate of Perceived Exertion scale, Basal $[La^-]_b$*: basal level of lactate,* $[La^-]b$ *peak: peak of lactate within 5 minutes after the test, HRpeak: peak of the heart rate calculated in the last 15 seconds of the shuttle, %HRmax: percentage of the maximum theoretical heart rate (theoretical HRmax= 208-(0.7*age)), calculated with the Tanaka formula* (Tanaka et al., 2001)*).*

2.2 Instrumentation

2.2.1. Stereophotogrammetric system

Figure 15-Camera of the motion analysis system, equipped with LED crown.

The stereophotogrammetric system, used for the analysis of the movement, is consisting of 8 cameras (Figure 15, Smart DX, BTS Bioengineering, Milan-Italy), equipped with *Charge Coupled Device* sensors, sensitive to infrared radiations (wavelength of 700-1000 nm of the electromagnetic spectrum). Around the lens, there is a ring of LED that emit a stroboscopic illumination, that radiates the passive photo-reflecting markers.

The system detects the position in time and space of the marker set; with a frequency of 100 Hz, it can acquire their positions every 10 ms. Each marker has to be framed by at least two non-parallel axis optical cameras, in order to guarantee its continuous three-dimensional reconstruction.

Before the acquisitions, the system is preliminary calibrated through two phases. The first lasts about 10 seconds; it is a static calibration, to establish the origin of the laboratory's global reference system (GRS), using a triad of three axes (X, Y, Z). The second phase is a dynamic calibration, in which an operator, using only the Z axis (wand), establishes the boundaries of the work volume within which the athletes will carry out the test. At the end of the calibration, the software crosses the various frames of the cameras, to reconstruct the spatial progress of the markers with respect to the origin of laboratory’s GRS.

2.2.2 Heart rate monitor

Figure 16-Heart rate monitor system with band.

The heart rate monitor (POLAR Electro, Finland, Figure 16) is an electronic device capable of measuring the number of heartbeats. From this measure, it determines the heart rate (HR) in real time and, for this reason, it is frequently used by athletes during training.

It consists of two elements: a transmitter, inside of a band, and an external receiver (watch). The band is wrapped around the chest; its electrodes, in contact with the skin, monitor the electrical tension of the heart. When a cardiac pulse is detected, a radio signal is emitted and used to determine the current heart rate. The signal can be a simple radio pulse or a signal coded by the transmitter; in the second case the user is avoided to receive signals from other transmitters (cross-talk interference).

2.2.3. Lactate meter

Figure 17-Lactate Pro 2 device.

This device shown in Figure 17 provides values within 15 seconds; it uses only 0.3 µl of whole blood, an automatic calibration and 3 different user settings. Up to 300 results can be exported from the device to a CSV file.

2.2.4. Electromyographic measure

Figure 18-EMG system and a probe.

A surface electromyography device with wireless probes (Figure 18, FreeEMG 1000, BTS Bioengineering, Milan-Italy) was used for the dynamic analysis of muscle activity during the shuttle run. This is a 4G technology device for surface electromyography (EMG); the absence of wires and the reduced size of the probes are all features that enable its use in analysis of every type of movement, for each body part, without altering natural actions. For signal acquisition, the probes are directly attached to the electrodes; thanks to its high accuracy, the system permits to detect also the weakest signals and to communicate them to a PC.

2.4. Data analysis

2.4.1. Electromyographic data processing

After the acquisition phase, it was necessary to process EMG data. Signal processing techniques are all those mathematical procedures, applied to extract useful information from the biomedical signal (Winter 2009).

In this study, to obtain a complete EMG processing and analysis, different software were used: Smart-Tracker (BTS Bioengineering, Milan-Italy), SmartAnalyzer (BTS Bioengineering, Milan-Italy), Visual3D (version 6.03.06, C-Motion, Inc.

Germantown, MD, USA) and Matlab (version R2019b, The MathWorks Inc, Natick, Massachusetts, USA).

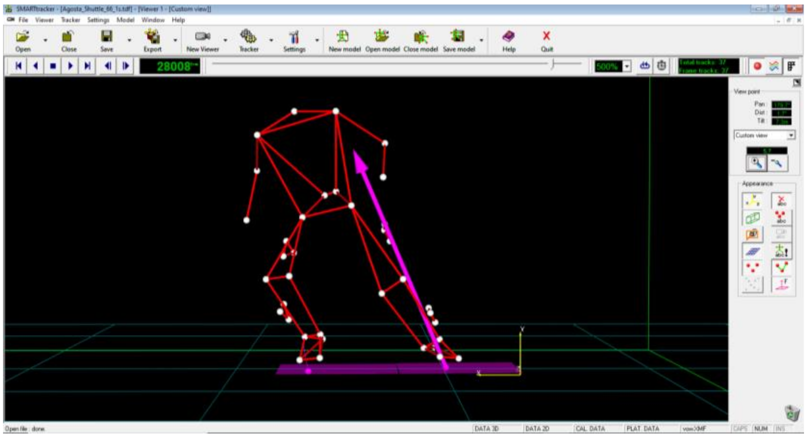
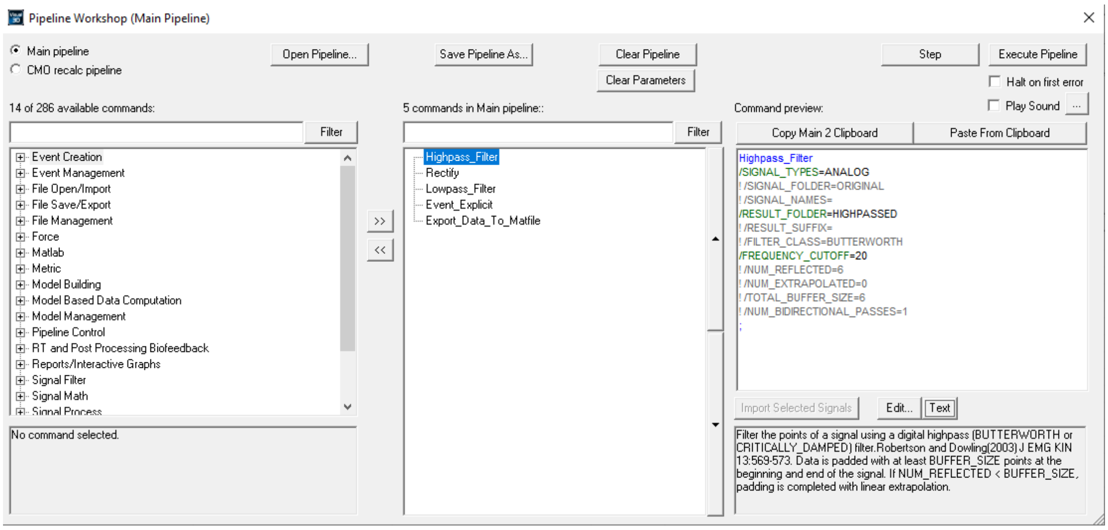


Figure 19-A CoD in SmartTracker.

In SmartTracker, after the tracking procedure for the creation of the model, the file relative to the shuttle test of the subject was cut in different temporal windows. Their length was of 500 ms (50 frames) and they consider the movement of the CoD, made by the athlete (Figure 19). In particular, these frames represented the landing phase (250 ms before the foot touch the ground) and the phase of load acceptance (250 ms following the contact), considering the limb of support. Every participant was characterized by a different total number of windows, since the duration of their test was various (the number of CoDs performed during the shuttle are reported in Table 6). According to the aim of our analysis, for each subject, we considered only the first five and the last five CoDs, to understand if fatigue generated any alteration of the movement or changes on the muscle activation patterns. We compared muscle activity during the initial phase of the test and how the athlete adapted her movement to a fatigue state in the last CoDs.



No command selected.

```
Highpass_Filter
Rectify
Lowpass_Filter
Event_Explicit
Export_Data_To_Matfile
```

```
Highpass_Filter
/SIGNAL_TYPES=ANALOG
! /SIGNAL_FOLDER=ORIGINAL
! /SIGNAL_NAMES=
/RESULT_FOLDER=HIGHPASSED
! /RESULT_SUFFIX=
! /FILTER_CLASS=BUTTERWORTH
/FREQUENCY_CUTOFF=20
! /NUM_REFLECTED=6
! /NUM_EXTRAPOLATED=0
! /TOTAL_BUFFER_SIZE=6
! /NUM_BIDIRECTIONAL_PASSES=1
;
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Filter the points of a signal using a digital highpass (BUTTERWORTH or CRITICALLY_DAMPED) filter.Robertson and Dowling(2003) J EMG KIN 13:569-573. Data is padded with at least BUFFER_SIZE points at the beginning and end of the signal. If NUM_REFLECTED < BUFFER_SIZE, padding is completed with linear extrapolation.

Figure 20-Pipeline created in Visual3D.

EMG data, related to each window, were then exported in .c3d format, in order to analyse and process them in Visual3D. This software enabled to process the analog electromyographic signal, through a pipeline, a list of several commands (Figure 20).

First of all, it was also possible to create an event at the half of the window, representing the ground contact of the dominant foot.

Then, to eliminate the low frequency noise and any interference, a high-pass filter was applied, with a cut off frequency of 20 Hz (Gokeler et al., 2010). After that, the filtered signal was rectified; the bipolar EMG signal was transformed into a unipolar one, thanks to a full wave rectifier, that reversed the polarity, maintaining the energy of the original signal (Besier et al., 2003; Cifrek et al., 2009; Neptunet, 1999; Subbu et al., 2015). Then, the EMG data were filtered again; the high frequency noise was deleted with a low-pass filter, with a cut off frequency of 10 Hz (Gokeler et al., 2010). This last filtering procedure created an envelope of the signal and enabled to consider the variation of its mean value, over time.

The last instruction of the pipeline was to export the processed signal as a Matfile, in order to make further calculation on Matlab.

Matlab was used to represent, in the same graph, a comparison between the behaviour of each muscle in two different phases, pre- and post-fatigue (at the beginning and at the end of the test). The muscle activity is represented by the mean and by the standard deviation of the signal, calculated both on the first five and the last five CoDs.

The electromyographic signal of each muscle, then, was divided by its maximum amplitude value, taken from the row signal. After this normalization process, it was possible to calculate the onset time values for the activation of each muscle, to understand if and when the organ was activated before the ground contact. To do this, a threshold was established; the level of activation was taken as the triple of the minimum value of the signal in the 250 ms before the foot strike.

In Matlab, it was also possible to study the signal in the frequency domain; with the support of SmartAnalyzer (Figure 21), for each muscle it was calculated the power spectrum of the EMG signal and its median value. It was important to analyse the frequency content of the signal, to understand if, due to fatigue, there is a shift towards lower frequency. The results obtained from this evaluation are at the basis of the statistical analysis, described below.

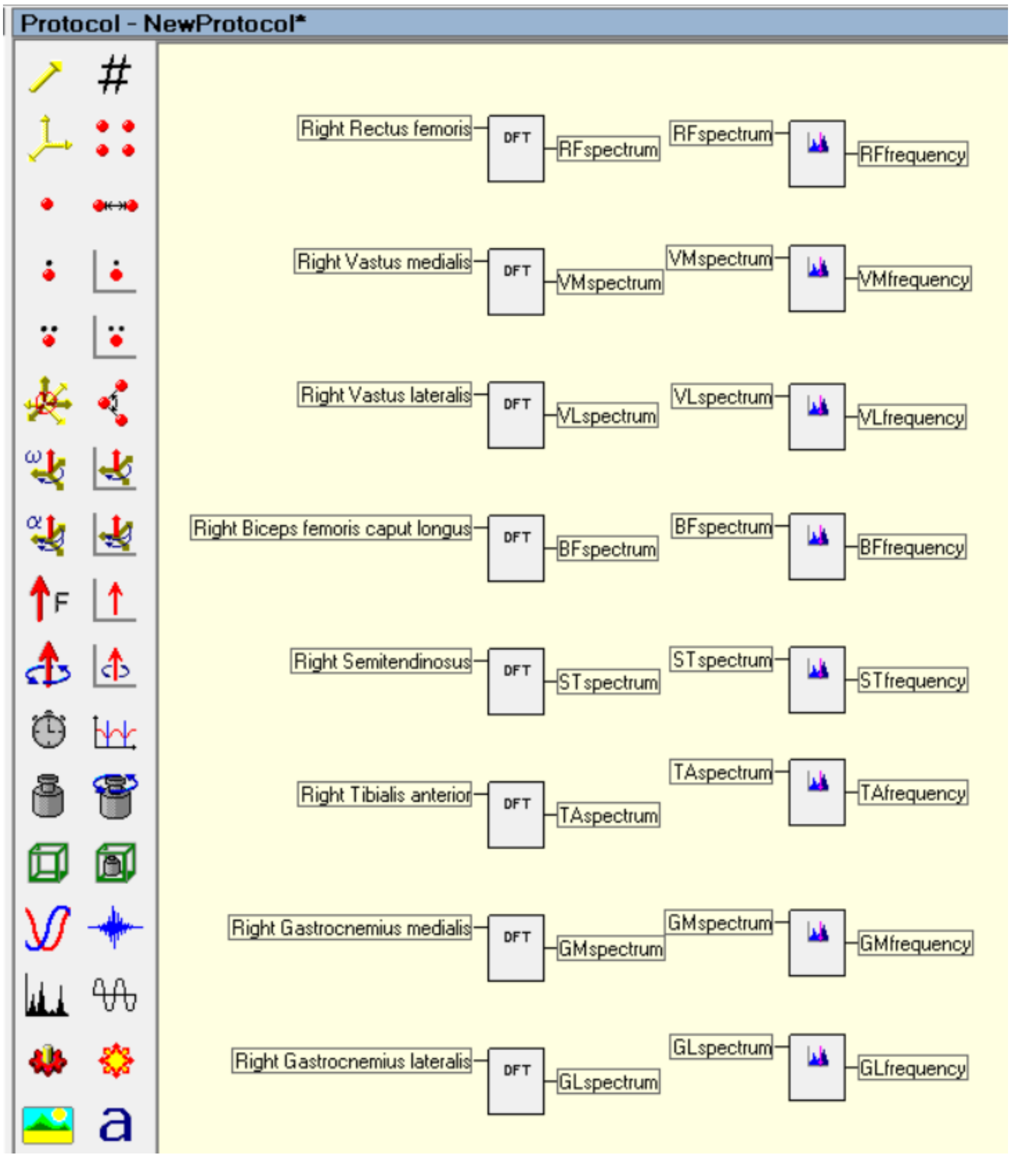


Figure 21-Protocol created in SmartAnalyzer to study the frequency content of the signal.

2.5. Statistical analysis

2.5.1. SPM

The time series relative to the muscles activity in the 250 milliseconds before and after the ground contact were analysed through Statistical Parametrical Mapping (SPM), in Matlab.

SPM enabled to perform statistical tests on sets of time series, taking into account the dependency relationships existing between the data at a certain instant of time and at the subsequent instants (temporal smoothness) (Pataky 2012).

In our case, this technique was applied for each muscle of the athlete, to understand if there is a correlation between the muscle activity in the pre-fatigue phase and in the post-fatigue one. For all the 500 temporal nodes, we obtain the statistical scalar output SPM {t}, indicating the p-values of the correlation at that moment.

If the black curve of these graphs exceeds considerably the upper limit, it means that, in that phase of the CoD, fatigue determines a lower activity of the muscle of interest, compared to the beginning of the shuttle. On the other hand, in the case the black line is under the lower limit, the muscle activity of the athlete in a fatigue condition is higher than in a fresh state.

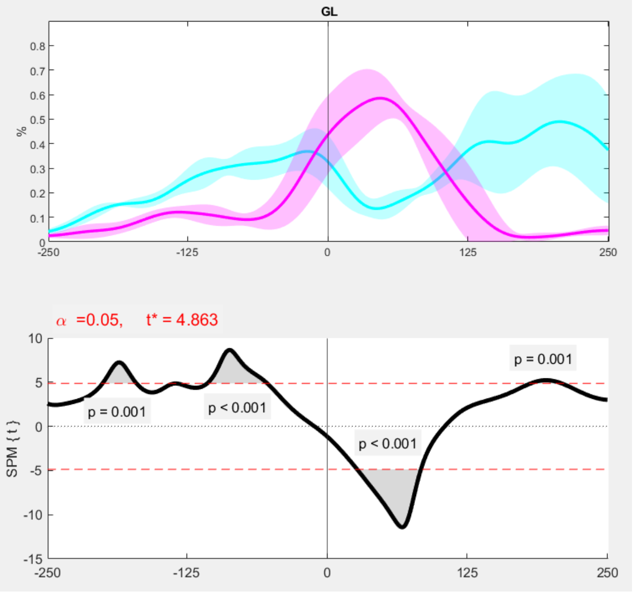


Figure 22-Example of statistical significance between pre- and post- fatigue.

An example is reported in the graph above (Figure 22). The lower panel shows the SPM {t} statistic, which allows to identify the intervals in which, in the upper panel, there is a correlation between the curves of muscle activity pre-fatigue (light blue) and post- fatigue (purple). There is a positive correlation if the significance cluster is above the upper limit, while a negative correlation exists if the cluster is below the lower limit.

For this analysis the package “spm1d”, version 0.4, was used; it is available at http://www.spm1d.org/index.html.

2.5.2. ANOVA2

A repeated-measure design Analysis of Variance (factors: subjects and pre- vs. post-condition, with subject x condition interaction) was conducted on the following dependent variables: frequency of the signal and onset times of the muscles. This was done to study the electromyographic signal of the eight muscles of the lower limb, both in the time domain (in term times of activation) and in the frequency domain (in term of spike frequency). The measures were repeated on all subject and the results are reported in term of mean and standard deviation.

The results are considered statistically significant only if the p-value is lower than an alpha level of 0.05. Statistical significance in the comparison between pre- and post-fatigue indicated that exercise-induced fatigue produced alterations in that dependent variable. On the other hand, if this statistical significance exists in the interaction between the subjects and the fatigue condition, it implies that the behaviour adopted due to fatigue is highly dependent on the individual athlete.

3. RESULTS

3.1. Time domain

Thanks to the application of SPM, from the graphs generated in Matlab we could quantitatively observe if there exist a significant change in the muscle activity due to fatigue. An example of the typical patterns of activation of the lower limb muscles is reported below, for the subject 10; the graphs of all the other subjects are reported in the appendix. In Figure 23, 24 and 25 hamstring muscles, quadriceps muscles and lower leg muscles are reported, respectively.

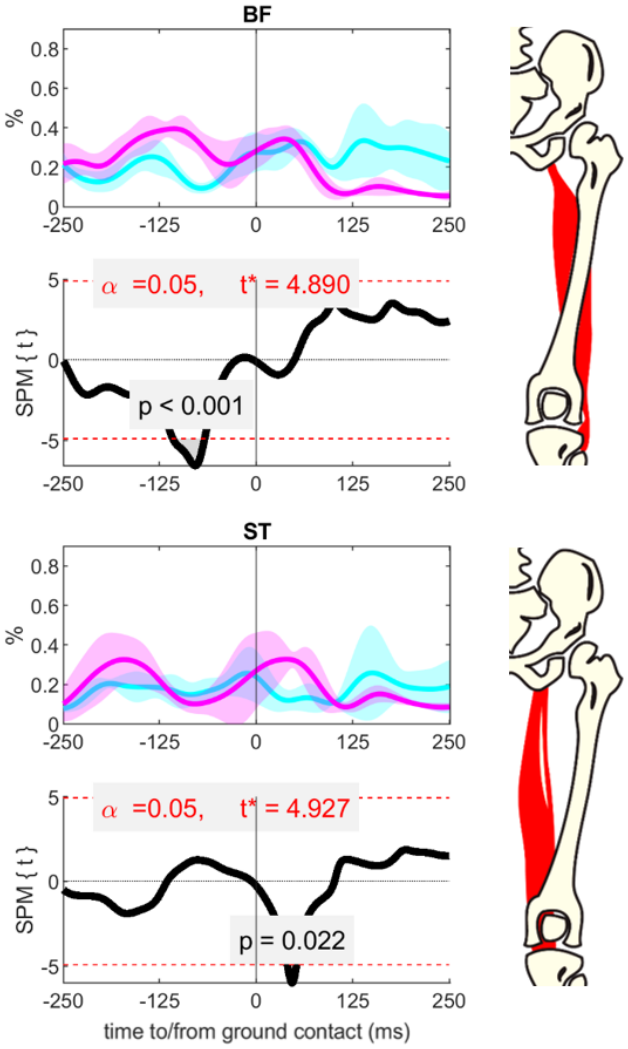


Figure 23- Hamstring (BF biceps femoris, ST semitendinosus) explanatory activation pattern.

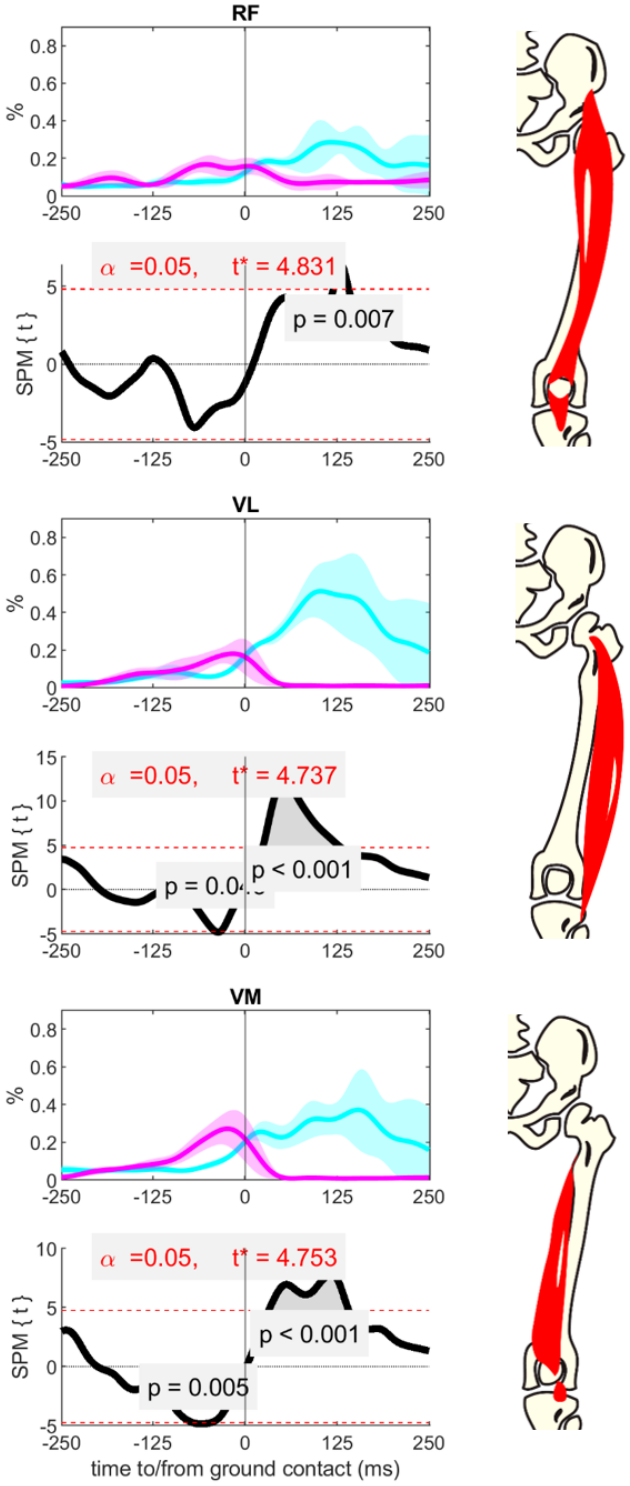


Figure 24-Quadriceps (RF rectus femoris, VL vastus lateralis, VM vastus medialis) activation patterns.

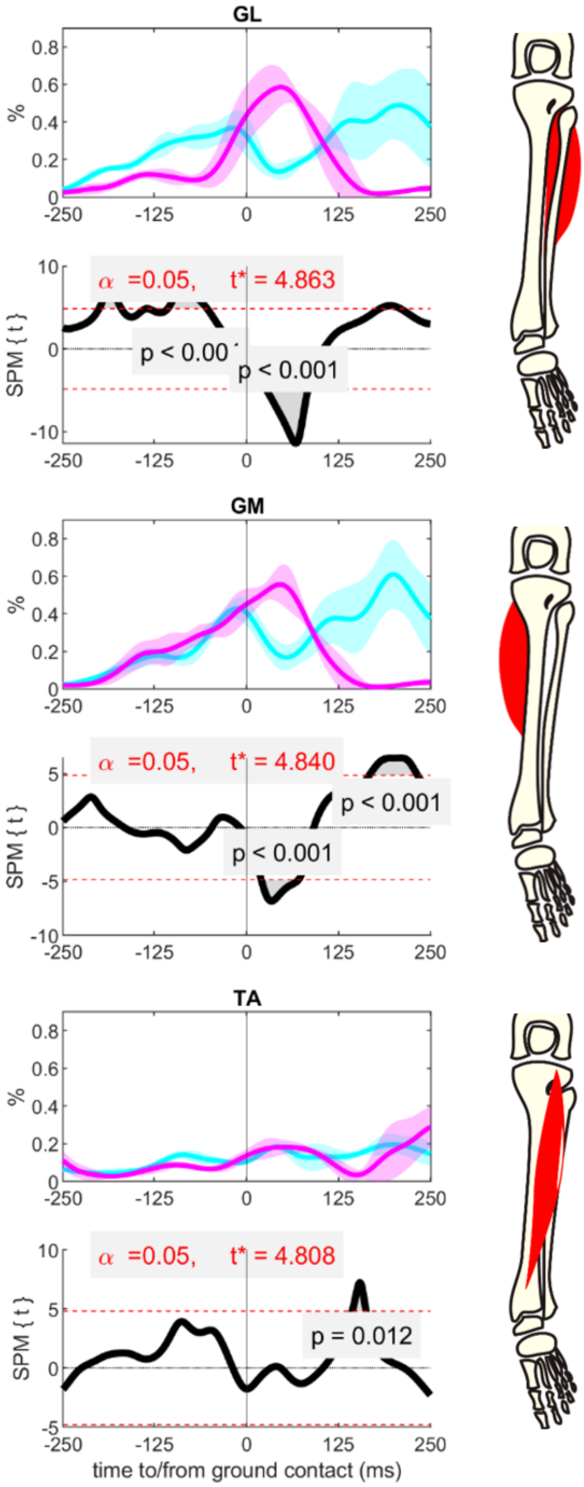


Figure 25-Lower leg muscle (GL gastrocnemius lateralis, GM gastrocnemius medialis, TA tibialis anterior) activation patterns.

In these plots, the signal trend is represented in term of mean and standard deviation; the light blue curve represents the muscle activity in the first five CoDs, while the line coloured in purple is related to the last five CoDs. Under the panel of each muscle, there are other graphs, that refer to the statistical significance.

A synthetic schematization of the significant changes observed for all the participants is shown in the following three tables, that refer to the activity of the quadriceps muscles (Table 7), the hamstring muscles (Table 8) and the lower leg muscles (Table 9). In these tables, there are the distributions of those subjects that increased (upper part of the graph) or decreased (lower part of the graph) the muscle activity due to fatigue; the distribution is relative to both the landing phase of the CoD (left part of the graph) and to the load acceptance after the ground contact (right part of the graph). Each subject is represented by a circle. For the vastus lateralis, the semitendinosus and the gastrocnemius medialis there are less than eighteen subjects represented; this is due to problems in the recording with the respective electromyographic probes.

Quadriceps muscles

For the rectus femoris, in the phase before the contact, there was not a significant trend in the altered use of this muscle, due to fatigue; in fact, its activity during the landing phase was decreased only for one subject. If we consider, instead, the instants of the CoD after the ground contact, in the load acceptance phase half of the subjects decreased the activity of the rectus femoris, with a statistical significance. A similar trend could be observed also in the case of the other quadriceps muscles, the vastus medialis and the vastus lateralis.

Hamstring muscles

Both for the biceps femoris and for the semitendinosus we did not observe a statistic significant trend, neither in the phase before the foot strike, nor after. After fatigue, only three subjects increased the amplitude of the electromyographic signal, while only two decreased it; the others maintained the same muscle activity. We can observe that some participants used less the lateral hamstring muscle (biceps femoris) in the phase after the ground contact.

Lower leg muscles

As concerns the muscles of the lower leg, there is not a univocal trend in the pre- and post- fatigue activity of none of them. Four subjects tended to use less the gastrocnemius medialis in the phase before the contact; in the same way, four participants activated less the tibialis anterior, in the phase following the foot contact. These muscles are a plantar and a dorsi flexor muscle of the ankle, respectively, and seemed to be reduced in the load acceptance phase, when the players were fatigued.

Finally, despite few statistically significant behaviours, we can say that, both in the landing phase and in the phase after the ground contact, most of the subjects maintained the same muscle activity, after fatigue.

Effects of Fatigue on Quadriceps Muscles

Rectus Femoris

n = 18 subjects

	-250 ms	Ground contact	+250 ms
Statistically Significant Increase ↑			
No Significant Change	●●●●● ●●●●● ●●●●● ●●	●●●●● ●●●●●	
Statistically Significant Decrease ↓	●	●●●●● ●●●	

Vastus Medialis

n = 18 subjects

	-250 ms	Ground contact	+250 ms
Statistically Significant Increase ↑	●	●	
No Significant Change	●●●●● ●●●●● ●●●●●	●●●●● ●●●●● ●	
Statistically Significant Decrease ↓	●●	●●●●● ●	

Vastus Lateralis

n = 13 subjects

	-250 ms	Ground contact	+250 ms
Statistically Significant Increase ↑	●●●		
No Significant Change	●●●●● ●●●	●●●●● ●●●	
Statistically Significant Decrease ↓	●●	●●●●●	

Table 7-Influence of fatigue on the quadriceps.

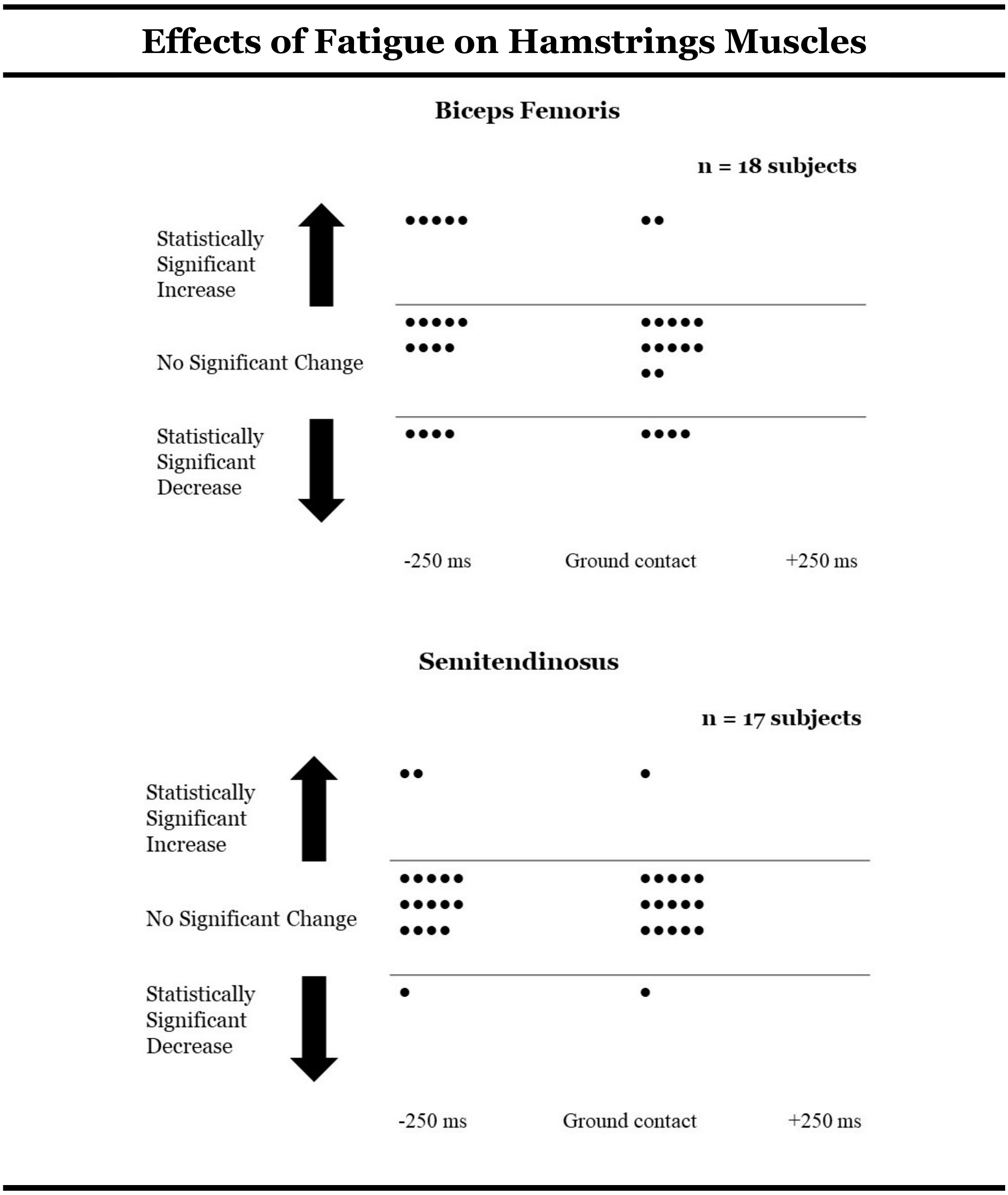


Table 8-Influence of fatigue on the hamstrings.

Effects of Fatigue on Lower Leg Muscles

Tibialis Anterior

n = 18 subjects

Statistically Significant Increase ↑	●●●	●●	
No Significant Change	●●●●● ●●●●● ●●●●	●●●●● ●●●●● ●●	
Statistically Significant Decrease ↓	●	●●●●	
	-250 ms	Ground contact	+250 ms

Gastrocnemius Medialis

n = 18 subjects

Statistically Significant Increase ↑		●●●	
No Significant Change	●●●●● ●●●●● ●●●●	●●●●● ●●●●● ●●	
Statistically Significant Decrease ↓	●●●●	●●●	
	-250 ms	Ground contact	+250 ms

Gastrocnemius Lateralis

n = 9 subjects

Statistically Significant Increase ↑	●	●●	
No Significant Change	●●●●	●●●●	
Statistically Significant Decrease ↓	●●●●	●●●	
	-250 ms	Ground contact	+250 ms

Table 9-Influence of fatigue on lower leg muscles.

3.1.1. Onset times

Another important aspect we analysed in the time domain was the instant of time at which a muscle activated during the landing phase of the CoD, before the ground contact. From this evaluation, we wanted to understand if fatigue delayed or anticipated the activity of the lower leg muscles or, on the other hand, if a fatigued state did not influence the moment muscle activation. In the table below (Table 10) all the results about the onset times of the muscles for all the subjectare reported.

For each muscle, comparing the columns relative to the mean values pre- and post-fatigue, we can notice that there is not the evidence of an increased or decreased trend: due to fatigue, some muscles seemed to delay its activation before the contact (e.g. biceps femoris, gastrocnemius medialis and tibialis anterior), while other muscles anticipated it (e.g. gastrocnemius lateralis, rectus femoris, vastus lateralis and vastus medialis). Most of the participants maintained the instant of activation of the semitendinosus unchanged during the test.

Finally, as we can see in the last column of the table, there is not a great interaction between the subjects and the fatigue condition; we had not enough elements to say that, as concern the instant of the muscle activation, the behaviour is highly subject-dependent.

3.2. Frequency domain

As regards the analysis of the frequency content of electromyographic signals, the results are reported in the table below (Table 11).

Observing the mean values, we observe that, when an athlete passed from a pre-fatigue condition to a post- fatigue state, there was a global decrease of the spike frequency in the activity of all her muscles. This decreasing trend is present in all the participants, for quite all the muscles (with the exception of the semitendinosus, that maintained the mean value unchanged pre- and post- fatigue).

Despite these observations, fatigue seemed not to produce an evident and repeated change in the patterns of muscle activities; as we can see in the graphs reported in appendix, the curves before and after the effort refer to different amplitude of the signal, but they maintain quite the same shape, as the shuttle run test proceed.

About the statistical significance of the interaction between subjects and the condition of fatigue, in the last column of the table there is a strong presence of p-values under 0.05.

	Pre		**Post**		**Pre-post factor**		**Condition x subjects**	
Muscle	**Mean [ms]**	**SD**	**Mean [ms]**	**SD**	**F**	**p**	**F**	**p**
RF	110.3	91.4	103.6	67.9	0,32	0,573	0,69	0,811
VM	153.5	110.5	149.9	85.6	0,08	0,783	0,89	0,589
VL	135.2	74.8	128.1	77.0	0,54	0,464	3,51	**>0.001**
BF	135.2	123.1	165.5	95.8	4,56	**0,034**	1,23	0,253
ST	160.7	128.1	162.9	124.3	0,02	0,892	1,04	0,420
TA	110.3	99.3	120.2	96.0	0	NaN	1,63	0,068
GM	177.0	110.5	180.9	115.0	0,04	0,846	1,46	0,120
GL	180.0	111.6	170.2	94.2	0,48	0,490	3,48	**0,002**

Table 10-Statistical analysis of the onset time of muscle activation,

	Pre		Post		Pre-post factor		Condition x subjects	
Muscle	**Mean [ms]**	**SD**	**Mean [ms]**	**SD**	**F**	**p**	**F**	**p**
RF	60.9	10.6	56.4	13.1	27.67	**<0.001**	4.54	**<0.001**
VM	60.1	14.2	57.0	13.3	12.51	**<0.001**	1.2	0.271
VL	54.7	14.4	52.9	13.5	2.06	0.154	1.29	0.237
BF	64.6	20.8	64.9	23.0	0.16	0.691	5.33	**0**
ST	59.9	26.2	58.4	26.2	1.27	0.263	2.89	**<0.001**
TA	97.1	16.8	89.4	17.2	57.68	**<0.001**	4.24	**<0.001**
GM	116.8	31.1	111.6	26.3	8.68	**0.004**	4.14	**0**
GL	93.8	21.8	89.8	20.5	5.15	**0.026**	3.95	**<0.001**

Table 11-Statistical analysis of the median frequency in muscle activity.

4. DISCUSSION

In our study, we investigated the lower leg muscle activity during a CoD; we focused on what happened in the short period of time that anticipates the movement, but also on the phase of load acceptance, following the ground contact. The interval of time of interest lasted about 500 ms. Our aim was to verify if fatigue induced by repeated changes of direction can produce a change in the muscle activation patterns of lower limbs muscles in young female élite football players fatigue.

A recent study (Maniar et al., 2019) investigated lower limb muscle function during a rapid sidestep cut, finding that vasti, gluteus maximus, soleus, gastrocnemius and the hamstrings were important for modulating anteroposterior progression during the stance phase of an unanticipated sidestep cut. These same muscles (except the hamstrings) were also important for supporting bodyweight, while the vasti, together with the glutei, played a critical role in accelerating the centre-of-mass towards the desired cutting direction.

Our results are commented below and are about different aspects, as concerns both the time domain and the frequency domain.

Activation intensity and fatigue

In general, there is not a univocal trend that shows a change in muscles activation intensity after fatigue. In response to a high muscle effort, athletes did not execute a modified and repeated muscle pattern. As a result, we can conclude that these 18 cohort subjects were not characterized by a common trend in their adaptation to a fatigue protocol.

At the same time, considering the single cases, some participants showed a behaviour in line with some considerations present in the existing literature, increasing muscle activity in the final part of the physical effort with respect to its beginning. Winter (2009) observed that the more the muscle strain increases, the more intensity and amplitude of the signal increase. In the graphs of these subjects, we notice an increment of the signal intensity in the last five CoDs; in other subjects, instead, there is a decrease after fatigue.

More specifically, we can consider what happened for the most tired players, who performed a moderately (> 6 mmol/L of $[La^-]_b$) or a highly (> 10 mmol/L) anaerobic exercise (Rampinini et al., 2007).

Most of them demonstrated a lower activity in their quadriceps muscles (knee extensors), especially in the load acceptance phase, in the first 250 ms that follow the ground contact. From the kinematic analysis conducted on the same subjects (unpublished results) we noticed that very fatigued athletes are characterized by a less knee flexion (Figure 26), confirming what is reported by Alentorn-Geli et al., (2009). A less flexed joint may generate a lower activity of the extensor muscle of the knee. Vastus medialis, together with vastus lateralis, are the main responsible for the stability of the tibiofemoral joint; their lower recruitment can have consequences on the more susceptibility to rupture of knee ligaments in very fatigued athletes.

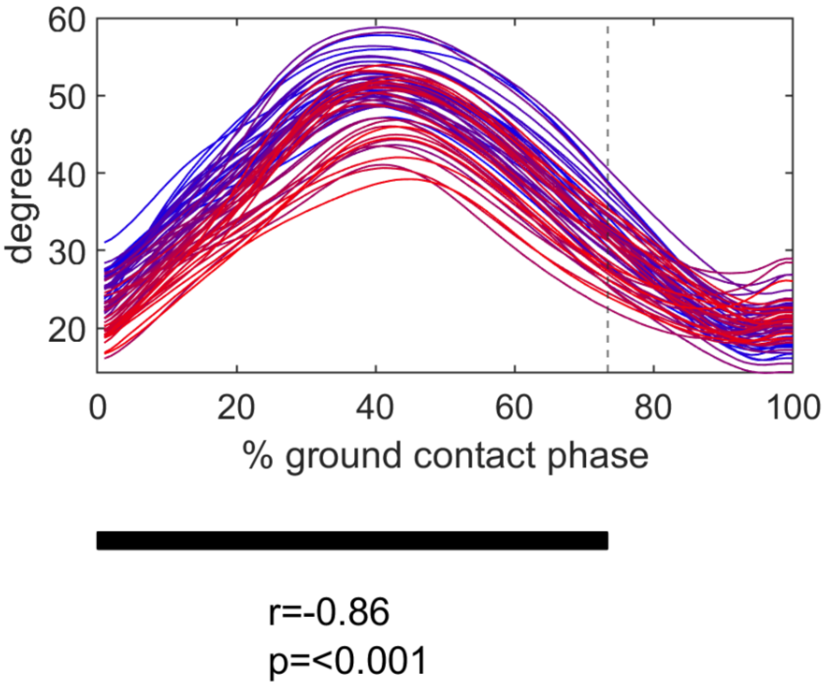


Figure 26-Sample plot showing the decrease of knee flexion angle from a fresh state (blue) to a fatigue condition (red) for a single participant (unpublished results).

To best support every movements, different groups of muscle act mutually, together with other joint ligaments. For a group that works less, there are other muscles that compensate with their activation.

As concern gastrocnemii and tibialis anterior, in the load acceptance phase, some very tired participants seemed to use these muscles more at the end of the exercise. This behaviour could be linked to the fact that, while in an athlete that perform an aerobic exercise the load is sustained more by the muscles of the thigh, in an exhausted one it is more controlled by the gastrocnemii (plantar flexors of the foot) and by tibialis anterior (dorsi flexor of the foot).

Finally, about the hamstring activity, there were some very fatigued athletes that improved it, but in other it decreased, both in the landing phase and in the acceptance after the contact.

Even though a reduction in muscle activity may be responsible for an overload of ligaments and other structures that compose the tibiofemoral joint, we cannot assume that fatigue is the only factor responsible for ACL tears.

As we see in Figure 27, quadriceps can produce varus or valgus moments on the knee joint, but there are other external loads on the tibia that contribute to the generation of a risky condition for the leg. For example, Olsen et al., (2004) stated that valgus loading in combination with external or internal knee rotation caused the injury and proposed notch impingement as a plausible cause of the excessive ACL loading.

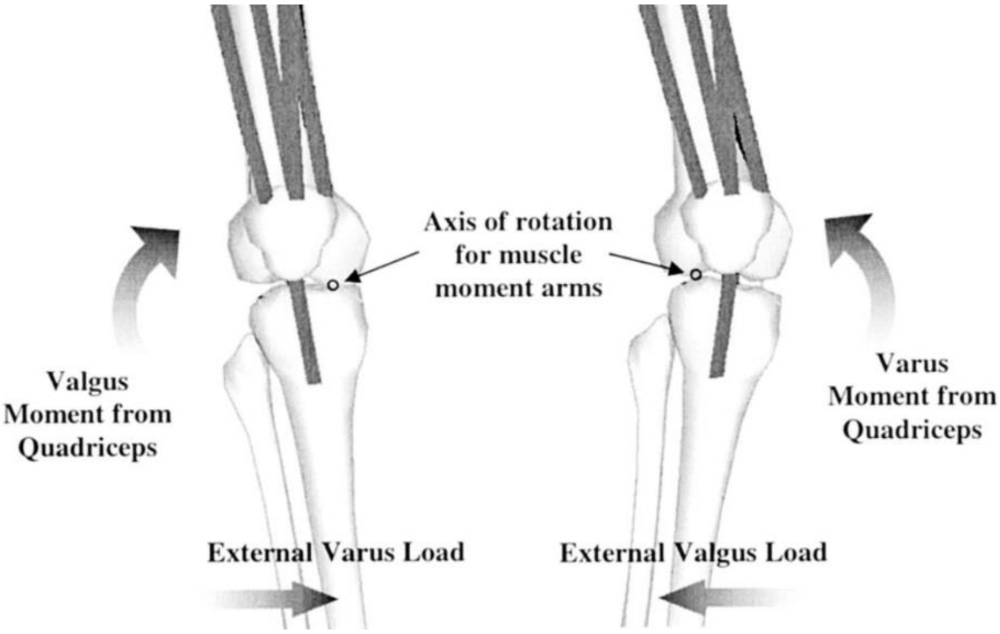


Figure 27-Biomechanics of the knee.

Onset times and fatigue

Another representative parameter of fatigue can be found in muscle activation timings; an exhausting physical exercise can induce a delay or an anticipation in the onset times of the muscles involved in the specific movement.

A study showed that muscle pre-activity increases the sensitivity of the muscle spindles, allowing joint perturbation to be detected more quickly (Dyhre-Poulsen et al., 1991); distinctive feedforward control strategy and preparatory muscle activity can increase joint stability (Tartakovsky and Broyda 2011).

In our case, among the 18 analysed subjects there was not an evident and common increment or decrease in muscle activation times due to fatigue. Every athlete was characterized by different onset time that they maintained quite unchanged during the shuttle run test. From a more specific analysis, we can notice that the muscles that anticipated their activation instant are the ones belonging to the quadriceps group; on the other hand, the muscle of the lower leg, in particular the gastrocnemius medialis and tibialis anterior, are characterized by a delay. Finally, for what concerns the hamstring group, only the biceps femoris seemed to be activated later when the athlete is fatigued.

The low statistical significance of these results (reported in the seventh column of Table 10) can be traced to the very high values of the standard deviations, both pre- and post- fatigue. In fact, analysing the onset times of the single athlete, we noticed that, in some cases, the onset times were registered at the beginning of the movement, and not just before the ground contact. In these situations, there is a delay in the shutdown of the muscle activity, not to be confused with the absence of the contraction in the last part of the landing phase.

Spike frequency and fatigue

As regards the frequency analysis of the surface myoelectric signal, Hakamada (1962) observed, for the first time, that in the power spectrum of the signal there was a shift towards lower frequency. According to (Winter 2009), the main change in the EMG signal during contraction is the compression of the signal spectrum towards the lower frequencies, associated to the metabolic fatigue of the muscle. These shifts of the mean and median frequency are determined by the speed of propagation of the action potential, whose decrease is connected to the production of acid catabolites (effects of metabolism) (Winter 2009). Spectral changes can also be assessed with the analysis of spikes (Winter 2009); the number of spikes of the raw EMG signal was seen to be dependent on the level of muscle activity (Porcellini et al. 2002).

This displacement is associated to the metabolic fatigue in the muscle, during fatiguing contractions (Winter 2009); the production of acid catabolites, in fact, lows the speed of propagation of the action potentials. Farina & Merletti (2004) found that, during fatigue, the changes in the frequency spectrum and in the speed of conduction of muscle fiber are mainly due to the accumulation of lactate, to the decrease of the pH value, to the increase in the concentration of potassium in the system of T tubules and to the exhaustion of substrates (phosphocreatine and ATP); moreover, there are some changes that occur also at the level of the central nervous system in the activation of the motor unit (especially the synchronization).

Our results are in line with these assumptions; the more all the athletes were fatigued, the more they reduced their spike frequency. This global decreasing trend is present in all the muscles of the leg, with the exception of the semitendinosus, whose frequency content seemed to be unchanged.

4.1. Limitations

Despite our effort to have the lowest number of influencing factors, still a number of them could have impacted results. First of all, the condition reproduced in laboratory does not corresponds to reality; a change of direction during a football match is much more dangerous, because unexpected and influenced by several external factors. The contact with other players and the movement of the ball can make the athlete less focused and divert his attention from a well-controlled movement. The joint loads during the shuttle run test are not as high as those during a football match.

Second, the group of subjects was heterogeneous, not only in term of age, but also for their playing role, and the level of competitions in which they participate; a goalkeeper, for example, is less accustomed to run for a long period during a match, with respect to a midfielder. Moreover, footballers belonging to Serie A level, used to more intensive and frequent trainings and they may resist better to ligaments stresses.

Contrasting results, together with the fact that most of the athlete did not show any change in their muscle pattern, may be also traced to a possible not adequate technique to induce fatigue. For example, the calculation of a too low running speed could not have exhausted the subject enough during the test. The inexistence of a common trend may be due to also to the fact that the number of subjects that took part in this study was relatively low.

When we deal with electromyographic measures, it is not possible to compare in the same graph the signals of different subjects, since there are highly dependent on so many factors, that affects the record of EMG. Another limitation that concern muscle activity measure, is that it is very difficult to obtain a clear signal, especially when the subject is performing a very dynamic movement, such as running.

4.2. Prevention programmes

The results of this study want to be a support for athletic trainers in designing prevention programs for athletes; the creation of a biomechanical profile would be helpful in ACL neuromuscular prevention training programs, with a quantifiable reduction the injury risk.

According to Renstrom et al., (2008), these programmes attempt to alter dynamic loading of the tibiofemoral joint through neuromuscular and proprioceptive training; they emphasise proper landing and cutting techniques, trying to avoid excessive dynamic valgus knee and decreasing peak landing forces. Training significantly enhance hamstring strength and power and reduce hamstring to quadriceps strength imbalances. It is also important to increase gluteus medius and hip abductor power and to address proper deceleration techniques.

Ideally, these prevention programmes should be introduced as early as possible in the training period. In some sports this would be at the age of 6–10 years (Renstrom et al., 2008).

Successful implementation of these programmes requires the collaboration of governing bodies, sports scientists, physicians, coaches and athletes.

Movement technique and performance are more stable under psychological and physical stress/fatigue when acquired with an implicit learning method (Benjaminse et al., 2011; Poolton et al., 2007). For example, during cutting and landing tasks, the adoption of a verbal or visual external focus of attention improves biomechanics, by increasing knee and trunk flexion angles (Benjaminse et al., 2017; Gokeler et al., 2015). Neuromuscular efficiency is enhanced with implicit motor learning strategies (Lohse & Sherwood, 2012; Zachry et al., 2005), without a reduction in performance, which is particularly necessary when fatigued.

An increase in overall physical fitness protects the athlete against injury and it decreases the perceived workload, reducing the injury risk (Windt and Gabbett 2017). More specifically, there is a significant probability of injury during intense training periods or during acute training loads changes (Jones et al., 2017). Moreover, exposing the athlete to a higher chronic workload provides protection against a spike in acute workload (McCall et al., 2018; Hulin et al., 2016).

4.3. Future directions

For a correctly performed movement there is a precise internal motor program, but unanticipated events can provoke a startle response within the central nervous system. This results in an involuntary change in neuromuscular activity (Davis 1984; Deangelis et al. 2015), inconsistent with the brain internal model of anticipated events. Therefore, high velocities of some movements require earlier cognitive planning, that can take several hundred milliseconds (Dunn et al, 1986; Kandler et al., 1991). It is fundamental to understand how external disruptions can influence the execution of a movement and how responsible they are in accidents and injuries, especially in sports.

During a football match, athletes cope with multiple simultaneous sensory stimuli, that slow their reaction times and diminish knee-stiffness–regulation strategies (Kim 2010); moreover visual-spatial disorientation may lead to attenuated muscle activity and poor coordination (Ford et al., 2005; Pashler, 1994; Woo et al., 2003).

Regardless of sex, the loss of neuromuscular control and a non-optimal regulation of knee-joint stiffness diminish dynamic stability, leading to unconstrained columnar buckling, associated with non-contact ACL injury pathomechanics (Boden et al., 2010; Dunning et al., 2015; Woo et al., 2003).

Yasuda et al., (1993) found that the ACL may tear in less than 70 milliseconds, but the earliest reflexive activity for dynamic restraint requires at least 35 milliseconds to begin(Shultz et al. 2001). More data are needed to establish the precise periods of time when individuals are vulnerable due to cognitive demands such as sensory integration, decision making, and motor planning.

Our preliminary results about muscle activity may guide researchers to pursue studies in several areas related to ACL injury. Sport-specific situations can be explored, with particular focus to visual attention in high-intensity, dynamic, complex environments. Various neuropsychological characteristics in injury propension should be investigated to enhance prevention and rehabilitation strategies.

4.4. CONCLUSION

This study identified the effects of fatigue on muscle activity during repeated changes of direction in a sample of élite female soccer players. We investigated the activation patterns of eight muscles of the supporting leg, belonging to the quadriceps (rectus femoris, vastus medialis and vastus lateralis), the hamstrings (biceps femoris and semitendinosus) and the lower leg (tibialis anterior, gastrocnemius medialis and gastrocnemius lateralis) muscle groups.

It was found that among the athletes did not exist a common adaptation and a univocal response to fatigue; the behaviour can be defined highly subject-dependent. For some subjects the intensity of muscle activity increased, for other it diminished. The reduction of muscle strength could be responsible for an overload of knee ligaments and may increase the risk of ACL non-contact injuries. In particular, we noticed that some players reduced the quadriceps muscle activity in the load acceptance phase; vastus medialis and vastus lateralis are the main responsible for the stability of the tibiofemoral joint. Neither as concern muscles onset timing there was a common trend in anticipating or delaying muscles recruitment.

In all the participants, fatigue produced a variation in the spectrum of the electromyographic signal. A shift towards lower frequencies is due to the metabolic production of acid catabolites, that low the speed of propagation of the action potential. Fatigue contractions determine also the accumulation of lactate, the decrease of the pH value, the rise in potassium concentration in the system of T tubules of muscles and the exhaustion of energy substrates, such as phosphocreatine and ATP.

In conclusion, fatigue can in principle increase the probability of non-contact ACL injury, but it cannot be identified as the only risk factor for this frequent damage (for example, external loads on the tibia contribute to generate a risky condition for the leg). However, training prevention programs are fundamental to improve the resistance of the athlete to a fatigue condition, reducing the risk of knee injuries. These programmes, through neuromuscular and proprioceptive training, try to avoid excessive dynamic valgus knee and decreasing peak landing forces; they enhance muscles strength and power and reduce hamstring to quadriceps strength imbalances.

In a complex environment such as a football match, a player is continuously subjected to different external stimuli, hardly repeatable in laboratory. Future studies are necessary to understand how visual and spatial disruptions can determine a highly dangerous situation for the soccer player. From a cognitive point of view, innovative prevention programs can train the athlete to deal with unexpected situation with less risk of damage.

5. APPENDIX

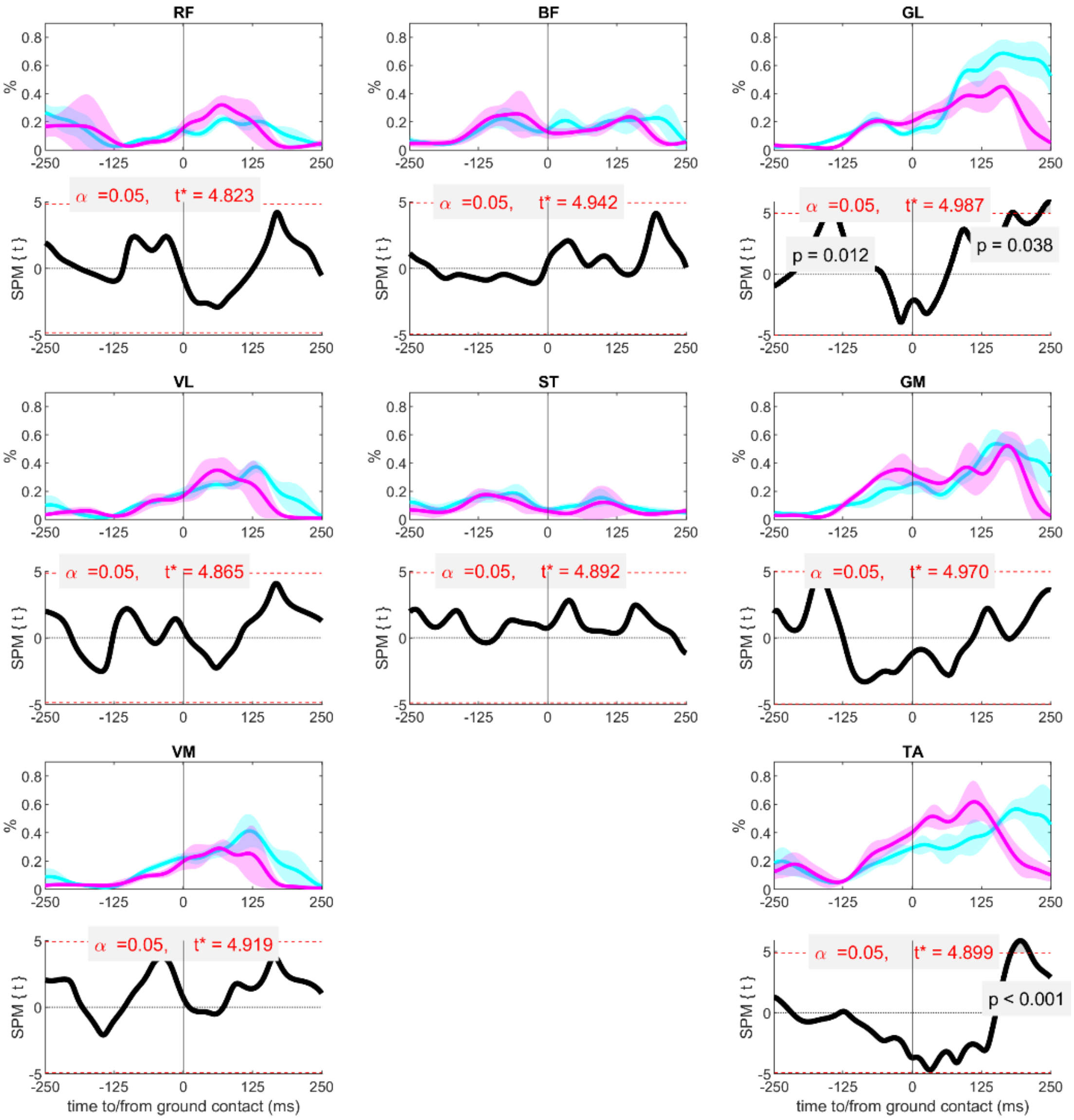
The following plots represent the muscles activity for each subject. They compare the intensity of the electromyographic signal in a fresh state (light blue) and after fatigue (purple). The three columns refer to the quadriceps muscles, to the hamstrings and to the lower leg muscle, respectively.

Subject: 1

Number of CoDs: 29

Running speed: 2.6 m/s

%HRmax: 92.9%

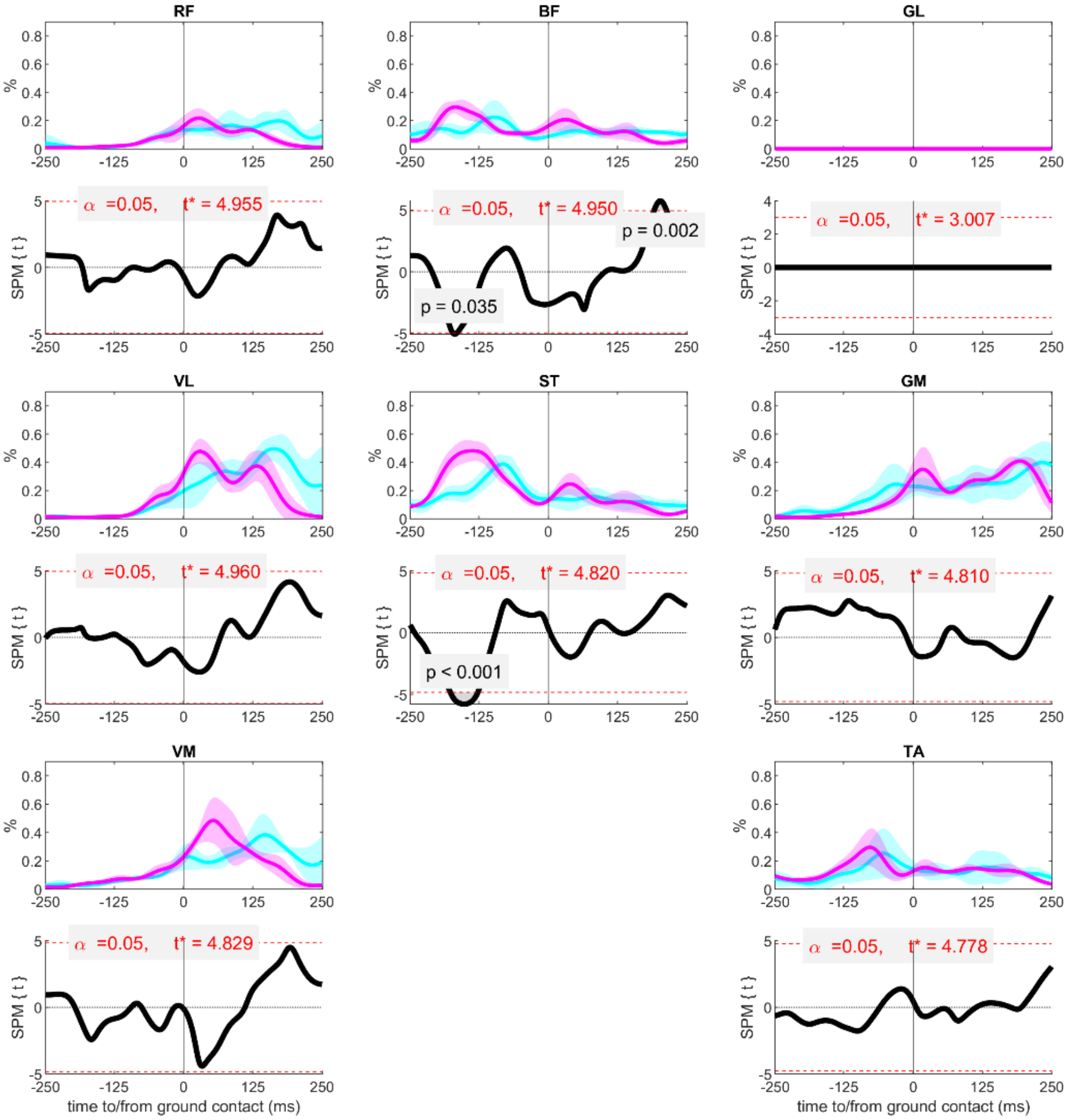


Subject: 2

Number of CoDs: 42

Running speed: 2.7 m/s

%HRmax: 98.0%

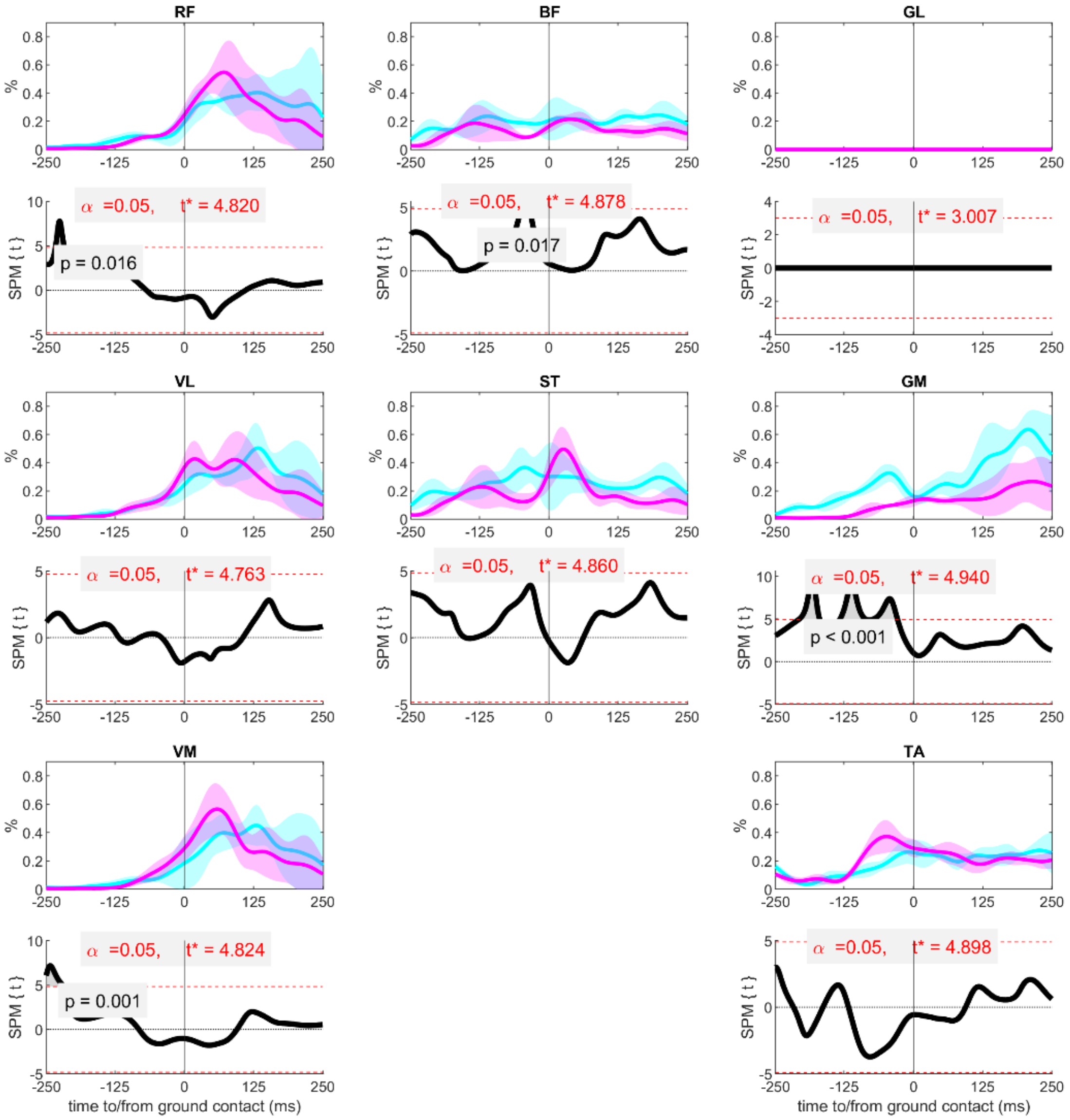


Subject: 3

Number of CoDs: 17

Running speed: 2.9 m/s

%HRmax: 92.9%

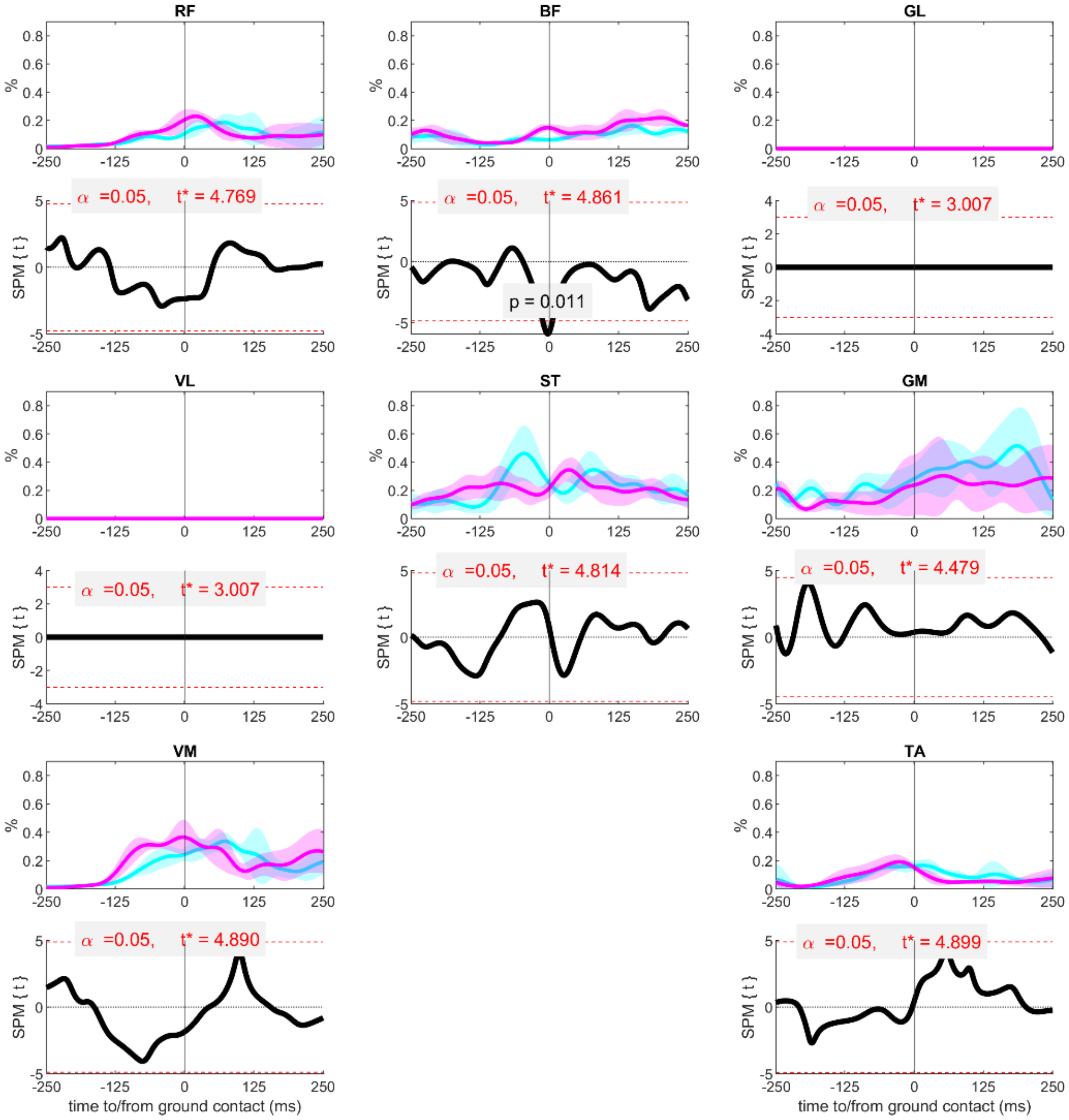


Subject: 4

Number of CoDs: 30

Running speed: 2.6 m/s

%HRmax: 89.2%

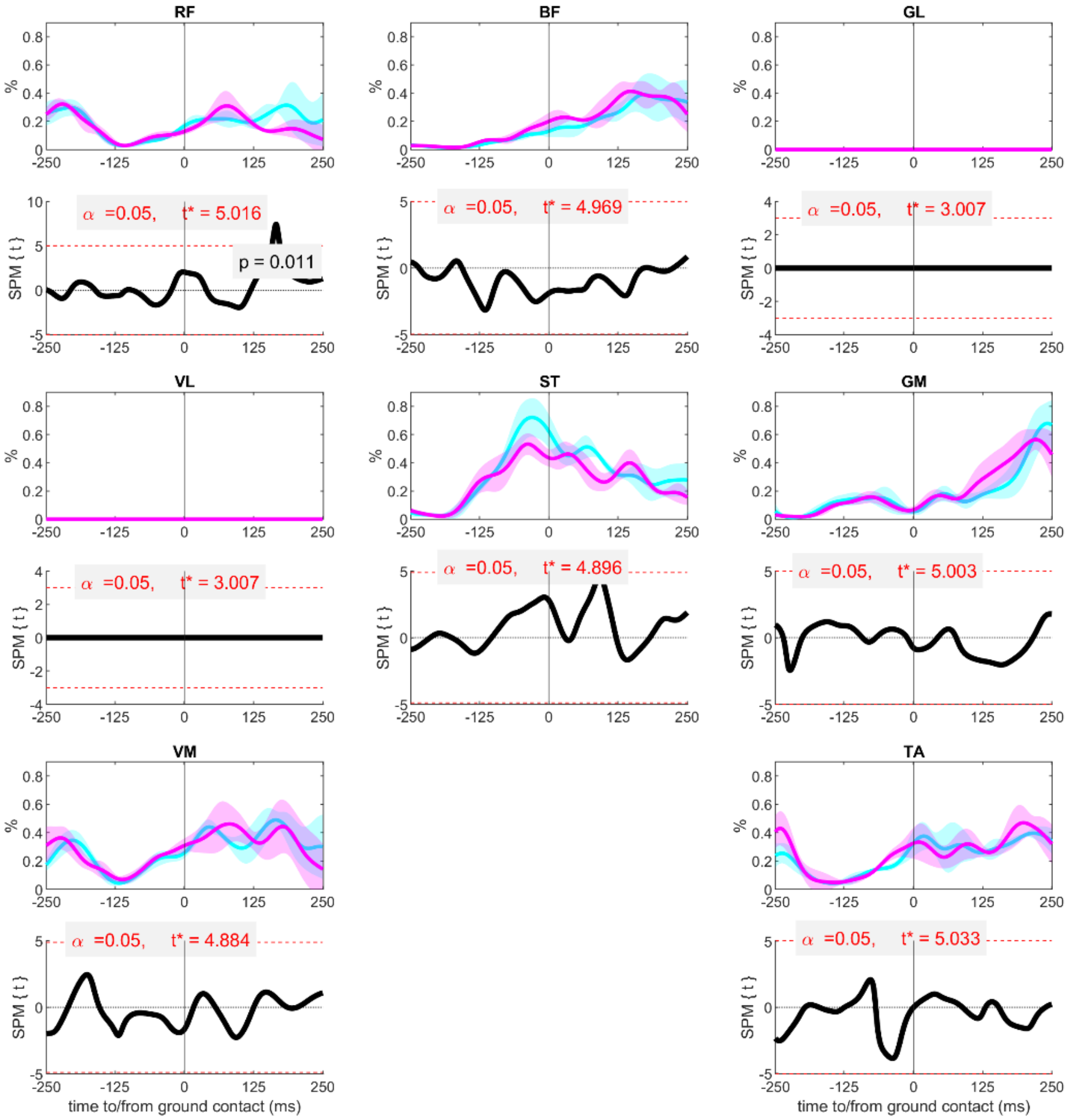


Subject:5

Number of CoDs: 55

Running speed: 2.3 m/s

%HRmax: 94.2%

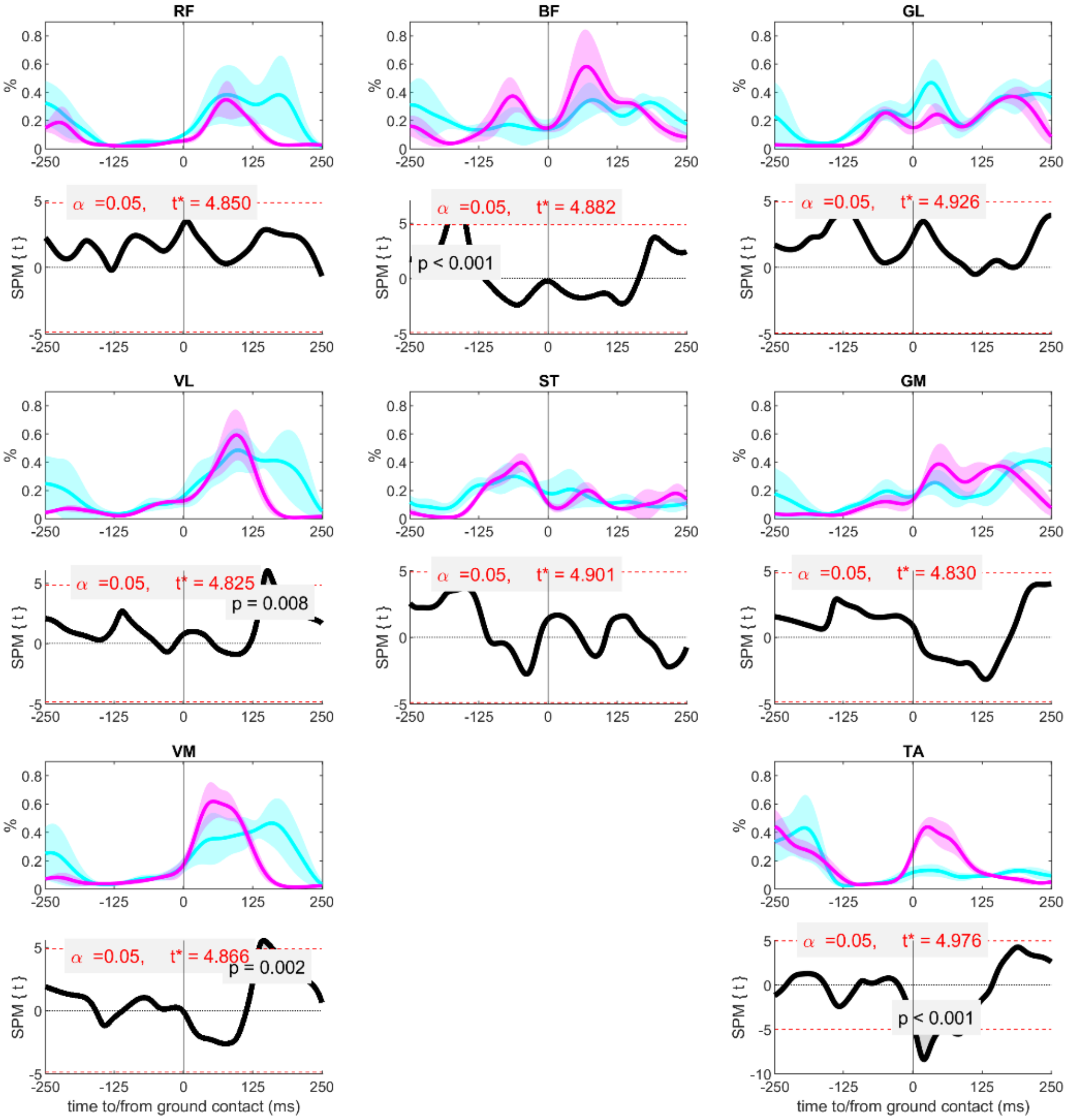


Subject: 6

Number of CoDs: 20

Running speed: 2.6 m/s

%HRmax: 97.6%

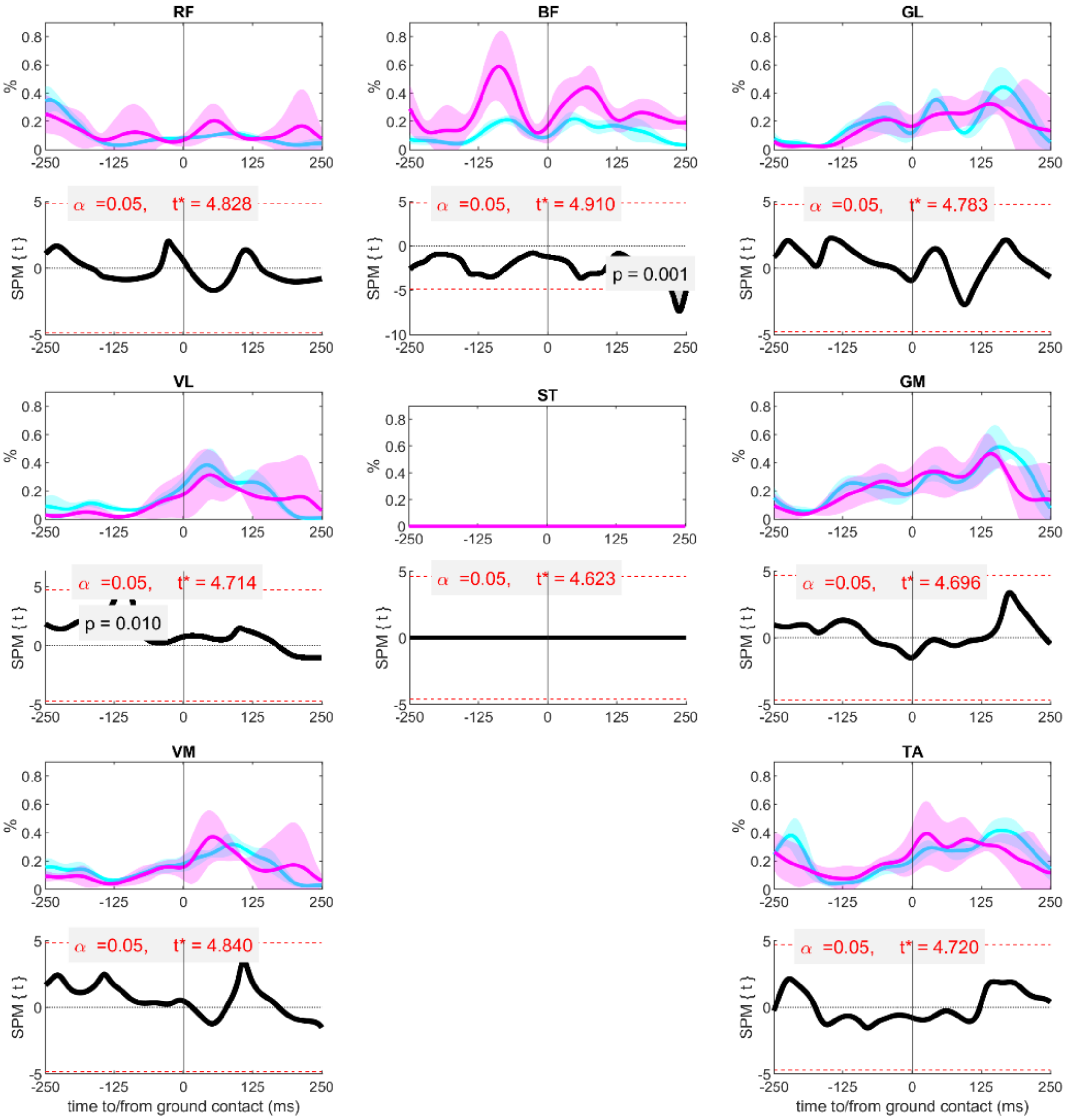


Subject: 7

Number of CoDs: 70

Running speed: 2.3 m/s

%HRmax: -

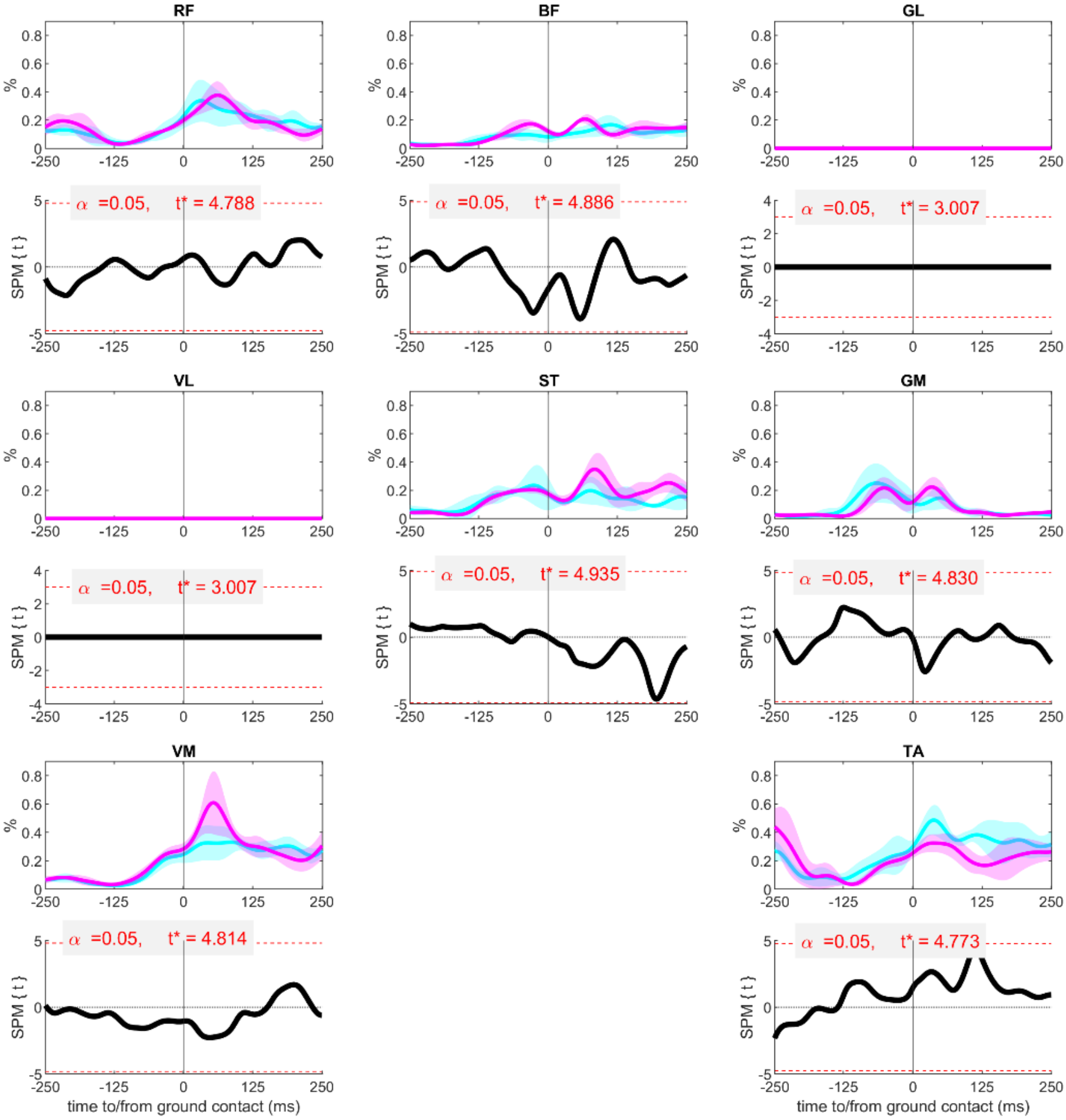


Subject: 8

Number of CoDs: 70

Running speed: 2.3 m/s

%HRmax: 95.2%

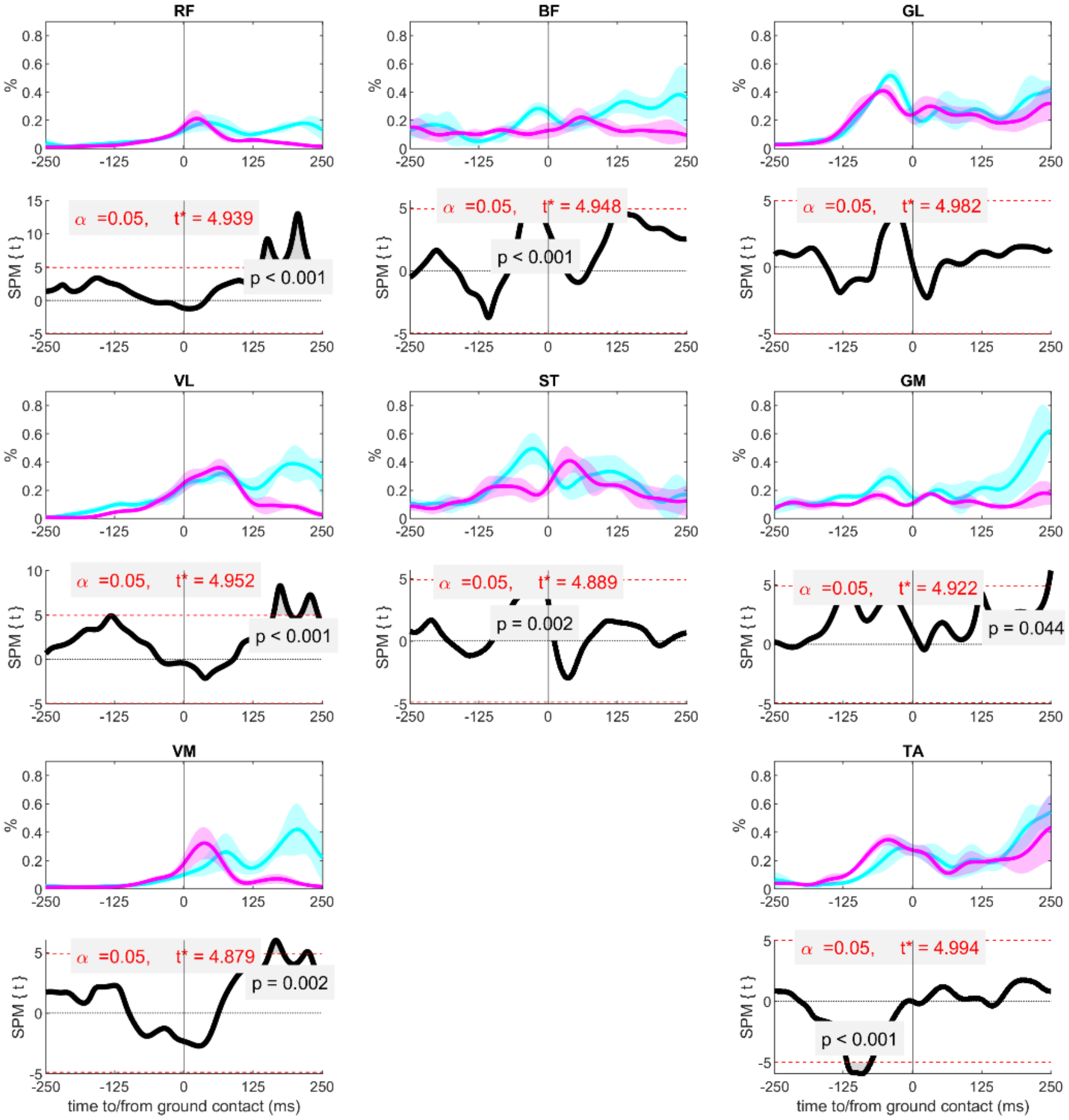


Subject: 9

Number of CoDs: 21

Running speed: 2.7 m/s

%HRmax: 97.8%

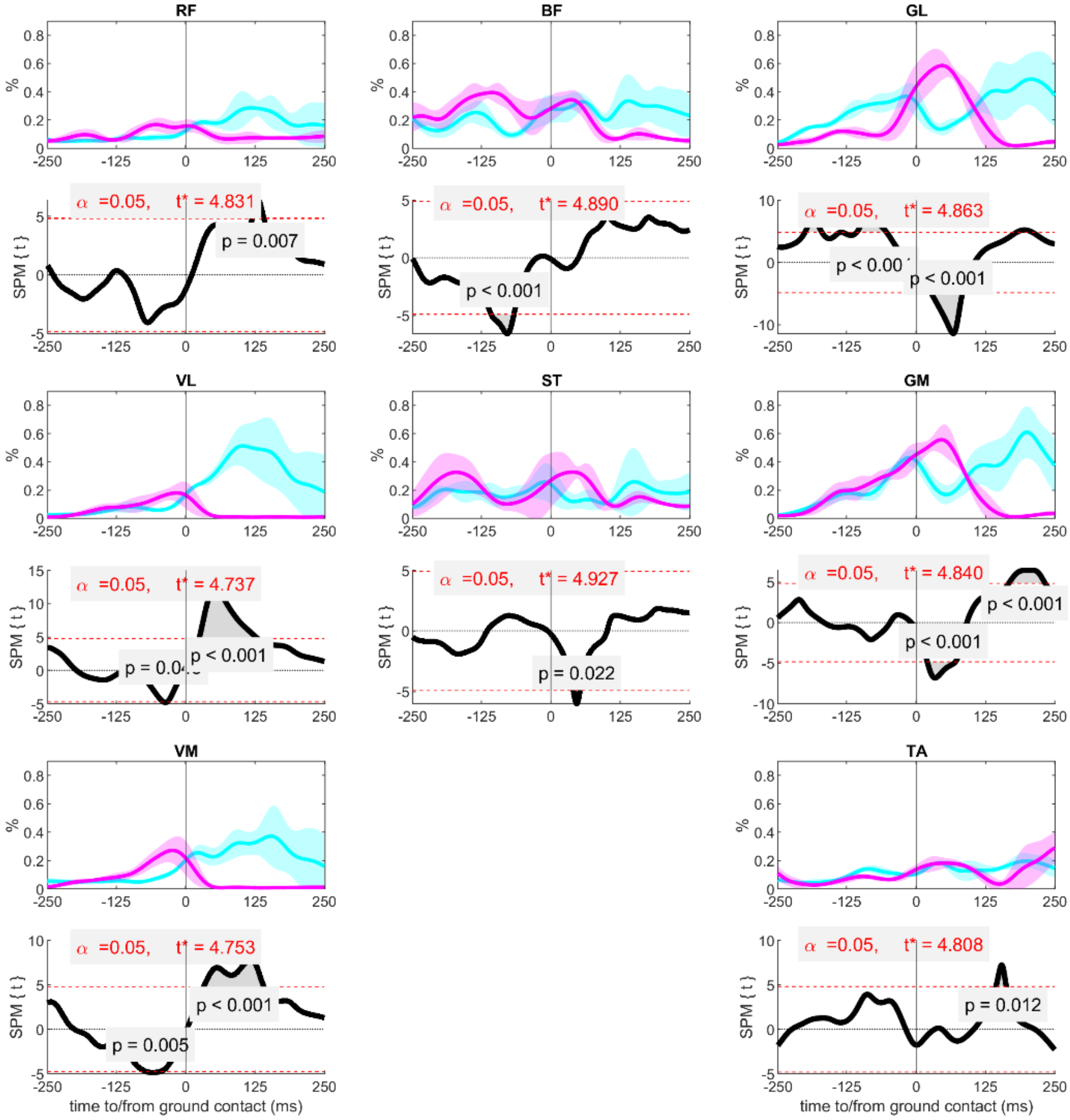


Subject: 10

Number of CoDs: 26

Running speed: 2.6 m/s

%HRmax: 97.3%

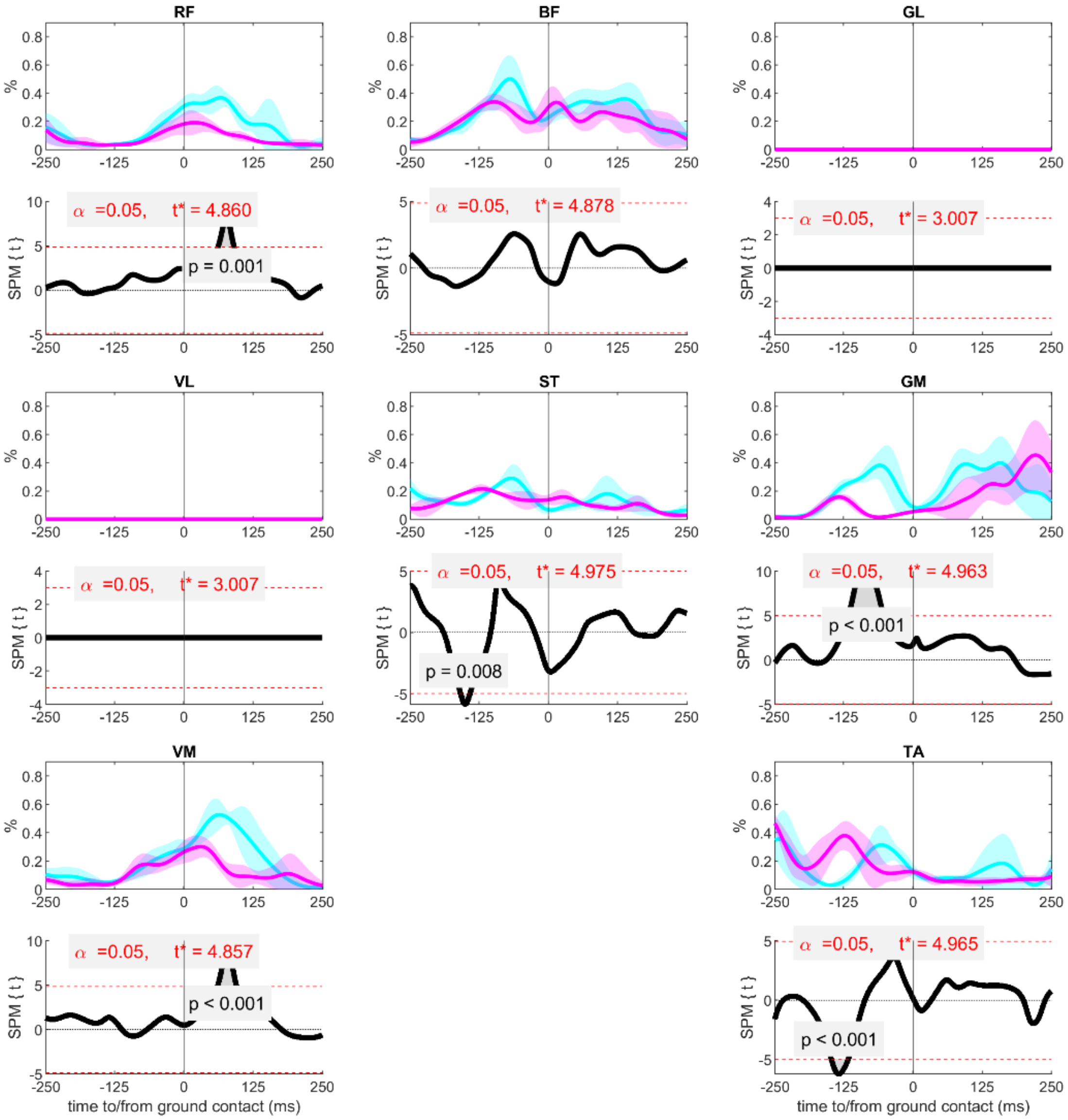


Subject: 11

Number of CoDs: 28

Running speed: 2.5 m/s

%HRmax: 91.1%

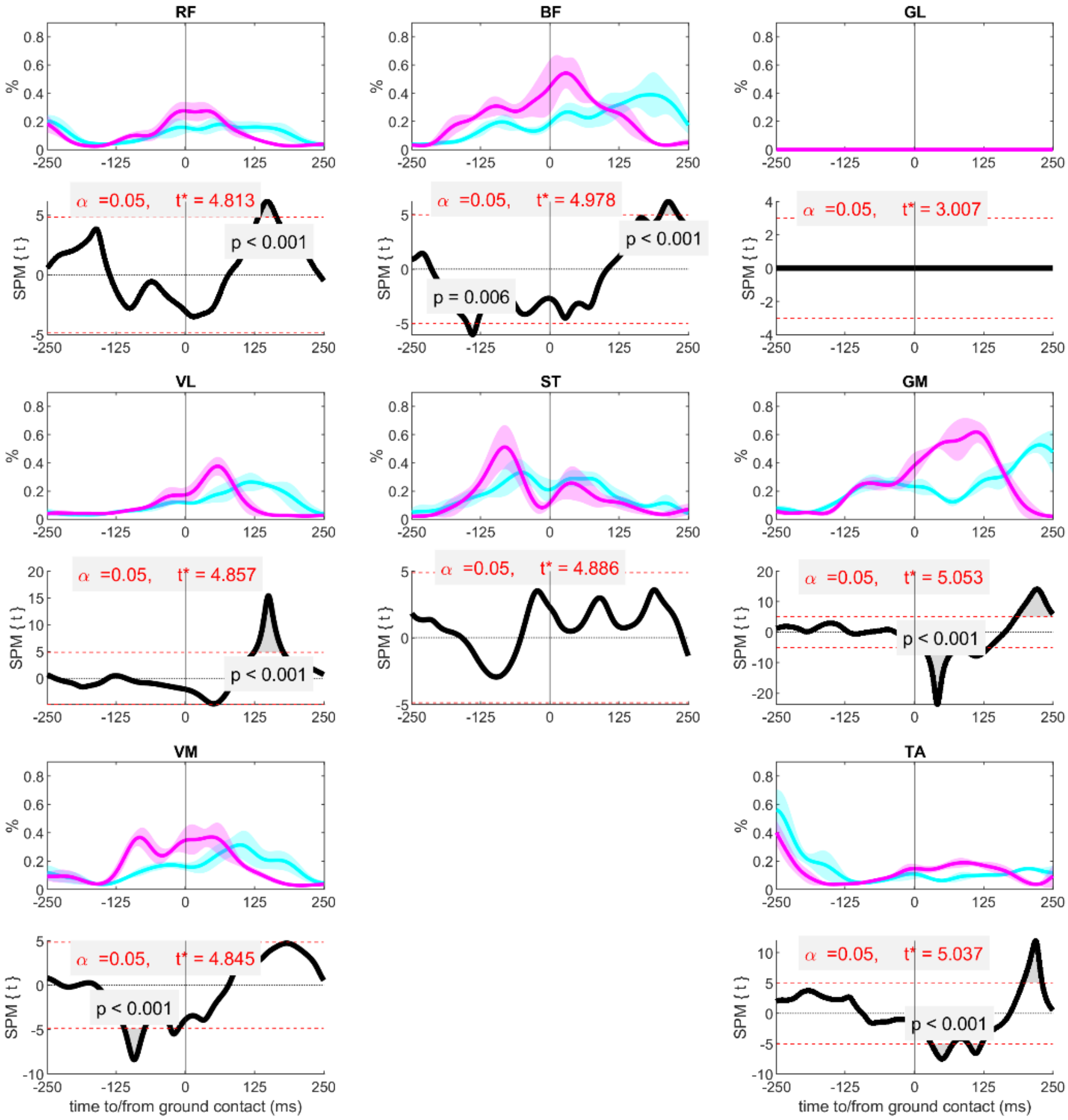


Subject: 12

Number of CoDs: 26

Running speed: 3.6 m/s

%HRmax: 92.4%

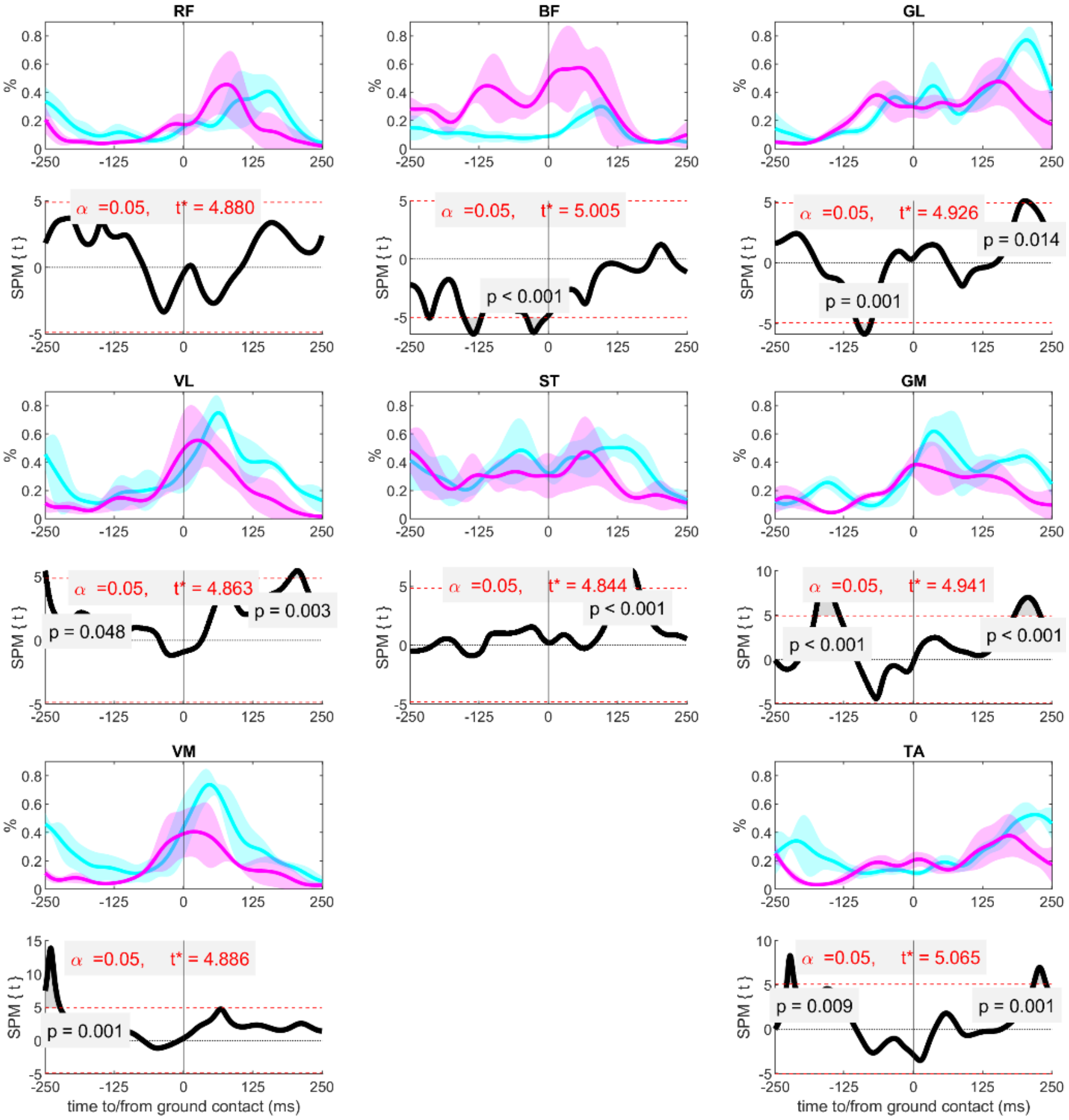


Subject: 13

Number of CoDs: 38

Running speed: 3.3 m/s

%HRmax: 94.3%

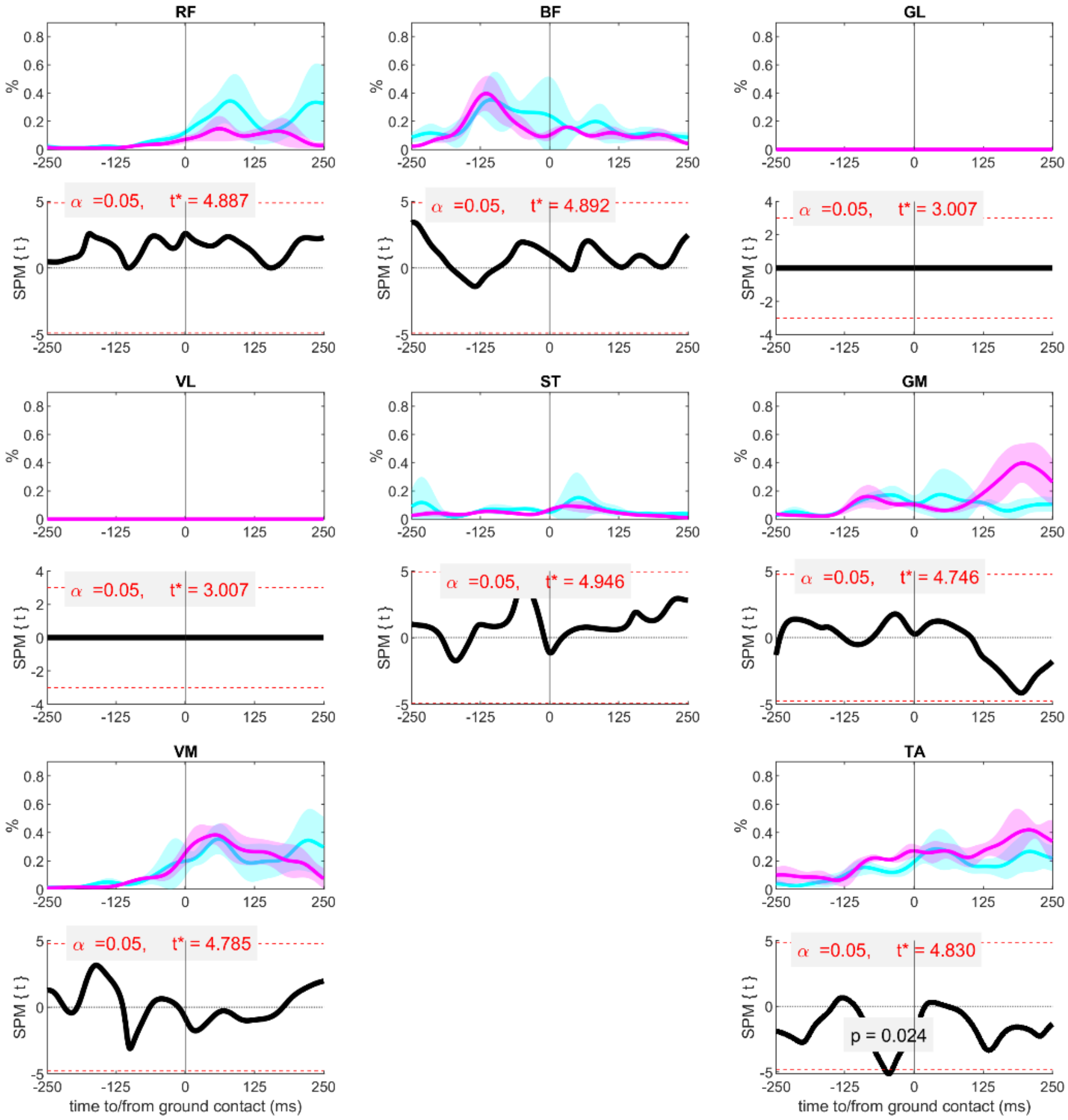


Subject: 14

Number of CoDs: 34

Running speed: 2.9 m/s

%HRmax: 92.8%

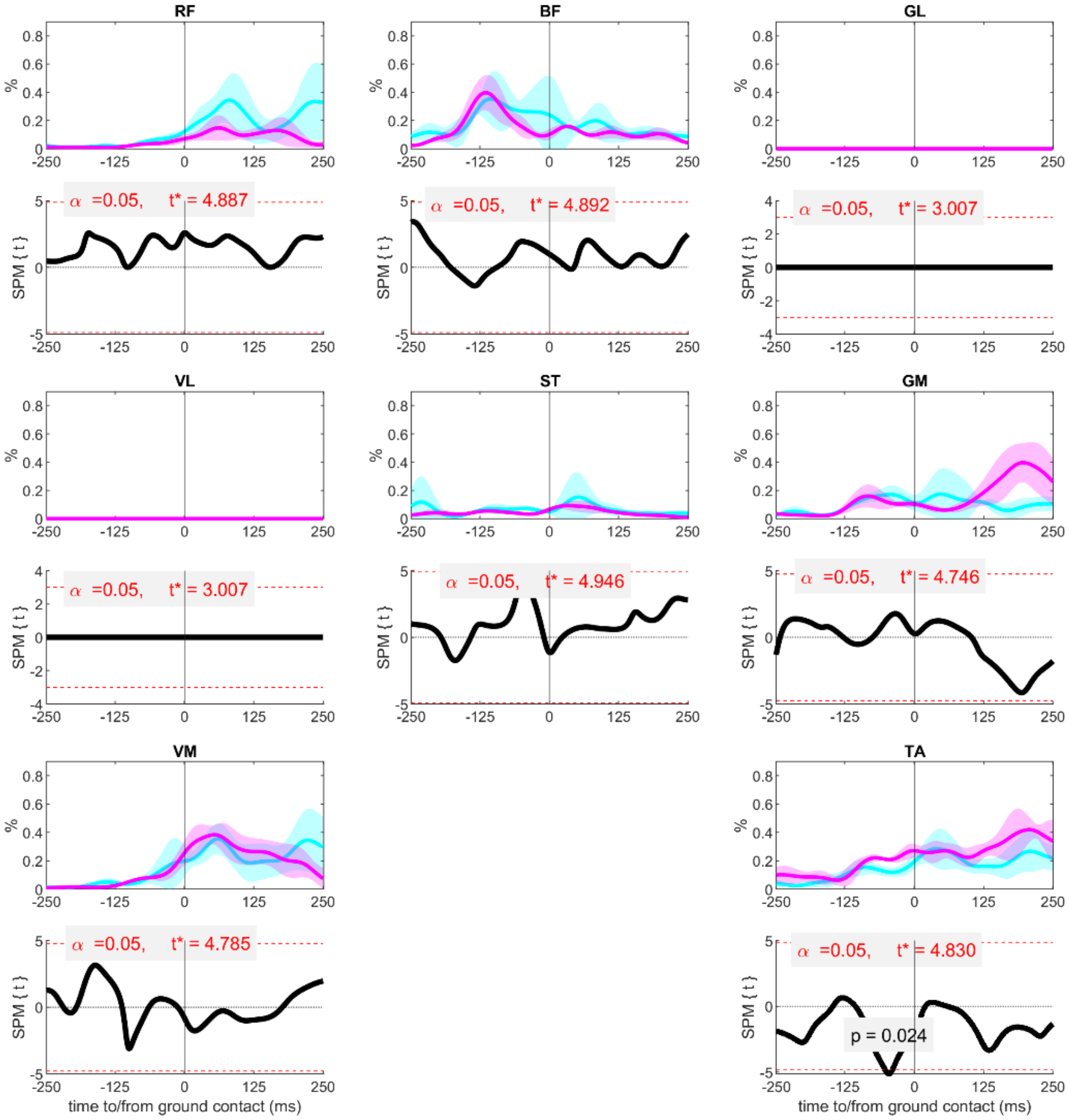


Subject: 15

Number of CoDs: 15

Running speed: 2.5 m/s

%HRmax: 99.3%

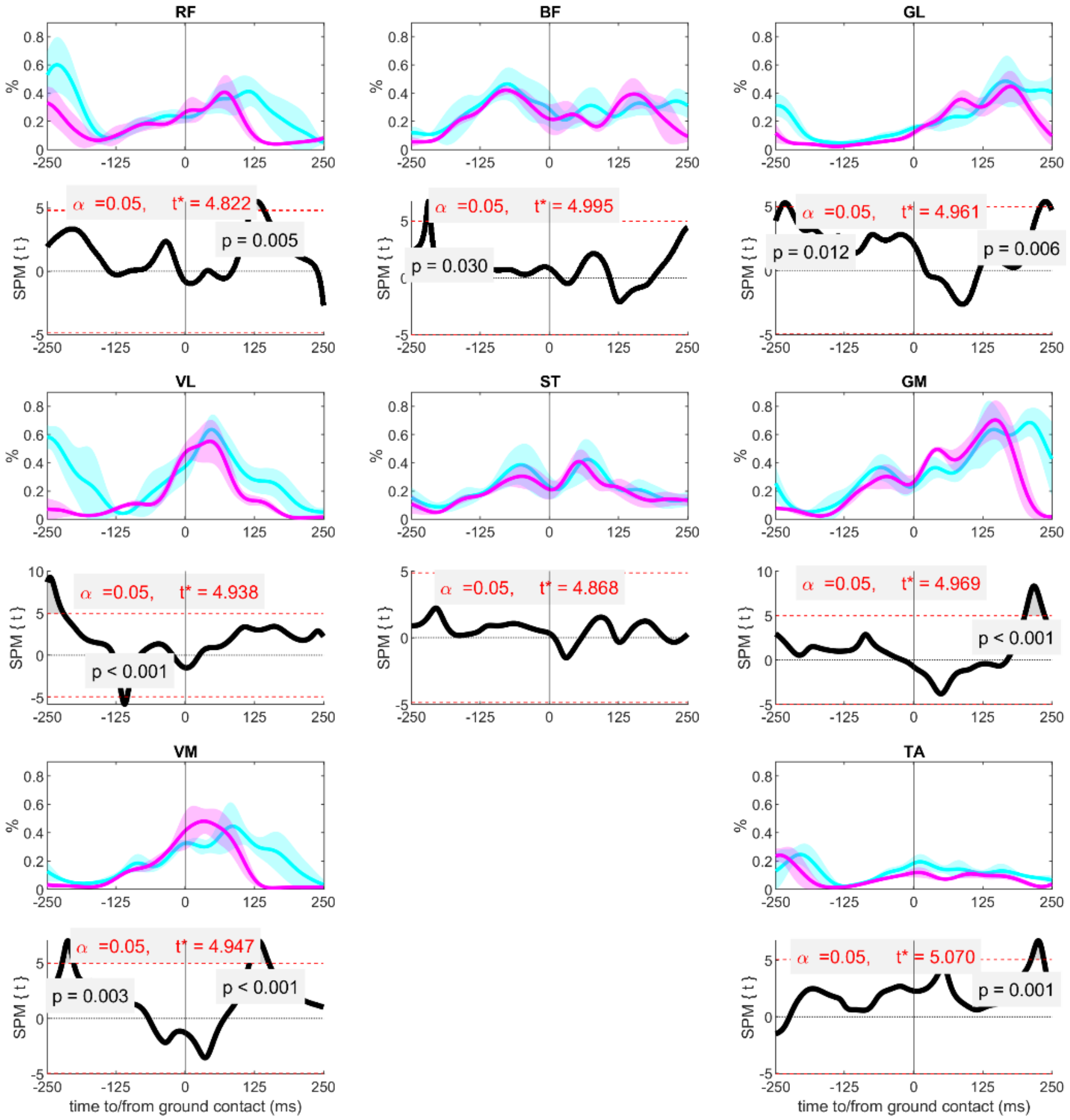


Subject: 16

Number of CoDs: 70

Running speed: 2.5 m/s

%HRmax: 91.8%

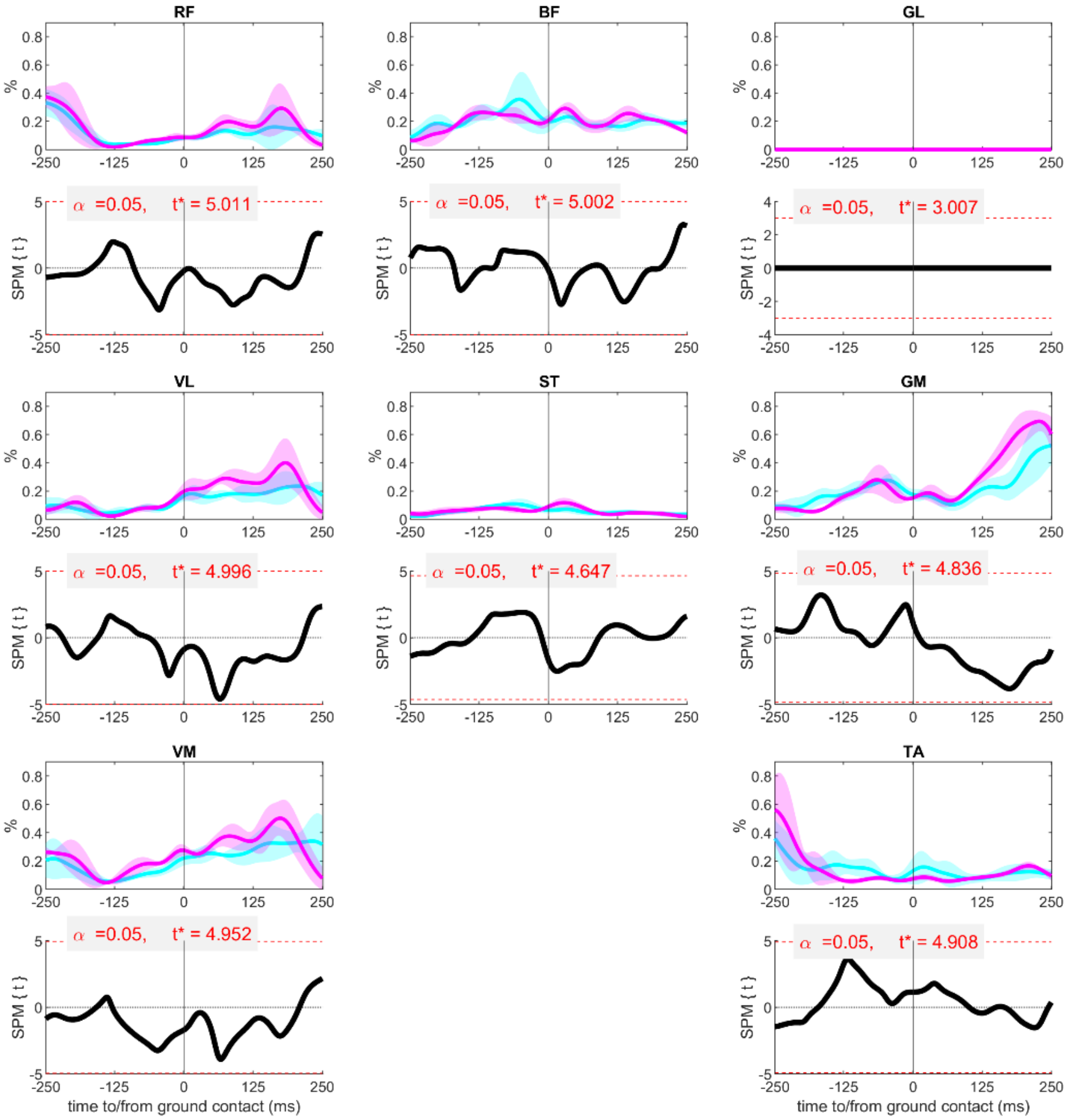


Subject: 17

Number of CoDs: 41

Running speed: 2.6 m/s

%HRmax: 96.4%

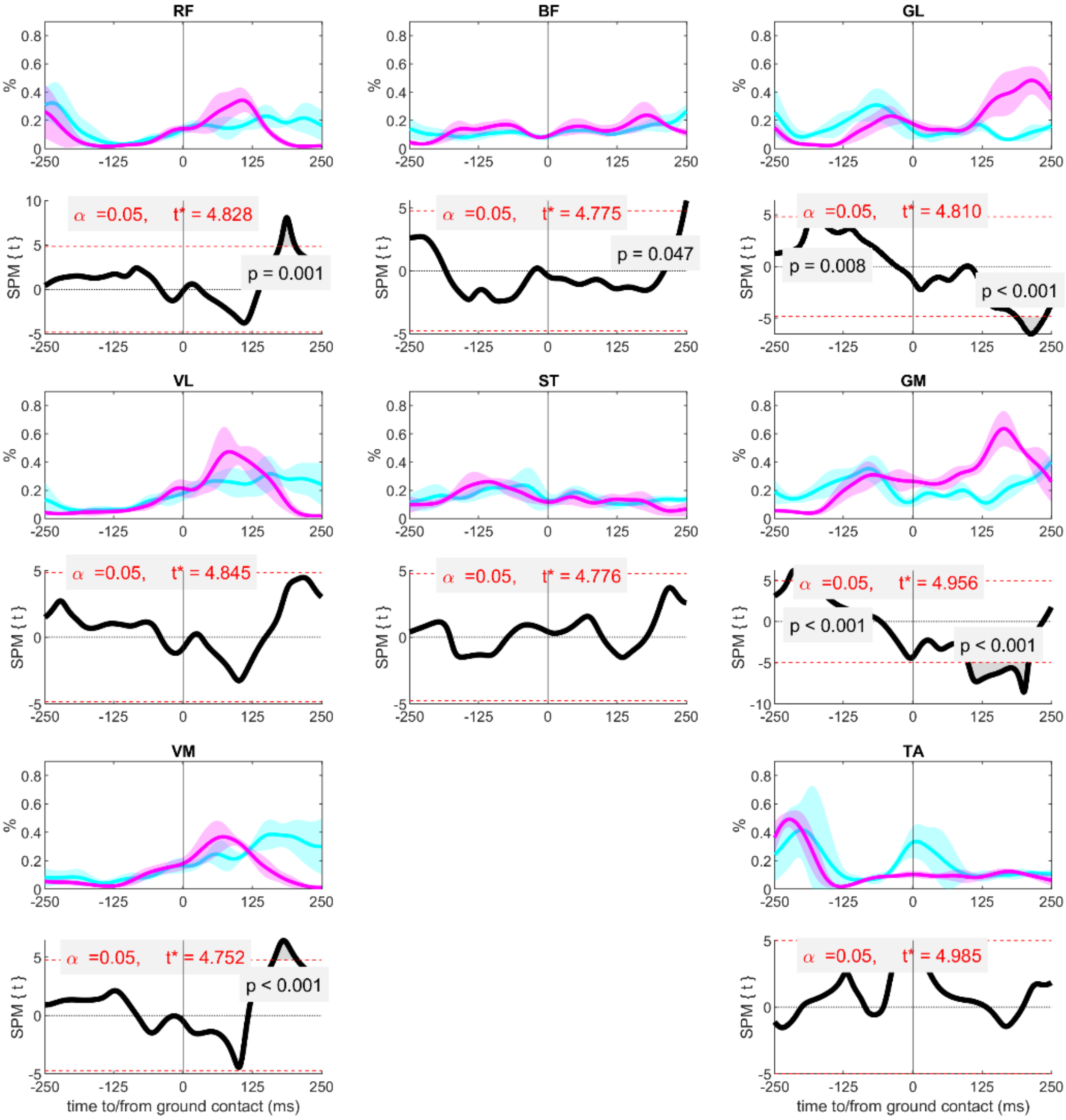


Subject: 18

Number of CoDs: 29

Running speed: 2.6 m/s

%HRmax: 98.4%



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