# LITHUANIAN SPORTS UNIVERSITY FACULTY OF SPORT BIOMEDICINE INTERNATIONAL MASTER IN PERFORMANCE ANALYSIS OF SPORT

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# MORPHO-MECHANICAL PROFILE CHARACTERIZATION OF COMPETITIVE CYCLISTS AND RUNNERS: RELATIONSHIPS WITH NEUROMUSCULAR FUNCTIONS AND SPRINT PERFORMANCE

FINAL MASTER'S THESIS

Scientific Supervisor:\_\_\_\_\_

Final thesis Supervisor recommends/ does not recommend the final thesis be assessed

Evaluation of the final thesis:

Secretary of the Assessment Committee:

KAUNAS 2021

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## ABBREVIATIONS

- ARC anaerobic running capacity
- CP critical power
- CSA cross-sectional area
- CV critical velocity
- FI fatigue index
- FL fascicle length
- H hamstring muscles
- MISS maximal intensity sprint session
- MT muscle thickness
- MTU muscle-tendon unit
- MVC maximal voluntary contraction
- MVIC maximal voluntary isometric contraction
- $MVIC_{5s}$  average torque during a 5s maximal voluntary contraction
- PA pennation angle
- PO<sub>15s</sub> average power 15s
- PPO peak power output
- Q quadricep muscles
- RPE rate of perceived exertion
- RTD rate of torque development
- SSC stretch-shortening cycle
- TL tendon length
- tPP time to peak power
- TT tendon thickness
- W' power duration curve curvature constant

## ABSTRACT

Different adaptive paths seem to delineate athletes' musculoskeletal morpho-mechanical profile and its functional performance implications. However, is not clear to which extent, chronic exposition to a specific exercise may determine morpho-mechanical differences. The aims of the present study were thus to characterize the morpho-mechanical profile of competitive cyclists and runners, to analyze the relationships between morpho-mechanical variables, neuromuscular and sprint performance, and to evaluate the performance decay associated with a maximal-intensity-sprintsession. Twenty-seven male subjects (competitive cyclists n=16, and runners n=11) voluntary participated to the study. After a vastus lateralis and patellar tendon musculoskeletal ultrasound evaluation and a knee extensors' muscle-tendon-unit (MTU) passive stiffness assessment, the subjects performed a knee extensors and flexors' maximal-voluntary-isometric-contraction test (3x5s, 60s recovery) before and after a session of 4x15s Wingate-tests. Cyclists displayed greater vastus lateralis pennation angle (PA) and thickness (MT) (p<0.05), greater patellar tendon CSA (p<0.05) and MTUs stiffness (p < 0.05). Significant differences emerged for knee extensors' neuromuscular functions (p < 0.05), while no differences were observed for the knee flexors. The vastus lateralis PA and the MTUs stiffness represented the main neuromuscular and sprint performance predictors for cyclists, while FL and MT the main for runners. Significant differences emerged from pre-to-post sprint-session (p < 0.05) while no interaction effect was observed between performance decay and discipline. Overall, the morpho-mechanical profile resulted to be different between competitive cyclists and runners, with cyclists taking advantage from greater PA and stiffer MTU compared to runners where the MT seem to better explain the maximal and explosive neuromuscular performance.

Keywords: performance analysis, muscle architecture, muscle-tendon unit, strength.

## SANTRAUKA

Tyrimo tikslas nustatyti ilgalaikio skirtingo treniruočių krūvio poveikį raumenų ir sausgyslių morfologinėms ir biomechaninėms savybėms ir įvertinti morfo-mechaninių savybių įtaką raumenų jėgos, galingumo ir darbingumo charakteristikoms. Uždaviniai: nustatyti dviratininkų ir bėgikų šoninio plačiojo raumens ir girnelės raiščio morfologinius, kelio tiesėjų pasyviųjų struktūrinių elementų standumo ir kelių tiesėjų ir lenkėjų jėgos ir jėgos augimo greičio skirtumus; įvertinti morfomechaninių kelio tiesėjų savybių įtaką kelio tiesėjų susitraukimo jėgai ir jėgos augimo greičiui; įvertinti morfo-mechaninių kelio tiesėjų savybių įtaką sportininkų galios ir darbingumo rodikliams atliekant maksimalaus intensyvumo pedaliavimo testa. Tyrime savanoriškai dalyvavo dvidešimt septyni tiriamieji vyrai (16 didelio ir vidutinio meistriškumo dviratininkų ir 11 vidutinio meistriškumo bėgikų). Šoninio plačiojo raumens ir kelio girnelės sausgyslės morfologiniai rodikliai buvo nustatomi echoskopijos metodu. Izokinetiniu dinamometru Biodex buvo išmatuotas kelių tiesėjų ir lenkėjų jėgos momentas maksimalaus izometrinio raumens susitraukimo metu ir atliktas keturgalvio raumens pasyvaus tempimo testas įvertinti kelio tiesėjų standumą. Maksimalaus intensyvumo pedaliavimo testas (4x15s) atliktas Monark veloergometru. Dviratininkų šoninio plačiojo raumens skaidulu krypties kampas, raumens storis, kelio girnelės sausgyslės skerspjūvio plotas ir kelio tiesėjų standumas buvo reikšmingai didesnis (p < 0.05) už bėgikų. Kelio tiesėjų jėgos ir jėgos augimo greitis turėjo reikšmingą sąsają su dviratininkų šoninio plačiojo raumens skaidulų krypties kampu ir pasyvių kelio tiesėjų standumu. Bėgikų jėgos rodikliai turėjo reikšmingą sąsają su šoninio plačiojo raumens storiu. Tiriamųjų specializacija galios ir darbingumo rodiklių pokyčiams atliekant maksimalaus intensyvumo pedaliavimo testa reikšmingos įtakos neturėjo. Apibendrinant, tyrimas parodė, kad raumenų ir sausgyslių biomechaninė adaptacija, morfo-mechaninės raumenų ir sausgyslių savybių sąsaja su raumenų jėgos ir jėgos augimo greičio rodikliais priklauso nuo ilgalaikio mechaninio krūvio ir sporto šakos specifikos.

**Raktiniai žodžiai**: *sportinio našumo analizė, raumenų architektūra, raumens ir sausgyslės vienetas, jėga.* 

#### **INTRODUCTION**

High-level physical performance is determined by the interaction of a wide range of factors, traditionally described as internal (i.e., physical capacity to produce work), and external (i.e., performance demands) (Joyner and Coyle, 2008; Jeukendrup and Martin, 2001). Among the internal factors, the musculoskeletal morphological (e.g., muscle and tendon architecture/dimensions), and biomechanical (e.g., muscle-tendon unit stiffness) profile has been considered as a performance descriptor, and in some cases, predictor (Kubo et al., 2011; Enomoto, Oda and Kaga, 2019, Kordi et al., 2020; Lee et al., 2021). Early observations, supported by theoretical frameworks, seem to underline specific adaptive paths arising from different exercise stimuli (e.g., mechanical load, movement patterns, exercise velocities), culminating in distinct morpho-mechanical properties and consequent performance implications (Franchi, Reeves and Narici, 2017). One explanatory hypothesis behind such differences, rely on the chronic exposition to specific mechanical loads and exercise specificities (Wilson et al., 1994; Bohm, Mersmann and Arampatzis, 2015; Franchi, Reeves and Narici, 2017). Possible differences in the skeletal muscle properties seem thus to depict, from one side the result of chronic exposition to specific mechanical stimuli, and on the other a functional profile that reflect the athletes' need in terms of performance (Franchi, Reeves and Narici, 2017). This is partly confirmed by interventional studies, in which different training protocols determined well-defined skeletal muscle architectural and biomechanical adaptations (Blazevich et al., 2003; Fouré et al., 2011; Kubo et al., 2006; Fouré et al., 2013; Morrissey et al., 2011), and by observational studies in which athletes belonging to a particular discipline or exposed chronically to a specific exercise, displayed specific morpho-mechanical profiles and singular relationships with performance markers (e.g., neuromuscular functions and sprint performance) (Kubo et al., 2011; Kordi et al., 2018; Enomoto, Oda and Kaga, 2019, Kordi et al., 2020; Lee et al., 2021; Kordi et al., 2021). Among the different sport disciplines, chronic exposition to cycling, as a concentric contraction predominant exercise, and running, characterized by the stretch-shortening cycles, may thus represent a valid model to analyze the possible differences in skeletal muscle profile and its performance implications (Kubo et al., 2011; Enomoto, Oda and Kaga, 2019, Kordi et al., 2020; Lee et al., 2021). However, although the theoretical frameworks and encouraging early observations, data are still discordant, and few or no studies have considered, in the same experiment, different athletic populations exposed chronically to distinct stimuli as cycling and running. A deeper characterization and understanding of the functional consequences of such processes, may support evidence-based approaches to target specific functional adaptations, and underpin training prescription procedures from athletes' staff and sport science practitioners.

Thus, the aim of the present study was to evaluate whether chronic exposition to different stimuli (cycling vs. running exercise) may reflect differences in the morpho-mechanical profile, and consequently, on performance markers.

The main objectives of the present investigation were:

- 1. To characterize and compare the morpho-mechanical profile of competitive, wellexperienced, cyclist and runner populations.
- 2. To explore the relationships between skeletal muscle morpho-mechanical variables, neuromuscular functions, and sprint performance, in cycling and runner populations.
- 3. To evaluate the impact of the discipline, and thus of the different morpho-mechanical profile, on performance decay dynamics induced by a maximal intensity sprint session.

Accordingly, the hypothesis, based on previous studies (observations in the two-population taken singularly), and theoretical frameworks (e.g., impact of mechanical load and contraction mode on musculoskeletal remodeling processes), is that cyclists may display a different morpho-mechanical profile compared to runners and that it may account differently in explaining athletes' performance.

#### **1. LITERATURE REVIEW**

The relationships between the skeletal muscle structure and its functions have been matter of research from a physiological, anatomical, and biomechanical point of view for long time (Narici, Franchi and Maganaris, 2016). Advances in these disciplines brought out new insights and defined the different fields in which a deeper knowledge of the musculoskeletal structural profile and its functional implications could be crucial. Among them, high-level sport performance, characterized by maximal physical expressions, represent a peculiar context that has been object of interest from both, skeletal muscle structure characterization, and evaluation of its functional implications on performance (Kubo et al., 2011; Watsford et al., 2010; Enomoto, Oda and Kaga, 2019; Kordi et al., 2020). Recent evidence seems to suggest how chronic exposition to mechanical load, muscle contraction mode, overloading or unloading, and more in general, an altered pattern of use of the skeletal muscle, may determine not only changes in muscle mass, but also its architecture and biomechanical properties (Narici, Franchi and Maganaris, 2016; Franchi, Reeves and Narici, 2017). Indeed, this is partly confirmed by interventional studies, in which different training protocols determined specific skeletal muscle architectural and biomechanical adaptations (Blazevich et al., 2003; Fouré et al., 2011; Kubo et al., 2006; Fouré et al., 2013; Morrissey et al., 2011), and by observational studies in which athletes' belonging to a particular discipline or exposed chronically to a specific exercise, displayed a definite morpho-mechanical profile and singular relationships with performance markers (Kubo et al., 2011; Kordi et al., 2018; Enomoto, Oda and Kaga, 2019, Kordi et al., 2020; Lee et al., 2021; Kordi et al., 2021). However, the mechanisms triggering skeletal muscle remodeling processes, determining the musculoskeletal profile and its functional implications remain unclear and still object of study.

#### 1.1 Musculoskeletal remodeling in response to different stimuli: theoretical frameworks

Different hypotheses, based on empirical observations and theoretical frameworks, have been raised to explain the differences in the muscle-tendon unit (MTU) morphological (fascicle length, pennation angle, muscle thickness) and mechanical (elongation-force relationship of the tendon and aponeurosis, muscle strength) profile observed in response to different stimuli and physical conditions. Animal experiments performed in the second half of the 20<sup>th</sup> century, firstly described the great skeletal muscle plasticity in response to regimes of overloading and unloading (Williams and Goldspink, 1971 and 1973; Tabary et al., 1972; Goldspink, 1985). In particular, early observations suggested how sarcomeres in series and in parallel can be either added or removed according to the conditions of chronic loading or unloading. This architectural remodeling process seemed to be related to the ability of the muscle to sense mechanical signals (change in tension) and convert these stimuli into biochemical events (mechanotransduction), regulating myofibrillar protein synthesis, and assembly of sarcomeres (Narici, Franchi and Maganaris, 2016; Agon et al., 2019). Costameres, and in particular the focal adhesion complexes, are considered the regions of the skeletal muscle specialized in mechanotransduction. In particular, within the focal adhesion complexes are cellsurface receptors known as integrins, connecting the ECM to the sarcomere through a chain of cytoskeletal proteins (Peter et al., 2011). Among the integrin associated factors, the focal adhesion kinase (FAK) has been shown to be highly sensitive to changes in mechanical load and recognized as an upstream modulator of the mechano-sensory pathway of p70S6K, that in parallel with the AktmTor signaling, represent key regulators of protein synthesis (Flück et al., 1999; Klossner et al., 2009). This, coupled with the evidence supporting the neural and muscular adaptations (i.e., increases in EMG activity, mRNA regulatory mechanisms leading to increased protein synthesis and proliferation of muscle satellite cells) driven by mechanical overload, seem to represent one main explanatory trigger of the musculoskeletal structural remodeling (Schiaffino et al., 2021) (figure 1).

Following a period of mechanical loading, also tendons are subjected to structural changes, as increases in stiffness and/or dimensions (Bohm, Mersmann and Arampatzis, 2015). Two mechanisms could account for an increase in tendon stiffness: i) changes in tendon material (i.e., increase of Young's modulus), and ii) changes of tendon morphological properties (i.e., thickness, length, or cross-sectional area) (Arampatzis et al., 2007; Kongsgaard et al., 2007; Seynnes et al., 2009; Arampatzis et al., 2010). Both, tendon material and morphological changes, may result from an increase in collagen synthesis but also from changes in collagen fibril morphology, and levels of collagen molecular cross-linking (Kjaer et al., 2009; Heinemeier and Kjaer, 2011). Kubo et al. (2001) first reported an increase in stiffness and Young's modulus of the patellar tendon, in humans following 12 weeks of isometric training. Region specific hypertrophy have been later observed on patellar and Achilles' tendons in the interventions performed in 2007 by Kongsgaard et al., and Arampatzis et al., respectively. Different studies also proven that adaptations in the passive MTU biomechanical properties are specific not only to the amount, but rather to the characteristics of the loading imposed (Blazevich, 2018). This, suggest possible alterations in the adaptive response to different patterns of muscle use and exercise specificities, culminating in a different MTU biomechanical profile (Blazevich, 2018). However, despise the significant research effort afforded to understand the effects of altered physical activity patterns and different exercise specificities, the mechanisms underpinning the adaptive path driving the MTU structural remodeling remain object of study.

Thus, although the evidence is consistent in describing the hypertrophic effect resulting from mechanical overloading, including the role of the mechanotransduction signaling, less is known regarding the exact features of the muscle-tendon unit remodeling processes linked to it. Indeed, the musculoskeletal structural remodeling appear to be not only driven by the amount of load but rather specific to the exercise specificities and the way this load is applied to the joint (e.g., movement patterns, exercise velocity, contraction mode). This have been observed either in interventional studies where different training strategies displayed different architectural (increase or decrease in FL and/or PA) and biomechanical properties adaptations (increase or decrease of the MTU stiffness) (Blazevich et al., 2003; Fouré et al., 2011; Kubo et al., 2006; Fouré et al., 2013; Morrissey et al., 2011), or in observational studies where populations exposed chronically to different disciplines (e.g., sprint vs. endurance running) were characterized by a different morpho-mechanical profile (Kubo et al., 2011; Kordi et al., 2018; Enomoto, Oda and Kaga, 2019, Kordi et al., 2020; Lee et al., 2021; Kordi et al., 2021). The increase in PA seems, for example, to be a remodeling consequence of concentric contraction loading and a functional adaptation arising from exercise conditions in which the athlete task is to develop maximum power in the shortest time against high loads (Franchi, Reeves and Narici, 2017; Kordi et al., 2020). Indeed, increases in PA, are thought to improve the force-generating capacity of a muscle by allowing a greater muscle mass to attach to a given tendon area (Kawakami, Abe and Fukunaga, 1985). On the other hand, changes in fiber length (FL) may influence the force production capacity of the muscle, largely because longer fibers contract at higher velocities than shorter and have been associated with eccentric load (Sacks and Roy, 1982; Akagi, Hinks and Power, 2020). Indeed, FL resulted to be greater in élite sprint runners compared with non-élite and endurance runners in the study of Abe, Kumagai and Brechue (2000), and of Kumagai et al. (2000). It is possible therefore that the training specificities, coupled to the amount of load, represent a trigger for musculoskeletal architectural remodeling. Indeed, one of the most accredited explanation behind the possible differences in the adaptative path arising from different exercises or training protocols, rely on the prevalence of muscle contraction mode (i.e., eccentric, concentric, isometric). Early observations by Wilson et al. (1994) firstly suggested how different muscle contraction features may induce different musculotendinous mechanical properties adaptations. As previously mentioned, more recent observations reported how concentric, differently from eccentric, contractions may promote a greater change (increase) in pennation angle (PA) reflecting a hypertrophy induced parallel

addition of sarcomeres, compared to the pronounced fascicle length (FL) increase (addition of serial sarcomeres) widely observed under eccentric contractions (Franchi, Reeves and Narici, 2017).

Taken together this seem to partially explain the differences observed in distinct athletic population or from different training interventions and suggesting a contraction-specific muscular remodeling and structural adaptation that can consequently determine the muscle functional properties (e.g., cycling vs. running populations, or sprinters vs. endurance athletes in the same discipline). However, to date, investigations failed to find any difference in metabolic response and protein synthesis signaling pathways arising from concentric vs eccentric contractions (Franchi, Reeves and Narici, 2017). Rather, has been suggested how during muscular structural remodeling, assembly of sarcomeres and so, architectural adaptations, may be independent of the quantity of new contractile material synthetized, and thus suggesting the existence of alternative mechanisms governing architectural remodeling. In this sense, other research focused on satellite cells activity to explain the arising point of these different morphological adaptations to different contractions and exercise specificities (Figure 1). Greater activity of satellite cells has been observed after single bouts of eccentric vs concentric exercise (Hyldahl et al., 2014) while chronic exposition to only concentric led to an increase in satellite cells pool (Farup et al., 2014). Different contractions may thus induce different satellite cells modulations. This may be linked with the observations of Fry et al. (2017) in which has been reported how the activation by mechanical overload of myogenic progenitor cells proliferation and the release of miRNA-containing exosomes into the extra cellular matrix niche (collagens, glycoproteins, proteoglycans, and elastin) may determine a reduction in collagen mRNA expression in fibrogenic cells, and thus, to regulate ECM deposition during the MTU complex structural remodeling. Thus, the cross-talk between mechanical stimuli, satellite cell activation and extracellular matrix remodeling could be differently modulated by concentric vs eccentric contractions, potentially determining different structural adaptations. Kostek et al. (2007), evaluated the differences in modulation of gene expression after eccentric vs. concentric resistance training, observing how fifty-one genes seemed to be differently expressed, genes mostly related with protein turnover, cellular stress, and sarcolemmal-structural remodeling, and suggesting that concentric and eccentric contractions and/or training modalities may trigger distinct and unique gene expression networks. Kubo, Yata and Tsunoda (2013) reported in their experiment how tendons' mechanical properties seem also to be explained by gene polymorphism as well as Flück et al. (2019) recently observed the role of gene polymorphisms in specifically trained (i.e., endurance and power) high level athletes. Thus, athletes' skeletal muscle morpho-mechanical properties may be the result of a combination of genetically and epigenetically determined factors, with specific exercise exposure and amount of exposure playing a pivotal role in the characterization of one to another feature.

The possible differences in the skeletal muscle properties of different athletic populations seem thus to depict, from one side the result of chronic exposition to a specific exercise stimulus and on the other a functional profile that reflect the athletes' need in terms of performance efficiency. Taken together, the evidence seems to suggest how the combination of the amount of mechanical load and the specific way by which the load is delivered to the MTU complex may determine the morpho-mechanical profile. However, no previous studies explored, in the same experimental conditions, morpho-mechanical characteristics involving populations exposed chronically to different mechanical load specificities. Among the different sport disciplines, chronic exposition to cycling, as a concentric contraction predominant exercise, and running, characterized by the stretch-shortening cycles, may thus represent a valid model to analyze possible differences in skeletal muscle profile and its performance implications (Kubo et al., 2011; Enomoto, Oda and Kaga, 2019, Kordi et al., 2020; Lee et al., 2021).



**Figure 1.** Different mechanical stimuli (e.g., different contraction mode or exercise specificities) may induce musculoskeletal remodeling adaptive paths through mechanotransduction. Different signaling cascades may originate from the mechanical stimulus and potentially inducing either hypertrophic and structural (i.e., architectural, and mechanical) changes in muscle and tendon tissues. From top to bottom and right side *MPC-dependent ECM remodelling*: satellite cells activated in response to a mechanical stimulus give rise to MPCs within the ECM that surrounds myofibers. This ECM is composed largely of collagens secreted by the interstitial fibrogenic cells as a result of the interactions with the MPCs. In addition, MPCs secrete exosomes containing miR-206 which represses Rrbp1, a master regulator of collagen biosynthesis, to prevent excessive ECM deposition (Fry et al., 2017). Left side from top to bottom, different mechanisms are hypothesized as regulator of MTU remodeling, among them, the TGF $\beta$ -mediated mechanotransduction has been observed as able to induce through different signaling cascades and molecular interactions, tenocyte differentiation and morphogenesis (Maeda et al., 2011; Subramanian et al., 2018). *Notes: MPS: muscle protein synthesis; EIMD: exercise-induced muscle damage; ECM: extra-cellular matrix; MPC: myogenic progenitor cells*.

#### Morpho-mechanical profile in running and cycling populations: an overview

Different studies, in the last two decades, aimed to characterize the musculoskeletal, morphological and/or biomechanical profile, of cyclists or runners and its possible relationship with performance. The stretch-shortening cycles (SSC) in running, determine and also require morphomechanical properties able to optimize the repeated lengthening and shortening actions of the MTU complex. In particular, longer muscle fascicles can exhibit higher shortening velocities and mechanical powers than shorter fascicles, as well as a muscle with a greater thickness is expected to exert a larger force on the ground and a consequent increased acceleration capacity (De Haan, Huijing and Vliet, 2003). Furthermore, the non-rigidity of the tendon and aponeurosis allows the muscle fibers to contract a lower shortening velocity than the MTU as a whole (Ettema et al., 1990) and, as a consequence of the force-velocity relationship, their force-generating potential will be higher (Bobbert, 2001; Hof, van Zandwijk and Bobbert, 2002). In addition, due to the non-rigidity (or compliance) of the tendon and aponeurosis, when the MTU is elongated, strain energy can be stored. In this way, all of the energy delivered during the shortening of the MTU can be enhanced, explaining the functionality of the musculoskeletal morphological and biomechanical specific properties (Alexander and Bennet-Clark, 1977; Bobbert, 2001). Observational studies, sustaining previous assumptions, reported how an increased MT and more compliant tendon structures of the lower limbs are characteristics of élite compared to recreational runners, and also how they can be explanatory factors of running performance, especially sprint (Kubo et al., 2011; Enomoto, Oda and Kaga, 2019; Stafilidis and Arampatzis, 2006). Abe, Kumagai and Brechue (2000) observed how sprint runners (100m) display a greater fascicle length (FL) and lesser pennation angle (PA) in vastus lateralis muscle compared to distance runners (5000-m) and how this would appear to favor shortening velocity as required for greater running speed. The importance of muscle morphology is further confirmed by the recent investigation of Monte and Zamparo (2019), in which emerged significant relationships between runners MT, FL and performance. An additional aspect to consider when comparing the stimuli to which runners are subjected than cyclists, is the joint loads, and in particular the knee joint, as a possible contributor to differences in the musculoskeletal morpho-mechanical profile. Indeed, differently from cycling, running is a weight-bearing exercise in which the athlete' lower limbs have freedom of motion, and where ground reaction forces represent the main joints load source and knee loads are believed to be higher compared to cycling exercise (Kutzner et al., 2012). Running technique and runner's characteristics will then determine how and to which extent the ground reaction forces will impact on knee joint (Lenhart et al., 2014; Maniar et al., 2018). In addition, as later described for cycling, increased running workload (i.e., speed or additional external load) result in higher in peak knee joint moments and dictating different morpho-mechanical adaptations (De David, Carpes and Stefanyshyn, 2014; Kujawa et al., 2020).

Taken together, the observational reports seem to support theoretical frameworks, in which from one side the SSCs may display for increased FL and more compliant MTU complexes, in order to increase muscle shortening velocity and to favor the storage and/or reuse of elastic energy. More in detail, what seem to be more clearly suggested is how MTU complexes for runners need to be stiff enough to cope with the high loads (i.e., ground reaction forces, knee and ankle joint loads, and SSCs contractions), but compliant enough to absorb and release energy.

Differently from running, cycling exercise is characterized by a mostly concentric and, in minimal part quasi-isometric muscle contraction mode. In addition, instead of facing the ground reaction forces, cyclists should face in this case the high loads displayed by the big gears used during cycling competitions and in which the ability to accelerate, reach a high power, and maintain it, represent the critical performance aspects (Kordi et al., 2021). In this sense, has been hypothesized how an increased PA, associated with an improvement of force output generation for concentric and/or isometric contractions against high loads (Kawakami, Abe and Fukunaga, 1985) may be more

advantageous compared to a greater FL. Accordingly, Kordi et al. (2020) observed a significant positive relationship between increased PA (vastus lateralis) and peak power output (PPO), while, contrary to what previously described for sprint running no correlations with FL emerged. More recently, Lee et al. (2021) confirmed similar observations with PA described, along with MT, as a potential performance predictor of cycling performance. In addition, has been suggested how, considering the reliance on concentric power and the importance of fast, high torque development during sprinting or transition to sprinting during cycling, a higher stiffness of the primary muscles involved in propulsion may improve the rate of torque development (RTD). The positive relationship between musculo-tendinous stiffness and concentric performance has been observed by Wilson et al. (1994), suggesting how a stiffer MTU may facilitate performances characterized by the prevalence of such contraction by improving the force production capabilities of the contractile component, due to a combination of improved length and rate of shortening (high contraction capacity), and enhancing the initial force transmission (RTD). This may thus result in direct benefits on RTD in a concentric prevalent muscle action exercise as cycling and consequently in higher power production (workability) and overall performance velocity. Indeed, relatively stiffer cyclists displayed superior crank torque development characteristics compared to more compliant ones in the study of Watsford et al, 2010.

As previously mentioned, knee load in cycling differs from running because of the intrinsic characteristics of the two disciplines. During steady state cycling the knee load moment is mainly flexing and counteracted by the knee extensors action. Considering a cycling sprint, the peak patellofemoral and tibiofemoral forces are expected to occur during and immediately after the standing start phases (i.e., quasi-isometric contractions first and concentric then) in which the athletes push has much as possible in order to move their bike equipped with big gears. Compared to other physical activities as walking, steady state cycling seems to display relative low levels of both shear and compressive tibiofemoral forces (Ericson and Nisell, 1986; Kutzner et al., 2012), this makes it for example, a good exercise for early rehabilitation phases from ACL injuries. Higher forces accounting for knee load during cycling, compared to shear and compressive tibiofemoral forces, have been observed on the patellofemoral joint (Ericson and Nisell, 1987). However, increased workload as for track sprints and bike position manipulation, especially extreme bike fitting, may determine marked changes in the amount and distribution of the load applied to the knee. Even if different hypotheses have been raised on the potential factors related to AKP development for cyclists the information about this topic are far than conclusive and no direct relationships with knee load have been described (Bini and Bini, 2018). On the other side, has been suggested how resistancetraining-induced patellar tendon hypertrophy (i.e.,  $+7\pm1\%$  in CSA) may lead to improvements in exercise economy for recreational cyclists (Rønnestad, Hansen, and Raastad, 2012).

Thus, cyclists seem to be exposed to low to moderate knee loads that, however, may become moderate to high at increased workload levels and when extreme bike fitting parameters are chosen. Prolonged exposure to proper workloads leading to functional adaptations, as an increased patellar tendon CSA seem to be positively related with cycling performance. However, considering the lack of information this can only lead to the speculation that training programs targeting such adaptations, in parallel with progressive monitoring, may represent a performance enhancing solution. Due to the increased knee joint load is expectable greater tendon morphological properties in runners compared to cyclists, however, different reports suggest how in this case, the amount of load rather than the specificities of the exercise may determine different adaptations (Bohm, Mersmann and Arampatzis, 2015).

In summary, cycling exercise compared to running display different characteristics and functional demands, this may be reflected in potential differences between the morpho-mechanical of these two populations, as the results of the adaptive paths arising from the distinct loading stimuli characteristics (Figure 2). In addition, the different musculoskeletal morpho-mechanical profile may

provide a functional interface between metabolic and mechanical power specific for the athlete's requirements and adapted to the athlete's tasks. Is thus expectable not only a different morphomechanical profile but also different relationships frameworks with performance markers.

#### 1.2 Performance implications associated with the morpho-mechanical profile

The musculoskeletal remodeling induced by different mechanical load and exercise specificities and culminating in different morpho-mechanical profile, has been also associated distinctly with physical performance markers.

The role of muscle morphology on cycling performance has been described by Coratella et al. (2020), Kordi, Menzies and Simpson (2018), Kordi et al. (2020) and Lee et al., (2021). In particular thigh muscles volume seem to be a primary explanatory factor of cyclists PPO (Corratella et al., 2020; Kordi, Menzies and Simpson, 2018; Kordi et al., 2020; Lee et al., 2021). PA seemed also to represent an explanatory factor for PPO (Corratella et al., 2020; Kordi et al., 2020; Lee et al., 2021), while FL has been related to a lower time to peak power by Corratella et al. (2020). To summarize, muscle architecture seems to be involved in power production dynamics during cycling, in particular sprinting. Both, Corratella et al. (2020) and Kordi et al. (2020), and more recently Lee et al. (2021) found quadriceps femoris muscle as the main predictor of sprint cycling performance as well as both authors found positive, significant relationships between vastus lateralis PA and PPO. Kordi et al. (2021), additionally, recently found positive significant correlations not only between morphological variables and PPO, but also with neuromuscular functions as maximal voluntary peak force (MVC), namely maximal neuromuscular strength, and the RTD, namely maximal explosive neuromuscular strength. Muscle mechanical properties, and in particular, quadriceps femoris MTU complex stiffness has been evaluated and associated with performance in the studies of Watsford et al. (2010) and Ditroilo et al. (2011). In the first intervention (Watsford et al., 2010) the authors divided the cyclists, involved in the study, in a stiff and a compliant group according to the knee extensors' stiffness. The results showed that stiff sprinters display a higher PPO and an increased RTD. Thus, the MTU complex stiffness has been positively associated with sprint performance. In the second study (Ditroilo et al. 2011), the authors further investigated the possible implication of knee extensors' MTU complex stiffness in fatigue accumulation during repeated sprints in cyclists, observing how fatigue may induce a progressive reduction in stiffness and a consequent sprint performance decrement. The authors confirmed the correlations between stiffness, RTD and sprint performance, and underlined the importance of a tailored training program to keep stiff cyclists' muscles during fatiguing conditions (e.g., repeated sprints) in order to better perform in final phases of competitions. Two additional studies evaluated the impact of morpho-mechanical properties on cycling performance, a case study by Klich, Krymski and Kawczyński (2020) and the study of Klich and colleagues (2020) that represent one of the few studies in which are described patellar and quadricep tendon morphology (i.e., thickness) of competitive cyclists. The authors additionally used myotonometry to assess mechanical properties changes before and after track cycling competitions and observed an increase in muscle stiffness after sprints compared to baseline, however without correlations reported by authors with changes in sprint performance. Interestingly, is possible to notice how Ditroilo et al. (2011) described opposite dynamics of changes in stiffness in relation to fatigue accumulation from Klich, Krymski and Kawczyński (2020) and Klich et al. (2020). This may be explained by the different methodological approaches (myotonmetry vs. dynamometry). In addition, the studies of Ditroilo et al. (2011) together with the results of Watsford et al. (2010), described the knee extensors MTU stiffness as a sprint performance determinant, associated with both PPO and RTD. On the other side the studies of Klich described an increase in stiffness as possible overuse injury marker and as a possible detrimental factor for performance. Notably, the studies of Klich compared to the others lacked a direct correlation analysis with athletes' performance, focusing primary on the changes between baseline and post exercise. This, show from one side the lack of data and interventions in this field and, possibly how different methodological approach may lead to difficulties in data comparison and interpretation.

Shifting to running, muscle morphology characterization and its possible relationships with running performance have been investigated by the studies of Abe, Kumagai and Brechue (2000), Abe et al., (2001), Kumagai et al. (2000) and Monte and Zamparo (2019). The results of these studies agree with the observation that increased FL seem to be related to running sprint performance, as a favoring factor for shortening velocity during sprinting. MT seem to be also higher in faster runners compared to slower. However, in the studies of Kubo et al. (2017), Kubo et al. (2020) no differences in muscle architecture emerged between competitive runners and untrained controls, and Stafilidis and Aramptzis (2007) reported no significant differences between muscle morphology characteristics between fast and slow runners. It has been also suggested how an increase in FL, may affect peak force production of a muscle group during maximal contractions and that thus, muscle architecture could have profound influences on neuromuscular functions, neuromuscular fatigue resistance and maximal performance (Akagi et al., 2020). Additionally, Kubo et al. (2000), Kubo et al., (2011) and Stafilidis and Aramptzis (2007) observed how faster runners displayed more compliant knee extensors' MTUs compared to slower runners or untrained subjects. While López Mangini and Fábrica (2016) as well as Paradisis et al. (2019), observed how faster runners displayed greater vertical stiffness. To summarize, fast runners compared to slower, or competitive runners compared to recreational, seem to be characterized by greater muscle FL and reduced PA and, by more compliant knee extensors' MTUs complexes and stiffer plantar flexor MTUs (Kubo et al., 2000; Stafilidis and Aramptzis, 2007; Kubo et al., 2011; Kubo et al. 2017; Kubo et al., 2020). However, is possible to recognize conflictual data, application of different methodological approaches and the needing of additional investigation to better characterize and understand the interplays between these factors and the possible relationships with performance markers.

In addition to neuromuscular functions and sprinting ability, Miura, Endo and Sato (2002) and more recently Kordi, Mensies and Simpson (2018), suggested also how thigh muscles morphomechanical properties seem to represent a main determinant of the curvature constant (W') defined as the muscular capacity to work above the athlete's critical power (CP) or critical velocity (CV), in the power output/velocity-duration relationship model. This hyperbolic relationship is characterized by the asymptote of this hyperbola (CP), representing the oxidative energy turnover workability of the athlete, while the curvature constant of the hyperbola (W'), can be viewed as the muscular store of energy that can be used at exercise intensity performed above the CP (Poole et al., 2016). W' represent thus, the main contributor of high intensity medium to short duration athletic performances, as sprinting, or high intensity endurance events, as track cycling pursuits. Beyond a merely physiological adaptations determined factor, some authors observed how exercising muscles, maximal isometric contraction (MVC), rate of torque development (RTD), muscle cross-sectional area (CSA), volume and morpho-mechanical properties may determine an increased W' workability (Miura, Endo and Sato, 2002; Kordi, Mensies and Simpson, 2018).

Taken together, the evidence seems to depict not only different morpho-mechanical profiles and characteristics as observed in the previous chapter, but also different performance implications from both morphological and biomechanical properties (Figure 2). However, no study involved cyclists and runners in the same investigation or compared these two athletic populations.



**Figure 2.** Cycling and running population may display different musculoskeletal morpho-mechanical profile, considering the exercise specificities to which they are chronically exposed.

#### **1.3 Evidence summary**

In summary, early studies and theoretical frameworks seem to suggest the mechanical load and the exercise specificities (e.g., muscle contraction mode) as the main drivers of the differences and functional implications observable in the musculoskeletal morpho-mechanical profile of different athletic populations. However, the complexity behind muscle remodeling dynamics, and the lack of studies covering these aspects in high-level athletic populations, leave different open questions. Based on the intrinsic differences and biomechanical prerogatives of the two disciplines and based on observational studies on these distinct populations, cycling and running exercises may represent a valid model to investigate both, the result of a chronic exposition to a particular load and exercise specificities and its functional performance implications. Indeed, the evidence seem to depict a different morpho-mechanical profile of athletes belonging to these disciplines, as the results of the stimuli arising from their activity and reflected in potential differences in performance outputs as the sprinting ability, the neuromuscular functions, fatigue accumulation dynamics and performance decay (Abe, Kumagai and Brechue 2000; Stafilidis and Aramptzidis, 2007; Watsford et al., 2010; Kordi et al., 2020; Akagi et al., 2020; Kordi et al., 2021).

Thus, in cyclists, the quasi-isometric action followed by a prevalent concentric action against the big gears, coupled to the additional external factors action (e.g., drag forces) seem to take advantage from a greater PA by packing more sarcomeres in parallel, associated with an improvement of force output generation for contractions against high loads, and a greater MTUs stiffness, leading to increased exercise efficiency (e.g., lower mechanical force dissipation). Globally, this profile seems to represent the results of functional adaptations leading to a musculo-tendinous complex able to produce high mechanical work in the shortest (e.g., RTD) (Watsford et al., 2010; Ditroilo et al., 2011), and most efficient way (e.g., reduced PPO decay and increased W' power) (Ditroilo et al., 2011; Kordi et al., 2018). On the other hand, running performance in which, the SSCs and different forces involved, seem to be reflected in a different morpho-mechanical profile, characterized by a greater FL, that appear to favor shortening velocity as required for greater running speed, and a MTUs complex, stiff enough to reduce energy dissipation and improve maximal and explosive strength, and compliant enough to take advantage from elastic energy storage and reutilization. These different combinations of morpho-mechanical characteristics seem to play different roles as possible determinants and/or contributor of the athlete' neuromuscular functions (e.g., maximal and explosive strength), sprinting ability and performance decay induced.

Taken together, the morpho-mechanical profile of athletes belonging to different disciplines, and in particular, when characterized by different mechanical loads and exercise specificities, may delineate different profiles, representing from one side the result of the adaptive path arising from exposition from a specific exercise and, from the other, a functional interface between metabolic and mechanical power, adapted for specific performance tasks.

However, the literature overview described in the previous chapters, brought out the wide heterogeneity of studies, the lack of comparison of different population in the same experiment, and different methodologies applied, and different terms used to identify similar parameters. All together this makes difficult to describe and account for clear evidence and assumptions and calls for additional studies performed covering this topic.

## 2. RESEARCH METHODOLOGY AND ORGANIZATION

#### 2.1 Research object and hypothesis

The aim of the present study was to evaluate whether chronic exposition to different stimuli (cycling vs. running exercise) may reflect differences in the morpho-mechanical profile, and consequently, on performance markers.

The main objectives of the present investigation were:

- 1. To characterize and compare the morpho-mechanical profile of competitive, wellexperienced, cyclist and runner populations.
- 2. To explore the relationships between skeletal muscle morpho-mechanical variables, neuromuscular functions, and sprint performance, in cycling and runner populations.
- 3. To evaluate the impact of the discipline, and thus of the different morpho-mechanical profile, on performance decay dynamics induced by a maximal intensity sprint session.

Accordingly, the hypothesis, based on previous studies (observations in the two-population taken singularly), and theoretical frameworks (e.g., impact of mechanical load and contraction mode on musculoskeletal remodeling processes), is that cyclists may display a different morpho-mechanical profile compared to runners and that it may account differently in explaining athletes' performance.

#### 2.2 Research design

In this observational, cross-sectional study performed on well-trained, medium-to-high level cyclists and runners (high-level amateurs and élite or U23 categories athletes), data on vastus lateralis muscle architecture, patellar tendon dimensions, maximal sprint performance, knee extensors' MTUs passive resistive torque and, extensors'-flexors' isometric torque production (MVIC and RTD) have been evaluated and analyzed (Figure 3 and Figure 5).



Figure 3. Study protocol flow diagram

#### 2.3 Study participants and research organization

Twenty-seven well-trained male subjects (élite and amateur cyclists n=16, and runners n=11;  $33.4 \pm 7.1$  years,  $80.2 \pm 12.4$  kg,  $181 \pm 5.3$  cm), voluntary participated to the study after signing informed consent forms. This research was designed in accordance with the Declaration of Helsinki (2008), with the Fortaleza update (World Medical Association, 2013) and approved by the local institutional review board (Lithuanian Sport University, NR. MNL-SVA(M)-2020-323).

#### 2.4 Sample size calculation and inclusion criteria

Well-trained cyclists and runners fulfilling at least two of Jeukendrup et al. (2000) training and race status description of "well-trained athlete", without any past or recent injury involving the lower limbs have been targeted as suitable study participants. Presence of overt cardiovascular, pulmonary, or metabolic disease as well as chronic joint pain represented additional exclusion criteria. Sample size have been calculated according to élite/sub-élite cyclists' and runners population in Lithuania (preliminary observation based on male national road championships participation (https://www.procyclingstats.com/race/nc-lithuania/2020/result/result), considering a 95% confidence level and 5% margin of error, lead to an approximative sample size of 20 participants, the same criteria applied for male track cycling 2020 national championships (https://www.uci.org/track/results) resulted in an approximate sample size of 8 athletes. The same criteria applied for 100m and 200m track and field sprint 2020 national championships participation

resulted in an approximate sample size of 15 and 12 participants respectively (http://lengvoji.lt/rezultatai/). The study ideal sample size calculated as in the previous descriptions was thus represented by 8 to 10 well trained cyclists and 8 to 10 well-trained runners.

#### **2.5 Research methods**

#### **Testing protocol**

After anthropometric assessment and the musculoskeletal ultrasound imaging procedures, in one testing session, after a standard warm up consisting in cycling at 100 W for 3 min and 150 W for further 3 min, the subjects performed in sequence i) a knee extensors' passive resistive torque test; ii)  $3 \times 5s$  dominant limb knee extensors' and flexors' MVIC test with 60s recovery between each contraction; ii) a maximal-intensity-intermittent-sprint session consisting in  $4 \times 15s$  maximal sprints interspersed with 2 min recovery, and iii)  $3 \times 5s$  dominant limb knee extensors' MVIC test with 60s recovery between each contraction (Figure 4). Additional three to five submaximal contractions were performed as familiarization and specific warm up before the MVIC test and an additional cycling warm up of 10 min including short accelerations was performed before the sprint session.



Figure 4. Testing protocol and procedures overview

#### Measurements

#### B-mode ultrasound imaging

Images of the vastus lateralis (VL) and patellar tendon (PT) were obtained through a gray scale Bmode ultrasonography linear array transducer (10-15 MHz transducer, Echoblaster 128, UAB; Telemed, Vilnius, Lithuania). The settings of the ultrasound system were standardized for all participants and kept identical for all measurements and recorded through a video-based software EchoWave II (Telemed). All the measurements were carried out following the recommendations from the European Society of Musculoskeletal Radiology and recent literature review on ultrasound imaging application in sports (Beggs et al., 2016; Sarto et al., 2021). Water-soluble transmission gel was used to coat the transducer that was positioned with minimal pressure over the subjects' skin. After body mass (Tanita TBF-300, Tanita Corp., Tokyo, Japan), and leg girth measurements (dominant leg in proximal, medial and distal position, measured with a standard, non-elastic, bendable tape with a sensitivity level of 0.1 cm), each subject was asked to lay on a physiotherapy bed in supine position with the probe applied to the mid-point of the VL, at 50% of femur length (from the knee joint space to the greater trochanter), first in transverse position and then slowly turned to longitudinal plane, with location slightly adjusted, if necessary, to obtain the clearest image of the fascicles and surrounding tissue (Hoffman et al., 2016; Kordi et al., 2020) (Figure 4). Parallel fascicle alignment was presumed when transducer orientation produced an image, whereby the aponeuroses and the fascicle perimysium trajectory were clearly identified with no visible fascicle distortion at the image

edges. In the same position, the subjects were asked to flex the knee at  $\sim 30^{\circ}$  to further acquire PT images (Klich et al., 2020). This knee position avoids possible anisotropy related to the concave profile as the result of posterior thigh muscles and PT extension (Skou and Aalkjaer, 2013). The linear transducer was thus placed first longitudinally, in order to evaluate tendon length and thickness (long-axis) and secondly on transverse plane to allow the tendon CSA evaluation (short-axis) (Arias-Buría et al. 2020). The images were later imported into two different analysis software (ImageJ, v.1.46; National Institutes of Health; and Tracker, v.5.1.5) to measure the VL muscle architecture [fascicle length (FL), pennation angle (PA), muscle thickness (MT)] and the PT dimensions [tendon length (L), tendon thickness (TT), tendon cross sectional area (CSA)] and echogenicity (Sarto et al., 2021).

#### Surface Electromyography (sEMG)

To monitor myoelectric activity, sEMG was used during the MVICs and the passive torque measurements, with 3 surface electrodes placed on the *rectus femoris*, *vastus lateralis* and *bicep femoris* muscles and one reference electrode on the patella, following the SENIAM (surface EMG for non-invasive assessment of muscles) recommendations (Hermens et al., 2000). Each location was shaved, lightly abraded, and then cleaned with a sterilized alcohol wipe before applying the self-adhesive pre-amplified electrodes (PGC10C; Fiab, Vicchio, Florence, Italy), with a 25-mm inter-electrode distance.

#### Passive resistive torque

Passive resistive torque, as the resistance against passive movement of the quadriceps MTU, was evaluated using the Biodex System 3 dynamometer (Biodex Medical Systems, Inc., Shipley, NY, USA) for three times with 60s of recovery between each test. Participants were placed in a standardized position on the seat of the dynamometer (Figure 4) with the pelvis and the thigh of the tested leg strapped and fixed to minimize secondary movements. The opposite hip was fixed at 90° flexion to limit pelvic and lumbar motion. The knee axis was aligned with the rotational axis of the dynamometer. To obtain the passive resistive torque, the lower leg was moved from full knee extension to maximal achievable knee flexion angle (120° starting from a knee extended position) with an angular velocity of 5°/s in passive mode of the dynamometer (Krause et al., 2019).

#### Maximal voluntary isometric contraction (MVIC) test

For the MVIC test, each subject was firmly strapped to a Biodex System 3 dynamometer (with two transversal shoulder-to-hip belts fixing the trunk, one hip belt, and one belt at the distal thigh. Subjects were in a seated position with 90° of flexion at the hip and, 90° for knee joints for isometric extensions, and  $60^{\circ}$  of knee flexion (0° represent full extension) for isometric flexions, with the femoral lateral epicondyle aligned to the dynamometer axis of rotation, and the lower leg fixed to the lever arm of the dynamometer just above the medial malleolus (Figure 4). The setting was further individually adjusted to ensure minimal hip and knee joint movement and to minimize vertical displacement between the lower back and the backrest during muscular force exertion. The subjects were carefully instructed to contract as fast and forceful as possible on a given signal from the test supervisor and during the test strong verbal encouragement was provided by the supervisor (Maffiuletti et al., 2016).

#### Data acquisition

During the passive resistive torque and MVIC tests, torque, angles and sEMG data were synchronized, sampled at 1000 Hz and recorded through the 12-bit analog-to-digital converter Biopac system (EL254S; BIOPAC Systems, Santa Barbara, CA, USA) and the Acknowledge software (version 4.1, Biopac Systems).

#### Maximal intensity sprint session (MISS)

The maximal-intermittent-sprint session consisted in 4 maximal Wingate test sprints of 15s duration interspersed by 2 min of recovery. Each sprint was performed through a Wingate ergometer (Monark 894E, Stockholm, Sweden) with a load corresponding to 7.5% of the subject's body mass (Driss and Vandewalle, 2013) (Figure 4). The subjects were carefully instructed to sprint as fast and forceful as possible on a given signal from the test supervisor and during each sprint strong verbal encouragement was provided by the supervisor.

#### Rate of perceived exertion (Borg 6-20 RPE)

The Borg 6-20 RPE scale data have been acquired from each subject respectively after each sprint and after the completion of the entire maximal-intensity-intermittent-sprint session, with a score of 6 indicating no exertion and a score of 20 indicating maximal exertion (Williams, 2017).

#### Data analysis

#### Muscle architecture measurements

The images were later imported into two different analysis software (ImageJ, v.1.46; National Institutes of Health; and Tracker, v.5.1.5) to measure the VL muscle architecture [fascicle length (FL), pennation angle (PA), muscle thickness (MT)] (Sarto et al., 2021) (Figure 5). The FL was measured as the length of the fascicular path between the superficial and deep aponeuroses and, when the transducer was not able to capture the whole length of the fascicles, the line of the fascicle was extrapolated beyond the frame of the image using the Tracker 5.1.5 software (Sarto et al., 2021; Blazevich et al., 2007). The PA, was measured as the angle between the fascicular path and the insertion of fascicles into the deep aponeurosis, while the MT was measured as the distance between the superficial aponeurosis and the deep aponeurosis (Sarto et al., 2021). Three different ultrasound images of the VL muscle and PT were recorded and analyzed for each participant, before averaging the measured values. Three different ultrasound images of the VL muscle were recorded and analyzed for each participant, before averaging the measured values. The intra-rater repeatability (Castro et al., 2019) have been calculated on all the variables from data acquired on the same voluntary four subjects (three students and one member of the University lab) and in different days, before the start of the experiment and revealed respectively: FL = ICC: 0.97; CV: 1.72%; PA = ICC: 0.97; CV: 3,78%; MT = ICC: 0.98; CV: 1.93%.

#### Tendon dimension measurements

The images were later imported into two different analysis software (ImageJ, v.1.46; National Institutes of Health; and Tracker, v.5.1.5) to measure the PT dimensions [tendon length (L), tendon thickness (TT), tendon cross sectional area (CSA)] and echogenicity (Sarto et al., 2021) (Figure 5). The length of the PT (L) was defined as the distance between the distal patellar pole to the bony ridge at the proximal tibia, at the level of the epiphysis (Gellhorn, Morgenroth and Goldstein, 2012). The thickness of the PT was assessed in four location as described by Klich et al. (2020), set at 5-10-15-20 mm inferior to the apex of the patella. The four measurements were then averaged for a single measure of tendon thickness. Tendon CSA was measured in the transversal plane with a region of interest chosen for each scan to include as much of the tendon as possible. After identifying the desired positions, short (approximately 5 to 10 s) ultrasound videos of the tendons CSA were captured. All captured videos were then converted into frames and in each video the frame with the best visibility of the tendon was selected for digitalization and CSA analysis. The tendon borders were defined inferiorly as the first hyperechoic region between the subcutaneous tissue and the deep fascia layer. Three different ultrasound images of the PT were recorded and analyzed for each

participant, before averaging the measured values. The intra-rater repeatability (Castro et al., 2019) have been calculated on all the variables from data acquired on the same voluntary four subjects (three students and one member of the University lab) and in different days, before the start of the experiment and revealed respectively: L = ICC: 0.96; CV: 3,82%; TT = ICC: 0.97; CV: 2.02%; CSA = ICC: 0.96; CV: 3.98%.

#### Echogenicity

Echo intensity measurements, have been obtained using the gray scale analysis, by which the greater the grayscale, the greater is the muscle damage (or the fat, connective tissue or in general fibrous tissues infiltration) and lower is the muscle quality (Lanferdini et al., 2019). Therefore, echogenicity measurements were made using ImageJ (National Institutes of Health) software, after selecting the muscle or tendon region of interest (ROI) and using the histogram function to analyze the image based on the gray scale analysis of each pixel and with the mean and SD of echogenicity produced (echo intensity between 0 and 255, with black=0 and white=255) (Lanferdini et al., 2019) (Figure 5).



Figure 5. From top to bottom: VL muscle architecture, PT dimensions, and echogenicity analysis.

#### Passive resistive stiffness calculation

Passive resistive stiffness was calculated from torque and angle measurements (slope of the curve between 80 and 100% of the total ROM) after correcting data for gravity and using a custom-made Microsoft Excel spreadsheet for calculations. Stiffness measure was obtained by dividing the change in passive torque by the change in knee joint angle:  $Stiffness: \left(\frac{\Delta T orque}{\Delta ROM}\right)$ . The passive resistive torque measurements have been accepted as valid only if the EMG activity of the considered muscles were lower than 5% of the maximum muscle activation recorded during the MVIC tests. EMG signals were filtered with a band-pass of 10-500 Hz using a fourth-order Butterworth filter and the EMG amplitude was transformed into a root mean square (RMS) for further calculations.

#### Maximal voluntary contraction (MVC) and rate of torque development (RTD) calculations

The MVC was defined as the peak isometric torque (Nm) exerted within the entire contraction phase. In accordance with the procedures described by Maffiuletti et al., contractile RTD was calculated as the average slope of the torque-time curve (Nms-1) in early contraction phase time interval (0-50 ms, and 0-100 ms) and late contraction phase time interval (0-200 ms, and 0-300 ms). Onset of contraction was defined as the time point where the knee extensor torque exceeded the 2.5% of the baseline to peak isometric torque difference (Maffiuletti et al., 2016). MVIC and RTD0-50, RTD0-100, RTD0-200, and RTD0-300, were averaged from the three contractions and the average value used for the analysis. All the RTD calculation were performed through a customized Excel spreadsheet using the raw data exported from the Acknowledge software.

#### Sprint performance variables acquisition and calculations

The peak power output (PPO), time to peak power (tPP), average 15s power (PO<sub>15s</sub>) data have been acquired from the Monark AnaErobic Test software. The Fatigue Index (FI):  $100 \times \left(\frac{[Sbest-Sworst]}{Sbest}\right)$ , and the Sprint Decrement Score (Sdec):  $\left\{1 - \frac{(S1+S2+S3+S4)}{Sbest \times 4}\right\} \times 100$ , have been calculated following the procedures described by Kerhervé et al 2020.

#### Critical power, critical velocity, and anaerobic capacity

A subgroup of the participants (n=20) additionally performed an in-field (outdoor) critical power (CP) (n=15) or critical velocity (CV) (n=7) test according to the respective discipline (cycling or running, with the exception of two subjects that shared both) and as part of their physical condition assessment procedures prescribed by coaches or teams' staff (Kordi et al., 2018; Galbraith et al., 2011). Additionally, and/or alternatively, competition files have been shared as indicator of maximal performance. Performance test data were voluntary shared by the participants as .fit or .tcx files captured using their own GPS computers and downloaded into the desktop software (Golden Cheetah training software) to determine the highest and continuous power output or velocity epoch achieved during the tests (Figure 6). The physical performance tests data were used to calculate the CP or CV (linear coefficients) and the anaerobic capacity (W' or ARC, slope coefficients) estimates using the linear work-time model [P=(W'/t)+CP], where P is the attainable power for time (t), given known W' and CP or [d=(CV×t)+ARC], where d=distance run (m), CV = critical running velocity (m·s<sup>-1</sup>), t = running time (s) and ARC = anaerobic running capacity (m) (Kordi et al., 2018; Galbraith et al., 2011).



Figure 6. Representation of the CP and W' chart obtained from the Golden Cheetah training analysis software

#### 2.6 Methods of statistical analysis

All data analyses were carried out using SPSS version 21.0 (IBM Corporation, Armond, NY) and GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). Descriptive statistics  $(mean \pm SD)$  were calculated for each variable and data have been stratified on discipline (cycling vs. running). Shapiro-Wilk test was used to assess the normality of distribution of the samples, leading to the choice of parametric or non-parametric tests. Therefore, an independent t-test test was performed in order to evaluate the differences between cyclists and runners for all morphomechanical parameters (FL, PA, MT, TT, TL, CSA, stiffness) and muscle contraction performance variables (MVIC, MVIC5s, RTD0-50, RTD0-100, RTD0-200, RTD0-300). ). Cohen's d effect size (ES), determined by calculating the mean difference between the two groups and dividing the result by the pooled standard deviation, was established according to the following criteria: 0 to 0.19, trivial; 0.20 to 0.59, small; 0.60 to 1.19, moderate; 1.20 to 1.99, large; 2.00 to 3.99, very large; >4.0; nearly perfect (Hopkins et al., 2009). Pearson correlation analysis with linear regression have been further conducted to evaluate the possible relationships between the criterion morpho-mechanical variables and the sprint performance and neuromuscular performance variables in the two groups (cycling and running). Additionally, a Fisher's r to z transformation was performed in order to statistically compare the z score (z test statistics) obtained from the same variables between the two groups (cyclists vs. runners) and a step-wise regression analysis to evaluate which variables was a better explanator of neuromuscular and sprint performance for cyclists and runners (Bernards et al. 2017). The following criteria were adopted to interpret the magnitude of correlations between measured variables: <0.09, trivial; 0.10 to 0.29, small; 0.30 to 0.49, moderate; 0.50 to 0.69, large; 0.70 to 0.89 very large; and >0.90, nearly perfect (Ferguson, 2009). A repeated measures ANOVA with time as within subjects' factor and discipline (cycling and running) as the between subjects' factor was performed to analyze whether the mean change in the outcome from pre to post MISS differed in the two groups (cyclists vs. runners). Partial eta squared (np2) was used as the repeated measures ANOVA effect size and interpreted as (<0.039 - no effect; 0.04 to 0.24 - minimum; 0.25 to 0.63 - moderate; >0.64 - strong (Ferguson, 2009). An alpha level of  $p \le 0.05$  was set to assess the statistical significance.

#### **3. RESEARCH FINDINGS**

#### 3.1 Morpho-mechanical profile characterization

Descriptive statistics (mean and standard deviation) of the anthropometric measurements, morpho-mechanical variables, and the results of the independent t-test conducted to examine the differences on *vastus lateralis* muscle architecture, *patellar tendon* dimensions and knee extensors' passive stiffness between cycling and running groups are described in the following subchapter.

	Cyclists (n=16)	Runners (n=11)	р
Age	$32.8\pm8.2$	$34.4\pm5.4$	ns
Body mass (kg)	$86.4\pm10.7$	$71.19\pm8.9$	***
Height (cm)	$182.5\pm4.5$	$178.63\pm5.7$	ns
BMI (kg/m <sup>2</sup> )	$26.03\pm3.7$	$22.27\pm2.2$	**
Leg girth prox. (cm)	$62.06\pm3.37$	$54.20 \pm 4.73$	***
Leg girth med. (cm)	$58.66 \pm 3.53$	$52.41 \pm 4.10$	***
Leg girth dist. (cm)	$49.72\pm2.37$	$46.05\pm2.62$	***
Calf girth (cm)	$40.22\pm2.48$	36.68 1.17	***
3.7 0.7 1.1			

**Table 1.** Subjects' characteristics (*mean*  $\pm$  *SD*) stratified by sport discipline

*Notes:*  $ns: p > .05; **: p \le .01; ***: p \le .001$ 

The results of the independent t-test performed on anthropometric variables between cycling and running groups, revealed a significantly greater body mass (kg) [t(25)=3.87, p=.001; mean difference: 15.27 kg; 95%CI: 7.13 to 23.39; ES: 1.52; large], and BMI (kg/m<sup>2</sup>) [t(25)=2.99, p=.006; mean difference: 3.77 kg/m<sup>2</sup>; 95%CI: 1.17 to 6.35; ES: 1.17; moderate] in cyclists compared to runners. In addition, lower limb girths measurements resulted to be significantly greater in cyclists compared to runners and in particular, the dominant leg proximal thigh girth (cm) [t(25)=5.05, p=.000; mean difference: 7.86 cm; 95%CI: 4.66 to 11.07; ES: 1.98; large], medial thigh girth (cm) [t(25)=4.23, p=.000; mean difference: 6.24 cm; 95%CI: 3.20 to 9.29; ES: 1.65; large]; distal thigh girth (cm) [t(25)=3.79, p=.001; mean difference: 3.67 cm; 95%CI: 1.68 to 5.67; ES: 1.48; large] and calf girth (cm) [t(25)=4.39, p=.000; mean difference: 3.53 cm; 95%CI: 1.87 to 5.19; ES: 1.72; large]. Non-significant differences emerged for age, and height.

**Table 2.** Descriptive statistics (mean and standard deviation) of the *vastus lateralis* muscle architecture parameters, and summary of the independent t-test means comparison between the two groups (cyclists and running).

		,				
	Cyclists (n=16)	Running (n=11)	р	Mean difference - 95%CI	ES	Interpretation
FL (mm)	$79.85 \pm 7.56$	$83.91 \pm 5.22$	ns	-4.06 (-9.48, 1.36)	-0.60	moderate
PA (mm)	$20.24 \pm 2.57$	$15.07 \pm 1.41$	***	5.17 (3.41, 6.92)	2.37	very large
MT (mm)	$26.63 \pm 4.10$	$21.59 \pm 4.03$	**	5.03 (1.74, 8.32)	1.23	large
EI (au)	$23.40\pm3.67$	$26.43 \pm 2.10$	**	-3.28 (-5.44, -1.12)	-1.22	large

Vastus lateralis muscle architecture

Notes: FL, fascicle length; PA, pennation angle; MT, muscle thickness; EI, echogenicity intensity; ns: p > .05; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

The results of the independent t-test performed on the VL muscle architecture variables and between cycling and running group are represented in Table 2. Statistically significant differences were observed for PA (°) [t(25)=6.05, p=.000], MT (mm) [t(25)=3.15, p=.004] and EI (au) [t(25)=-3.13, p=.004] between cyclists and runners, while non-significant differences emerged between FL [t(25)=-1.54, p=.136].

**Table 3.** Descriptive statistics (mean and standard deviation) of the *patellar tendon* parameters, and summary of the independent t-test means comparison between the two groups (cyclists and running).

	Patellar tendon dimensions									
	Cyclists (n=16)	Running (n=11)	р	Mean difference - 95%CI	ES	Interpretation				
TT (mm)	$4.78\pm0.84$	$4.19\pm0.67$	ns	0.59 (-0.03, 1.22)	0.76	moderate				
<i>L</i> (mm)	$61.30\pm7.98$	$55.27 \pm 9.20$	ns	6.02 (-0.83, 12.87)	0.71	moderate				
CSA (mm <sup>2</sup> )	$97.93 \pm 14.26$	$85.78 \pm 11.32$	ns	12.15 (1.53, 22.76)	0.92	moderate				
EI (au)	$48.75\pm3.49$	$53.45 \pm 3.22$	**	-4.07 (-7.43, -1.97)	-1.38	large				

Notes: TT, tendon thickness; L, tendon length; CSA, cross sectional area; EI, echogenicity intensity; ns: p > .05; \*\*:  $p \le .01$ .

The results of the independent t-test performed on the PT dimension variables and between cycling and running group are represented in Table 3. Statistically significant differences were observed for the CSA (mm<sup>2</sup>) [t(25)=2.35, p=.027], and EI (au) [t(25)=-3.54, p=.002], while non-significant differences emerged between TT (mm) [t(25)=1.94, p=.063], and between TL (mm) [t(25)=1.18, p=.082] between cyclists and runners.

**Table 4.** Descriptive statistics (mean and standard deviation) of the knee extensors' MTUs passive stiffness, and summary of the independent t-test means comparison between the two groups (cyclists and running).

Knee extensors' MTUs passive stiffness								
Cyclists (n=16) Running (n=11) p Mean difference - 95%CI ES Interpretation								
Stiffness (Nm/deg) $0.94 \pm 0.26$ $0.69 \pm 0.17$ *** $0.209 (0.09, 0.32)$ $1.46$ large								

*Notes:* \*\*\*: *p*≤.001

The results of the independent t-test performed on the knee extensors' MTUs passive stiffness measurements (Nm/deg) and between cycling and running group are represented in Table 4. Statistically significant differences emerged between the two groups [t(25)=3.72, p=.001].

**Table 5.** Descriptive statistics (mean and standard deviation) of the maximal isometric strength parameters, and summary of the independent t-test means comparison between the two groups (cyclists and running).

Waxmar and 55 average isometric strength (WVIC, WVIC55)							
	Cyclists (n=16)	Running (n=11)	р	Mean difference - 95%CI	ES	Interpretation	
QMVIC (Nm)	$347.24\pm73.43$	$299.75\pm68.07$	***	91.19 (39.38, 143.01)	1.42	large	
HMIVC (Nm)	$150.08\pm28.31$	$139.55\pm24.68$	ns	11.93 (-12.73, 36.61)	0.39	small	
QMVIC <sub>5s</sub> (Nm)	$283.46\pm60.69$	$237.35\pm49.42$	**	72.47 (29.40, 115.53)	1.36	large	
HMVIC <sub>5s</sub> (Nm)	$126.17\pm25.64$	$113.33\pm24.41$	ns	15.41 (-6.48, 37.32)	0.57	small	

Maximal and 5s average isometric strength (MVIC, MVIC<sub>5s</sub>)

Notes: Q, quadriceps; H, hamstrings; MVIC, maximal voluntary isometric contraction; MVIC<sub>5s</sub>, average 5s maximal voluntary isometric contraction; ns: p > .05; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

The results of the independent t-test performed on the knee extensors (Q) and knee flexors (H) MVIC and MVIC<sub>5s</sub> values and between cycling and running group are represented in Table 5. Significant differences emerged between knee extensors MVIC (Nm) [t(25)=3.62, p=.001] and between knee extensors MVIC<sub>5s</sub> (Nm) [t(25)=3.466, p=.002] of cyclists and runners, while non-statistically significant differences were observed knee flexors MVIC (Nm) [t(25)=0.996, p=.329] and between knee flexors MVIC<sub>5s</sub> (Nm) [t(25)=1.45, p=.160] of cyclists and runners.

**Table 6.** Descriptive statistics (mean and standard deviation) of the explosive parameters, and summary of the independent t-test means comparison between the two groups (cyclists and running).

	Cyclists (n=16)	Running (n=11)	р	Mean difference - 95%CI	ES	Interpretation
QRTD <sub>0-50</sub> (Nm·s <sup>-1</sup> )	$1796.38 \pm 440.71$	$1278.12 \pm 355.38$	**	456.41 (170.77, 742.06)	1.29	large
HRTD <sub>0-50</sub> (Nm·s <sup>-1</sup> )	$1219.31 \pm 279.50$	$924.86\pm128.55$	*	254.58 (64.39, 444.78)	1.08	moderate
$QRTD_{0-100} (Nm \cdot s^{-1})$	$1293.15 \pm 308.82$	$1000.50 \pm 235.23$	**	296.39 (87.94, 504.83)	1.15	moderate
HRTD <sub>0-100</sub> (Nm·s <sup>-1</sup> )	$780.83\pm202.17$	$612.01 \pm 112.28$	ns	145.59 (-4.04, 295.22)	0.78	moderate
QRTD <sub>0-200</sub> (Nm·s <sup>-1</sup> )	$998.54 \pm 184.83$	$784.60\pm143.37$	**	226.27 (86.16, 366.38)	1.30	large
$HRTD_{0-200} (Nm \cdot s^{-1})$	$569.02 \pm 146.00$	$451.77\pm93.35$	ns	92.39 (-32.28, 217.08)	0.59	small
QRTD <sub>0-300</sub> (Nm·s <sup>-1</sup> )	793.81 ± 134.71	630.73 ± 122.29	***	184.09 (80.16, 288.02)	1.43	large
HRTD <sub>0-300</sub> (Nm·s <sup>-1</sup> )	427.33 ± 123.74	$349.91 \pm 82.75$	ns	70.22 (-28.82, 169.26)	0.57	small

Explosive isometric strength (RTD<sub>0-50</sub>, RTD<sub>0-100</sub>, RTD<sub>0-200</sub>, RTD<sub>0-300</sub>)

Notes: Q, quadriceps; H, hamstrings; RTD, rate of torque development, 0-50, 0-100, 0-200, 0-300, ms from the onset of muscle contraction; ns: p > .05; \*:  $p \le .05$ ; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

The results of the independent t-test performed on the knee extensors (Q) and knee flexors (H) RTD early phase values (0-50, 0-100 ms) and late phase values (0-200, 0-300 ms) and between cycling and running group are represented in Table 6. Significant differences emerged between knee extensors RTD<sub>0-50</sub> (Nm·s<sup>-1</sup>) [t(25)=3.29, p=.003], RTD<sub>0-100</sub> (Nm·s<sup>-1</sup>) [t(25)=2.93, p=.007], RTD<sub>0-200</sub> (Nm·s<sup>-1</sup>) [t(25)=3.32, p=.003] and RTD<sub>0-300</sub> (Nm·s<sup>-1</sup>) [t(25)=3.65, p=.001] but only the RTD<sub>0-50</sub> (Nm·s<sup>-1</sup>) [t(25)=2.75, p=.011] resulted in statistically significant differences between cyclists and runners, while non-significant differences were observed for knee flexors RTD<sub>0-100</sub> (Nm·s<sup>-1</sup>) [t(25)=2.00, p=.056], RTD<sub>0-200</sub> (Nm·s<sup>-1</sup>) [t(25)=1.52, p=.140] and RTD<sub>0-300</sub> (Nm·s<sup>-1</sup>) [t(25)=1.46, p=.157].

Descriptive statistics and results of the independent t-test between the sprint performance markers of cyclists and runners are represented in table 7, while the descriptive data of the functional performance descriptors are presented in table 8.

**Table 7.** Descriptive statistics (mean and standard deviation) of the sprint performance data, FI and RPE, and summary of the independent t-test means comparison between the two groups (cyclists and running).

	Cycling (n=16)	Running (n=11)	р	Mean difference - 95%CI	ES	Interpretation
PPO (W)	$1159.42 \pm 220.00$	$920.71 \pm 139.25$	***	238.71 (75.13; 83.96)	1.24	moderate
tPP (s)	$1.32\pm0.34$	$1.54\pm0.31$	ns	-0.21 (-0.47; 0.05)	1.43	large
PO <sub>15s</sub> (W)	$815.68 \pm 139.89$	$652.73\pm89.53$	***	162.95 (47.89; 64.32)	1.33	large
FI (au)	$15.75\pm10.75$	$19.83 \pm 11.32$	ns	-4.08 (-12.94; 4.78)	1.14	moderate
RPE (au)	$17.00 \pm 1.82$	$17.73 \pm 1.19$	ns	-0.72 (-2.02: 0.56)	0.45	small

*Notes: PPO, peak power output; tPP, time to peak power; PO*<sub>15s</sub> average power output 15s; FI, fatigue index; RPE, rate of perceived exertion; ns: p > .05; \*\*\*:  $p \le .001$ 

Significant differences emerged between the PPO (W) [t(25)=3.17, p=.004], and the PO<sub>15s</sub> (W) [t(25)=3.40, p=.002] of cyclists and runners, while non-significant differences were observed for the other parameters (Table 7).

**Table 8.** Descriptive statistics (mean and standard deviation) of the functional performance data, stratified for discipline (cycling and running groups).

	Cycling (n=15)	Running (n=7)	
CP (W)	$331.10\pm46.26$		
W' (kj)	$23.51\pm6.15$		
CV (m·s <sup>-1</sup> )		$3.77\pm0.25$	
ARC (m)		$103.8\pm30.22$	
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Notes: CP, critical power; W', total work over CP until failure; CV, critical velocity; ARC, anaerobic running capacity.

# **3.2** Relationships between musculoskeletal morpho-mechanical variables, neuromuscular functions, and sprint performance

**Table 9.** Bivariate relationship (r) between maximal and explosive neuromuscular strength, sprint performance variables and *vastus lateralis* FL, z-score comparisons between the two groups and respective levels of significance (p).

	FL (mm)									
		Cyc	ling (n=16)		Run	ning (n=11)	Cycling vs.	Running		
	r	р	Relationship	r	р	Relationship	z-score	р		
MVIC	.60	*	large	.48	ns	moderate	.375	.354		
MVIC <sub>5s</sub>	.44	ns	moderate	.42	ns	moderate	.063	.475		
RTD <sub>0-50</sub>	.50	*	moderate	.16	ns	small	.853	.197		
RTD <sub>0-100</sub>	.56	*	large	.59	*	large	108	.457		
RTD <sub>0-200</sub>	.52	*	large	.39	ns	small	.366	.357		
RTD <sub>0-300</sub>	.54	*	moderate	.39	ns	small	.832	.203		
PPO	.52	*	large	.47	ns	moderate	.169	.433		
tPP	.45	ns	moderate	.19	ns	small	.648	.258		
$PO_{15s}$	.66	**	very large	.55	ns	large	.371	.355		
FI	.38	ns	moderate	.19	ns	small	.459	.323		

*Notes: ns:* p > .05; \*:  $p \le .05$ ; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

Very large or large positive bivariate relationships between FL and performance variables emerged for cyclist group when considering the MVIC [p=.013; r=.60 (95% CI: 0.15 to 0.84); R<sup>2</sup>=0.36], RTD<sub>0-100</sub> [p=.022; r=.56 (95% CI: 0.10 to 0.83); R<sup>2</sup>=0.32], RTD<sub>0-200</sub> [p=.039; r=.52 (95% CI: 0.03 to 0.81); R<sup>2</sup>=0.27], PPO [p=.036; r=.53 (95% CI: 0.17 to 0.85); R<sup>2</sup>=0.22] and PO<sub>158</sub> [p=.005; r=.66 (95% CI: 0.25 to 0.87); R<sup>2</sup>=0.45] while for the other parameters moderate relationships were observed. For running group, the vastus lateralis FL was largely correlated with the RTD<sub>0-100</sub> [p=.050; r=.59 (95% CI: 0.01 to 0.88); R<sup>2</sup>=0.35] and the PO<sub>158</sub> [p=.073; r=.56 (95% CI: 0.06 to 0.86); R<sup>2</sup>=0.31] while for the other parameters small to moderate relationship emerged. The z-score comparison revealed no-significant differences between the correlation coefficients of the two groups (Table 9).

**Table 10.** Bivariate relationship (r) between maximal and explosive neuromuscular strength, sprint performance variables and *vastus lateralis* PA, z-score comparisons between the two groups and respective levels of significance (p).

				PA	A (°)				
		Cycl	ing (n=16)		Runi	ning (n=11)	Cycling vs.	Cycling vs. Running	
	r	р	Relationship	r	р	Relationship	z-score	р	
MVIC	.69	**	large	.11	ns	small	1.65	.049	
MVIC <sub>5s</sub>	.79	***	very large	.09	ns	trivial	2.16	.015	
RTD <sub>0-50</sub>	.43	ns	moderate	.31	ns	moderate	.322	.374	
RTD <sub>0-100</sub>	.40	ns	moderate	.38	ns	moderate	.375	.354	
RTD <sub>0-200</sub>	.46	ns	moderate	.15	ns	small	.965	.167	
RTD <sub>0-300</sub>	.58	*	large	.17	ns	small	1.06	.143	
PPO	.63	**	large	.28	ns	small	1.01	.157	
tPP	.45	ns	moderate	.09	ns	trivial	.878	.190	
$PO_{15s}$	.51	*	large	.06	ns	trivial	1.12	.133	
FI	.04	ns	trivial	.44	ns	moderate	-0.96	.168	

*Notes: ns:* p > .05; \*:  $p \le .05$ ; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

Very large or large positive bivariate relationships between PA and performance variables emerged for cyclist group when considering the MVIC [p=.005; r=.69 (95% CI: 0.25 to 0.87); R<sup>2</sup>=0.44], MVIC<sub>5s</sub> [p=<.001; r=.79 (95% CI: 0.48 to 0.92); R<sup>2</sup>=0.62], RTD<sub>0-300</sub> [p=.020; r=.57 (95%

CI: 0.11 to 0.83);  $R^2=0.33$ ], PPO [p=.009; r=.63 (95% CI: 0.19 to 0.86);  $R^2=0.39$ ] and PO<sub>15s</sub> [p=.044; r=.51 (95% CI: 0.02 to 0.80);  $R^2=0.26$ ], while for the other parameters trivial to moderate relationships were observed. For running group all the parameters resulted in trivial to moderate relationship. The z-score comparison revealed significant differences between the correlation coefficients of the two groups for MVIC (z=1.65, p=.049), MVIC<sub>5s</sub> (z=2.16, p=.015) (Table 10).

	MT (mm)										
		Cyc	ling (n=16)		Runr	ning (n=11)	Cycling vs. Running				
	r	р	Relationship	r	р	Relationship	z-score	р			
MVIC	.52	*	large	.50	ns	moderate	.051	.480			
MVIC <sub>5s</sub>	.56	*	large	.29	ns	small	.759	.224			
RTD <sub>0-50</sub>	.30	ns	moderate	.24	ns	small	.130	.448			
RTD <sub>0-100</sub>	.39	ns	moderate	.69	*	large	973	.165			
RTD <sub>0-200</sub>	.40	ns	moderate	.58	ns	large	544	.293			
RTD <sub>0-300</sub>	.47	ns	moderate	.59	*	large	379	.352			
PPO	.56	ns	large	.48	ns	moderate	1.11	.133			
tPP	.09	ns	trivial	.01	ns	trivial	.185	.426			
PO <sub>15s</sub>	.59	ns	large	.16	ns	small	1.14	.127			
FI	.01	ns	small	.33	ns	moderate	748	.227			

**Table 11.** Bivariate relationship (r) between maximal and explosive neuromuscular strength, sprint performance variables and *vastus lateralis* MT, z-score comparisons between the two groups and respective levels of significance (p).

*Notes: ns:* p > .05; \*:  $p \le .05$ ; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

Large positive bivariate relationships between MT and performance variables emerged for cyclist group when considering the MVIC [p=.038; r=.52 (95% CI: 0.03 to 0.81);  $R^2=0.27$ ], MVIC<sub>5s</sub> [p=.113; r=.50 (95% CI: -0.13 to 0.78);  $R^2=0.25$ ], PPO [p=.025; r=.56 (95% CI: 0.10 to 0.82);  $R^2=0.31$ ] and PO<sub>15s</sub> [p=.016; r=.59 (95% CI: 0.13 to 0.84);  $R^2=0.35$ ] while for the other parameters trivial to moderate relationships were observed. For running group, the vastus lateralis MT was largely correlated with the RTD<sub>0-100</sub> [p=.019; r=.69 (95% CI: 0.15 to 0.91);  $R^2=0.48$ ], the RTD<sub>0-200</sub> [p=.060; r=.58 (95% CI: 0.02 to 0.87);  $R^2=0.34$ ] and the RTD<sub>0-300</sub> [p=.056; r=.59 (95% CI: 0.02 to 0.88);  $R^2=0.35$ ], while for all the other parameters resulted in trivial to moderate relationship. The z-score comparison revealed no-significant differences between the correlation coefficients of the two groups (Table 11).

**Table 12.** Bivariate relationship (r) between maximal and explosive neuromuscular strength, sprint performance variables and the knee extensors' MTUs passive stiffness, z-score comparisons between the two groups and respective levels of significance (p).

		Stiffness (Nm/deg)										
		Cycli	ng (n=16)		Run	ning (n=11)	Cycling vs. Running					
	r	р	Relationship	r	р	Relationship	z-score	р				
MVIC	.79	***	very large	.18	ns	small	1.97	.024				
MVIC <sub>5s</sub>	.74	***	very large	.10	ns	small	1.89	.030				
RTD <sub>0-50</sub>	.67	**	large	.12	ns	small	1.51	.065				
RTD <sub>0-100</sub>	.62	*	large	.37	ns	moderate	.741	.229				
RTD <sub>0-200</sub>	.71	**	very large	.00	ns	trivial	1.73	.042				
RTD <sub>0-300</sub>	.75	***	very large	.01	ns	trivial	2.14	.016				
PPO	.64	**	large	.06	ns	trivial	1.54	.062				
tPP	.17	ns	small	.21	ns	small	109	.457				
PO <sub>15s</sub>	.59	*	moderate	.23	ns	small	1.01	.157				
FI	.55	*	moderate	.13	ns	small	1.05	.141				

*Notes: ns:* p > .05; \*:  $p \le .05$ ; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

Very large or large positive bivariate relationships between stiffness and performance variables emerged for cyclist group when considering the MVIC [p=<.001; r=.79 (95% CI: 0.48 to 0.93); R<sup>2</sup>=0.63], RTD<sub>0-50</sub> [p=.005; r=.67 (95% CI: 0.26 to 0.87); R<sup>2</sup>=0.44], RTD<sub>0-100</sub> [p=.025; r=.56 (95% CI: 0.10 to 0.82); R<sup>2</sup>=0.31], RTD<sub>0-200</sub> [p=.002; r=.71 (95% CI: 0.33 to 0.89); R<sup>2</sup>=0.51], RTD<sub>0-300</sub> [p=<.001; r=.75 (95% CI: 0.40 to 0.91); R<sup>2</sup>=0.56], and PPO [p=.008; r=.64 (95% CI: 0.21 to 0.86); R<sup>2</sup>=0.40], while for the other parameters small to moderate relationships were observed. For running group all the parameters resulted in trivial to moderate relationship. The z-score comparison revealed significant differences between the correlation coefficients of the two groups for MVIC (z=1.97, p=.024), MVIC<sub>5s</sub> (z=1.88, p=.030), RTD<sub>0-200</sub> (z=1.73, p=0.042), and RTD<sub>0-300</sub> (z=2.14, p=0.016) (Table 12, Figure 14).

**Table 13.** Bivariate relationship (r) between critical power, W' and the morpho-mechanical variables (cyclists n=15) and respective levels of significance (*p*).

Cyclists (n=15)	Stiffn	less (	Nm/deg)		FL	(mm)		PA	(deg)			MT (mm)
	r	р		r	р		r	р		r	р	
CP (W)	.57	*	large	.26	ns	small	.35	ns	moderate	.36	ns	moderate
W' (kj)	.55	*	large	.33	ns	moderate	.46	ns	moderate	.54	*	large
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*Notes:*  $ns: p > .05; *: p \le ..05.$ 

Large positive bivariate relationships emerged between stiffness, CP [p=.027; r=.56 (95% CI: 0.09 to 0.84); R<sup>2</sup>=0.33], and W' [p=.034; r=.56 (95% CI: 0.09 to 0.84); R<sup>2</sup>=0.31]. Large positive correlation was observed also between the MT and W' [p=.037; r=.54 (95% CI: 0.05 to 0.82); R<sup>2</sup>=0.30]. Small to moderate relationships emerged between the other parameters (Table 13).

**Table 14.** Bivariate relationship (r) between critical velocity, ARC, and the morpho-mechanical variables (cyclists n=15) and respective levels of significance (*p*).

Runners (n=7)	Sti	iffness	(Nm/deg)		FL (n	nm)		PA	(deg)	]	MT (r	nm)
	r	р		r	р		r	р		r	р	
CV (m·s <sup>-1</sup> )	53	ns	large	.06	ns	trivial	.35	ns	moderate	.20	ns	small
ARC (m)	73	ns	very large	.18	ns	small	.10	ns	small	.27	ns	small

*Notes: ns: p>.05* 

Large and very large negative bivariate relationships emerged between stiffness, CV [p=.217; r=-.53 (95% CI: -0.91 to 0.36); R<sup>2</sup>=0.28], and ARC [p=.062; r=-.73 (95% CI: -0.96 to 0.05); R<sup>2</sup>=0.53]. Trivial to moderate relationships emerged between the other parameters (Table 14).

The PA, FL, MT and Stiffness measurements have been included in the stepwise regression analysis in order to predict the MVIC, RTD<sub>0-50</sub>, RTD<sub>0-200</sub> and PPO for cycling and running groups. With this set of predictors, the collinearity diagnostic exploration resulted in allowable inflation factors variance and tolerance, as indicators of an acceptable levels of multicollinearity (Hair et al., 1995). Thus, for the cycling group the regression analysis found 78% of the variability in MVIC between cyclists (F=20.15, p=.000) explained by two variables, stiffness (59%) and PA (19%), 44% and 55% of the variability respectively for RTD<sub>0-50</sub> (F=11.25, p=.005), and RTD<sub>0-200</sub> (F=14.31, p=.002) accounted by stiffness, and 62% of the variability of the PPO (F=10.56, p=0.003) explained by stiffness (36%), MT (16%) and PA (10%). For the running group, the regression analysis found 40% of the variability of MVIC (F=6.515, p=0.007) explained by the MT (25%) and the FL (15%), a non-significant predictive effect on RTD<sub>0-50</sub> and RTD<sub>0-200</sub> and 23% of the variability in PPO (F=6.225, p=0.021), explained by the MT. Trivial to moderate correlation and mostly non-statistically significant emerged between the tendon dimension measurements (TT, TL, CSA and EI) and the performance parameters stratified per discipline group (cycling and running).

#### 3.3 Impact of the MISS on performance decline (fatiguing effect) in cyclists vs. runners

The results of the repeated measures ANOVA with time (neuromuscular and sprint performance variables pre and post MISS) as within subjects' factor, and discipline (cycling and running) as the between subjects' factor, performed to analyze whether the mean change in the outcome from pre to post MISS differed in the two groups (cyclists vs. runners) are presented in the following subchapter. Descriptive statistics of the neuromuscular and sprint performance (pre and post MISS) are reported in table 15.

**Table 15.** Descriptive statistics (mean and standard deviation) of the neuromuscular and sprint performance data pre and post MISS, stratified for discipline (cycling and running groups) and with the respective pre  $\rightarrow$  post  $\Delta$ % (performance decay).

	Cyc	cling (n=16)		Run	ning (n=11)	
	PRE	POST	$\Delta\%$	PRE	POST	$\Delta\%$
QMVIC (Nm)	$347.24\pm73.43$	$299.75\pm68.07$	-13,68	$256.04\pm47.15$	$212.39\pm60.48$	-17,05
HMIVC (Nm)	$150.08\pm28.31$	$139.55\pm24.68$	-7,02	$138.15\pm10.15$	$122.73\pm35.61$	-11,16
H:Q ratio	$0.44\pm0.10$	$0.48\pm0.09$	9,09	$0.55\pm0.12$	$0.59\pm0.11$	7,27
QMVIC <sub>5s</sub> (Nm)	$283.46\pm60.69$	$237.35 \pm 49.42$	-16,27	$210.99\pm39.98$	$161.01\pm39.87$	-23,69
HMVIC <sub>5s</sub> (Nm)	$126.17\pm25.64$	$113.33 \pm 24.41$	-10,18	$110.75\pm29.26$	$93.54\pm21.43$	-15,54
$QRTD_{0-50} (Nm \cdot s^{-1})$	$1796.38 \pm 440.71$	$1278.12 \pm 355.38$	-28,85	$1339.96 \pm 148.75$	$941.92\pm177.60$	-29,71
$HRTD_{0-50} (Nm \cdot s^{-1})$	$1219.31 \pm 279.50$	$924.86\pm128.55$	-24,15	$964.73 \pm 147.63$	$682.57 \pm 113.11$	-34,84
$QRTD_{0-100} (Nm \cdot s^{-1})$	$1293.15 \pm 308.82$	$1000.50 \pm 235.23$	-22,63	$996.76 \pm 154.48$	$723.03\pm141.36$	-27,46
HRTD <sub>0-100</sub> (Nm·s <sup>-1</sup> )	$780.83 \pm 202.17$	$612.01 \pm 112.28$	-21,62	$635.24 \pm 157.19$	$477.33\pm80.52$	-24,86
QRTD <sub>0-200</sub> (Nm·s <sup>-1</sup> )	$998.54 \pm 184.83$	$784.60 \pm 143.37$	-21,43	$772.27 \pm 155.49$	$566.90\pm86.89$	-26,59
HRTD <sub>0-200</sub> (Nm·s <sup>-1</sup> )	$569.02 \pm 146.00$	$451.77\pm93.35$	-20,61	$476.62 \pm 166.58$	$378.84\pm73.49$	-20,52
$QRTD_{0-300} (Nm \cdot s^{-1})$	$793.81 \pm 134.71$	$630.73 \pm 122.29$	-20,54	$609.72 \pm 119.48$	$449.55\pm59.05$	-26,27
HRTD <sub>0-300</sub> (Nm·s <sup>-1</sup> )	$427.33 \pm 123.74$	$349.91 \pm 82.75$	-18,12	$357.10 \pm 121.32$	$290.98\pm61.41$	-18,52
PPO (W)	$1159.42 \pm 220.00$	$963.34 \pm 153.20$	-16,91	$920.71 \pm 139.25$	$738.52 \pm 161.62$	-19,79
tPP (s)	$1.32\pm0.34$	$2.13\pm0.61$	61,36	$1.54\pm0.31$	$2.40\pm0.38$	55,84
$PO_{15s}(W)$	$815.68 \pm 139.89$	$704.87 \pm 114.83$	-13,58	$652.73\pm89.53$	$519.24 \pm 136.36$	-20,45
FI (au)	15	.75 ± 10.75		19.	83 ± 11.32	
RPE (au)	17	$7.00 \pm 1.82$		17	$1.73 \pm 1.19$	

**Table 16.** Significance level (p) and partial eta-squared  $(np^2)$  of the comparisons pre and post MISS for the maximal and 5s average isometric strength and for the time\*discipline interaction.

	Maximal and 5s average strength									
	QM	IVC	HM	VC	H:Q ratio		QMVIC <sub>5s</sub>		HMV	/IC <sub>5s</sub>
	р	$n_p^2$	р	$n_p^2$	р	$n_p^2$	р	$n_p^2$	р	$n_p^2$
Time (pre to post MISS)	***	.71	***	.37	**	.29	***	.77	***	.60
Time*Discipline Interaction	ns	.02	ns	.04	ns	.02	ns	.14	ns	.11

*Notes: ns: p>.05; \*: p* $\leq$ *.05; \*\*: p* $\leq$ *.01; \*\*\*: p* $\leq$ *.001* 

No significant interactions between time and discipline on the MISS-induced performance decline emerged, while a significant effect of time (pre to post variations) emerged for the QMVC ( $F_{(1,25)}=79.85$ , p=.000), HMVC ( $F_{(1,25)}=15.80$ , p=.001), H:Q ratio ( $F_{(1,25)}=15.24$ , p=.001), QMVIC<sub>5s</sub> ( $F_{(1,25)}=86.05$ , p=.000), and HMVIC<sub>5s</sub> ( $F_{(1,25)}=27.85$ , p=.000) (table 15 and table 16).

**Table 17.** Significance level (p) and partial eta-squared  $(np^2)$  of the comparisons pre and post MISS for the early explosive isometric strength and for the time\*discipline interaction.

	Early explosive strength									
	QRT	D <sub>0-50</sub>	HRT	D <sub>0-50</sub>	QRTD <sub>0-100</sub>		HRT	D <sub>0-100</sub>		
	р	$n_p^2$	р	$n_p^2$	р	$n_p^2$	р	$n_p^2$		
 Time (pre to post MISS)	***	.65	***	.53	***	.72	***	.65		
Time*Discipline Interaction	ns	.00	ns	.01	ns	.04	ns	.05		
	1									

*Notes: ns:* p > .05; \*:  $p \le .05$ ; \*\*:  $p \le .01$ ; \*\*\*:  $p \le .001$ 

No significant interactions between time and discipline on the MISS-induced performance decline emerged, while a significant effect of time (pre to post variations) emerged for the QRTD<sub>0-50</sub> ( $F_{(1,25)}$ = 58.27, p=.000), HRTD<sub>0-50</sub> ( $F_{(1,25)}$ =42.68, p=.000), QRTD<sub>0-100</sub> ( $F_{(1,25)}$ =79.66, p=.000), and HRTD<sub>0-100</sub> ( $F_{(1,25)}$ = 41.32, p=.000) (table 15 and table 17).

**Table 18.** Significance level (p) and partial eta-squared  $(np^2)$  of the comparisons pre and post MISS for the late explosive isometric strength and for the time\*discipline interaction.

		Late explosive strength									
		QRTD <sub>0-200</sub>		HRTI	HRTD <sub>0-200</sub>		D <sub>0-300</sub>	HRT	D <sub>0-300</sub>		
		р	$n_p^2$	р	$n_p^2$	р	$n_p^2$	р	$n_p^2$		
	Time (pre to post MISS)	***	.71	***	.72	***	.69	***	.53		
_	Time*Discipline Interaction	ns	.04	ns	.12	ns	.04	ns	.05		
								-			

*Notes:*  $ns: p > .05; *: p \le .05; **: p \le .01; ***: p \le .001$ 

No significant interactions between time and discipline on the MISS-induced performance decline emerged, while a significant effect of time (pre to post variations) emerged for the QRTD<sub>0-200</sub> ( $F_{(1,25)}$ = 71.69, p=.000), HRTD<sub>0-200</sub> ( $F_{(1,25)}$ =30.25, p=0.001), QRTD<sub>0-300</sub> ( $F_{(1,25)}$ =67.62, p=.000), and HRTD<sub>0-300</sub> ( $F_{(1,25)}$ = 21.72, p=.000) (table 15 and table 18).

**Table 19.** Significance level (p) and partial eta-squared ( $np^2$ ) of the comparisons pre and post MISS sprint performance variables and for the time\*discipline interaction.

	Sprint Performance									
	PPO tPP			PO	$PO_{15s}$					
	р	$n_p^2$	р	$n_p^2$	р	$n_p^2$				
Time (pre to post MISS)	***	.58	***	.71	***	.55				
 Time*Discipline Interaction	ns	.03	ns	.01	ns	.03				

*Notes:*  $ns: p > .05; *: p \le .05; **: p \le .01; ***: p \le .001$ 

No significant interactions between time and discipline on the MISS-induced performance decline emerged, while a significant effect of time (pre to post variations) emerged for the PPO ( $F_{(1,25)}=45.23$ , p=.000), tPP ( $F_{(1,25)}=99.61$ , p=.000), and the PO<sub>15s</sub> ( $F_{(1,25)}=53.07$ , p=.000). (table 15 and table 19).

Additionally, an independent t-test was performed to compare the FI and the Sdec induced by the MISS between the two groups, revealing a non-statistically significant difference (FI: t<sub>(25)</sub>=-.95,

p=.352; Sdec:  $t_{(25)}=.82$ ; p=.871), with lower values for cycling (FI: 15.75±10.75 au; Sdec: -7.67±5.51%) compared to running (FI: 19.83±11.32; Sdec: -9.37±4.81%) group.

#### 4. CONSIDERATIONS

The present investigation aimed to characterize the morpho-mechanical profile of competitive cyclists and runners, to analyze the possible relationships between the morpho-mechanical variables, the neuromuscular and sprint performance, and to evaluate the performance decay associated with a maximal intensity sprint session.

The analysis of the morpho-mechanical characteristics highlighted how the cyclists' profile seem to be represented by an increased vastus lateralis PA and MT and by an increased knee extensors' stiffness, while runners displayed greater FL. The present results are in line with our hypotheses and are supported by previous theoretical frameworks and observations by which runners, due to the eccentric contraction phases part of the repeated SSCs may be exposed to muscle remodeling processes leading to increased FL rather than PA (Franchi, Reeves and Narici, 2017). On the other side, in cycling, the predominant concentric contractions may potentially predispose to adaptations resulting in PA increases rather than FL (Franchi, Reeves and Narici, 2017). Indeed, has been described how concentric, differently from eccentric, contractions may promote a greater increase in PA reflecting a hypertrophy induced parallel addition of sarcomeres, rather than the FL increase (addition of serial sarcomeres) widely observed under eccentric contractions (Franchi, Reeves and Narici, 2017). This suggests a contraction-specific muscular remodeling and structural adaptation and further determine muscle functional properties, explained from one side (runners) by the fact that increased fascicle length may confer greater velocity capacity during identical tendon excursion, because a fiber containing more sarcomeres in series may contract at a greater velocity than one containing less and resulting in a better running performance (Abe, Kumagai and Brechue 2000, Monte and Zamparo, 2019), while on the other (cycling), a greater PA is thought to be associated with an improvement of force output generation for contractions against high loads, by packing more sarcomeres in parallel (Kordi et al., 2020). The greater loads (gear combinations) to which cyclists, especially sprinters, are used to be exposed and combined with the peculiarities of the pedaling biomechanics may explain the increased vastus lateralis MT observed in comparison with runners, in which anyway a difference in MT between faster and slower athletes have been previously observed, with first displaying greater MT (Hug et al., 2006; Kubo et al., 2011; Enomoto, Oda and Kaga, 2019). Similarly, the increased MTUs stiffness observed for cyclists is in line with what in part, previously observed, that is for cyclists an elevated MTUs stiffness may provide a greater tensile force per unit of length change, with a resultant elevation of explosive and maximal strength capacity (Watsford et al., 2010), while the MTUs of runners need to be stiff enough to cope with the high loads (i.e., ground reaction forces during sprinting and SSC contractions), but compliant enough to absorb and release energy (Kubo et al., 2010; Kubo et al., 2015).

Indeed, relative highly levels of stiffness have been related to augments in contractile properties enhancing the magnitude and rate of force output (Hannah and Folland, 2015; Watsford et al., 2010; Ditroilo et al, 2011). MTU stiffness is thought thus, to influence the time course of force production by affecting the efficacy of force transmission from muscle to bone. A stiffer muscle would in theory be more effective in transferring force to bone, and this would be reflected by a shorter time taken to achieve a given level of force and a potential more efficient gesture (Watsford et al., 2010; Spurrs et al., 2003). Hill's model type (1938) suggests the stiffness of the MTU series elastic component (SEC) as composed by a passive part (i.e., mainly tendon and aponeuroses) and an active part (i.e., contractile elements) (Huxley and Simmons, 1971). Training strategies have been observed as able to modulate changes in both passive and active components of the MTU stiffness, however, there is conflicting evidence showing that both increases and decreases in stiffness may

lead to improvement in performance and that a training strategy rather than others may induce prevalently one or the other adaptation. During passive stretches, the intrinsic subcellular cytoskeletal proteins (i.e., titin and desmin) (Waterman-Storer, 1991), the associated connective tissues (epimysium, perimysium, and endomysium), and tendons (Herbert et al., 2002) are probably the main tissues lengthened (Gajdosik et al., 2004) and the assessment of the passive stiffness may represent a way to characterize the functions of these complex structures. It was previously suggested that passive joint torque may play an important role in both, daily activities (Silder, Heiderscheit and Thelen, D. G., 2008) as well as a determinant of functional performance outcome that indeed, seem to be influenced by elastic properties of the passive musculo-articular and musculotendinous complexes, including structures as muscles, tendons, skin, subcutaneous tissue, fascia, ligaments, joint capsule, and cartilage (Wright, 1973).

Despite different studies aimed to evaluate the impact of training strategies (e.g., plyometric, eccentric, isometric, concentric) on musculo-tendinous and musculo-articular stiffness, the difficulties in the standardization of the study design and examined population make the available results incongruent and inconsistent (Fouré et al., 2011; Kubo et al., 2006; Fouré et al., 2013; Morrissey et al., 2011). However, current evidence supports our hypothesis and findings, with long term exposure to an exercise with peculiar characteristics (e.g., muscle action) and task-specific demands, potentially explaining the differences observed between cyclists and runners, with the different mechanisms of force generation at the contractile protein level involved by different contractions constituting probably the main reason of different force production and consequent remodeling processes (Franchi, Reeves and Narici, 2017).

Mechanical loadings within thigh muscles may affect not only muscle dimensions, architecture, and biomechanical properties, but also tendons (Malliaras et al., 2013; Bohm, Mersmann and Aramptzidis, 2015). However, although differences emerged between the vastus lateralis architecture and the knee extensors' MTUs stiffness, the only observed difference emerged among the patellar tendon dimension parameters was related to the tendon CSA. This can be considered in line with the conclusions of the systematic review with meta-analysis performed on this topic by Bohm, Mersmann and Aramptzidis (2015) by which emerged that an evidence supported high responsivity of tendons to mechanical loading, however, the magnitude of load represents the main trigger rather than the muscle contraction type. Thus, our results displaying similar results between the groups may be related to the homogeneous high-level of training characterizing the subjects involved in the present investigation.

From the present experiment emerged also significant differences in the echogenicity of both, the *vastus lateralis* muscle and the *patellar tendon* with cyclists displaying lower values in both anatomical areas. The echo intensity is considered an index of muscle quality as it may reflect muscle composition (Sarto et al., 2021). The explanation behind a higher EI has been linked indeed with an increased presence of connective tissue and fat as they are more reflective compared to muscle tissue (Sarto et al., 2021). This may potentially explain from one side the concomitant reduced EI values of cyclists in the *vastus lateralis* muscle and greater maximal and explosive strength and better sprint performance (greater muscle quality = better performance). On the other it may represent one of possible explanator of the increased or reduced MTU stiffness, as greater echogenicity was found in runners that displayed lower stiffness while increased echogenicity was found in cyclists that displayed greater stiffness. However, although EI measurement represent promising field of application in sport sciences, it can be considered a novel application on this field, with few or no studies covering the present topic, and with the majority of studies present to date are related to aging or clinical settings.

Moving to the differences in neuromuscular maximal and explosive performance (MVIC and RTD), significant differences emerged for knee extensor muscles for all the examined parameters, while no differences (expect for the HRTD0-50) were observed for the knee flexor muscles. Kordi et

al. (2019) found the quadricep muscles, compared to hamstrings as the main explanatory variables of cycling sprint performance, in addition has been demonstrated how quadricep muscles are among the primary involved muscles as well as the primary contributors of cycling performance and also how an imbalance between hamstrings and quadriceps exists in cycling (Turpin and Watier, 2020; McIntyre, Mawston, Cairns, 2012). Differently, a more balanced H:Q ratio have been reported in runners, as well as more data are present on the role of the knee flexors in running performance (Schache et al., 2012; Morin et al., 2015). This is confirmed by our results not only in terms of neuromuscular performance output but also by the differences in the H:Q, with runners displaying greater values.

However, the primary focus of the discussed comparison was to underline the observed differences (in this case considering only knee extensors') on the light of the differences in the morpho-mechanical variables. Thus, to deeper investigate if the differences observed in the vastus lateralis architecture and MTUs stiffness accounted for the variation in the neuromuscular performance we evaluated the relationships between the morpho-mechanical variables and the neuromuscular and sprint performance among the two groups, as the second aim of the present study.

From the aforementioned analysis, emerged that FL and the MT explained similarly both, neuromuscular and sprint performance (z-score comparisons: p>.05) while the MTUs stiffness and the vastus lateralis PA resulted to be a better performance predictor for the cycling group compared to the running (p<.05). The results emerged for cyclists are in line with recent observations (Kordi et al., 2020; Kordi et al., 2021; Lee et al., 2021) by which the vastus lateralis PA seem to represent a valid predictor of sprint and neuromuscular performance and confirming the assumptions described previously. The largest correlations emerged with stiffness as the criterion variable and for almost all the considered performance indicators, confirm previous observations by Watsford et al. (2010) and Ditroilo et al. (2011) and bring additional data and insights to a little investigated topic. Taken together, the present results confirmed our hypothesis for cyclists and reinforced previous observations on the importance of morpho-mechanical variables as performance determinants. On the other side, for runners, the present results confirm in part previous observations, with the FL representing a moderate explanator of the neuromuscular performance while has been confirmed the role of the MT as performance indicator for runners (Enomoto, Oda and Kaga, 2019; Monte and Zamparo, 2019). The MTUs was slightly correlated with the neuromuscular and sprint performance for runners, and in addition, negative large correlations emerged with CV and ARC. On the contrary large positive correlations emerged between CP, W' and the MTUs stiffness for cyclists, confirming the previous assumptions and previous observations (Kordi, Menzies and Simpson, 2018; Kordi et al., 2021).

Additionally, we aimed to evaluate the possible impact of the discipline, and thus of a different morpho-mechanical profile, on the performance decay (sprint and neuromuscular) induced by a MISS. The FI and the Sdec, emerged from the sprint session as well as the fatigue perception (RPE), did not differ between the two groups. Although performance declined significantly either for sprint, maximal and explosive neuromuscular tasks, in both groups, no interactions of the discipline emerged. This may suggest that athletes are subjected to fatigue accumulation and the associated performance decay independently on their morpho-mechanical profile. Training status, physical condition and performance adaptations may potentially represent better explanator of performance decay and may be further considered as additional variables for future investigations (Abt et al., 2011, Rønnestad et al., 2020).

Overall, the main findings of the present investigation contributed to depict the morphomechanical profile of cyclists and runners, making the two groups in comparison, and evaluating the relationships with performance among these two different disciplines. The morpho-mechanical profile resulted to be different between competitive cyclists and runners with cyclists seeming to take advantage from a more pennated muscle and stiffer MTU compared to runners where the MT seem to better explain the maximal and explosive neuromuscular performance. Implementing performance analysis tests with morpho-mechanical evaluations as musculoskeletal ultrasound and MTU biomechanical properties may provide additional insights able to potentially determine consistent changes in the athlete's physical expression and allowing the coach of sport science practitioner to have a wider view of the athlete's performance profile. Indeed, this may bring evidence to coaches, sport science practitioners and athletes' team staff on potential variables to monitor along the season giving support to evidence-based choices in training planning strategies and decision-making, once considered specific adaptative targets. However, although the results of the present investigation are promising, additional studies will be necessary to better clarify the role of the different variables associated with the morpho-mechanical profile in determining athletes' performance.

To improve the present experiment, future studies may include larger populations (especially of runners), should consider involving an additional group as control (e.g., unactive or moderately active people not involved in any competitive sport or exercise), and including additional and/or different performance tasks. Although, previous studies used similar set-ups by using the Wingate ergometer, runners or more in general, athletes' different from cyclists, may feel not fully-comfortable and not able to express their maximum performance level when sprinting or exercising differently from daily performance-tasks.

## CONCLUSIONS

- 1. The morpho-mechanical profile of competitive cyclists differed from that of competitive runners. Cyclists displayed increased *vastus lateralis* PA, MT, and knee extensors' MTU stiffness, while runners a greater *vastus lateralis* FL. Cyclists displayed greater maximal and explosive strength (MVIC and RTD) for knee extensors but not for knee flexors, compared to runners.
- 2. *Vastus lateralis* FL and the MT explained similarly both, neuromuscular and sprint performance while the *vastus lateralis* PA and knee extensors' MTU stiffness resulted to be a better performance predictor for the cycling group compared to the running group.
- 3. No interactions of the discipline on athletes' performance decay emerged, despite performance declined significantly either for sprint, either for maximal and explosive neuromuscular tasks, in both groups.

## SUGGESTIONS AND RECOMMENDATIONS

The main findings of the present experiment may suggest the following practical remarks:

• Implementing performance analysis models with morpho-mechanical profile evaluations to assess cyclists and runners' physiological and biomechanical conditions.

• Supporting evidence-based choices on targeting and selecting training strategies for coaches and sport science practitioners.

• Brining new evidence and remarks on unresolved research open-questions, underlying the need of deeper information on muscle remodeling adaptations in high-level athletes and its related performance implications.

The main methodological remarks related to the present investigation:

• Although is notably difficult to involve high-level athletes the sample size could be improved by recruiting more participants (especially runners).

• Any testing task may be tailored according to athlete discipline specifications.

• A control group may be included in future studies to allow further comparisons not only ascribed to the groups of high-level athletes.

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