The effects of a strength exercise intervention on the gait biomechanics and gastrocnemius muscle-tendon unit behavior in the elderly

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Para o meu Avô Filipe.

A saudade não se traduz. Nem noutra língua, nem em palavras.
Preface

The work presented in this thesis was performed at the Human Movement Biomechanics Research Group, Department of Movement Sciences of the Katholieke Universiteit Leuven (Leuven, Belgium), during the period February-October 2019, under the supervision of Prof. Benedicte Vanwanseele, and within the frame of the Erasmus programme. The thesis was co-supervised at Instituto Superior Técnico by Prof. Carlos Quental.

Declaration

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.
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Abstract

The interaction between the medial gastrocnemius (MG) muscle and the Achilles tendon (AT), i.e. muscle-tendon unit (MTU) interaction, is crucial to minimize the energy cost of walking. The fascicles follow a different length-change pattern from those of MTU and tendon during walking, leading to beneficial contractile conditions for MG. Aging is associated with a decreased AT stiffness, which impacts walking performance. Older adults tend to decrease their preferred walking speed, adapting muscle fascicle behavior as a strategy to decrease the cost of energy. Nonetheless, aged AT can increase its stiffness through training interventions, which has been related to more optimal muscle fascicle lengths during walking. To our knowledge, the impact of a training-induced increase in AT stiffness in the interaction of MG MTU during walking in the elderly was not assessed. Thus, we investigated the effect of a 12-week strength exercise on the walking kinetics and kinematics and MG MTU behavior in older adults. MG muscle fascicles of 11 older male adults were tracked using dynamic ultrasonography during treadmill walking trials. Kinetic and kinematic data were collected, and musculoskeletal models used to determine joint angles and moments and to estimate MTU lengths. No length and velocity changes in MG MTU, fascicle and tendinous tissues were observed after training. Additionally, a similar walking pattern before and after training was found, with no significant differences in joint kinematics and kinetics. Questions remain regarding the MTU training-induced adaptations of the aged MG during walking and further research is warranted to draw more conclusions.

Keywords: Walking, Muscle-Tendon Unit, Medial Gastrocnemius, Older Adults, Ultrasound
Resumo

A interação entre o gastrocnémio medial (GM) e o tendão de Aquiles (TA), i.e. interação da unidade músculo-tendão (UMT), minimiza o custo de energia da marcha. Ao andar, o padrão de mudança de comprimento dos fascículos difere do seguido pela UMT, permitindo condições benéficas de contração para o GM. Envelhecer origina perda de rigidez do TA, influenciando o desempenho da marcha. Os idosos tendem a andar mais devagar, adaptando o comportamento fascicular do músculo para diminuir o custo de energia. Porém, o TA envelhecido consegue aumentar a sua rigidez através de treino físico, o que está relacionado a comprimentos fasciculares ótimos durante a marcha. É desconhecido o impacto que o exercício tem para aumentar a rigidez do TA na interação da UMT do GM durante a marcha em idosos. Assim, investigou-se o efeito de 12 semanas de exercícios de força sobre a cinética e cinemática da marcha e o comportamento da UMT do GM em idosos. Os fascículos do GM de 11 idosos do sexo masculino foram rastreados usando ultrassonografia durante testes de marcha numa passadeira. Dados da cinemática e cinética foram obtidos, e modelos músculo-esqueléticos usados para determinar ângulos e momentos das juntas e estimar o comprimento da UMT. Não foram observadas diferenças significativas no comprimento e velocidade dos fascículos, tecidos tendinosos e UMT do GM pós-treino. Adicionalmente, os ângulos e momentos das juntas foram semelhantes pré e pós-treino. Estudos futuros são necessários para melhor compreender as adaptações induzidas pelo exercício físico na interação da UMT dos idosos durante a marcha.

Keywords: Marcha, Unidade Músculo-Tendão, Gastrocnémio Medial, Idosos, Ultrassom
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# Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>MTU</td>
<td>Muscle-Tendon Unit</td>
</tr>
<tr>
<td>MG</td>
<td>Medial Gastrocnemius</td>
</tr>
<tr>
<td>LG</td>
<td>Lateral Gastrocnemius</td>
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<td>SOL</td>
<td>Soleus</td>
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<td>AT</td>
<td>Achilles Tendon</td>
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<td>SEE</td>
<td>Series Elastic Elements</td>
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<tr>
<td>3D</td>
<td>Three-Dimensional</td>
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<tr>
<td>MTJ</td>
<td>Muscle-Tendon Junction</td>
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<tr>
<td>PCSA</td>
<td>Physiological Cross-Sectional Area</td>
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<tr>
<td>AGR</td>
<td>Architectural Gear Ratio</td>
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<tr>
<td>CE</td>
<td>Contractile Element</td>
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<tr>
<td>SEC</td>
<td>Series Elastic Component</td>
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<tr>
<td>PEC</td>
<td>Parallel Elastic Component</td>
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<td>TT</td>
<td>Tendinous Tissues</td>
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<td>ROM</td>
<td>Range of Motion</td>
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<tr>
<td>GRF</td>
<td>Ground Reaction Force</td>
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<tr>
<td>IK</td>
<td>Inverse Kinematics</td>
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<td>ID</td>
<td>Inverse Dynamics</td>
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<tr>
<td>1RM</td>
<td>One-Repetition Maximum</td>
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<tr>
<td>ROI</td>
<td>Region of Interest</td>
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<tr>
<td>dof</td>
<td>Degree of Freedom</td>
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<tr>
<td>KF</td>
<td>Kalman Filter</td>
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<tr>
<td>SD</td>
<td>Standard Deviations</td>
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<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<td>3DUS</td>
<td>Three-Dimensional Ultrasound</td>
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Chapter 1

Introduction

1.1 Motivation

Humans possess the capacity to produce an extensive diversity of postures and movements. For this, structures of the human body are required to cooperate in producing and responding to forces responsible for creating and controlling movement. From a mechanical point of view, the primordial function of the skeletal muscle is to convert chemical energy into mechanical energy in order to generate force and power. In turn, the main task of tendon is to transfer the force produced by the skeletal muscles to the skeleton, creating movement of the joints.

Muscle and tendon are combined in a complex structure - muscle-tendon unit (MTU) - and work together to produce movement [Lichtwark and Wilson 2007]. The MTU has always been a topic of interest for anatomists and physiologists, who firstly relied on anatomical dissections. The latest technological improvements, especially in the field of image acquisition and processing, allowed a better insight of the MTU behavior and function.

The triceps surae muscles - medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SOL) - are connected to the Achilles Tendon (AT), which is the largest and strongest tendon of the human body. These muscles allow stabilization of the human body during locomotion, restraining it from falling and providing propulsion in the late stance phase of walking. The tendon allows energy storage and return during functional activities, due to its elastic properties. As a result, the fascicles follow a different length-change pattern from those of MTU and tendon during walking [Fukunaga et al. 2001, Lichtwark et al. 2007], which allows beneficial contractile conditions for the triceps surae muscles and favors economic force production [Roberts et al. 1997]. There is a consensus in the literature that during walking, stretch and recoil of the fascicles are limited and elongation of the MTU is mainly accompanied by the stretching of series elastic elements (SEE; i.e. tendinous tissues), which allows for elastic energy storage.

In the elderly, maximal physiologic capacities are highly diminished. For instance, aged triceps surae experience loss of strength and AT stiffness appears to be decreased through altered AT material properties [Delabastita et al. 2019]. These factors have been found to be associated with decreased
walking performance (Stenroth et al., 2015). In fact, older adults tend to walk slower than younger adults. Stenroth et al. (2017) proposed that walking slower for older adults improves triceps surae contractile conditions, allowing muscle fascicles to operate at a narrower range and performing less mechanical work. In addition, a lesser role of ankle plantar flexors in gait, found for older adults compared to young adults (Boyer et al. 2017), may contribute to the reduction in their preferred walking speed. Hence, the reduction in preferred walking speed allows a change in muscle fascicle behavior, which can reduce the energy cost of walking and be a strategy to compensate for decreased plantarflexor strength.

It is well accepted that tendons are able to adapt to mechanical loading through various forms of resistance training (Wiesinger et al., 2015). Likewise, and despite the age-related alterations, older tendons preserve their ability to increase its stiffness in response to a long-term training intervention (McCrum et al. 2018). Since the elastic properties of the AT allow the decoupling of muscle fascicle behavior from that of the MTU during walking, changes in AT stiffness are expected to influence MG fascicle behavior. Simulation studies using a two-dimensional model of the Achilles tendon in series with the gastrocnemius muscle (Lichtwark and Wilson 2007; 2008) agree on the existence of an optimal value for tendon stiffness related to a shortening pattern of muscle fascicles that minimizes the energy cost of walking.

While several studies assessed the impact of a training-induced increase in AT stiffness in the behavior of MG MTU during dynamic tasks, such as running (Albracht and Arampatzis 2013; Werkhausen et al. 2019) and landing (Werkhausen et al. 2018), to our best knowledge, there is no information in literature regarding this effect during walking in older adults. Poor mobility in older adults is connected to adverse health outcomes, such as an increased risk of falling, which can lead to mortality (Soriano et al., 2007). Thus, it is of great interest to access the complex interactions in aged muscle-tendon units during daily tasks. Findings could lead to the design of exercise interventions to be applied in health care systems, aiming to improve walking performance to reduce the loss of function in the elderly.

### 1.2 Scopes and Objectives

Achilles tendon decouples muscle fascicle behavior from that of the MTU during walking, allowing beneficial contractile conditions for the triceps surae muscles (Roberts et al., 1997). It is expected that changes in AT stiffness influence MG fascicle behavior (Lichtwark and Wilson 2007; 2008). Thus, theoretically, a training-induced increase in AT stiffness in the elderly (McCrum et al. 2018) would lead to changes in the stretch and recoil pattern of the muscle fascicles, possibly leading to a more favorable walking strategy.

The main purpose of the present study was to understand if a 12-week strength exercise intervention would have a significant impact on the typical behavior of the medial gastrocnemius MTU in older adults. Since MTU behavior is highly influenced by the walking pattern (joint angles and moments), these parameters were also investigated pre- and post-training intervention. A three-dimensional (3D) musculoskeletal model of the human body allows the accurate estimation of joint angles and joint moments and MTU lengths throughout movement. On the other hand, the human fascicular kinematics can
be measured using real-time ultrasonography, a reliable and non-invasive scanning method (Maganaris et al., 1998).

Bearing this in mind, several goals were set:

- To obtain and compare joint angles and joint moments between pre- and post-training intervention sessions.
- To obtain and compare muscle fascicle, MTU and SEE length changes of the MG between pre- and post-training intervention sessions, using the model described by Fukunaga et al. (2001).
- To obtain and compare pennation angles of the medial gastrocnemius between pre- and post-training intervention sessions.
- To obtain and compare fascicle, MTU and SEE shortening velocity changes of the MG between pre- and post-training intervention sessions.

1.3 Literature Review

Muscles and tendons play a central role in producing movement. As such, questions regarding the interaction of these structures during everyday tasks, e.g. walking, or how they are influenced by aging or training interventions, and how in turn these changes affect physical performance, arise.

During the last two decades, the structural and functional measures of the MTU have received remarkable help from the technical progress in ultrasonography and such improvements allowed understanding in vivo tendon behavior. Until then, much of the knowledge regarding muscle and tendon interaction during movement had come from studying animal locomotion. However, anatomical differences between animals and humans led researchers to draw several approaches for studying human locomotion with focus on muscle-tendon energetics.

In earlier studies on this topic (e.g. the extensive work of Cavagna, 1977), no distinction between muscle and tendon was made and their interaction was practically ignored. In 1984, Griffiths (1984) was one of the first implying that muscle fascicles and the whole MTU could be decoupled from each other, leading to different length changes to enhance muscle performance. In this work, Griffiths also highlighted the important contribution of the tendon to elastic energy storage. However, it must be noted that the tendon is not the only tissue possessing elastic properties. The muscle itself is an elastic structure which produces passive tension when stretched beyond its resting length. In addition, recent studies stress the importance of the different roles of connective tissue in muscle-tendon interaction (Maas and Sandercock, 2010; Roberts and Azizi, 2011).

Some years after publishing his extensive work, Griffiths (1987) confirmed his hypothesis on decoupled length changes between muscle and tendon, by applying ultrasound transit times in cats walking to calculate muscle fascicle length changes. This technique was only successfully applied on humans more than one decade later to measure fascicle length changes during dynamic movement (Fukunaga et al., 2001). Between these two events, more work on the muscle and tendon behavior during locomotion was performed using sonomicrometry, which allowed to obtain information on the
muscle fascicle length changes (Griffiths, 1991; Roberts et al., 1997). However, this technique could only be used in animals due to its highly invasive nature. Ultrasound measurements started to gain popularity later since they were non-invasive and allowed the same detail of information. Following the work by Fukunaga et al. (2001), several studies investigating the interaction between muscle and tendon during various tasks, such as walking (e.g. Ishikawa et al., 2005), running (e.g. Lichtwark and Wilson, 2006) or jumping (e.g. Kurokawa et al., 2001) were published. MTU interaction kept being studied mainly in less explosive tasks and later on Farris and Sawicki (2012a) showed that this interaction differs when performing different tasks, such as walking and running, and is also affected by the speeds within tasks.

As stated earlier, the contribution of tendons for economical movement was not always recognized. This tissue, besides acting as a force transmitter between the MTU and the skeleton, allows energy storage and return during functional activities due to its elastic properties. As a result, the tendon decouples the muscle fascicle length changes from the total MTU length changes (Fukunaga et al., 2001; Lichtwark et al., 2007). During walking, there is evidence of MTU elongation throughout the beginning of the stance phase. This is mainly accompanied by the stretch of SEE, while muscle fibers operate near-isometrically and perform almost no mechanical work. Then during late stance, SEE recoil rapidly and release elastic energy while fascicles produce positive work. This decoupling between muscle and tendon allows the overall muscle-tendon unit to operate with high power output and efficiency during walking (Brennan et al., 2017; Farris and Sawicki, 2012a; Fukunaga et al., 2001; Ishikawa et al., 2005; Ishikawa et al., 2007; Lichtwark and Wilson, 2006).

The mechanism by which muscle fascicle length changes are decoupled from the length changes of the MTU optimize the muscle force production through the force-length and force-velocity relationships (Alexander, 2002; Biewener and Roberts, 2000; Lichtwark and Barclay, 2010). These relationships are crucial to understand the effects that muscle architecture and tendon properties have on muscle fiber length and velocity and, hence, in force and power production output. Pennation angle can also influence muscle function, since it reduces the amount of force applied to a tendon (and the skeleton) by a factor of cosine of pennation angle. Furthermore, greater pennation angles allow a higher number of parallel sarcomeres in a given certain muscle volume leading to an increase in the physiological cross-sectional area of the muscle (Lieber and Fridén, 2000). The mechanical tendon properties are expected to influence the mechanics, energetics and even neural control of several functional activities (Roberts and Azizi, 2011).

Muscle-tendon morphology and interaction do not remain constant throughout human life. The scientific opinion is consensual when it comes to the fact that aging and mechanical loading might lead to structural and behavioral changes in the MTU. However, the potential role of age-related alterations has received only a limited amount of research interest.

In the elderly, maximal physiologic capacities are highly diminished, thus there is a limitation in the ability of performing physical tasks at the same level of energy expenditure or muscular force. Activities that once were performed easily, such as rising from a chair or crossing a street, become maximal or impossible efforts. For instance, aging is associated with loss in muscle force (Morse et al., 2005c), power (Reid et al., 2012) and altered muscle architecture and tendon mechanical properties.
A reduction in pennation angle and fascicle length was reported with aging which has implications for
force and power muscle production (Kubo et al., 2003a; Morse et al., 2005b). Additionally, studies as
the one performed by Stenroth et al. (2012) showing muscle architecture and tendon properties to be
significantly different between young and old adults, turn these factors into potential explanations for
age-related decline in mobility.

Achilles tendon is the longest tendon in the human body and its energy storage capacity, together
with the influence on the gastrocnemius and soleus muscle work, may have a great impact in functional
performance. As highlighted by Roberts and Azizi (2011), tendon stiffness is a key parameter for elastic
mechanisms associated with muscle-tendon interaction and, indeed, AT stiffness was found to be related
with energy cost of walking and running in experimental (Kubo et al., 2000; Arampatzis et al., 2006)
and simulation (Lichtwark and Wilson, 2007) studies. These findings were explained by the crucial
role of the gastrocnemius and soleus MTU during walking (Neptune et al., 2001; 2008). Due to what
was previously stated and the fact that tendon properties are different between young and old adults,
several studies have investigated Achilles tendon stiffness in the elderly. The contrasting results on AT
stiffness and its functional impact led to a systematic review and meta-analysis (Delabastita et al., 2019)
which suggested that AT stiffness is decreased in the elderly through altered AT material properties.
Nonetheless, authors refer the need for more research in order to understand the mechanisms behind
the association between decreased AT stiffness and decreased walking performance in older adults.

Furthermore, advanced age is related with changes in the neuromuscular system which are trans-
lated in altered balance, joint mobility and cardiovascular fitness (Judge et al., 1996; Karamanidis and
Arampatzis, 2005; Kang and Dingwell, 2009; Graf et al., 2005). Neuromuscular system changes can
lead to a reduction in mobility and performance of daily activities by impacting gait biomechanics (Mcgib-
on, 2003). Over the past 30 years, differences in gait kinematics and kinetics between young and older
adults and how gait changes in relation to age-related physiological changes have been investigated
extensively. The literature has a consensual opinion on a lesser role of ankle plantar flexors in gait for
older adults. Stenroth et al. (2017) proposed that a decreased preferred walking speed for older adults
could be a strategy to compensate for decreased plantarflexor strength. Humans tend to walk at a speed
that minimizes the gross energy cost of walking per unit distance (Farris and Sawicki, 2012b). For older
adults, walking slower leads to a change in muscle fascicle behavior, allowing them to operate within a
narrower range and, hence, performing less mechanical work. Thus, a reduction in preferred walking
speed seems to provide a mechanism to improve triceps surae contractile conditions and minimize the
energy cost of walking (Stenroth et al., 2017).

Previous studies have reported strong correlations between the net ankle moment and power
generated by the ankle plantarflexors and walking performance (e.g Winter et al., 1990). However,
biochemical analyses investigating only the net ankle and knee joint moments do not allow con-
clusions on the mechanical contributions of each individual monoarticular and biarticular plantarflexor
muscle throughout movement. This happens since it is difficult to rigorously quantify the contribution of
a muscle to the individual body segment energetics. In spite of this difficulty, loss or impairment of force
generation by either the soleus or gastrocnemius muscle clearly impacts walking performance.
Age-related alterations can be predicted in individual aspects of muscle-tendon architecture and mechanical properties and how they impact independently contractile function. Nonetheless, it is much more difficult to predict age-related impact on muscle-tendon behavior during a complex, multi-joint locomotor task, such as walking. This happens since numerous sources of complication can be found, besides the interactions between the effects of aging on the architectural and mechanical aspects of the MTU. Multi-joint nature of the task, muscle activation patterns and prior contractile history within a certain stretch-shortening cycle are some of the factors to be accounted. Thus, studies to gain insights on how aging impacts the muscle-tendon behavior during activities of human daily routine are needed.

In a pioneer work, Mian et al. (2007) investigated the behavior of fascicular and tendinous components of the lateral gastrocnemius MTU. Even though no age-related difference in the lengthening of the LG MTU during stance was found, authors reported a relatively shortened position of the gastrocnemius fascicles on older adults whilst tendinous tissues lengthened more compared to the young adults. A proposed explanation for this behavior was an increased compliance of tendinous tissues found in older adults (Kubo et al., 2003b; Morse et al., 2005b). The latter factor would in turn increase the lengthening of tendinous tissues, hence reducing the need for fascicle lengthening to achieve the same level of MTU lengthening.

According to the systematic review performed by Wiesinger et al. (2015), there is an adaptability of tendons to the mechanical loading. Several were the works reporting an increased Achilles tendon stiffness after different types of resistance training (e.g. Arampatzis et al., 2007). In addition, tendinous adaptations seem to occur in parallel with muscular adaptations (Seynnes et al., 2009), which suggests the influence of the latter on work production during locomotor activities.

Bearing this in mind, and in addition to biological changes, mechanical loading is expected to influence aging tendon. Previous in vivo studies have shown that chronic inactivity (20–90 days bed rest) can lead to a reduction in tendon stiffness (Kubo et al., 2004; Reeves et al., 2005). Therefore, aging tendon is also affected by a reduction on the habitual loading due to decreased physical activity and muscle strength (Iannuzzi-Sucich et al., 2002; Lauretani et al., 2003). In their recent work, McCrum et al. (2018) aimed to review previously reported age-related differences in human triceps surae and quadriceps femoris MTU biomechanical properties and to understand if, and to what extent, mechanical loading interventions could interfere with these changes in older adults.

In the review of McCrum et al. (2018), six intervention studies conducted with older adults were analyzed (Reeves et al., 2003a; Reeves et al., 2003b; Onambele-Pearson and Pearson, 2012; Grosset et al., 2014; Karamanidis et al., 2014; and Epro et al., 2017). Each intervention consisted on mainly resistance-based exercises performed during a total of 12-14 weeks, with one of the works also reporting a 1.5 year long intervention (Epro et al., 2017). The adopted training protocols contained exercises ranging from highly specific and controlled protocols (Epro et al., 2017) to more mixed strength exercises herewith running or hopping (Onambele-Pearson and Pearson, 2012; Grosset et al., 2014; Karamanidis et al., 2014). All protocols, apart from the one of Grosset et al. (2014), were designed to impose high mechanical loads and they were performed two or three times a week with partial or complete supervision. Grosset et al. (2014) compared low and high intensity training groups, whereas
Pearson and Pearson (2012) performed a gender-based comparison in older adults. The study of Epro et al. (2017) focused exclusively in the AT, four articles assessed exclusively patellar tendons (Reeves et al., 2003a; 2003b; Onambele-Pearson and Pearson, 2012; Grosset et al., 2014) and Karamanidis et al. (2014) studied both human triceps surae and quadriceps femoris MTUs. All studies except the one by Grosset et al. (2014) reported increase in muscle strength (13.4-25.5% for the triceps surae and 9.2-25.4% for the quadriceps femoris). Furthermore, all but one reported significant increases in the AT or patellar tendons stiffness (19.6-22.5% and 10.1-82.5%, respectively).

According to the reported works, older tendons seem to preserve their adaptability to mechanical loading with age. However, the results must be evaluated keeping in mind the effectiveness of the interventions. Epro et al. (2017) showed that 14 weeks of resistance exercise is a sufficient time period to lead to adaptative responses in the biomechanical properties of the AT. These changes were maintained for 1.5 years of continued training sessions, which suggests that a non-linear time-response relationship between aging and adaptation to mechanical loading could exist. Moreover, the intervention with the lowest exercise intensity (Grosset et al., 2014) was the only one that did not find significant changes in the tendon biomechanical properties, which can suggest that tendon adaptation is triggered only when a specific threshold is exceeded during the loading exercise. The previous findings are consistent with the ones found in younger adults, so it appears that the leg extensor MTUs of older adults change when subjected to increased mechanical loading in a similar way to what happens with younger adults.

Tendon stiffness is clearly crucial for both muscle power and efficiency. However, the relationship between tendon stiffness and the force generating capacity varies from muscle to muscle (Roberts, 2002). Furthermore, if the compliance of SEE is varied, the shortening pattern of the muscle fascicles is required to change in order to achieve the same force output. Simulations studies, using a two-dimensional model of the AT in series with the gastrocnemius muscle, were developed to investigate the influence of human MG fascicle and AT stiffness in muscle efficiency for walking and running (Lichtwark and Wilson, 2007; 2008). The same authors have shown the existence of an optimal value of SEE stiffness that maximizes muscle fascicle efficiency in different gait conditions. Moreover, Lichtwark and Wilson (2007) reported that at high tendon stiffness values the muscle fascicle behavior would be similar to that of the MTU, lengthening throughout most of the stance phase until it shortens rapidly. In turn, with low tendon stiffness, the muscle fascicles are predicted to shorten throughout the stance phase and then lengthen rapidly.

Previous studies assessed the behavior of fascicular and tendinous components of the MG MTU during running (Albracht and Arampatzis, 2013; Werkhausen et al., 2019) and landing (Werkhausen et al., 2018) after a resistance training intervention to increase AT stiffness. Nonetheless, to our best knowledge, there is no information in the literature regarding those aspects during walking in older adults.

Hence, the purpose of the present study was to investigate the effect of 12-week strength exercise on the behavior of fascicular and tendinous components of the medial gastrocnemius MTU during walking. MTU behavior is largely influenced by the walking pattern, thus joint angles and moments were also assessed pre- and post-training intervention. It was hypothesized that while 1) joint kinematics and kinetics would remain similar before and after training, identical to what was reported by Werkhausen et al., 2019.
et al. (2019), due to an increased AT stiffness post-intervention and subsequently decreased tendon strain, 2) the tendon would undergo less stretch and recoil and, thus, 3) fascicles could experience higher length changes.

1.4 Contributions

The main contribution of this work is the assessment of the MG MTU behavior during walking, after a training intervention performed by older adults to increase AT stiffness.

Although previous studies assessed the impact of a training-induced increase in AT stiffness in the behavior of MG MTU during dynamic tasks, none focused on older adults during walking. Aging is associated with several changes in muscle and tendon properties, such as reduced AT stiffness, which impacts walking performance. Thus, this study is expected to provide further insights into how mechanical loading interventions can counteract these changes in the elderly.

Moreover, findings from this study can lead to the design of exercise interventions to be applied in health care systems, aiming to improve walking performance to reduce the loss of function in the elderly.

1.5 Thesis Organization

This dissertation is divided into 6 main chapters:

Chapter 1 contains the motivation behind the developed work, the scopes and objectives of the dissertation and its contributions to research field. Moreover, a brief literature review on the work performed by different authors throughout time is presented. This literature review allows contextualization within the scope of muscle-tendon unit function and interaction and its alterations with aging and training.

Chapter 2 comprises the theoretical background indispensable to the comprehension of the study. Firstly, MTU and its components (i.e. muscle and tendon) are described in terms of morphological and architectural properties. Since the work focus on the medial gastrocnemius, triceps surae muscle group and Achilles tendon are briefly presented. Thereafter, MTU function is reported. Muscle and tendon mechanics are reviewed separately and then their interaction is accounted. Lastly, the human gait cycle is presented with a focus on its kinematics, kinetics and muscle activity.

Chapter 3 provides the workflow of this dissertation. The details on how data acquisition during the walking trials of the older male adults was performed is presented. Moreover, the strength exercise intervention to increase AT stiffness is reviewed. Thereafter, each step of data processing is detailed.

Chapter 4 presents the obtained results. This chapter is divided in subjects characteristics, effects of training on walking kinematics and kinetics and effects of training on MG MTU behavior during walking.

Chapter 5 aims to discuss the results. Moreover, limitations regarding the developed work are presented.

Chapter 6 summarizes the conclusions of the dissertation and points out directions for future work.
Chapter 2

Theoretical Background

Movement of the human body is continuously achieved by a complex and highly coordinated mechanical interaction between the components within the musculoskeletal system, which is under control of neuronal commands. The musculoskeletal system describes the connection between the muscular and skeletal systems. It includes bones, muscles, tendons, ligaments, joints and other connective tissues of the body that work together to provide body stability and limb control, allowing for precise movement. Furthermore, muscle and tendon come together to form a complex unit with distinct mechanical and morphology function, yet a shared purpose of generating and transmitting forces to the skeletal system. To generate movement, the muscle and tendon rely on each other in a sort of catapult mechanism, allowing them to use their full potential. Tendons are passive elements and they cannot actively generate force, yet they are able to develop passive tension by stretching. On the other hand, muscles can actively produce force, however they are limited in power output by functional relationships, force-length and force-velocity relationships. Since both muscle and tendon have performance limitations, by combining their specialized functions, they are able to overcome these limitations and achieve high power outputs at the level of the muscle-tendon unit.

To understand the mechanical function of the complex unit formed by the muscle and tendon during movement, it is relevant to be familiarized with the anatomy and physiology of both muscle and tendon. Thus, an overview of the anatomy, physiology and architecture of the muscle, as well as an introduction to tendinous tissues is presented. Moreover, and since the present work focuses on one muscle of triceps surae muscle group, the medial gastrocnemius muscle, triceps surae muscle group and the Achilles tendon are presented. The rationale behind the choice of the medial gastrocnemius as the main focus of the study is multi-factorial and the reasons will be presented throughout the rest of the chapter. Muscle-tendon function is described first through the separation of concepts regarding muscle mechanics and tendon mechanics, and then by the details of the interaction between these two tissues. In addition, and since task performed by the older adults was walking, the gait cycle is introduced together with its kinematics, kinetics and muscle activity.
2.1 Muscle-Tendon Unit Structure

The muscle-tendon unit can be considered the functional unit for generation of movement of joints and is defined by the muscle and the connective tissue structures, such as tendons. Figure 2.1 illustrates a simplified model of a MTU.

![Figure 2.1: Simplified model of a muscle-tendon unit with pennate architecture. The MTU is formed by the muscle fibers and the connective tissue structures in the distal and proximal ends of the muscle. Muscle fibers/fascicles are represented by the gray lines. The tendon structure can be divided into external, free tendon, and internal part, aponeurosis. Muscle fascicles are aligned at an angle \( \alpha \) denominated pennation angle. Adapted from Zajac (1989).](image)

In the illustration, the muscle (often referred as muscle belly) concerns the part of the MTU that extends from distal to proximal muscle-tendon junction (MTJ). As is possible to observe, the muscle is attached at both ends to tendons which connect MTU to bones. Muscle and tendons do not abruptly join into each other. Rather, the tendon continues along the muscle forming aponeuroses, which serve as attachment surfaces for the muscles fascicles. The distal and proximal free tendons will then attach to the bones.

2.1.1 Muscle Anatomy

Skeletal muscle (Figure 2.2) is composed of parallel bundles of long and multinucleate fibers, fascicles, which, in most cases, extend to the entire length of the muscle. Each of these fibers contains one cell membrane, denominated sarcolemma, and is surrounded by connective tissue. The connective tissue found in muscle structures provides strength, structural support and elasticity to the muscle and exists in three different sets: epimysium, perimysium and endomysium. The first one consists in a tough structure which encloses the entire muscle arrangement and separates it from others. This type of connective tissue possesses high resistance to stretch conferred by its tightly interlinked bundles of collagen fibers. The perimysium, also thick and resistant to stretch, can be found beneath the epimysium and separates the large bundles of muscle fibers into fascicles, providing space for enervation and blood vessels. Lastly, the endomysium forms the immediate external environment for each individual muscle fiber. Its meshwork of collagen fibrils is partly connected to the perimysium and, due to lateral connections to the muscle fiber, this connective tissue transfers contractile force to the tendon.
Furthermore, another thick layer of connective tissue, denominated fascia, stabilizes and encloses each individual muscle. The three categories described behave as a continuous sheet of connective tissue and are combined at each extremity of the muscle to form the tendons.

On a microscopic scale, a muscle fiber is composed of cylindrical, thread-like shaped myofibrils, each one consisting of several elements connected in series, sarcomeres. The sarcomere, represented in Figure 2.3 is the smallest contractile unit of the skeletal muscle’s structure and one is separated from another through the Z-membranes. The composition of sarcomeres consists in three major myofilaments, or simply filaments, which are long chains of protein subunits. In the middle of the sarcomere, myosin gives rise to the thick filaments, whereas actin constitutes the thin filaments and can be found at either side of the sarcomere. Thick filaments are attached to the Z-membranes through highly extensible filaments made of a protein called titin. The thin myofilaments are composed by two chain-like strings of actin and the globular protein troponin is found in notches between the two chains of actin. Attached to each troponin, there is the protein tropomyosin. These two proteins are responsible for controlling muscle contraction.
To ease identification concerning the arrangement of the thick and thin filaments, the areas of the sarcomere are divided into bands or zones. The anisotropic or A band includes both thick and thin filaments. Its central portion, consisting only in thick filaments, is called the H zone. In turn, the central portion of the H zone, denominated M band, concerns the wide middle portion of the thick filaments. Areas containing only actin filaments are the isotropic or I bands.

There is a close yet intricate connection between the central nervous system and the musculoskeletal system. Even though the sarcomere is the basic contractile unit of a muscle, it is part of a greater structure called the motor unit, which contains the alpha motor neuron and the fibers that it innervates. The stimulus received by the muscle fibers to initiate the contractile process, is transmitted through an alpha motor neuron, located in the anterior horn of the spinal cord, as illustrated in Figure 2.4. The nerve cell, axon, extends from the cell body of the alpha motor neuron until the muscle, where it divides into a few or many thousands of smaller branches. Each one of these smaller branches ends in a motor end plate, which is close to the sarcolemma of a single muscle fiber.

The contraction of the entire muscle occurs when several motor units fire asynchronously and repeatedly (Levangie and Norkin 2005). The number of motor units within a muscle, as well as their structure, varies from muscle to muscle. The magnitude of a contraction can be changed either by altering the number of motor units activated or their frequency of activation. Each variation in the structure of the motor unit or muscle fibers will affect the function of the motor unit itself.

The size of the motor unit is determined by the size of the motor nerve axon and by the number of muscle fibers it contains. Therefore, as depicted in the figure 2.4, large motor units or small motor units can be found in the human body. Units containing small cell bodies have axons with small diameters and nerve impulses will take longer to travel through them. Thus, in the small-diameter units, a stimulus will need more time to reach the muscle fibers. Muscles that control fine movements or that are used to make small adjustments in the human body have small-size motor units. Muscles that are used to produce large movements and large increments of force usually contain large-size motor units.
2.1.2 Muscle Physiology

The journey leading to muscle contraction is initiated in the central nervous system, when a nerve impulse is sent to the motor end plate. Then, an electric impulse, called action potential, is released and travels along the muscle fiber leading to release of calcium ions. In turn, these calcium ions begin the replacement of tropomyosin molecules, by troponin ones, in a way that receptor sites on the actin are free for binding with the head groups of the myosin. This connection is known as cross-bridges and the tension across the sarcomere results from the continuous disengagement and engagement at successive sites of these cross-bridges, which in turn pull on the actin filaments. Thus, shortening of muscle fibers is achieved when actin filaments slide alongside myosin filaments, which depends on ATP hydrolysis by the myosin. Actin and myosin are considered to be contractile proteins due to their behavior in muscle contraction.

The latter mechanism explaining muscle contraction is known as Sliding-Filament Theory and was born from two papers published by Huxley and Niedergerke (1954) and Huxley and Hanson (1954).

Shortening of a muscle fiber is only achievable if one or both ends of it are free to move and if there are sufficient number of sarcomeres actively shortening. Muscle contraction might involve or not change in sarcomere length, leading to different classifications: concentric contraction, eccentric contraction and isometric contraction. The first one refers to the active shortening of a muscle, the most popular type of contraction, in which the thin filaments are pulled towards the thick filaments causing length changes. In contrast, in eccentric contraction the thin filaments are pulled away from the thick filaments, leading to muscle lengthening. Lastly, isometric contraction is the type of contraction without length changes since there is an active resistance to motion by the muscle and the cross-bridge cycling is matched by an external force (Levangie and Norkin 2005).

2.1.3 Muscle Architecture

Muscle architecture consists in the macroscopic arrangement of muscle fibers and is a major determinant of muscle function. According to Lieber and Fridén (2000), the understanding of structure-function relationship is of great importance as not only allows explanation about force production and movement but also clarifies, for instance, the mechanical basis of muscle injury. It can also provide guidelines for the placement of electrodes for measuring muscle activity. There exist many variations of architectural arrangements as the number of muscles themselves. Nevertheless, for simplicity purposes, muscles are generally divided into two broad classes of fiber architecture: parallel and pennate muscles.

A muscle is said to have a parallel architecture when its fibers run parallel to each other and converge to the central tendon at one or both ends. In a pennate architecture the fibers are positioned obliquely to the line of pull. The latter classification can be divided into unipennate, bipennate and multipennate depending on the amount of similar angled fibers that converge to the central tendon. Figure 2.5 illustrates three different muscle architectures.
Furthermore, muscle fibers can also contain different forms, being the two main types called spanning and nonspanning fibers. Within the first type, fibers span along the entire fascicle, connecting the tendons or aponeuroses at the ends of the fascicle and in muscles containing nonspanning fibers, fibers are too short to reach the tendons at the ends, ending somewhere in the middle of the fascicle.

The most important architectural parameters consist in physiological cross-sectional area (PCSA), pennation angle and muscle length, muscle fiber length or muscle fascicle length. The first refers to the amount of contractile protein within the muscle which generates force, while the second reflects the orientation angle between the muscle fibers and the tendon. Muscle length is commonly defined as “the distance from the origin of the most proximal muscle fibers to the insertion of the most distal fibers” (Lieber 1992), while fiber length refers to the number of sarcomeres found in series. Muscle length and muscle fiber length might be different due to a variable degree of offset present in muscle fibers. Since separating individual muscle fibers can be technically challenging, fascicle length is typically measured during research using ultrasonography and magnetic resonance imaging (Blazevich 2006). Nevertheless, Zatsiorsky and Prilutsky (2012) recall that in muscles containing nonspanning fibers, fascicle length and fiber length differ from one another, and thus especial care is needed when dealing with this matter.

A good example of how architecture influences muscle function is the comparison between two hypothetical muscles with similar muscle fiber composition and same muscle mass. The amount of force produced by a muscle is directly proportional to the number of sarcomeres in parallel within the muscle and is approximated by PSCA. Hence, the muscle composed with shorter muscle fibers has more sarcomeres in parallel and can produce greater amounts of force. In turn, the muscle with longer fibers will have faster maximal shortening velocity and higher excursion range. For instance, the quadriceps muscles have a larger PSCA, whereas the hamstrings have longer fibers. This arrangement allows the first muscles to produce higher forces and the hamstrings to achieve movements requiring larger ranges of motion. Since the hamstrings muscles span two joints, hip and knee, the muscles need longer fibers to allow greater excursion during movements of the both joints (Levangie and Norkin, 2005).

Fascicle and tendon behavior during movement are also influenced by muscle architecture. For example, there is a consensus in the literature that alterations in these architectural features with aging,
mainly reduction in the fascicle length, might lead to crucial changes in force and power generation of a muscle. For instance, a decreased fascicle length would tend to lower maximum fascicle shortening velocity. However, this can be partly compensated by a diminution in the pennation angle, which would in turn increase the shortening velocity along the line of pull of the tendon (Narici et al., 2003). Pennate muscles and their characteristics are discussed further.

2.1.3.1 Pennate Muscles

As mentioned earlier, in pennate muscles, fascicles can be found attached to the tendon at a certain angle and fiber shortening and tendon movement occur at different directions. This angle is called pennation angle and defines the direction of muscle fibers with either the line of action of the muscle (external part of the tendon) or the aponeurosis (internal part of the tendon). In muscles with higher architectural complexity, such as the gastrocnemius muscle, this angle is often measured between the superficial aponeurosis and the deep aponeurosis of the muscle, similar to that performed by Fukunaga et al. (2001).

Pennation angle does not remain constant throughout life. For instance, it decreases when muscle size decreases, which can be caused by aging or disuse atrophy, or increases with muscular hypertrophy promoted by strength training or during growth. Since, strength training leads to an increased muscle thickness and pennation angle, a positive correlation between muscle force and pennation angle is usually associated to pennate muscles.

In a geometrical analysis, the existence of a pennation angle in a muscle’s architecture would suggest a negative effect on the force generation capacity. In fact, a fraction of the force generated by the muscle is directed perpendicular to the muscle line of action. Nonetheless, this effect is counterbalanced by the impact of pennation angle on PCSA. As a greater number of fibers can be filled into a certain volume of muscle, the force produced by all the muscle fibers can be greater than in other muscles with the same volume.

Assuming that fascicle pennation angle is fixed, it would also have a negative impact on muscle shortening velocity and excursion range. The pennation angle would require a higher muscle fascicle shortening to allow a given muscle shortening (reduction between the proximal and distal muscle-tendon junctions), which is a function of the cosine of the pennation angle. Yet, fascicles rotate in the muscles, increasing their pennation angle while the muscle is shortening (Maganaris et al., 1998). The ratio between the shortening velocity of the whole muscle belly and the velocity of the fascicles is known as muscle architectural gear ratio (AGR) or muscle belly gearing (Azizi et al., 2008). Since fascicle rotate, the gear ratio exceeds the unity, which means that muscle shortening velocities are higher than would be due to fascicle shortening only (muscle shortening is higher that of the fascicle). In pennate muscles, AGR equals 1/cosine of pennation angle, which could suggest a drawback in force production due to increasing shortening velocities when the pennation angle increases. However, the gear ratio has been shown to vary according to the force requirements (Azizi et al., 2008).

Thus, gear-ratio will favor the force generation capacity of the muscle by means of the force-velocity and force-length relationships. These relationships are detailed further.
2.1.4 Tendinous Tissues

The tendon is a type of connective tissue, rich in collagen, which provides the mechanical link between muscle and bone. The tendon can continue inside a tissue, in the form of a strong sheet of fibrous tissue, called aponeurosis. In turn, the aponeurosis behaves as an attachment surface for the muscle fibers, allowing them to exert force on to the tendon. Despite being an extension of the tendon, the mechanical properties of the aponeurosis may differ from the ones of the free tendon (Finni et al., 2003a). Tendon shape and size can differ significantly, however the overall internal structure is conserved across tendons. The internal structure is imposed by hierarchical organization of the extracellular collagen matrix (Figure 2.6).

![Figure 2.6: Tendon structural hierarchy. Adapted from Handsfield et al. (2016).](image)

Collagen molecules are bound together by covalent cross-links and form fibrils. In turn, fibrils give rise to fibers, which are grouped together to form fascicles and at last the whole tendon. Tendons are highly specialized for the type of tasks which they are meant to perform. In general, tendons located more proximally in the lower limbs make up a relatively small part of the total length of the MTU. These tendons have lower capacity for deformation, thus less capacity for storage of energy. Their main function is to effectively transfer force (Farris and Sawicki, 2012b). In turn, tendons located more distally in the lower limbs are more compliant, longer, and make up a larger part of the total MTU length. Lower extremities MTUs need to be versatile and meet the demands of a broad range of locomotion (Doral et al., 2010). Since tendon is responsible by transfer force from the muscle to the bone, it will have to adapt to the loading it experiences. Usually, adaptations in the tendon due mechanical loading occur by changes in its stiffness. A well-known important role of the tendon is storage and release of energy. However, due to their viscoelastic properties, Roberts and Azizi (2011) also listed energy conservation, power amplification and power attenuation as crucial contributions of the tendon to human locomotion.

2.1.5 Triceps Surae Muscle

A useful muscle-tendon unit to review in the scope of this work is that of human triceps surae muscle group and Achilles tendon. This muscle group contain fibers of three muscles - medial gastrocnemius,
lateral gastrocnemius and soleus - which range in length from to 30 to 70 mm. The MTUs span the length of the tibia, approximately 400 mm for a full grown man, thus the tendon and aponeurosis comprise the majority of the length of each muscle. The three muscles composing triceps surae are the main synergists for ankle plantarflexion (Fukunaga et al., 1997) and attach distally to the Achilles tendon. However, the three muscles have separate proximal origins. SOL lies under the gastrocnemius and originates from the head and upper third of the dorsal surface of the fibula. While medial and lateral heads of gastrocnemius originate from the superior surfaces of medial and lateral femoral condyles, respectively.

In their work, Morse et al. (2005a) reported that the relative contribution of these muscles to the overall PCSA of the triceps surae is different, the lateral gastrocnemius contributing \(~13\%\), the medial gastrocnemius \(~24\%\) and the soleus \(~60\%\). Thus, soleus is the strongest muscle among triceps surae muscles. Gastrocnemius and soleus are pennate muscles, however the structure of soleus is more complex. This muscle is composed by two compartments, a posterior compartment with unipennate structure while the anterior compartment is bipennate (Chow et al., 2000).

In addition, the gastrocnemius muscles are biarticular, meaning that they span both knee and ankle joints, whilst the soleus muscle is monoarticular, only influencing the ankle joint. Bearing all the previous aspects in mind, is is likely that architectural properties and muscle behavior to be highly specific for each individual muscle.

The gastrocnemius muscle is the most superficial muscle of the dorsal aspect of the lower leg and is what gives the calf its characteristic bulge. Although medial gastrocnemius and lateral gastrocnemius comprise one muscle unit, the two heads present distinct architectural and force-generating characteristics. The LG presents the longest fascicle lengths in the triceps surae muscles, having the highest number of sarcomeres in series and, hence, showing eminent velocity potential. In turn, MG comprises shorter fascicle lengths and larger fascicle angles, packing more fibers within a certain volume and, thus, bearing higher force potential. The architectural characteristics of MG turns it into a relatively easy muscle to image using ultrasonography, from a technical aspect. Furthermore, the validity of ultrasound imaging in this muscle is well established encouraging future studies with it (Kwah et al., 2013).

The muscle-tendon unit of the gastrocnemius is crucial to human locomotion and structural variation in it is predicted to affect the efficiency of locomotion. It supplies the necessary propulsive force to walk and run, thus is a central focus for several areas, including clinical biomechanics, exercise physiology and human evolutionary biology. Furthermore, this muscle has a high tendon-to-muscle length ratio compared with other muscle-tendon units (e.g. vastus lateralis), which confers unique advantages, namely a high capacity for elastic energy storage. On its turn, the energy capacity of a tendon is influenced by its stiffness, which is affected mainly by the properties of the tissue (Lichtwark and Wilson, 2008).
2.1.6 Achilles Tendon

Achilles tendon is the largest and strongest tendon of the human body. When the calf muscles flex, AT pulls on the heel. This movement allows humans to stand when walking, running or jumping.

The Achilles tendon consists of tendon fascicles arising from each head of the triceps surae and enclosed in a connective tissue sheet. These tendon fascicles twist along their course from the muscle to their insertion at the calcaneus. Fascicles from the LG insert on the lateral to anterior side of calcaneus, from MG on the posterior and lateral side and fascicles arising from the soleus on the medial and anterior side. Bojsen-Møller and Magnusson (2015) suggested that the twisting of the tendon fascicles along the tendon length may have a functional role, for instance, allowing high strains and energy storage.

This tendon has the capacity to sustain high forces and high strains. Thus, AT has a high capacity for elastic energy storage, turning this tendon indispensable for energy saving during locomotion.

2.2 Muscle-Tendon Unit Function

Muscle-tendon unit function is highly dependent on the intrinsic properties of its muscle fibers, muscle architecture and tendon mechanical properties. In the context of the present work, selected aspects of
the MTU mechanical function are reviewed. Firstly, fundamental mechanical properties of muscle and tendon are reviewed individually. Then, the whole MTU interaction is considered and the different roles of the tendinous tissues are explained.

2.2.1 Muscle Mechanics

Muscle function is greatly dependent on the mechanical properties of its muscle fibers. Since there is a variation in the overlap of actin and myosin filaments to produce muscle contractions, muscle force production is length dependent. This dependence has been described as the force-length relationship of muscles (asymmetric Gaussian form; Gordon et al., 1966). However, length is not the only factor that influences the force producing capacity of a muscle. In one of his works Hill (1938) also demonstrated the dependency of force production on contraction velocity (hyperbolic curve).

2.2.1.1 Force-Length Relationship

Muscle force production capacity is dependent on muscle length, which can be identified at sarcomere, fiber, MTU and at joint level (Rassier et al., 1999). The force-length dependency at any level derives from the one observed at sarcomere level and was first shown in 1966 by Gordon and his colleagues (Gordon et al., 1966). The identification of this force-length relationship constitutes the primary evidence supporting the sliding-filament theory of muscle contraction. Muscle force generation is dependent on the overlap of the actin and myosin filaments, which establishes the number of cross-bridges that are possible to be formed between the filaments. In turn, the overlap of actin and myosin is defined by sarcomere length, thus muscle force generation is dependent on muscle length (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954).

The muscle sarcomere has a specific optimal length for maximal force production, i.e. the sarcomere length for which the optimal amount of cross-bridges occur. There is no single optimal sarcomere length value, but rather a small range of sarcomere lengths, called plateau region, for which the greatest values of force can be produced. According to Rassier et al. (1999), the human muscle plateau region is estimated to be between values of 2.64 and 2.81 \( \mu \text{m} \). Experimental findings support this estimation with value found between 2.54-2.78 \( \mu \text{m} \) (Gollapudi and Lin, 2009).

Figure 2.8 represents the inverted parabola-shaped force-length relationship (Gordon et al., 1966). To be noted that the inverted parabola represents active force production by the muscle, originated by the contractile elements of the muscle when cross-bridges are being formed and thick and thin filaments slide along each other. Passive force relates to the force created in the parallel elastic components of the muscle, therefore arises from the lengthening of the muscle. The combination of both active and passive force forms the total muscle force. Active force generation by human sarcomeres or muscle fibers is achievable within lengths ranging from approximately 0.5 to 1.5 times the optimal length (Zajac, 1989; Hill, 1938).
Within a sarcomere, where only the interaction between myofilaments is important, the previous inverted parabola-shaped force-length curve is translated by four straight segments as represented in Figure 2.9.

This curve is well explained by the theory of muscle contraction. During the ascending limb phase, the sarcomeres are completely shortened due to the total overlapping of actin and myosin filaments and the overlapping between actin filaments from the opposite sides of the sarcomere. The latter aspect hampers the formation of cross-bridges, preventing force production. Muscle contraction ends when myofilaments start appending to the Z-discs and cross-bridges start to be formed, increasing sarcomere length until it reaches its optimal length, with maximal force being produced. The descending limb phase starts when the length of the sarcomere surpasses the optimal one, hence filaments start to not overlap and force output decreases.
2.2.1.2 Force-Velocity Relationship

The capacity of a muscle to generate force is also dependent on the velocity muscle contraction and the force-velocity relationship was first described by Hill in 1938 (Hill, 1938). This relationship is presented in Figure 2.10 and can be translated in a curve with hyperbolic shape. In resemblance to the force-length relationship, the general shape of the force-velocity relationship can be observed at all levels, from sarcomere, fiber, MTU to joint level (Finni et al., 2003b; Bobbert, 2012). When the muscle velocity is negative, i.e. muscle is lengthening, force production capacity strongly increases.

Muscle power production capacity, i.e. the rate at which work can be done, depends on muscle contraction velocity. As a result, the maximum power production capacity of a muscle is at about one-third of the maximum contraction velocity. However, in order to maximise force output and economy of the whole muscle-tendon unit, muscles tend to operate isometrically or at slower velocities when producing high forces. The force-velocity relationship is dependent on the muscle fiber type (Larsson and Moss, 1993), unlike the force-length relationship. This happens due to a dependency of the force-velocity relationship on the cycling rate of myosin cross-bridges established by the myosin ATPase activity. For example, type II muscle fibers present higher ATPase activity, thus are able to generate greater force at a given shortening velocity (Larsson and Moss, 1993).

Figure 2.10: Force-velocity relationship for a fully activated muscle. Adapted from Zajac (1989).

2.2.2 Tendon Mechanics

The mechanical properties that describe how tendons react to physical forces include stress and strain at a specific force, stiffness, Young's modulus and hysteresis. These parameters are usually measured with tensile tests in which deformation is applied and subsequent resisting force is measured. Traditionally, tendon tensile tests were performed on dissected tendons from human cadavers or animals. Nonetheless, improvements in imaging methods offered the possibility to perform these tensile tests without invasive procedures and in a physiological environment. The most used methods nowadays are ultrasonography (Fukashiro et al., 1993) and magnetic resonance imaging (Shin et al., 2008).
Tendons are viscoelastic, thus they have both elastic, spring-like properties, and viscous, fluid-like properties (Butler et al., 1978). Hysteresis is a parameter that describes the viscosity of a tendon whereas stiffness and Young's modulus are related with the elastic properties. Figure 2.11 shows the typical data from a tensile test of a tendon, strain-stress and force-elongation curves.

When a tensile force is applied to a material, e.g. a tendon, it will elongate. Stiffness of a material is then the ratio between the applied force and elongation \((\Delta F/\Delta L)\) and is measured from the slope of the linear part of the force-elongation curve. Tendon stiffness depends mainly on its length, material properties and PCSA. For instance, if the resting length of a tendon is doubled, tendon stiffness will reduced to its half, since with the same elongation of the tendon, it will resist with half of the force compared to the original situation. In contrast, if the PCSA of a tendon is doubled, tendon stiffness is expected to also be doubled (Butler et al., 1978).

![Figure 2.11: Schematic example of typical curves from tensile test of a tendon to determine stiffness, Young's modulus and hysteresis. Solid lines represent loading and dotted lines the unloading phase of the tensile test results. (a) Force-elongation curve and stiffness is defined as the slope of the curve. (b) Stress-strain curve and young's modulus is defined as the slope of the curve. Hysteresis consists on the percentage of energy lost during a loading-unloading cycle. To be noted that stiffness and Young's modulus are determined from the linear part of the curves.](image)

When information regarding mechanical properties of a tendon as a material is needed, tensile test results are normalized to the dimensions of the tendon. The variable that normalizes tendon stiffness to its dimensions is denominated Young's modulus. Young's modulus is a measure of the stress-strain relationship of the material, whilst stiffness is a measure of the force-elongation relationship. Stress is obtained diving tensile force by PCSA \((\sigma = F/A)\) and strain is calculated by dividing elongation by initial length \((\epsilon = \Delta L/L_0)\). Since both stress and strain do not depend on the dimensions of the material tested, Young's modulus will be independent on the dimensions but only on the properties of the material (Butler et al., 1978). Thus, Young's modulus is a useful parameter when the aim is to establish comparisons between individuals or different tendons.

Lastly, tendons show some degree of viscous properties when physically loaded giving to its viscoelastic properties. Hysteresis consists in one of the manifestations of tendon viscosity. When cyclical loading is applied to the tendon, a loop in the force-elongation or stress-curve is formed. This loop is denominated hysteresis and concerns the amount of energy that is converted to heat during
the loading cycle. Normally, hysteresis is expressed as a percentage and is obtained from the area under the loading curve. Finni et al. (2008) reported *in vivo* values of hysteresis ranging from 3% to 38% to the human tendon.

### 2.2.3 Muscle-Tendon Interaction

The mechanical characteristics of the whole muscle during contraction and passive extension are influenced by the tendons and the connective tissues in and around the muscle belly. Hill (1970) showed that tendons consist in a spring-like elastic component located in series (series elastic component, SEC) with the contractile component (CE; i.e. muscle), whilst the epimysium, perimysium, endomysium, and the sarcolemma represent a second elastic component that is located in parallel with the contractile element (parallel elastic component, PEC). During active contraction or passive extension, the parallel and series elastic components stretch, tension is produced and elastic energy is stored. When they recoil with muscle relaxation, the energy once stored is released. Even though there are two types of elastic elements, tendon and aponeurosis have the greatest capacity for elastic energy storage (Zajac, 1989; Roberts, 2016). Moreover, the compliance (the reciprocal of stiffness) of the tendon and aponeurosis will be much greater than that of the fascicles (Wilson and Lichtwark, 2011). Hence tendon and aponeurosis can be treated as the most important elastic components in regards of MTU function and the fascicles can be approximated to be only contractile in nature.

Bearing this in mind, it is common to consider that mechanical interaction of the MTU is determined by the interaction between the muscle and the series elastic components. In studies regarding the MTU interaction and function, it is widespread the use of a mechanical model containing the tendon and aponeuroses (collectively denominated tendinous tissues, TT, or SEE) connected in series with the muscle fascicles. In this model, all muscles fascicles in parallel are represented by a single contractile component (Fukunaga et al., 2001). To be noted that in this model, SEE is only a model representation of the series elastic element and not a specific tissue or combination of tissues. This model is quite useful in *in vivo* studies, due to the possibility to estimate length changes in the series elastic components based on joint kinematics and measurements of a single muscle fascicle length.

As depicted in section 2.2.1, mechanical behavior at fascicle or fiber level is constrained by two functional relationships, force-length (Gordon et al., 1966) and force-velocity (Hill, 1938) relationships. Furthermore, architectural aspects play an important role in the mechanical function of a muscle, expanding versatility of this tissue. On the other hand, SEE mechanical function is determined by their inherent mechanical properties, such as stiffness. The way that active muscle and passive SEE interact with each other is the key to the fulfillment of different functional demands by the MTUs (Wilson and Lichtwark, 2011). For instance, the elasticity of the SEE is used to decouple muscle length and velocity from that of MTU (Fukunaga et al., 2001), allowing the muscle to overcome the impositions of force-length and force-velocity relationships. Regarding energetics of MTU, Roberts and Azizi (2011) suggested SEE may have three different roles - energy conservation, power amplification and power attenuation. As the main interest of this work was to assess the muscle-tendon interactions during walking...
in the elderly, the main focus is the power amplification effect of the so-called catapult mechanism for storage and release of elastic energy due to decoupling of the MTU. Nonetheless, energy conservation and power attenuation are briefly described.

2.2.3.1 Energy Conservation

Efficiency of human locomotion is highly improved by energy conservation using SEE elasticity. The basic mechanism of energy conservation by SEE consists on the temporary storage of kinetic and potential energy related to the movement and subsequently release to power the movement (Cavagna, 1977). The cornerstone of improving efficiency of locomotion by utilizing the elasticity inherent to SEE is the energy cycling between the body and SEE elastic energy. This effect allows the muscle to not perform external work or to work at slow shortening velocities, improving efficiency.

To be noted that muscle not performing mechanical work, does not mean that energy cost is zero. Elastic energy storage by SEE implies muscle to generate force. Nonetheless, Fletcher et al. (2013) proposed that energy cost of force generation is lower in isometric contractions rather than in concentric contraction, which allows energy to be saved compared to a situation where muscle had to perform MTU work.

2.2.3.2 Power Amplification

Muscle capacity to power production is at about one-third of the maximal contraction velocity. Nonetheless, in some movements where fast joint rotations occur, MTU velocity exceeds the optimal velocities for muscle power generation. In these cases, joint power can exceed the power that would be theoretically possible to achieve even if the muscle was contracting at the optimal velocity (Sawicki et al., 2015). This effect of high power production, exceeding the limits of muscle capacity, is called power amplification. Power amplification is accomplished through a mechanism similar to a catapult, allowing storage and release of elastic energy due to decoupling of the MTU.

Decoupling of the MTU

Using sonomicrometry, Griffiths (1987) was able to measure the length changes of the muscle fibers and the whole muscle-tendon unit in cats. Moreover, he was able to show that muscle fiber length was decoupled from the length changes occurring in the muscle-tendon unit, both due to changes in pennation angle and the elastic behavior of the tendinous tissues. Later, Fukunaga et al. (2001) confirmed this muscle-tendon behavior in humans.

During locomotion, muscle fascicles actively contract to generate the necessary forces to move forward. The muscle fibers of the human medial gastrocnemius have been shown to operate over the descending limb of the force-length relationship during a large part of the stance phase and then move over the optimal region onto the ascending limb during the plantar flexion phase (Arnold and Delp, 2011).

If this mechanism of decoupling did not exist, muscle fascicles would need to be subjected to substantial length changes, up to 25mm as suggested by the work of Lichtwark and Wilson (2006). Length changes of these dimensions would lead the muscle sarcomeres to work at very short lengths.
and they would be unable to produce the required force. Moreover, these length changes would mean an increase in sarcomere shortening velocities, thus lower force production capacity.

**Catapult Mechanism**

Ultrasound imaging allowed for the quantitative assessment of the contribution of the ankle joint for the mechanisms of elastic energy storage and return during human walking (e.g. [Ishikawa et al., 2005] and [Lichtwark et al., 2007]). The plantar flexor muscles and their long compliant tendon, Achilles tendon, behave similarly to a catapult throughout movement. During stance phase of the human gait, the muscle-tendon unit lengthens and the fascicles undergo isometric contraction which allows for the stretching of the tendinous tissue, leading to a storage of energy. This energy is later released with the combination of the tendinous tissues shortening very fast and the further shortening of the muscle fascicles, allowing for high power outputs in the second half of the stance phase.

The catapult mechanism limits the stretch and recoil cycle of the muscle-tendon unit, but allows the muscle fascicles to work nearly isometrically, producing high forces with very little mechanical work. [Rall, 1985] suggested that muscles spend less metabolic energy when producing force isometrically compared to shortening contractions. Therefore, the elastic contribution at the ankle joint possible saves a significant amount of metabolic energy during walking.

Most studies in humans regarding energy usage in muscle-tendon units focus on the biarticular gastrocnemius muscles ([Fukunaga et al., 2001], [Lichtwark and Wilson, 2006], [Ishikawa et al., 2005], [Farris et al., 2016]), although the effect of energy storage and release has also been reported for the mono-articular soleus ([Farris et al., 2016], [Lai et al., 2015]). In their work, [Farris and Sawicki, 2012a] have suggested that the gastrocnemius muscle has a higher capacity for elastic energy storage than the soleus muscle due to the fact that, during squat jumping, simultaneous extension of the knee prolongs the stretch of this muscle unit during plantar flexion.

### 2.2.3.3 Power Attenuation

SEE are passive tissues with generally low hysteresis, hence the task of absorb large amounts of energy in MTUs is carried out by the muscle. Nonetheless, SEE can attenuate power, i.e. decrease the rate at which muscle needs to dissipate energy. This ability is of special interest, for instance in landing situations when the mechanical energy of the body decreases rapidly. Moreover, power attenuation can prevent eccentric muscle injury ([Thelen et al., 2005]). If SEE did not have this ability, dissipation of a great amount of energy in a short period of time would require a forceful eccentric contraction which could lead to muscle damage.

### 2.3 Human Walking Gait Cycle

Gait consists in the most important method of human locomotion and allows numerous activities of daily living. Normal human walking can be defined as "a method of locomotion involving the use of the two legs, alternately, to provide both support and propulsion" ([Whittle, 2007]). Walking differs from running
since one foot, at least, is in contact with the ground at all times.

Walking constitutes probably the most studied movement of human being. The amount of disciplinary perspectives allied with technology, has produced a complex range of literature. In terms of biomechanical investigation, gross motor functions, ranging from walking to sport activities, are analyzed to investigate musculoskeletal function and to assess how the musculoskeletal system is reorganized and adapted to cope with these functions. Clinical gait analysis can be defined as the process of recording and analyzing biomechanical data of walking. In an overview on guidelines for managing a clinical gait analysis, Baker et al. (2016) indicate that it is often performed to diagnose between disease entities, to assess the severity, extent or nature of a certain injury or even to predict the outcome of an intervention. Moreover, the ultimate goal of gait analysis is to understand the relationship between functional capabilities and limitations of an individual and establish a gait pattern, aiming to enhance performance while preventing injury.

Exploring terminology, gait cycle is characterized as the temporal interval between two consecutive occurrences of one of the events of walking. Selecting the instant at which one foot contacts the ground to define the beginning of gait cycle, know as initial contact (or heel strike), the cycle will continue until that foot contacts the ground again. Logically, the other foot follows the same series of events, but displaced in time by half cycle. The gait cycle is subdivided to identify the major phases and events in it, however the used nomenclature varies appreciably from one publication to another. Therefore, to facilitate further analysis and comparison of results, the nomenclature presented in the upcoming subsections follows the one used by Lichtwark et al. (2007). An individual is able to walk at different speeds and maintain the essential characteristics of the gait cycle. Definition of some temporal-spatial gait parameters, such as stride time, step time, stride length, step length, gait velocity and cadence, allow objective reports of both walking and running. Stride time defines the time from the initial contact of one foot to initial contact of the same foot while step time refers to the same period however between opposite feet. Stride length is the distance covered from initial contact of one limb to the initial contact of the same limb and step length defines the latter distance between opposite limbs. Gait velocity consists in stride length divided by stride time, usually expressed by meter per second or kilometers per hour. Lastly, cadence refers to the number of steps taken in a unit of time, measured as steps per minute. These characteristics are typically acquired with force platforms and 3D motion analysis camera systems.

This being clarified, the gait cycle is divided into two main phases: a long stance phase, during which some part of the foot is in contact with the ground, and a short swing phase, when the foot is moving forward through the air. Generally, stance phase lasts about 60% of the cycle, while the swing phase lasts about 40%. During the walking gait cycle two periods of double support occur between the time that one foot makes the initial contact and the other foot leaves the floor, lasting about 10% of the cycle. However, the effect of speed in walking must be considered. As shown by Murray (1967) with the increase of speed, the stance and double support phases become shorter and the swing phase becomes longer. The late disappearance of the double support phase sets the transition between walking and running. A representation of the right gait cycle is presented in Figure 2.12.
The stance phase, in the traditional terminology, is characterized by the following events:

1. *Heel strike* is the instant at which the heel of the leading foot touches the ground. Usually, the heel is the first point of contact and the absence of this event can indicate abnormalities.

2. *Foot Flat* occurs after heel strike and consists in the first instant when the foot is flat on the ground.

3. *Midstance* refers to the point at which the reference lower extremity supports the total body weight.

4. *Heel-off* designates the point at which the heel of the reference foot leaves the ground. Push-off, period when the lower limb is pushing away from the ground, starts at this point and ends with the next phase.

5. *Toe-off* denotes the ending of stance phase and refers to the point at which the toe of the foot leaves the ground.

The swing phase, also in the traditional terminology, comprehends the following phases:

1. *Acceleration* has its beginning when the toe leaves the ground and continues until midswing or until the moment at which the limb is directly under the body.

2. *Midswing* occurs when the limb passes directly beneath the body.

3. *Deceleration* refers to the moment when the extremity is decelerating to initiate heel strike.

![Figure 2.12: Representation of the gait cycle. Adapted from Levangie and Norkin (2005).](image)

Each phase and event of the gait cycle is highly determined by the adjustments made by the hip, knee and ankle, powered by the lower extremity muscles, throughout the entire movement.

### 2.3.1 Gait Kinematics

Gait kinematics is mostly studied with the aid of 3D motion analysis systems which reconstruct the body of a subject as a multi-body system. To capture the position of the body throughout time, infrared markers are placed at specific anatomic landmarks and then their position is triangulated by cameras to obtain the multi-body system. This process allows the estimation of joint angles, angular velocities and
accelerations. Usually, measurements are collected for each joint in the three cardinal planes of motion: sagittal, coronal and transverse.

The approximate range of motion (ROM) of a normal gait is described next (Levangie and Norkin 2005). However, to be noted that the presented values consist in approximations of reported results that vary in age, gender, walking speed of the subjects and method of analysis. In the anatomical position, the hip, knee and ankle are approximately at 0°, therefore, flexion for the hip and knee and dorsiflexion for the ankle will be treated as positive values. Figure 2.13 presents the main changes during walking in the sagittal plane.

![Figure 2.13](image)

Figure 2.13: Variation in the joint angles in degrees at the hip, knee and ankle joints on the sagittal plane of motion. Adapted from Levangie and Norkin (2005).

The hip shows peak flexion (~20°) around initial contact and peak extension (~-20°) just before the toe-off phase. The knee is straight (0°) at initial contact and nearly straight just before heel-off, approximately at 40% of the gait cycle. A small knee flexion (~15°) occurs at around 10% of the gait cycle and during swing, 70% of the gait cycle, knee reaches maximum flexion of ~60°. Lastly, the ankle shows maximum dorsiflexion of ~7° around heel-off and reaches maximum plantarflexion of ~-25° at toe-off. Therefore, in normal walking, a hip ROM of approximately 20° of extension to 20° of flexion, a knee ROM from 0° to 60° flexion and ankle range from 25° plantarflexion to 7° dorsiflexion is needed. If these ranges of motion are not achieved, the gait pattern is considered to have a deviation from the norm.

### 2.3.2 Gait Kinetics

Gait kinetics reflects the cause of movement and allows the estimation of forces, power and energy. During walking, ground reaction forces (GRFs), measured usually with force plates incorporated in treadmills or ground, translate the forces that act on the body throughout the stance phase. When processing the
GRFs together with the joint kinematics, one can estimate joint kinetics, or joint moments, which show how external GRFs, inertia and gravity interact with the recruitment of muscles, tendons, ligaments that span the joint. Hence, internal moments are created to counteract the external forces acting on the body. For instance, in the late stance phase the weight of the body is applied on the forefoot, thus the calf muscle produces an internal ankle plantarflexor moment. This moment will oppose to the external dorsiflexion moment created by the GRF and other forces. As a convention ankle plantarflexor, knee extensor and hip extensor will be treated as positive internal moments.

An important concept to take into account when discussing gait kinematics is the support moment. The support moment was introduced by Winter (1991) and describes the sum of all sagittal plane moments acting at the hip, knee and ankle. This sum is a positive, or extensor internal moment, consistent for all walking speeds and for all individuals, whether they are considered normal or with disabilities. The support moment prevents the leg from collapsing during the stance phase and can be reached through different combinations of moments acting at the three joints, as hip and knee moments can vary considerably among individuals. This sum of internal moments provides the backdrop to understand the joint moment profiles for each joint and ranges from a net internal extensor to a net internal flexor in late stance that continues into swing.

During the swing phase, moments are very small compared to the ones experienced during the stance phase, thus, they are not presented. As shown in the figure 2.14 in early stance hip extension starts to provide all of the positive moment being soon joined by an internal knee extensor moment. As stance phase proceeds there is an increasing support by the ankle plantarflexors, until they become the only support in most of late stance.

Figure 2.14: Variation in the joint moments at the hip, knee and ankle joints on the sagittal plane of motion. Adapted from Levangie and Norkin (2005).
2.3.3 Muscle Activity

Several muscles of the lower limbs participate in the gait cycle. Nonetheless, depending on the phase of the cycle, different groups of muscles are activated. During the gait cycle, the majority of the movements of the ankle occur in the sagittal plane, which requires the activation of dorsiflexors and plantarflexors muscles. As mentioned previously, the prime ankle dorsiflexor is the tibialis anterior, while the main ankle plantarflexors are the medial and lateral gastrocnemius and the soleus.

The tibialis anterior, since it is a dorsiflexor, is activated mainly at the end of swing to keep the foot dorsiflexed. Its activity reaches a maximum immediately after the heel-strike when it generates the necessary force to lower the foot on the ground, opposing to the plantarflexing ground reaction forces. The activity and function of medial and lateral gastrocnemius is quite similar. Their activity initiates just prior to heel-strike and reaches a maximum around heel-off, about 50% of the gait cycle. Then, the muscle shortens along with the other plantarflexors, leading to the active plantarflexion of the foot and to the generation of the most important impulse of energy. Activity drops until the toe-off and remains lowered during swing, acting as knee flexor. The soleus also behaves as an ankle plantarflexor and it is mainly activated during the stance phase. This muscle initially controls the forward rotation of the leg and, between 40% and 60% of the stride period, allows the explosive push-off.
Chapter 3

Methodology

Figure 3.1 provides a schematic representation of the framework followed throughout the present work. Musculoskeletal modelling allows the quantification of the MTU length changes and walking kinematics and kinetics, whilst the use of ultrasound provided measurement of the length changes of muscle fascicles. This framework is similar to the ones used in muscle-tendon unit behavior research during dynamic tasks (e.g. Aeles et al., 2018; Swinnen et al., 2019; Lai et al., 2018). This chapter is divided in eight main sections, as seen in the framework.

Figure 3.1: Descriptive framework to obtain the parameters to assess gait biomechanics and the MG MTU behavior after training.

The subjects studied are presented in the Data Acquisition section, which also includes simultaneous collection of kinetic, kinematic and ultrasound data during walking. Data acquisition was performed before and after the subjects followed a strength exercise intervention program for 12 weeks, to understand the effects of this training on the MG MTU behavior and on joint angles and moments during
walking. Hence, the details of the training intervention are presented in the second section.

Once data are acquired, they need to be processed for further analysis. The dark blue box in the framework presents each step of data processing to estimate the parameters of interest. To obtain MG muscle fascicle length changes and pennation angles during walking, the ultrasound video images captured by the ultrasound system were tracked with a semiautomatic tracking algorithm, UltraTrack 4.2 (Farris and Lichtwark, 2016). A brief description of the algorithm and its usage is provided in the Ultrasound Processing section. Next, the musculoskeletal model used to estimate the remaining parameters is described. To allow the computation of accurate results, the model needs to be scaled according to the anthropometry and physical dimensions of each subject, hence the step of scaling using OpenSim (Delp et al., 2007) is also presented in section Musculoskeletal Modelling. Having the musculoskeletal model scaled, joint angles and joint moments during walking were obtained performing inverse kinematics and inverse dynamics, respectively. Since a Kalman Smoother algorithm was preferred to the OpenSim inverse kinematics (IK) tool, it is introduced in the fifth section. The sixth section focuses on the inverse dynamic (ID) analysis using the OpenSim tool and the estimated joint angles as an input. MG MTU lengths were obtained through the muscle analysis tool available in OpenSim, also with the estimated joint angles as input, described in the seventh section.

After processing all data, they can be analyzed. The last section, represented by the last box in the framework, reports how the several parameters retrieved from the processing steps were organized and statistically analyzed. Boxes delimited by dashed-lines concern parameters to assess the effect of training on gait kinematics and kinetics, whereas boxes delimited by full lines are related to the parameters used to evaluate the effect of training in the MG MTU behavior.

### 3.1 Data Acquisition

Eleven older male adults (Age = 67.9 ± 2.6 years; body mass = 82.3 ± 10.1 kg; body height = 169.9 ± 5.4 cm; mean ± SD) gave their written informed consent to participate in the study. In order to be included, the participants had to be between 65 and 75 years old at the start of the study and to have a clean history of cardiovascular, neurological or cognitive diseases. Moreover, the subjects did not present any knee, ankle or hip prostheses and AT injuries. No participation in any resistance training in the last six months prior to the study was also required. The experimental design was approved by the ethical committee of Medical Ethics Committee Universitair Ziekenhuis/Katholieke Universiteit Leuven, which evaluates medical and health research.

During measurements, subjects were asked to walk on a motorized force-measuring treadmill (Motekforce Link, Amsterdam, The Netherlands) for about 5 to 8 minutes at different walking speeds: 3.0 km/h, which will be referred as SLOW speed, 5.0 km/h (FAST speed), comfortable walking speed (COMF speed), and 6-minute walking distance speed (6MWD speed). The 6MWD speed is the walking speed of each subject while walking as fast as possible for a period of six minutes (6-minute walking test). The utility of this test was first described by Enright (2003). Before the experimental testing, participants executed, barefoot, overground walking tests of their comfortable speed as a warm-up and
then to measure the 6MWD speed. These walking speeds were used on the treadmill to obtain the
data at each given pace. Likewise, a familiarization session on the treadmill was performed for about
20 minutes. In this session subjects practiced walking at different speeds, including the speeds used
for data collection. Kinetic, kinematic and ultrasound data were collected during the last minute of each
walking speed to have data for at least three strides.

To allow the dynamic ultrasound imaging, a B-mode ultrasound system (Telemed Echo Blaster
128 CEXT system) with a sampling frequency of 60 Hz was used with a 128-element linear transducer
(LV 7.5/60/128Z-2; Telemed, Vilnius, Lithuania) measuring at 8 MHz to visualize MG muscle fascicles
of the left leg of each participant. The transducer had a 60 mm field of view and was securely attached
to the calf with tape and elastic bandages and placed on the midbelly of the muscle so that it was
approximately in the same plane as the muscle fascicles. The transducer was placed according to the
study of Lichtwark and Wilson (2006), as depicted in Figure 3.2. All ultrasound recordings were obtained
using the Echowave II 3.4.0 software.

Figure 3.2: Example of the placement of the ultrasound transducer on the midbelly of MG muscle of the left leg,
securely attached with tape and elastic bandages. To be noted that the observed electromyography electrodes were
not used in the present study.

For the motion acquisition, an extended full body gait marker set, consisting of 65 markers with
9 marker clusters attached to the body of each participant was used to track the subject’s body seg-
ments. Thirteen infrared motion-capturing cameras (Vicon, Oxford Metrics) captured the trajectory of
these markers working at a sampling rate of 100 Hz. A static trial was executed before the walking mea-
surement and ground reaction forces during trials were measured using the force plates integrated in the
treadmill with a sampling frequency of 900 Hz. All data were measured in the Movement Analysis Lab-
oratory Leuven. After the measurements, the markers were labelled offline using Vicon Nexus (Vicon,
Oxford Metrics, UK). The full body gait marker set is described in detail in Annex A and is represented
in Figure 3.3.
Both ultrasound and motion capturing were performed at the same time and a trigger pulse was used at the start of the ultrasound imaging acquisitions to synchronize it with the 3D motion-capture system and the force-plates.

### 3.2 Strength Exercise Intervention

Participants were measured before and after a 12-week strength exercise intervention program. The program consisted on the performance of strength exercises three times a week under close supervision to increase muscle strength and AT stiffness. Between training sessions, a day of rest was planned. At each session, subjects performed seated ankle plantarflexion contractions, seated leg press and knee extension contractions on weight machines. The strength intervention was divided in three blocks. In the first block exercises were completed in a high-volume regime. As the subjects progressed in the program, we increased exercise intensity following the principles of progressive overload. Thus, the training volume was as follows:

- **Week 1-4**: 2 sets of each 12-15 repetitions per exercise;
- **Week 5-8**: 3 sets of each 10-12 repetitions per exercise;
- **Week 10-12**: 4 sets of each 8-10 repetitions per exercise.

Subjects had 2 minutes of rest between exercises and 1 minute of rest between each set of exercises. Before starting the study, the subjects performed an one-repetition maximum (1RM) test, which is simply defined as the maximal weight an individual can lift for only one repetition and with a correct technique. Based on the 1RM test, their 12-15 RM, i.e. maximal weight that could be lifted 12-15 times, was calculated. The 12-15 RM was the starting weight. As suggested by Arampatzis et al. (2007) high strain magnitudes are needed to induce mechanical and morphological adaptive responses on the muscle-
tendon unit. Hence, when the subjects could perform the exercises correctly and without too much of an effort, the training load was adjusted by increasing the weight on the machines. On the other hand, the training was performed in a way that participants had always to perform the number of assigned repetitions. For instance, if a participant in week 3 could only perform the exercise 10 times, the weight was decreased. The participants together with the team decided which weight was appropriate. The study team also ensured that quality of exercise was high enough.

3.3 Ultrasound Processing

After the ultrasound dynamic imaging, all the video images captured by the ultrasound system were converted to MATLAB® files. Then, a semiautomatic tracking software, UltraTrack 4.2, fully described by Farris and Lichtwark (2016) was used. This software package is freely available and its algorithm was specially designed to track the behavior of muscle fascicle and aponeuroses positions on ultrasound images.

The software is of easy usage, reducing the time consumption and the subjectivity inherent to this type of processing. Moreover, Cronin et al. (2011) previously reported robust and viable reliability and accuracy of this semiautomatic software in comparison to manual tracking.

The algorithm implemented in the software is based on an affine extension to an optic flow algorithm to track the movement of muscle fascicle end-points throughout consecutive images dynamically obtained. For that, the algorithm covers the image sequence, one frame at a time, computing the optic flow between consecutive images and applying the affine transformation in order to obtain the new xy-coordinates of the muscle fascicle end-points and its length. The complete description of the algorithm can be found in Farris and Lichtwark (2016).

Figure 3.4 represents the interface of UltraTrack 4.2.

Figure 3.4: Snapshot of the UltraTrack GUI after the image sequence has been loaded and one ROI and the MG fascicle have been defined. Numbers 1 to 4 represent important steps from the tracking process.
To begin the tracking, an image sequence must be given as an input. Next, is the responsibility of the user to define one or more regions of interest (ROI), step (1), according to what suits the tracking, and the end-points (points A and B) of the representative fascicle of the studied muscle, step (2). These end-points are defined in a way that the line intersecting them must follow the orientation of the fascicles to track. Each ROI is delimited by red broken lines and the fascicle by a red full line.

From that point on, the user can process automatically the image sequence, step (3), obtaining for each frame time the fascicle position and length. Since the process is iterative, errors appearing in individual frames are susceptible to accumulate over time, leading to a misplacing of the fascicle position and a miscalculation of its length. A misplacement of the fascicle is visible when the full red line is not aligned with the orientation of the MG muscle fascicles. To correct this, the software offers the user the option to correct any potential error manually, step (4), between frames.

In the present work, to obtain the MG fascicle lengths and pennation angles, three regions of interest containing one tracking line each were drawn as observed in Figure 3.5 (a). The first line was drawn along the orientation of MG muscle fascicles, the second in the inner surface of the superficial aponeurosis and the third in the inner surface of the deep aponeurosis. Each pair of letters - A,B; C,D and E,F - correspond to the end-points of the MG fascicle, superficial aponeurosis and deep aponeurosis, respectively. After processing, the xy-coordinates, in the reference frame of the image coordinate system, of each end-point at each frame of the image sequence were exported to MATLAB®. A custom-made MATLAB® code was written in order to compute the two main parameters of interest. In short, using their xy-coordinates, the end-points were placed in the image frame. Then, through interpolation, the MG fascicle and aponeuroses were reconstructed as they were drawn in UltraTrack. After having the reconstructed fascicle and aponeuroses, extrapolation was used on them and intersection points were calculated as depicted in Figure 3.5 (b).

![Figure 3.5: Example of the tracking procedure of an ultrasound image of the Medial Gastrocnemius. (a) Representation of the three regions of interest and respective tracking lines drawn. ROIs are delimited by red broken lines, each one containing a full red line that represents: 1 muscle fascicle orientation, 2 superficial aponeurosis and 3 deep aponeurosis. The pair of letters (A,B; C,D and E,F) correspond to the end-points of each tracking line. (b) Representation of the extrapolation used on fascicle and aponeuroses to calculate the intersection points. The straight line 1 was used to calculate fascicle length, whilst pennation angle, represented by $\alpha$, was defined as the angle between the deep aponeurosis, line 3, and the MG fascicles, line 1.](image-url)
The muscle fascicle length is then calculated based on the straight line between the intersection points of the fascicle with both aponeuroses. Pennation angle was determined as the angle between the fascicle and the deep aponeurosis as suggested by Bolsterlee et al. (2015). In the previous study, authors reached the conclusion that deep pennation angles and fascicle lengths in the MG are unbiased and avoid underestimation.

Fascicle lengths and pennation angles were then filtered using a recursive fourth-order Butterworth filter with a cut-off frequency of 12 Hz (MATLAB R2015a, Aeles et al., 2018). To differentiate between the MG muscle fascicles, MTU and tendinous structures behavior, the SEE length was calculated using a Hill-type muscle model described by Fukunaga et al. (2001). The model is represented in Figure 3.6.

Figure 3.6: Hill-type model used to estimate the tendon length changes. $L_m$ is fascicle length, $\alpha$ pennation angle, $L_{pt}$ proximal tendon (free tendon and aponeurosis), $L_{dt}$ distal tendon (free tendon and aponeurosis), and $L_{MTU}$ is MTU length. The entire tendinous structures length ($L_{pt}+L_{dt}$) equals $L_{MTU} - L_m \times \cos(\alpha)$. Adapted from Fukunaga et al. (2001)

In this model, muscle fascicle lengths and pennation angles from the ultrasound images were combined with the MTU lengths retrieved from musculoskeletal model (detailed further), to obtain SEE lengths ($L_{SEE}$):

$$L_{SEE} = L_{MTU} - L_m \times \cos(\alpha)$$  \hspace{1cm} (3.1)

where $L_{MTU}$ is muscle-tendon unit length, $L_m$ is fascicle length, $L_{SEE}$ is series elastic elements length and $\alpha$ is the pennation angle. SEE represents all tendinous tissues, which includes the free tendon, aponeuroses, and connective tissues. The velocities of the muscle fascicle, MTU and SEE were calculated as the first derivative of their lengths with respect to time. Since the resting lengths of MTU, muscle fascicle and SEE were not measured, MTU and muscle fascicle data were normalized by the initial length value of the muscle fascicle at heel strike ($L_{m0}$), SEE length was normalized by the length of the SEE at heel strike ($L_{SEE}$) (Lai et al., 2015).

### 3.4 Musculoskeletal Modelling

Musculoskeletal modelling allows the estimation of several parameters in the study of human movement biomechanics. For that, a musculoskeletal multibody model described by rigid elements (bones) con-
connected between each other by joints, which in turn are actuated by muscle tendon actuators, is used. In the present work, a stepwise workflow was followed with the aim of computing joint angles, joint moments and MTU lengths. The software used in this stage was OpenSim 3.2 - an open-source software for musculoskeletal modelling (Delp et al., 2007). To be noted that the information with respect to this software was retrieved from its online documentation.

The first task was the customisation of a generic model. For this purpose, the Hamner model (Hamner et al., 2010) was used. This three-dimensional (3D) model contains 12 segments, 29 degrees of freedom (dof) and the lower extremity and back joints are actuated by 92 MTU actuators. Moreover, the default weight is 82.57 kg. Each lower extremity has five degrees of freedom, the hip is a ball-and-socket joint defined by three dofs (flexion/extension, adduction/abduction and internal/external rotation), the knee is modeled as a custom joint with one dof (flexion/extension) and the ankle is a revolute joint that allows one dof (dorsiflexion/plantarflexion). Lumbar motion is modeled as a ball-and-socket joint, defined by three dofs (flexion/extension, bending and rotation). Each arm presents five degrees of freedom, the shoulder is modeled as a ball-and-socket joint, thus allowing 3 dofs (flexion/extension, adduction/abduction and rotation) and the elbow and the forearm are defined as revolute joints with 1 dof each (flexion/extension and pronation/supination, respectively). Furthermore, the model possesses a virtual marker set based on the markers experimentally placed on specific anatomical landmarks during the motion acquisition. This virtual marker set is extremely useful when scaling, i.e. when altering the anthropometry of a model so that it matches that of a particular subject as closely as possible. The step of scaling is described further and the musculoskeletal model used is shown in Figure 3.7.

![Figure 3.7: Used musculoskeletal model. (a) -X view (b) X view, (c) Z view, (d) -Z view. The axes are organized so that the x-axis represents the anterior/posterior axis, the y-axis the axial axis and z-axis the medial-lateral axis. The pink points correspond to the virtual marker set placed at the same anatomical locations as the experimental markers during motion acquisition. Retrieved from OpenSim 3.2.](image_url)

Hence, there are two distinct marker sets, the experimental and the model (or virtual) markers. The first set refers to the markers attached at specific anatomical landmarks of the body of each subject to track anatomical positions (Figure 3.3 Annex A). The model markers are the ones placed in the musculoskeletal model at the same location as the experimental markers.
3.4.1 Scaling of the model

The customised model needs to be scaled according to the anthropometry and physical dimensions of each subject. The scaling was performed with the scaling tool available in OpenSim \cite{Delp2007}.

The input needed to perform scaling consists of the computational model to be scaled, the body mass of the individual subject, the model marker set and the experimental measured markers from the static pose of the subject. The static pose data file is in the .trc format (Track Row Column), a format created to specify the marker positions in time, during the motion acquisition trial.

The first step in scaling is computing the scale factors for each body segment. Each segment in the musculoskeletal body is defined by two or more virtual markers, thus each scale factor can be computed using two or more virtual markers. In measurement-based scaling, scale factors are estimated by comparing the distances between the virtual markers on the model and the ones between the experimental markers in the .trc file. Figure 3.8 presents the experimental and virtual markers, and the distances between pair \( p_1 = (RKNEL, RANKL) \) and \( p_2 = (LKNEL, LANKL) \), to compute the scale factors for the lower leg (left and right tibia).

![Figure 3.8: Experimental (dark blue) and virtual (pink) markers to compute the scale factors for the lower leg. \( e_{1,2} \) represent the distances between the experimental marker pairs and \( m_{1,2} \) the distances between virtual marker pairs. Retrieved from OpenSim 3.2.](image)

The distance between the pairs in the model \( (m_i) \) is obtained by placing the model in the default position, i.e. all the joint angles in the default values. The distance between the experimental pairs \( (e_i) \) is computed by averaging all the distances between the pairs at each frame of the experimental data in a specified time range. Then, the scale factor for \( p_1 \) is \( s_1 = e_1/m_1 \) and for \( p_2 \), \( s_2 = e_2/m_2 \). Since both lower legs need to have the same dimension, the overall scale factor is \((s_1 + s_2)/2\). Each segment is defined by its own combinations of markers, thus, it has assigned its own scale factor.

After this first step, the geometry was scaled based on the computed factors, i.e. the dimensions of each segment were based on the scale factor computed for it. For instance, a scale factor of 1.2
for the lower leg indicates that the model has a segment smaller than the reality. Moreover, joint frame locations, mass center locations, muscle attachment points and force application points were scaled according to the scaling factor of the corresponding segment (e.g. MG muscle attachment point was scaled according to the lower leg segment).

To scale the masses of the segments, the scaling tool uses the computed scale factors, mass input, i.e. body weight of each subject, and preserve mass distribution. Preserve mass distribution was aimed, in order to the mass of the scaled model to be equal to the mass of the subject, while the relative masses of its body segments were preserved. Since the mass input was provided for each subject, each body mass was scaled by the product of its scale factors and then each body was scaled again by the ratio of the mass input to the sum of the scaled masses.

To scale muscle actuators, a scale factor, computed as the ratio of the length before scaling to the length after scaling, is used. Muscle scaling is rather complicated, since the length of a muscle is configuration dependent. Therefore, scaling tool of OpenSim tries to maintain the model configuration across the whole process of scaling.

After scaling, a static pose was obtained by positioning the model in a pose that best matched the experimental marker and coordinate values, similarly to inverse kinematics. This step is detailed further. Figure 3.9 presents the model in the static pose before the step of inverse kinematics with both set of markers, experimental (dark blue) and the model markers (pink), shown.

![Figure 3.9: Static pose of the musculoskeletal model applied. In dark blue, experimental marker positions captured with the motion capture equipment and in pink, model marker positions. For the sake of simplicity, muscle actuators are hidden. (a) - X view. (b) X view. The axes are organized so that the x-axis represents the anterior/posterior axis, the y-axis the axial axis and z-axis the medial-lateral axis. Retrieved from OpenSim 3.2.](image)

To be noted that in this step each marker also contains a weight associated and the higher the weight given, the higher the degree at which the match should be satisfied for that marker. For instance, to markers applied in important anatomical landmarks for walking study (e.g ankle, knee, pelvis) the weight given was higher than to markers placed in the shoulders.
3.5 Inverse Kinematics: Kalman Smoother Algorithm

The next step in the designed workflow was an inverse kinematics analysis. Information regarding joint angles is crucial for a better understanding of the behavior of each joint during the movement in question and to point out differences between subjects, or in the scope of this work, between sessions. Therefore, IK allows the estimation of joint kinematics by positioning the model, at each time frame, in the pose that best matches the experimental marker and coordinate values, in order to reconstruct the analysed movement. The best match is expressed by the weighted least squares equation, which aims to minimize the distance between an experimental marker and the marker on the model (marker error) and the difference between an experimental coordinate value and the coordinate value obtained by inverse kinematics (coordinate errors). The weighted least squares equation is presented as follows:

$$\min_q \left[ \sum_{i \in \text{markers}} w_i \| x_i^{\exp} - x_i(q) \|_2^2 + \sum_{j \in \text{unprescribedcoords}} j (q_j^{\exp} - q_j)^2 \right]$$ (3.2)

Where the first summation corresponds to the marker errors and the second to the coordinate ones. Moreover, $q_j$ is equal to $q_j^{\exp}$ for all coordinates whose trajectory is known and which will not be computed. These coordinates are referred to as locked or prescribed. The vector $q$ corresponds to the vector of generalized coordinates being solved for, $x_i^{\exp}$ is the experimental position of marker $i$, $x_i(q)$ is the position of the corresponding model marker (dependent on the coordinate values) and $q_j^{\exp}$ is the experimental value for coordinate $j$.

However, IK may be hampered by experimental errors, such as ambiguous marker data, markers missing from certain cameras or even phantom markers, and soft tissue artefacts, which are not taken into account by most used methods, such as the segmental optimisation method (Spoor and Veldpaus, 1980) and the global optimization method (Lu and O’Connor, 1999). In 2003 Cerveri and his colleagues (Cerveri et al. (2003b); Cerveri et al. (2003a)), used for the first time a Kalman filter (KF) to estimate joint kinematics. This filter uses a series of time measurements from a biomechanical model and a process model that includes prior knowledge about the smoothness of the motion. The biomechanical model allows correspondence between the measured and model marker positions, whilst the process model guarantees the smoothness of the motion by assuming that the n-th derivative of generalized coordinates is constant. Therefore, there are no discontinuities in positions, velocities or accelerations. Nonetheless, a drawback pointed to KF is that for each time instant the filter estimates are based only on the measured marker trajectories until the considered time instant, leading to asymmetrical use of data.

A Kalman smoothing algorithm, which allows the estimates at each time instant to be based on the complete marker trajectories instead of only on partial marker trajectory information, was proposed by De Groote et al. (2008). Due to several advantages, namely the ability to produce more physiologically realistic changes in joint angles, removing spikes in the joint angle data and allowing for smoother transitions in joint angles between consecutive frames, it was used to perform inverse kinematics analysis.
The Kalman Smoother algorithm is defined by two consecutive steps: application of KF to estimate the joint kinematics at a given time instant, \( t_i \), using marker trajectory information until that time instant; and a backward recursion going from the last instant of measured marker trajectory until \( t_i \). Hence, all the available information regarding the movement is used to compute the result. This information ranges from the complete marker trajectory, the process model, with the main function of describing the expected time evolution of the joint kinematics for each degree of freedom, and the measurement model, which relates the joint kinematics to the measured marker position.

To perform Kalman Smoother analysis, the experimental marker data acquired during the motion capture trials must be given as an input. Moreover, it is also mandatory to provide a scaled musculoskeletal model with the definition of the marker set used, as shown in Figure 3.3 and the weights associated to each marker. As mentioned before, the higher the weight given to a marker, the higher the degree that it will try to match the position of the experimental marker. Nonetheless, marker weights are relative and in dynamic tasks the relative movement of the markers due to skin motion or the clothes used by the subjects makes it difficult to predict the actual motion of markers. Therefore, for this step of IK a value of 1 was given to all weights. The Kalman Smoother algorithm handles markers missing from certain cameras or phantom markers better than other IK methods, however if a marker presented a considerable number of gaps between time frames, it would be removed from the analysis to prevent incorrect estimations of its position throughout movement.

### 3.6 Inverse Dynamics

The next step consisted in an inverse dynamics analysis to obtain the joint moments, performed through the ID tool available in OpenSim (Delp et al., 2007). This tool computes the generalized forces (e.g. net forces and torques) at each joint responsible for a given movement, when given the kinematics (states or motions) and the kinetics (external loads) applied to the model. During this type of analysis the classical equations of motion, are solved to yield the generalized forces at each joint. Mathematically, the equations of motion are written as:

\[
M(q)\ddot{q} + C(q, \dot{q}) + G(q) = \tau \tag{3.3}
\]

where \( q, \dot{q}, \ddot{q} \in \mathbb{R}^N \) are the vectors of, respectively, generalized positions, velocities and accelerations, \( M(q) \in \mathbb{R}^N \) is the system mass matrix, \( C(q, \dot{q}) \in \mathbb{R}^N \) is the vector of Coriolis and centrifugal forces, \( G(q) \in \mathbb{R}^N \) is the vector of gravitational forces, \( \tau \in \mathbb{R}^N \) is the vector of generalized forces. The \( N \) represents the number of degrees of freedom.

Since the generalized positions, velocities and accelerations define absolutely the motion of the model, all the terms on the left-hand side of the equation \( 3.3 \) must be known whereas the term on the right-side remains unknown. Therefore, the inverse dynamics uses the known motion of the model to solve the equations of motion for the unknown generalized forces (net forces and torques) for each joint.

To perform inverse dynamic analysis in OpenSim, three types of input files are needed. A motion
file with the joint angles that describe the movement of the model computed by inverse kinematics, external load data (i.e. ground force reactions, moments and center of pressure location) and the scaled musculoskeletal model with the definition of the marker set used.

### 3.7 Muscle Analysis

Muscle-tendon unit lengths were estimated through the Analyses Tool, available in OpenSim. This tool offers several types of analyses to the user, such as kinematics (generalized coordinates, generalized speeds, and the accelerations), joint reaction loads and generalized force, speed, and power developed by each actuator of the model. On the scope of this study, the analysis used was the muscle analysis type.

The muscle analysis requires a scaled musculoskeletal model and motion file with the joint angles that describe the movement as inputs. Then, it estimates parameters of musculoskeletal geometry for each time frame and for the desired muscles (actuators) defined in the model. To the present study muscle analysis was used to estimate MG MTU lengths.

### 3.8 Data Analysis and Statistics

Due to the variability of the acquired data during the gait cycles, all data were splined to an equal number of 100 data points per stride. Data from one participant was excluded due to poor marker visibility during treadmill walking trials. All data are organized and presented as means ± standard deviations (SD) for all the subjects. All data were analysed statistically using an open-source SPM1d code (v0.4.2; www.spm1D.org) in MATLAB®, which consists in a package for one-dimensional Statistical Parametric Mapping (SPM). Usually, for zero-dimensional datasets, statistical interference derives from a model of randomness, in most cases the Gaussian distribution. The goal in these datasets is to quantify the probability that random data would exceed a certain test statistic value. SPM applies the same idea, but to datasets at the continuum level, by quantifying the probability of that data would produce a test statistic continuum (equivalent of a statistical parametric map) with a maximum that exceeds a certain test statistic value.

The data extracted for statistical analysis were assessed to be normally distributed using a SPM Shapiro-Wilk normality test (P > 0.05). To test our initial hypotheses, SPM paired \( t \)-tests were performed, comparing the joint kinematics and joint kinetics, pennation angles, MG muscle fascicle, MTU and SEE lengths and their respective contraction velocities between pre- and post-training intervention session. The probability level accepted for statistical significance was P < 0.05.

Paired \( t \)-test consists in a statistical procedure applied to determine whether the mean difference between two sets of observations is zero. Thus, in a paired sample \( t \)-test, each subject or entity is measured twice, which results in pairs of observations.
Chapter 4

Results

Following the workflow of data acquisition and processing, the results obtained are described. They are separated in subjects characteristics, effects of training on walking kinematics and kinetics and effects of training on the MTU interaction of the medial gastrocnemius muscle during walking. As stated previously, data of one participant was excluded, thus, the results of the remaining subjects were analyzed (n=10). All results were analyzed according to their statistical significance as mentioned in Section 3.8.

Due to the high number of statistical analyses and for the sake of simplicity, the results for SPM analysis are not reported. Nevertheless, Figure 4.1 exemplifies how the lack of statistically significant difference was determined for one parameter. Upper graph shows MG fascicle lengths for the SLOW speed between pre- (black solid line) and post-training intervention session (blue dash-dotted line). Bottom graph shows the Statistical Parameter Mapping for the paired t-test (SPM \( t \)) as a function of the gait cycle. The critical threshold \( t^* \) was not exceeded therefore no significant difference was found for MG muscle fascicle lengths after the training intervention.

Figure 4.1: Upper graph, MG muscle fascicle length changes for the pre-training intervention session (black solid line) and post-intervention session (blue dash-dotted line) for the SLOW walking speed. Bottom graph shows the Statistical Parameter Mapping for the paired t-test (SPM \( t \)) as a function of the gait cycle.
4.1 Subjects Characteristics

The mean age of the subjects was 68.1 ± 2.7 years and the mean body height consisted in 169.2 ± 5.1 cm. Body weight decreased significantly in older adults between sessions (P = 0.001) as detailed in Table 4.1. Furthermore, body mass index (BMI) decreased significantly between sessions (P<0.001, Table 4.1). The mean comfortable and 6-minute walking speed did not significantly change after the intervention.

<table>
<thead>
<tr>
<th>Table 4.1: Subjects characteristics</th>
</tr>
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<tbody>
<tr>
<td>N Pre-intervention</td>
</tr>
<tr>
<td>Body weight (kg) 10</td>
</tr>
<tr>
<td>BMI (kg/m$^2$) 10</td>
</tr>
<tr>
<td>COMF (m/s) 10</td>
</tr>
<tr>
<td>6MWD (m/s) 10</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Significant difference with paired sample t-test (P<0.05). BMI, body mass index; COMF, comfortable walking speed; 6MWD, 6-minute walking distance walking speed.

4.2 Effects of training on walking kinematics and kinetics

Figure 4.2 presents the results for ankle (A), knee (B) and hip (C) joints. For each subfigure, top rows report joint angles, whilst bottom rows show joint moments. Joint moments are normalized by body mass.
Figure 4.2: Joint angles and moments for ankle (A), knee (B) and hip (C) joints for the pre-training intervention session (black solid line) and post-intervention session (blue dash-dotted line) for walking at the four different speeds. The vertical black line denotes the end of stance phase and beginning of swing phase. The results are presented as mean ± SD, where the shaded regions represent ± SD of the joint angles and moments.

SPM analysis did not reveal significant differences in walking kinematics and kinetics for the four walking speeds pre. and post-training intervention sessions (P > 0.05).

4.3 Effects of training on MG MTU behavior during walking

Medial gastrocnemius muscle fascicle, MTU and SEE length changes are presented, respectively, in Figures 4.3, 4.4 and 4.5 and pennation angles in Figure 4.6.

Length changes are presented together with their respective shortening velocity. Just as the previous section, each figure presents the results for the four walking speeds. MG muscle fascicles and MTU lengths were normalized by the muscle fascicle length at heel strike and SEE length was normalized by SEE length at heel strike (Lai et al., 2015). This normalization means that, for instance, a
change of 0.2 \( (L_{m}) \) in the MTU fascicle length during walking indicates that the MG MTU varied in length by 20% of the MG fascicle length measured in the beginning of the stride.

Figure 4.3: MG muscle fascicle length changes and velocities for the pre-training intervention session (black solid line) and post-intervention session (blue dash-dotted line) for walking at the four different speeds. Muscle fascicle lengths and velocities were normalized to their corresponding length at heel strike \( (L_{m}) \). Negative and positive velocities denote shortening and lengthening, respectively. The vertical black line denotes the end of stance phase and beginning of swing phase. The results are presented as mean ± SD, where the shaded regions represent ± SD of the fascicle length changes and velocities for the MG muscle.

Figure 4.4: MG MTU length changes and velocities for the pre-training intervention session (black solid line) and post-intervention session (blue dash-dotted line) for walking at the four different speeds. MTU lengths and velocities were normalized to their corresponding corresponding MG muscle fascicle length at heel strike \( (L_{m}) \). Negative and positive velocities denote shortening and lengthening, respectively. The vertical black line denotes the end of stance phase and beginning of swing phase. The results are presented as mean ± SD, where the shaded regions represent ± SD of the MTU length changes and velocities for the MG muscle.
Figure 4.5: SEE length changes and velocities for the pre-training intervention session (black solid line) and post-intervention session (blue dash-dotted line) for walking at the four different speeds. SEE lengths and velocities were normalized to their corresponding length at heel strike ($L_{\text{SEE}}$). Negative and positive velocities denote shortening and lengthening, respectively. The vertical black line denotes the end of stance phase and beginning of swing phase. The results are presented as mean ± SD, where the shaded regions represent ± SD of the SEE length changes and velocities for the MG muscle.

Figure 4.6: MG muscle pennation angles for the pre-training intervention session (black solid line) and post-intervention session (blue dash-dotted line) for walking at the four different speeds. The vertical black line denotes the end of stance phase and beginning of swing phase. The results are presented as mean ± SD, where the shaded regions represent ± SD of the pennation angles the MG muscle.

No distinct training-induced differences in the mechanical behavior of the MG MTU were observed for the four walking speeds. Pre- and post-training intervention sessions did not significantly differ in MG muscle fascicle lengths, shortening velocities and pennation angles ($P > 0.05$), respectively in Figures 4.3 and 4.6.

The fact that MG fascicle lengths and pennation angles remained unaffected by the training intervention, while the ankle and knee joint angles did not suffer any change (i.e., same MG MTU length), led to the stretch and recoil of the SEE also remaining unchanged. Shortening velocities of the MG muscle fascicles, MTU and SEE were estimated as the first derivative of their lengths with respect to time. Thus, unchanged lengths result in similar shortening velocities after training.

Hence, paired sample $t$-tests revealed no significant differences in the stretch and recoil pattern for MTU and SEE lengths and their respective shortening velocities before and after the intervention ($P > 0.05$), respectively in Figures 4.5 and 4.4.
Chapter 5

Discussion

The present work examined the effect of a 12-weeks training intervention on the kinematics and kinetics of walking and on the medial gastrocnemius muscle-tendon interactions in older adults. An increase in AT stiffness would result in less strain at a given force acting on the tendon. Given the fact that, during walking, MG muscle fascicles act quasi-isometrically while MTU is lengthening, less strain of the tendon was hypothesized to result in higher MG muscle fascicle length changes during lengthening of the MTU (Lichtwark and Wilson, 2007).

To our knowledge, this study is the first to examine the influence of a resistance training program on the muscle-tendon interactions during walking in the elderly. The first hypothesis that joint kinematics and kinetics would remain similar before and after training during walking was confirmed. However, the remaining hypotheses were rejected. Contrary to the predictions, tendon stretch and recoil was not reduced after training and MG muscle fascicles did not present significant differences in the stretch and recoil patterns. Likewise, MG muscle fascicle velocity remained unchanged after the training intervention.

5.1 Effects of training on walking kinematics and kinetics

The first hypothesis that joint kinematics and kinetics would remain similar before and after training during walking was confirmed. Similar results were reported for resistance training interventions in younger adults during running (Werkhausen et al., 2019; Albracht and Arampatzis, 2013). The fact that joint moments did not change suggest that the force applied to the AT did not increase (Werkhausen et al., 2019). In their work, Hof et al. (2002) have studied the effect of AT stiffness on walking and running and found that even though tendon stiffness could vary considerably among subjects, their ankle kinematics would remain similar. This is not a surprise considering that, at midstance, the base of the foot is in contact with the ground and the ankle angle is mainly dependent on sweep of the distal leg. This sweep is a function of speed and thus, any effect would be related to the period of plantar flexion in later stance. In addition, knee angle would have a small influence on leg angle and MTU length (Lichtwark and Wilson, 2007). The results obtained for the joint angles and the joint moments are
supported by the type of training protocol adopted in the study. This training protocol was designed to induce structural and mechanical changes in the MTU, by increasing MG tendon stiffness and muscle strength (e.g. Blazevich 2006; Karamanidis et al. 2014; Epro et al. 2017). Thus, higher joint range of motion was not aimed.

5.2 Effects of training on MG MTU behavior during walking

Aging is associated with changes in muscle architectural characteristics and tendon mechanical properties (e.g. Stenroth et al. (2012)), which has implications in the force and power generation capacity of a muscle. Moreover, an age-related decrease in AT stiffness would suggest a change in SEE elongation patterns for older adults. However, when comparing MG MTU behavior between young and older adults, Stenroth et al. (2017) did not report differences in either MG muscle fascicle or SEE length change pattern for older adults at matched walking speeds. These authors suggested that the stiffness of the aponeuroses could preserve the SEE length changes despite the differences of AT stiffness.

Hence, the overall behavior of the MG muscle fascicles and the SEE during walking for the four different walking speeds showed consistency with earlier findings for young adults (Brennan et al. 2017; Farris and Sawicki 2012a; Fukunaga et al. 2001; Ishikawa et al. 2005; Ishikawa et al. 2007; Lichtwark and Wilson 2006). In fact, during the whole gait cycle, the MG fascicles endured different length changes than those experienced by the MTU and the tendinous tissues. The MG fascicle length change showed a relatively isometric behavior during midstance, followed by shortening in late stance. In turn, SEE stretched during the major part of stance and recoiled in push-off. Pennation angles were also consistent with the literature (Lichtwark et al. 2007; Lichtwark and Wilson 2006). When MG muscle fascicles acted relatively isometrically, pennation angles showed little change and the shortening of the fascicles throughout the stance phase was accompanied by increases in the pennation angles.

It is well accepted in literature that tendons are able to adapt to mechanical loading and several studies reported an increased Achilles tendon stiffness after resistance training (e.g. Arampatzis et al. 2007). Likewise, the study of Epro et al. (2017) provided evidence that the aged human AT is still mechanosensitive and preserves the ability to increase its stiffness in response to a long-term training intervention. Since the elastic nature of AT allows the decoupling of muscle fascicle behavior from that of the MTU during walking, it is expected that changes in AT stiffness have influence on both muscle contractile behavior and tendon elastic energy storage. Moreover, simulation studies using a two-dimensional model of the Achilles tendon in series with the gastrocnemius muscle support this theory (Lichtwark and Wilson 2007; 2008). Lichtwark and Wilson (2007) reported that at high tendon stiffness values the muscle fascicle behavior would be similar to that of the MTU, lengthening throughout most of the stance phase until it shortens rapidly. In turn, with low tendon stiffness, the muscle fascicles are predicted to shorten throughout the stance phase and then lengthen rapidly.

However, contrary to our predictions, the length changes pattern of MG muscle fascicles did not differ significantly after training for the four walking speeds. Likewise, pennation angles did not suffer significant training-induced alterations. The fact that MG fascicle lengths, pennation angles and ankle
and knee joint angles were not affected by the training intervention, i.e., same MG MTU lengths, led to similar stretch and recoil of the SEE. Therefore, our second hypothesis could not be accepted. Since velocities of the muscle fascicle were estimated as the first derivative of their lengths with respect to time, unchanged muscle fascicle lengths resulted in similar shortening velocities after training.

The interpretation of the obtained results is subjected to several aspects. Firstly, the design of the performed training intervention, the loading intensities applied during the exercises and the duration of the intervention itself affect tendon adaptations. A meta-analysis performed by McCrum et al. (2018) on the alterations of MTU biomechanical properties with aging and mechanical loading, found that exercise interventions should implement high and repetitive mechanical loading to induce adaptive responses in the aged AT. Furthermore, the same authors suggested that training interventions should be endured up to 3 or 4 months to successfully counteract age-related changes in MTU properties. Since AT stiffness was not measured in our study, it is not possible to quantify the effectiveness of the training in increasing it. Thus, the possibility that the adopted training intervention did not impact AT stiffness significantly remains.

Furthermore, the stiffness of a tendon in relation to the force producing capacity of the attached muscle varies between muscles. Roberts (2002) suggested that this variation is greatly explained by the muscle and tendon architecture. Moreover, the study of Lichtwark and Wilson (2008) has shown that different muscle fascicle length will compensate for a more stiff or compliant tendon to maintain high efficiency. For instance, efficiency of a muscle could be high with a compliant tendon as long as the muscle fascicles are much shorter. Thus, the lack of adjustment in MG muscle fascicles behavior may also be attributable to the architectural characteristics and biarticular nature of this muscle. Ishikawa et al. (2005) firstly suggested that MG and soleus fascicles behaved differently during human walking, even though SEE showed similar length change patterns for both muscles. This study reported that, while the MG fascicles remained at the same length during the major part of stance phase, the soleus fascicles lengthened continuously. Moreover, the soleus fascicles lengthened with increasing in muscle activation, whereas MG fascicles remained at the same length or even tended to shorten in the opposite direction. In comparison with the gastrocnemius muscles, the soleus muscle contributes more to the ankle moment, uses more elastic energy storage and is more metabolically efficient in walking (Krishnaswamy et al., 2011). In addition, Mian et al. (2007) reported that the lateral gastrocnemius fascicle lengthening contributed less and the SEE lengthening more to the lengthening of the MTU in older adults compared to young adults. The lateral head of the gastrocnemius muscle presents the longest fascicle lengths in the triceps surae muscles and has been shown to undergo higher length changes than those experienced by the medial head of the gastrocnemius (Maganaris et al., 1998). Hence, length changes in the LG or in the soleus muscle may be more affected by a training-induced increase in tendon stiffness.

Lastly, the obtained results might suggest that the walking strategy adopted by the older adults already provides optimal cost of energy, with no further adaptations of the muscle-tendon behavior after training interventions. The Hill-type muscle model proposed by Lichtwark and Wilson (2007) to predict MG muscle energetics suggests that there is an optimal value of SEE stiffness that maximises muscle fascicle efficiency in different gait conditions. Nonetheless, the model also reported that this efficiency
could be achieved across a broad range of SEE stiffness values during walking and only low stiffnesses would be detrimental to the capacity of a muscle to produce force. The training-induced changes in AT stiffness in our study may be within the optimal range, thus allowing MG fascicles to behave in a similar way as before the training intervention.

Moreover, according to the force-length relationship, the force generation capacity of a muscle is affected by the length at which the muscle fascicles are generating force. The muscle fibers of the human MG have been shown to operate over the descending limb of the force-length relationship during a large part of the stance phase and then move over the optimal region onto the ascending limb during the plantar flexion phase (Arnold and Delp 2011). Higher length changes for the MG muscle fascicles would imply the muscle sarcomeres to work at very short lengths and they would be unable to produce the required force. Moreover, these length changes would mean an increase in sarcomere shortening velocities, thus lower force production capacity. Hence, the hypothesized higher changes in MG muscle fascicle lengths could shift the operating length of the muscle to a less favorable portion to produce force. This supports the idea that the walking strategy adopted by the older adults is already optimal.

As a final remark, it should be noted that walking is a complex locomotion task with complex muscle-tendon interactions that might not be fully explained by in-series models. In addition, several muscular strategies can be adopted when walking by older adults (Stenroth et al. 2017). Boyer et al. (2017) support the hypothesis of a reduction in hip and ankle joint movements, peak ankle moments, power generation and ground reaction forces, in older adults compared to young adults. Furthermore, Franz et al. (2015) proposed that older adults might limit propulsion in push-off on purpose to improve balance. MTU interaction is influenced by the adopted walking pattern, hence, other aspects besides the ones mentioned throughout the discussion may influence the obtained results.

### 5.3 Study Limitations

Certain limitations in the study should be addressed. Firstly, our hypotheses relied on a training-induced increase in AT stiffness when it was not assessed in the present study. Instead, it was assumed that AT would increase after training according to previous results in literature (McCrum et al. 2018). The lack of information about AT stiffness does not allow to draw conclusions regarding the overall MTU behavior after training.

Secondly, MG muscle activation during the task performance was not assessed. Biewener and Roberts (2000) proposed that an alteration in the pattern of muscle activation (timing, duration and intensity) when speed, environment or gait task change can lead to different interaction between muscle fascicles and SEE. Hence, results on muscle activation could lead to a better understanding of the results.

Another limitation of the present study is the small sample size measured. A higher number of subjects could reduce the variability of the obtained results (i.e., decrease in the SD) and possibly lead to statistically significant differences.

The designed strength exercise intervention could have also included more exercises specifically
for the ankle plantarflexors. From the three exercises performed by the subjects, two were used for knee extension and flexion (seated leg press and knee extension contractions), which might not affect MG MTU as much as exercises to increase the strength of the plantarflexors.

Moreover, the MG was modeled as an individual MTU with individual SEE rather than a common Achilles tendon to the triceps surae muscle. The influence of this design on muscle fascicle and tendon behavior remains uncertain and to be researched in the future. To be noted, nonetheless, that previous in vivo studies assessing non-uniform Achilles tendon deformation have suggested that tendon fascicles arising from the plantarflexors may function independently during walking (Franz et al., 2015).

Lastly, ultrasound imaging is a two-dimensional measurement, despite the three-dimensional nature of the MG muscle studied. Hence, this method only translates the true behavior of the muscle fascicles when it occurs in the same two-dimensional plane as the ultrasound image. According to Blazevich (2006), when the transducer is not oriented in the plane of the fascicles, large errors may arise (2.4-14.0%). On the other hand, the method of fascicle tracking employed in the present study involved drawing a line parallel to the visible portion of the fascicle in the mid-muscle belly from its insertion at the superficial and deep aponeurosis. Then, the whole fascicle length was estimated using extrapolation in the analyses. Errors of 2-7% have been associated to MG muscle when employing this method (Blazevich, 2006). Recently, Raiteri et al. (2016) were able to measure geometrical changes of the human tibialis anterior muscle and its central aponeurosis during isometric contractions using three-dimensional ultrasound (3DUS). Nonetheless, this method still cannot be applied to assess MTU behavior during dynamics task, such as walking and running. Until now, two-dimensional ultrasound and the method applied for tracking are the more accurate and reliable options to assess MG MTU behavior during dynamic tasks (Cronin et al., 2011; Aeles et al., 2017).
Chapter 6

Conclusions and Future Work

The present study aimed to investigate the effect of 12-week strength exercise intervention on the MG MTU behavior in older adults. Since MTU behavior is largely influenced by the walking pattern, joint angles and moments were also assessed. In conclusion, we showed that this training intervention did not affect the gait biomechanics and the medial gastrocnemius muscle-tendon unit behavior during walking in the elderly. As predicted, joint kinematics and kinetics remained unchanged after training. However, contrary to our remaining hypotheses, the patterns of MG muscle fascicle and SEE lengthening did not present significant changes after training. It is well accepted in the literature that aged-AT preserves its mechanosensitivity to applied mechanical loading, thus an increase in AT stiffness would affect the stretch and recoil pattern of MG muscle fascicles and SEE and their respective shortening velocities. Nonetheless, questions remain regarding the MTU training-induced adaptations of the aged MG during walking and further research is warranted to draw more conclusions.

To gain further insight into the effects of a strength intervention in the MG MTU behavior in older adults, future studies should include AT stiffness and electromyography measurements. Furthermore, future research should incorporate measurements of both soleus and lateral gastrocnemius. The study of Lichtwark et al. (2007) provided evidence that the MG and soleus muscle do not have a similar mechanical behavior of the fascicles during walking, despite being commonly accepted as synergists. Moreover, the different muscle architectural and force-generating characteristic from the triceps surae muscles may lead to different adaptations of the triceps surae MTU after a training intervention (e.g. LG presents the longest fascicle lengths in the triceps surae muscles having the highest number of sarcomeres in series and, hence, showing eminent velocity potential).

Triceps surae MTU behavior after training interventions during walking warrant more attention. In particular, it would be interesting to examine whether the adaptation after training of MG MTU behavior is different between young adults and the elderly. The fact that AT stiffness is higher in young adults compared with older adults can lead to different effects on the fascicular and tendinous behavior of the MG MTU after a strength exercise intervention. In future studies, it may also be interesting to design a training intervention with different exercise types (e.g. resistance, endurance, stretching) and understand how and to what extent the type of training affect the triceps surae MTU behavior during walking.
References


Appendix A

Marker Placement

Table A.1 gives a detailed description of the markers considered for the acquisition of motion.

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Definition</th>
<th>Anatomical Landmark</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(L)FHD</td>
<td>Right (Left) front head</td>
<td>Located approximately over the temple</td>
</tr>
<tr>
<td>R(L)BHD</td>
<td>Right (Left) back head</td>
<td>Placed on the back of the head, on the horizontal plane of the front head markers</td>
</tr>
<tr>
<td>C7</td>
<td>7th cervical vertebrae</td>
<td>Spinous process of the 7th cervical vertebrae</td>
</tr>
<tr>
<td>T10</td>
<td>10th thoracic vertebrae</td>
<td>Spinous process of the 10th thoracic vertebrae</td>
</tr>
<tr>
<td>CLAV</td>
<td>Clavicle</td>
<td>Jugular notch where the clavicles meets the sternum</td>
</tr>
<tr>
<td>STERN</td>
<td>Sternum</td>
<td>Xiphoid process of the sternum</td>
</tr>
<tr>
<td>RBAK</td>
<td>Right back</td>
<td>Middle of the right scapula</td>
</tr>
<tr>
<td>R(L)SHO</td>
<td>Right (Left) shoulder</td>
<td>Acromioclavicular joint</td>
</tr>
<tr>
<td>R(L)UAA</td>
<td>Right (Left) upper arm anterior</td>
<td>Anterior part of marker cluster of the upper arm between elbow and shoulder</td>
</tr>
<tr>
<td>R(L)UAP</td>
<td>Right (Left) upper arm proximal</td>
<td>Proximal part of marker cluster of the upper arm between elbow and shoulder</td>
</tr>
<tr>
<td>R(L)UAM</td>
<td>Right (Left) upper arm medial</td>
<td>Medial part of marker cluster of the upper arm between elbow and shoulder</td>
</tr>
<tr>
<td>R(L)ELB</td>
<td>Right (Left) elbow</td>
<td>Over the lateral epicondyle of the humerus</td>
</tr>
<tr>
<td>R(L)LAA</td>
<td>Right (Left) lower arm anterior</td>
<td>Anterior part of marker cluster of the lower arm between elbow and wrist</td>
</tr>
<tr>
<td>R(L)LAP</td>
<td>Right (Left) lower arm proximal</td>
<td>Proximal part of technical cluster of the lower arm between elbow and wrist</td>
</tr>
<tr>
<td>R(L)LAM</td>
<td>Right (Left) lower arm medial</td>
<td>Medial part of marker cluster of the lower arm between elbow and wrist</td>
</tr>
<tr>
<td>Marker Name</td>
<td>Definition</td>
<td>Anatomical Landmark</td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>R(L)WRA</td>
<td>Right (Left) wrist anterior</td>
<td>Lateral epicondyl of the radius</td>
</tr>
<tr>
<td>R(L)WRP</td>
<td>Right (Left) wrist proximal</td>
<td>Medial epicondyle of the ulna</td>
</tr>
<tr>
<td>R(L)FIN</td>
<td>Right (Left) finger</td>
<td>Dorsum of the hand just below head of 2nd metacarpal</td>
</tr>
<tr>
<td>R(L)ASI</td>
<td>Right (Left) anterior superior iliac spine (ASIS)</td>
<td>Directly over ASIS</td>
</tr>
<tr>
<td>R(L)PSI</td>
<td>Right (Left) posterior superior iliac spine (PSIS)</td>
<td>Directly over PSIS</td>
</tr>
<tr>
<td>SACR</td>
<td>Sacrum</td>
<td>Midpoint between left and right proximal superior iliac spines (PSIS)</td>
</tr>
<tr>
<td>PELA</td>
<td>Pelvis Anterior</td>
<td>Anterior part of marker cluster of</td>
</tr>
<tr>
<td>PELP</td>
<td>Pelvis Proximal</td>
<td>Proximal part of marker cluster of</td>
</tr>
<tr>
<td>PELM</td>
<td>Pelvis Medial</td>
<td>Medial part of marker cluster of</td>
</tr>
<tr>
<td>R(L)THIA</td>
<td>Right (Left) thigh anterior</td>
<td>Anterior part of marker cluster of the proximal-third of the thigh</td>
</tr>
<tr>
<td>R(L)THIP</td>
<td>Right (Left) thigh proximal</td>
<td>Proximal part of marker cluster of the proximal-third of the thigh</td>
</tr>
<tr>
<td>R(L)THIM</td>
<td>Right (Left) thigh medial</td>
<td>Medial part of marker cluster of the proximal-third of the thigh</td>
</tr>
<tr>
<td>R(L)KNEL</td>
<td>Right (Left) knee lateral</td>
<td>Lateral epicondyle of the femur</td>
</tr>
<tr>
<td>R(L)KNEM</td>
<td>Right (Left) knee medial</td>
<td>Medial epicondyle of the femur</td>
</tr>
<tr>
<td>R(L)TIBA</td>
<td>Right (Left) tibia anterior</td>
<td>Anterior part of marker cluster of the posterior surface of the middle-third of the calf</td>
</tr>
<tr>
<td>R(L)TIBP</td>
<td>Right (Left) tibia proximal</td>
<td>Proximal part of techical cluster of the posterior surface of the middle-third of the calf</td>
</tr>
<tr>
<td>R(L)TIPM</td>
<td>Right (Left) tibia proximal</td>
<td>Medial part of marker cluster of the posterior surface of the middle-third of the calf</td>
</tr>
<tr>
<td>R(L)ANKL</td>
<td>Right (Left) ankle lateral</td>
<td>Lateral malleolus of the fibula</td>
</tr>
<tr>
<td>R(L)ANKM</td>
<td>Right (Left) wrist medial</td>
<td>Medial malleolus of the fibula</td>
</tr>
<tr>
<td>R(L)MTP1</td>
<td>Right (Left) 1st metatarsophalangeal</td>
<td>Medial aspect of 1st metatarsophalangeal (MTP) joint</td>
</tr>
<tr>
<td>R(L)MTP5</td>
<td>Right (Left) 5th metatarsophalangeal</td>
<td>Lateral aspect of 5th MTP joint</td>
</tr>
<tr>
<td>R(L)HEE</td>
<td>Right (Left) heel</td>
<td>Distal aspect of bisection of proximal calcaneum</td>
</tr>
</tbody>
</table>