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Epilogue

Thesis summary

Force generated by sarcomeres within skeletal muscle is transmitted to the skeleton predominantly via the myotendinous pathway. That is via myotendinous junctions located at the ends of skeletal muscle fibers. However, sarcomere force can also be transmitted to other structures via connective tissue structures surrounding muscle bellies. Such force transmission is called epimuscular myofascial force transmission. In addition, tendons of neighboring muscles can merge into a common tendon. The experiments described in this thesis have resulted in more insight into the mechanical relevance of these intermuscular linkages in the rat hindlimb for physiological ranges of muscle lengths and relative positions.

The experiments were performed in two muscle compartments of the rat hindlimb. The first compartment studied was the posterior crural compartment. It contains the mono-articular soleus (SO) muscle spanning the ankle joint, and the bi-articular gastrocnemius (GA, comprising the lateral head (LG) and the medial head: (MG)) and plantaris (PL) muscles spanning the ankle and knee joints. While these muscles have separate origins, distally they merge into the Achilles tendon and connect to the calcaneus as a common tendon. Therefore, for this muscle compartment, we assessed the mechanical effects of not only epimuscular myofascial pathways, but also of the shared Achilles tendon. The second compartment studied was the anterior crural compartment. It contains the mono-articular tibialis anterior (TA) muscle spanning the ankle joint, and the poly-articular extensor hallucis longus (EHL) and extensor digitorum longus (EDL) muscles both spanning the ankle and interphalangeal joints of the toes, with EDL spanning also the knee joint. In contrast to the posterior crural compartment, muscles in the anterior compartment do not share a common tendon. Therefore, the effects of only epimuscular myofascial pathways were assessed.

In this thesis, effects of intermuscular connections on various outcome measures were assessed. In Chapters 2, 3 and 4, the main outcome measures were 3D ankle moments exerted by the muscles in the posterior (Chapters 2 and 3) and anterior (Chapter 4) crural compartments. In Chapter 5, the outcome measures were mean sarcomere length and sarcomere length distribution of passive mono-articular SO and TA muscles. In Chapter 6, the outcome measures were individual tendon forces of muscles in the posterior crural compartment.

3D ankle moments

The aims of the experiments described in Chapter 2 were (i) to assess 3D ankle moment summation between SO and GA muscles for a range of ankle angles (70°-150°) while the knee was kept at a constant angle of 90°, and (ii) to assess the effects of muscle activation on the length of the Achilles tendon. At each ankle angle, SO and GA were first excited separately, and subsequently both muscles were excited simultaneously (SO&GA). The magnitude as well as the direction of ankle moments exerted by SO, GA and SO&GA were assessed. The mathematical sum of individual SO and GA moments was compared with the moment exerted by SO&GA to assess nonlinearity of ankle moment summation. We found limited values of nonlinear magnitude summation (<4%) and nonlinear direction summation (<3°) that were not dependent on ankle angle. In addition, changes in Achilles tendon length caused by SO contraction were significantly lower than those during contraction of GA and SO&GA, which could at least partially explain the nonlinear magnitude summation. We hypothesized that the mechanical consequences of epimuscular myofascial force transmission at the joint level may be acting in opposite direction compared to the mechanical consequences of the common Achilles tendon, which could explain the limited degree of nonlinear summation found in this chapter.

In Chapter 3, we aimed to assess effects of knee angle (i.e. proximal muscle-tendon unit (MTU) length changes of GA and PL, exclusively) on 3D ankle moments exerted by SO muscle, and on 3D ankle moment summation of SO and GA. Ankle angle was kept constant (90°), while knee angle was varied between 60° and 130°. At each knee angle, a similar stimulation protocol was used as in Chapter 2: SO and GA were excited separately as well as simultaneously (SO&GA). We found that knee angle did not affect the magnitude of the SO ankle moment. Although joint moment directions in the transverse and frontal planes were affected by knee angle, dissection indicated that this was not caused by length changes of GA and PL. Limited nonlinear summation of SO and GA ankle moments was found in magnitude (<2%) and in direction (<1°), which did not change with knee angle. While SO&GA contraction increased Achilles tendon length compared to rest, this change in length was not knee angle dependent. Because epimuscular myofascial force transmission may be masked by the shared Achilles tendon, such transmission

of force could not be excluded. Nonetheless, we concluded that, the mechanical effects of epimuscular myofascial connections between rat ankle plantar-flexors are not functionally relevant *in vivo*.

In Chapter 4, we assessed (i) effects of knee angle (i.e. changes in MTU length of EDL and position relative to TA and EHL) on TA ankle moment, (ii) interaction effects between knee angle with fasciotomy and proximal EDL tenotomy on TA ankle moment, and (iii) effects of knee angle on TA and EDL ankle moment summation. Knee angle was varied between 60° and 130°, while ankle angle was kept constant (90°). TA and EDL were excited individually and simultaneously (TA&EDL). The mathematical sum of individual TA and EDL moments was compared with the moment exerted by TA&EDL to assess nonlinearity of ankle moment summation. The magnitude of TA ankle moment was not affected by knee angle, but frontal plane moment direction was. However, dissections indicated that this was not caused by the compartmental fascia or EDL length changes. Moment summation was nonlinear in magnitude (~1%) and direction (~1°). However, as the net effects found were very limited, we conclude that myofascial connections between muscles in the anterior crural compartment have limited mechanical relevance during normal movement.

Sarcomere lengths

Results from recent imaging studies as well as previously used finite element models suggest effects of epimuscular myofascial force transmission on sarcomere lengths in series within muscle fibers. However, experimental evidence is lacking. Therefore, in Chapter 5 we evaluated effects of intermuscular mechanical interaction on (i) mean sarcomere length and (ii) sarcomere length distribution within passive fibers of the rat TA and SO. In this study, we positioned rat hindlimbs in predefined knee (55°, 90°, 125°, 160°) and ankle angles (either 90° or 125°), and fixed them in a formaldehyde solution. Varying knee joint angle causes length changes of synergistic MTU's, but not of SO and TA. Whole fibers were taken from SO and TA and photographed along their length. Mean sarcomere length was assessed for the entire fiber and for the proximal and distal thirds (fiber segments), separately. Mean fiber sarcomere length was not affected by knee angle, neither for SO nor for TA. Only for TA, a significant interaction between knee angle and fiber segment (proximal vs distal) was found, indicating changes in the distribution of lengths of sarcomeres in-series. Thus, while

epimuscular myofascial force transmission did not cause mean sarcomere length changes within passive SO and TA, it did alter the length distribution of sarcomeres within passive TA. This suggests that the epimuscular myofascial forces exerted onto TA were higher than those exerted onto SO. Therefore, effects of myofascial connections cannot be generalized to other muscles and other species.

Tendon forces

Force transmission between rat ankle plantar-flexors has been found for physiological lengths and relative positions of the involved muscles. However, the effects of this at the joint level appear to be limited. This may be explained by the extent of synergistic co-activation. The aims of this study, described in Chapter 6, were to assess intermuscular mechanical interactions between ankle plantar-flexors during (i) fully passive conditions, (ii) excitation of SO, (iii) excitation of LG, and (iv) during co-activation of SO and LG (SO&LG). In addition, the consequences of such mechanical interaction for Achilles tendon force were investigated in the condition that only SO was excited. We assessed effects of proximal lengthening of LG and PL muscles (i.e. simulating knee extension) on forces exerted at the distal SO tendon (F_{SO}) and distal LG-PL tendon (F_{LG-PL}) of the rat. Active F_{SO} and F_{LG-PL} were summed to obtain Achilles tendon force (F_{AT}). We found that the increase in F_{SO} due to LG-PL lengthening was higher during LG excitation than during fully passive conditions. The change in F_{SO} due to LG-PL lengthening was also higher during SO&LG excitation than during SO excitation only. The change in active F_{AT} due to LG-PL lengthening was, however, lower than the change in active F_{SO}. This study showed that epimuscular myofascial force transmission between rat ankle plantar-flexors is enhanced by muscle activation. However, the magnitude of this interaction was limited (<0.07 N) and only partially reflected in Achilles tendon force.

These results can explain the limited intermuscular mechanical interaction between rat ankle plantar-flexors found in Chapters 2 and 3. Proximal LG-PL lengthening minimally affects SO force output, and, although force is transmitted between these muscles, both muscles transmit force to the calcaneus via the shared Achilles tendon. In contrast to our hypothesis stated in Chapter 2, the limited nonlinear summation can, therefore, largely be ascribed to the common Achilles tendon.

General discussion

In the following section, the effects of muscle activation and muscle-specificity on the extent of intermuscular mechanical interaction will be discussed as well as the advantages and limitations of expressing mechanical interaction as changes in joint moments. Subsequently, some implications and functional relevance of intermuscular mechanical interaction will be addressed. But first, an important challenge of our experimental approach will be discussed.

Hindlimb fixation

Experiments for the studies described in Chapters 3 and 4 revealed a measurement bias introduced by securing the hindlimb in the set-up using a femur and foot fixation. Initially, we allowed some compliance in the ankle and knee joints to compensate for possible misalignment of the joint axes of rotation with the rotational axes of the set-up. Although non-absolute fixation has been shown to only minimally affect maximal muscle torque exertion in mice (Gorselink *et al.*, 2000), this was assessed for only one position of the hindlimb. When changing the knee joint angle we observed changes in both the magnitude and direction of the ankle moments exerted by the mono-articular ankle muscles, suggesting mechanical interaction with their synergistic muscles. However, very similar moment changes were still present after proximal tenotomy of the synergists, despite the fact that the MTU length of these muscles was no longer affected by knee joint angle. Thus, the changes in ankle moments could not be ascribed to mechanical interaction with synergistic muscles.

We hypothesized that bone reaction forces between the femur and the tibia were dependent on knee joint angle as the knee joint is not a perfect hinge joint. These forces may have resulted in translation and/or rotation of the tibia, which could have affected the length and orientation of the mono-articular muscle and, therefore, its moment exerted at the ankle joint. To minimize such movements, we reduced the compliance by increasing the compression force on the lower limb. In addition, we aimed to keep the compression force constant for all knee angles. We found that the change in the ankle moment magnitude exerted by the mono-articular muscles disappeared, but changes in its direction were still present, even after proximal tenotomy of their synergists. Therefore, we performed our experimental

protocol before and after proximal tenotomy of the bi- and poly-articular synergists. The absence of an interaction effect between tenotomy and knee angle indicated that any changes in ankle moments exerted by the mono-articular muscles could not be attributed to mechanical interaction with their synergists. However, mechanical interaction could still be attributable to other structures spanning the knee joint (e.g. neurovascular tract).

These results indicate that it is important to realize that allowing for some compliance in the joints can affect moment-angle relationships of muscles. Such effects can also occur in human studies in which extremities cannot be fixed as tight as in animal studies. Therefore, it is important to acknowledge that, if the relevance of intermuscular mechanical interaction is assessed using comparable fixation techniques as used in Chapters 3 and 4, results can be influenced by bone movements..

Intermuscular mechanical interaction between passive and active muscles

In the present thesis, effects of intermuscular connections were assessed for passive as well as active muscle conditions. Changes in knee angle resulted in substantial sarcomere length distributions in a passive mono-articular muscle (Chapter 5), however, during active muscle conditions, only limited changes in ankle moment (Chapters 3 and 4) and tendon forces (Chapter 6) were found. If changes of sarcomere length as found in passive conditions had occurred in active muscle conditions, changes in active muscle force production would have been expected. The limited changes in ankle moment and tendon force during active muscle conditions implies, therefore, limited changes in sarcomere length distribution. This may be related to differences in the stiffness of muscle fibers. At equal muscle fiber lengths, the stiffness of a muscle fiber during muscle activation is higher than in a passive muscle. Therefore, a certain myofascial load will cause smaller sarcomere length changes in an active fiber than in a passive fiber. Such a hypothesis would be in agreement with our results in Chapter 5, showing a reduced effect of knee angle on TA sarcomere length distribution at 125° ankle angle compared to 90° ankle angle. At 125° ankle angle, TA muscle fibers are longer than at 90° ankle angle, and, according to the passive length-force curve, their stiffness will be increased. This implies that effects found in passive muscles cannot be generalized to active muscle conditions.

If effects of epimuscular force transmission on sarcomere lengths within muscle fibers of active muscles are reduced, epimuscular force transmission found in previous studies (Huijing, 2009; Maas & Sandercock, 2010) may also have occurred predominantly via the extracellular matrix and connective tissue network, without affecting sarcomere lengths. This was indeed suggested by a finite element model in a study in which rat EDL was kept at a constant low length (i.e. 5 mm below optimum length) while its relative position was changed (Yucesoy *et al.*, 2006). Unequal forces were found at the proximal and distal tendon of EDL, which were dependent on EDL position. However, the finite element model predicted only limited changes in sarcomere length due to changes in EDL position. In contrast, the finite element model did predict changes in sarcomere lengths as a result of epimuscular myofascial force transmission if EDL muscle was kept at a constant high length (i.e. 2 mm beyond optimum length (Yucesoy *et al.*, 2006). To date, however, no studies have been performed that have assessed effects of epimuscular myofascial loads on sarcomere lengths during muscle excitation, to test these predictions.

The effects of epimuscular pathways are affected not only by the stiffness of muscles fibers, but also by other factors. In the present thesis, the mono-articular muscles were excited maximally, while their synergists were either passive or activated sub-maximally. In contrast, in many studies that previously reported epimuscular myofascial force transmission between synergists, muscles were excited both maximally and simultaneously (Huijing, 2009). In Chapter 6, we found stronger intermuscular mechanical interactions if levels of muscle activation were increased (passive SO with either passive or submaximally active LG and active SO with either passive or submaximally active LG). Therefore, the magnitude of myofascial loads may have been smaller in some of the conditions studied in the present thesis. The reduced effects we found could also partially be explained by the reduced range of muscle lengths and relative displacements as a result of imposing changes in joint angles. In addition, in Chapters 2, 3 and 4 intermuscular mechanical interaction was assessed at the joint level, while in earlier studies such effects on individual tendon forces were assessed. The mechanical consequences of such an approach will be discussed in the next paragraph.

Joint moments versus tendon forces

In Chapter 6, we found clear evidence of force transmission from the active SO muscle to the tied distal tendons of passive LG and PL muscles for a physiological range of muscle lengths and relative positions. However, using an identical stimulation protocol, results of Chapter 2 indicate no effects of such force transmission on the moment exerted by SO at the ankle joint. The most obvious explanation for this discrepancy is that, in an intact hindlimb, the distal SO and LG tendons merge into the Achilles tendon and insert on the skeleton as a common tendon. Any force generated within these muscles will eventually be transmitted via this shared insertion site, resulting in similar moments at the ankle joint. Thus, from the lack of any changes in active SO ankle moment in response to changes in knee angle it cannot be concluded that epimuscular myofascial force transmission is absent.

This shared Achilles tendon also acts as a pathway for intermuscular mechanical interaction. In Chapters 2 and 3, the role of the Achilles tendon for the interaction between SO and GA was discussed based on the observed negative nonlinear summation of ankle moments. It was proposed that GA contraction caused elongation of the distal SO tendon. Upon activation of SO, SO muscle belly would attain a shorter length compared to the condition in which GA was passive, which would decrease SO muscle force. This decrease in SO muscle force can (at least partly) explain the negative nonlinear summation of SO and GA ankle moments. As we did not test this hypothesis, further research should determine to what extent the tendons of SO and GA within the Achilles tendon are merged and to what extent the negative nonlinear summation can be ascribed to such a common tendon.

In contrast with the calf muscles, muscles in the anterior crural compartment of the rat do not share a common tendon. TA, EHL and EDL muscles have separate insertion sites on the skeleton. Because any force transmitted between TA and EDL will result in changes in moments exerted at the ankle joint, larger effects of intermuscular mechanical interaction on joint moments can be expected than in the posterior crural compartment. Nevertheless, our results indicated that myofascial connections between TA and EDL have limited mechanical relevance at the level of the ankle joint (Chapter 3). This appeared to be in agreement with previous studies measuring tendon forces in which it was found that force transmission between TA-EHL and EDL was substantial if EDL was lengthened distally (Huijing & Baan,

2003, 2008), but limited if EDL was lengthened proximally (Huijing & Baan, 2001*a*, 2003, 2008; Huijing *et al.*, 2003). Because we imposed changes in knee angle in our studies, EDL length was changed proximally. The present study is, however, the first study that assessed intermuscular mechanical interaction between these muscles in terms of joint moments.

Assessing the effects of intermuscular interactions in terms of joint moments allows more physiological experimental conditions: the muscle compartment can be kept as intact as possible and muscle length changes and relative muscle positions are limited to a range that is physiologically possible. However, the major limitation of this approach is that only the net-effect of such force transmission can be detected and not the presence per se. Such information would be available if tendon forces of the individual muscles are measured. For future studies, the use of buckle force transducers placed around the tendons of SO, LG, and PL muscles might allow determining the extent of myofascial force transmission in an intact hindlimb. Nonetheless, it is the net effect that is essential for understanding the relevance of intermuscular mechanical interaction during movement.

Effects of intermuscular mechanical interaction are muscle and species specific
Previous studies have investigated epimuscular myofascial force transmission for a wide variety of muscle groups. In general, such force transmission was always found, although the extent of it was variable (Huijing, 2009). In contrast, we found that effects of epimuscular myofascial force transmission on the distribution of sarcomere lengths were muscle specific (Chapter 5). In Chapter 5, the different effects were ascribed to potential differences in myofascial loads. This suggests that effects of epimuscular myofascial connections cannot be generalized to other muscles and other species. For example, while no effects of intermuscular connections on sarcomere lengths within passive SO in rats were found (Chapter 5), recent imaging studies in human SO do suggest such effects (Bojsen-Møller *et al.*, 2010; Huijing *et al.*, 2011; Tian *et al.*, 2012). As rat SO is parallel fibered and human SO is multi-pennated, it is also possible that the muscle architecture plays an important role in the consequences of intermuscular connections. Therefore, for a complete understanding of intermuscular mechanical interactions, more muscle groups should be studied in a variety of animal species. The combination of mono-articular

and bi-/poly-articular muscles within a muscle compartment, as used in the present thesis, enables the assessment of mechanical interaction for physiological lengths and relative positions. Possible locations for such a combination of mono-articular and bi-articular muscles in humans are: (i) the short head of the biceps femoris (mono-articular) and semitendinosus, semimembranosus and the long head of the biceps femoris (bi-articular); (ii) vasti muscles (mono-articular) and rectus femoris (bi-articular); (iii) the medial and lateral head of triceps brachii (mono-articular) and the long head of the triceps brachii (bi-articular); (iv) the brachioradialis (mono-articular) and the biceps brachii (bi-articular).

Other mechanisms of mechanical interaction between adjacent muscles

The focus of this project was to assess the mechanical relevance of linkages that interconnect skeletal muscles. However, mechanical interaction can potentially also occur via mechanisms that do not involve direct connective tissue linkages. During muscle contractions, the length of a muscle belly decreases but its diameter increases, resulting in an increased transversal load onto neighboring muscles. It has been shown that an increase in transversal load decreases forces measured at the distal tendon of the rat medial gastrocnemius muscle (Siebert *et al.*, 2014). In addition, muscle bulging could increase the moment arm relative to a joint (Maganaris *et al.*, 1998), thereby increasing the joint moment exerted by that muscle. If the mechanical effects of muscle bulging would be of sufficient magnitude, this should be dependent on muscle length (muscle bulging is larger at shorter muscle lengths). However, the magnitude of nonlinear summation found in Chapters 2, 3, and 4 was not dependent on joint angle. This indicates that the mechanical effect of muscle bulging was limited in our studies.

Implications

The results of this thesis are important for understanding the contribution of the various pathways of muscular force transmission during movement, which is important for motor control. Chapter 5 has shown that mechanical interaction via inter- and extramuscular pathways can result in substantial distribution of sarcomere lengths and Chapter 6 showed significant force transmission between SO and GA if muscle lengths and relative displacement are within physiological ranges. Despite this

evidence of mechanical interaction in both the posterior (Chapter 6) and anterior (Chapter 5) crural compartments of the rat, the moment exerted at the ankle joint was affected only to a limited extent (Chapters 3 and 4) and summation of ankle moments exerted by multiple muscles within each compartment was nearly linear (Chapters 2, 3 and 4). These outcomes are important for biomechanical models of the musculoskeletal system and the application of methods such as functional electrical stimulation. In both cases, it is assumed that muscles are independent actuators, which means that (i) the moment exerted by a muscle is not affected by surrounding muscular and non-muscular structures; and (ii) that summation of muscle moments is linear. Based on the results of the present thesis, we can conclude that only limited errors will be made by making such assumptions.

From the work in the present thesis, we can conclude that epimuscular myofascial force transmission may not be mechanically relevant at the level of the ankle joint. Nonetheless, some myofascial force transmission was found within physiological ranges of muscle lengths and positions (Chapter 6), although the extent was only minimally affected by knee angle. During hindlimb movements, it is the change in knee angle that causes relative displacements of muscles in the anterior and posterior crural compartments. Such changes in muscle relative position have been shown to be the main determinant of the extent of intermuscular mechanical interactions (Maas *et al.*, 2004; Rijkelijkhuizen *et al.*, 2007; Huijing & Baan, 2008). The results of this thesis imply that connective tissues are organized in such a way that they allow independent proximal movement of the bi- and poly-articular synergists. We also showed that, even if there is epimuscular force transmission between the distal tendons of SO and LG (Chapter 6), it has only minimal effect on the moment exerted by SO at the ankle joint (Chapter 3). Therefore, epimuscular myofascial pathways may be relevant for other mechanisms. As already mentioned in Chapter 6, epimuscular myofascial force transmission between SO to GA may result in a distribution of stress between the distal tendons of SO and GA, thereby decreasing peak stresses (Bojsen-Møller *et al.*, 2010). In addition, the distribution of sarcomere lengths, as described in Chapter 5, can be detected by sensory receptors within the muscle belly, such as muscle spindles, thereby potentially affecting sensory encoding of muscle conditions (Smilde *et al.*, 2014).

As most of our current knowledge on epimuscular myofascial force transmission, the results of the present thesis are based on experiments using isometric contractions exclusively. During many motor tasks, however, muscle fibers are actively shortening or lengthening. Such contractions were not examined in the present thesis. To date, only one study has assessed effects of epimuscular myofascial force transmission during concentric contractions (Maas & Huijing, 2005). This study found significantly higher tendon forces exerted by the 3rd head of the rat EDL muscle, than would be expected from its cross-sectional area. Although this suggests force transmission from other structures than from muscle fibers within the 3rd head, more research is necessary to assess the importance of epimuscular myofascial force transmission during dynamic muscle contractions.

While the mechanical relevance of epimuscular myofascial force transmission may be limited in a healthy muscular system, it may be more important in pathological conditions. For example, after a muscle injury, growth of scar tissue can result in connective tissue that is stiffer than in normal conditions, which could enhance the extent of epimuscular myofascial force transmission. Increased stiffness of connective tissue has also been suggested to a play a role in spastic paresis (Huijing, 2007; De Bruin *et al.*, 2014). Children with cerebral palsy often develop contractures in the hand and develop a limited range of motion in the knee during gait. To improve function of the upper and lower extremities, tendon transfers can be performed: the tendon of an affected muscle can be cut and placed on the antagonistic site. However, mechanical function of the transferred muscles is not always as expected (Smeulders & Kreulen, 2007). In children with cerebral palsy, it was found that the rectus femoris muscles still exerted a knee extension moment after it was transferred to the flexor site of the knee (Riewald & Delp, 1997). In rats, it has been found that force exerted by the flexor carpi ulnaris, which was transferred to the extension site of the wrist, was still transmitted to the tendon of the palmaris longus muscle (Maas & Huijing, 2011, 2012), a flexor of the wrist. These results could be explained by epimuscular myofascial force transmission, connecting the transferred muscle to its former synergists and, thereby, partially maintaining its original function. The above-described examples indicate that epimuscular myofascial force transmission plays a substantial role in pathological conditions.

Conclusions

From the work in the present thesis, we conclude that intermuscular mechanical interactions are present within physiological ranges of muscle lengths and relative position of synergistic muscles. The effects of such mechanical interactions seems dependent not only on the activation level of synergistic muscles, but also on their architecture. However, intermuscular connections have limited effect on the mechanical muscle function at the joint level.