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Review

Ultrasonography as a tool to study afferent feedback from the muscle–tendon complex during human walking

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ABSTRACT

In humans, one of the most common tasks in everyday life is walking, and sensory afferent feedback from peripheral receptors, particularly the muscle spindles and Golgi tendon organs (GTO), makes an important contribution to the motor control of this task. One factor that can complicate the ability of these receptors to act as length, velocity and force transducers is the complex pattern of interaction between muscle and tendinous tissues, as tendon length is often considerably greater than muscle fibre length in the human lower limb. In essence, changes in muscle–tendon mechanics can influence the firing behaviour of afferent receptors, which may in turn affect the motor control. In this review we first summarise research that has incorporated the use of ultrasound-based techniques to study muscle–tendon interaction, predominantly during walking. We then review recent research that has combined this method with an examination of muscle activation to give a broader insight to neuromuscular interaction during walking. Despite the advances in understanding that these techniques have brought, there is clearly still a need for more direct methods to study both neural and mechanical parameters during human walking in order to unravel the vast complexity of this seemingly simple task.

Contents

1. Introduction

In humans, one of the most common tasks in everyday life is walking, and the average human takes several thousand steps per day. It is generally accepted that the complex motor control of walking involves the integration of information from

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supraspinal structures (e.g. Armstrong, 1988), rhythm generating structures at the spinal level (Grillner, 1975) and sensory afferent feedback from peripheral receptors (for reviews see Duysens et al., 2008; Rossignol et al., 2006; Zehr and Stein, 1999). With regard to the latter component, the importance of afferent feedback is clearly articulated when examining Ian Waterman, an individual who lacks proprioception from large myelinated afferent nerve fibres, and thus relies on other sensory cues to determine the orientation of his limbs (af Klint et al., 2008). Relying heavily on visual feedback, Ian is unable to move in the dark (Cole and Sedgwick, 1992), and all movement requires complete visual attention due to the lack of sensory input to the central nervous system that usually helps to control movement accurately.

During walking, afferent feedback has been suggested to play three broad but distinct roles: contribute to ongoing muscle activity during unconstrained walking, to contribute to corrective responses to sudden perturbations and to trigger phase transitions in the gait cycle, (e.g. Donelan and Pearson, 2004; Pearson, 2004; Grey et al., 2004; Sinkjaer et al., 2000). In addition, afferent feedback plays a crucial role in motor program adaptation and novel motor learning. In recent years, we have investigated the roles of various afferents that contribute to the normal background activation that occurs in unconstrained walking, as well as those involved in corrective responses to sudden perturbations. Since our data have enabled us to rule out the contribution of certain afferents, including cutaneous afferents and proprioceptors of the foot (e.g. Grey et al., 2002, 2004), much of our work has focussed on two receptors: the muscle spindles and the Golgi tendon organs (GTO). Muscle spindles are mechanoreceptors located among the muscle fibres, and are capable of monitoring muscle length, velocity and acceleration. There are two types of sensory afferents originating in the spindles: primary and secondary. Primary muscle spindle afferents, referred to as group Ia fibres, predominantly sense velocity, but are also capable of detecting length changes, whereas secondary muscle spindle endings, referred to as group II fibres, predominantly sense changes in muscle length (Edin and Vallbo, 1988; Edin and Vallbo, 1990a,b; Kakuda, 2000; Matthews and Stein, 1969). Golgi tendon organs are force-sensitive mechanoreceptors that are innervated by fast-conducting Ib afferent fibres. GTOs are mostly located at points where muscle fibres connect to tendinous tissue, including deep intramuscular tendons or aponeuroses (Crago et al., 1982; Houk and Henneman, 1967; for a review see Jami, 1992).

Until recently, investigations of the motor control of walking have used mechanical or electrical perturbations of the leg combined with the measurement of muscle activity as an experimental model. Many of the existing methods, such as surface electromyography (sEMG), are necessarily indirect. Although direct neural recording techniques do exist, it has not yet been possible to implement them during human walking. For example, microneurography is a valuable method that has yielded advances in our understanding of motor control (for a review see Mano et al., 2006). However, with this method data are obtained from humans *in situ*, and it has yet to be successfully applied during walking. Some studies have measured muscle–tendon unit (MTU) length and used it to infer about muscle fibre and even muscle spindle activity. However, the MTU consists of both muscular and tendinous tissues, which complicates this estimation. Indeed, direct data measured in cats suggest that this approach is likely to be erroneous as MTU length diverges from muscle fibre length in some portions of the stance phase (Griffiths, 1991; Hoffer et al., 1989). As an alternative to measuring MTU length, it is now possible to estimate muscle fascicle (or fibre) length in humans *in vivo* using ultrasonography, a non-invasive imaging modality that has been successfully employed during walking and other movements.

The use of imaging to study muscle and tendon behaviour in humans has dramatically increased our understanding of muscle–tendon architecture, and it has become increasingly apparent that precise knowledge of the anatomic features of muscle, aponeurosis and tendon is necessary to understand how a muscle–tendon unit (MTU) generates force and accomplishes length changes (for a review see Finni, 2006). For example, in the human triceps surae complex, muscles are attached to long tendons that exhibit elasticity, which can have a decisive influence on the pattern of length changes that muscles undergo during movement and can thus also influence movement efficiency (for reviews see Magnusson et al., 2008; Roberts, 2002). Furthermore, there is an increasing realisation that changes in muscle–tendon mechanics, which influence the firing behaviour of sensory afferent receptors, may in turn affect motor control.

The purpose of this review is to summarise research that has incorporated the use of ultrasound-based techniques to study muscle–tendon interaction, predominantly during walking. We then review recent research that has used ultrasound imaging to study the broader issue of neuromechanical control during human walking.

2. Animal studies as the basis of knowledge about neuromechanics during movement

For several decades it has been recognised that due to the effects of tendon compliance, a movement imposed at a joint is not necessarily faithfully imposed upon muscle fibres. Consequently, sensory receptors located among extrafusal muscle fibres (muscle spindles) may ‘sense’ an attenuated version of the stretch imposed on the joint (e.g. Rack et al., 1983). As a result, it seems intuitive to examine length changes at the muscle fascicle (or fibre) level. As the gamma system is known to influence muscle spindle sensitivity, and this effect is not directly measurable *in vivo*, fascicle length does not provide a direct indication of muscle spindle output, but it is likely to yield a better estimate of this parameter than measuring MTU length. Before examining data obtained in humans, it is important to first highlight findings in animals; due to the relative ease of employing invasive methods in many animal species, a large number of studies have been published concerning muscle–tendon interaction, many of which have served as a basis for non-invasive study in humans.

From the late 1980s onwards, a series of papers were published using a method that enabled direct measurements of muscle fibre length in animals during movement (Griffiths, 1987, 1991; Hoffer et al., 1989). Sonomicrometry uses piezoelectric crystals to measure distances within an aqueous medium. Pairs of crystals are inserted at both ends of a muscle fibre, and these crystals generate waves of ultrasound that travel at known velocities in biological tissue. One crystal acts as a transmitter and the other as a receiver. Distances between the two crystals are calculated based on the transit time of the ultrasound waves between them, combined with knowledge of the speed of sound through the tissue.

Using sonomicrometry, data obtained from walking cats have provided convincing evidence that length changes at the MTU level are not necessarily representative of length changes of the muscle fibres (Griffiths, 1987, 1991; Hoffer et al., 1989). The first studies in this field showed that at some phases of the step cycle, length changes of the muscle fibres actually occurred in the opposite direction to those of the MTU, which is effectively due to the compliance of tendinous tissues. These findings had obvious implications for the motor control of walking, and raised concerns over the use of MTU length as a predictor of muscle fibre or even spindle activity (see Maas and Lichtwark, 2009; Maas et al., 2009).

In animals, the subsequent use of sonomicrometry, in combination with the assessment of directly measured tendon forces and recording of muscle activity (Higham et al., 2008; McGuigan et al., 2009; Roberts et al., 1997), has provided valuable information about neuromechanical interactions during walking in response to changes in surface slope (Gregor et al., 2006; Higham et al., 2008; Maas et al., 2009; McGuigan et al., 2009; Roberts et al., 1997) and walking speed (Prilutsky et al., 1994), as well as information about synergistic muscle behaviour (Higham et al., 2008; Maas et al., 2009; Prilutsky et al., 1994). During cat locomotion, for example, medial gastrocnemius (MG) and soleus (Sol) contribute differently to the generation of force and mechanical energy (Fowler et al., 1993; Gregor et al., 1988; Herzog et al., 1993; Prilutsky et al., 1994, 1996; Walmsley et al., 1978), and this may influence the sensory input to the central nervous system from each muscle (Prilutsky et al., 1996). For example, Sol fascicles have been shown to lengthen after ground contact, whereas MG fascicles shortened (Maas et al., 2009). Accordingly, the authors suggested that the potential role of length feedback (from muscle spindle type II afferents) may be greater in Sol than in MG. They also noted that this would be consistent with the observation that spindle density is greater in Sol than gastrocnemius (Chin et al., 1962). Regarding spindle Ia afferents, these structures are predominantly velocity-sensitive (but do still sense length), so differences in fascicle lengthening velocity between muscles may also influence the relative importance of Ia afferents in a given muscle.

3. Making the transition from animals to humans: Ultrasonography as a non-invasive tool to study muscle–tendon behaviour

As stated, there is a clear need to investigate fascicle length changes during human movement since this parameter would almost certainly provide a better estimate of muscle spindle activity than length changes at the MTU level (e.g. Maas and Lichtwark, 2009). Given that tendon compliance can influence the stretch of muscle fibres, and thus presumably muscle spindles, it is pertinent to note that in humans, tendon length often greatly exceeds muscle fibre length. This is particularly true in the gastrocnemius muscle, and further emphasises the potential mismatch between muscle fascicle and MTU length changes.

Due to the invasive nature of sonomicrometry, it has been very difficult to apply this technique to humans. Although limited Achilles tendon strain data have been presented (Arndt et al., 2006), these experiments were stopped prematurely due to subject pain and difficulties obtaining valid data (Arndt, 2009 – Personal communication). However, a non-invasive alternative, which is also based on the transmission of ultrasound waves, has been applied to the study of dynamic human movement in the last two decades.

Ultrasonography (US) works on the echo location, or sonar principle (see Fig. 1). A linear array probe is positioned on the skin surface over the structure of interest, and sound waves are emitted into the tissues. When the sound waves hit different structures (e.g. muscle fibres), they are reflected (or echoed) back to the

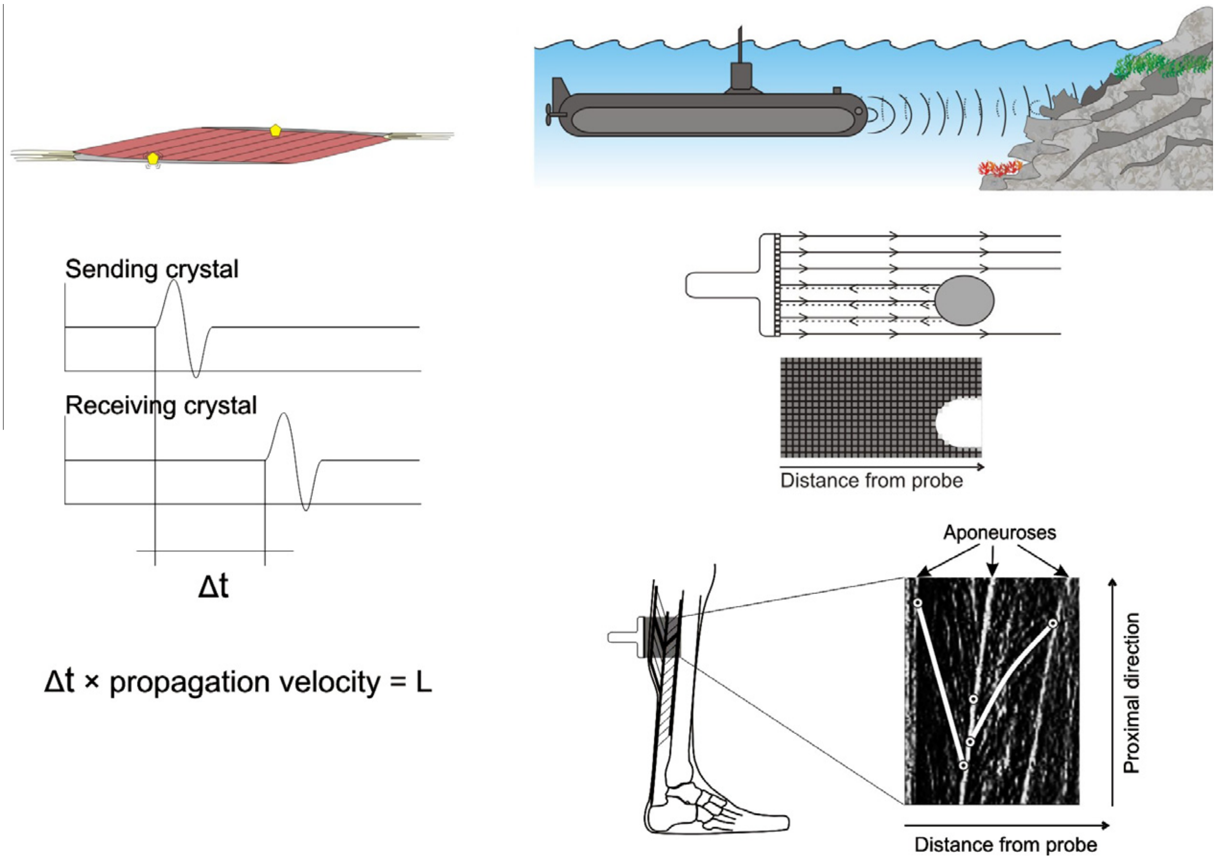

Fig. 1. Left: Illustration of the sonomicrometry technique, showing a pair of crystals implanted at either end of a muscle fascicle (top), as well as the resulting sound waves and the method used to calculate the distance between the crystals (bottom). Δt denotes the time between the pulse being sent from the transmitting crystal and arriving at the receiver crystal, and L represents fascicle length. Right: Demonstration of the sonar principle in a submarine, whereby sound waves are emitted, and the resulting echoes are used to form an image of the surrounding environment (top); the same principle in an ultrasound probe where the sound waves travel over much shorter distances (middle); positioning of the probe over the human lower limb to enable the MG and Sol fascicles to be visualised (bottom). In theory, a single fascicle is identified and tracked frame by frame throughout a given movement or contraction cycle.

probe. As with the sonomicrometry method, knowledge of the speed of sound through the tissue combined with information about the send and receive times of the sound waves enables the distance to each visualised structure to be calculated, and an image can then be reconstructed. As this process occurs at a high frame rate, it is possible to use this method to study the length changes of muscle and tendinous tissues during human movement, provided that some form of stabilising support device is used to keep the probe in position.

Despite the relative infancy of the use of US to examine muscle–tendon mechanics, a wealth of data has been presented using this method. As the number of publications concerning the use of US for these purposes is so voluminous, we have not attempted to include a comprehensive list of publications. Instead, to illustrate the versatility of this technique, we will briefly summarise some of the many ways in which the US method has been used to study muscle–tendon interaction in humans.

The technique was initially used during isometric contractions, with the aim of examining muscle fascicle lengths and pennation angles at different joint configurations and contraction levels (e.g. Fukashiro et al., 1995; Henriksson-Larsen et al., 1992; Herbert and Gandevia, 1995; Kuno and Fukunaga, 1995; Narici et al., 1996; Rutherford and Jones, 1992). These early studies confirmed that even in isometric conditions, muscle fascicle length was clearly modulated with changes in force level, so that for a given MTU length, fascicle length and pennation angle could markedly differ depending on the level of force production (e.g. Narici et al., 1996). More recently, numerous studies have used US to examine muscle and tendon behaviour during human movement. To date, the range of movements examined includes running (Ishikawa and Komi, 2007; Ishikawa et al., 2007; Lichtwark et al., 2007), hopping (Lichtwark and Wilson, 2005a; Peltonen et al., 2010), cycling (Wakeling et al., 2006), various forms of jumping (Kubo et al., 1999; Kurokawa et al., 2001; Sousa et al., 2007; Finni et al., 2003; Galindo et al., 2009), including drop jumps (Hoffren et al., 2007; Ishikawa et al., 2003, 2005; Ishikawa and Komi, 2004), other forms of counter movement exercise (Kawakami et al., 2002) and stair climbing and descent (Chleboun et al., 2008; Spanjaard et al., 2007, 2008, 2009).

More importantly in the context of this review, the US method has been applied to the study of human walking (af Klint et al., 2010; Aggeloussis et al., 2010; Chleboun et al., 2007; Cronin et al., 2009a,b; Fukunaga et al., 2001; Ishikawa et al., 2005; Lichtwark and Wilson, 2006; Lichtwark et al., 2007; Mian et al., 2007), and this is discussed in more detail below with reference to the behaviour of lower limb muscles. Although the focus of this review is on walking, the reader is also referred to several reviews of the use of ultrasound in different experimental settings (Fukashiro et al., 2006; Fukunaga et al., 2002; Ishikawa and Komi, 2008; Kawakami and Fukunaga, 2006).

4. Muscle–tendon interaction during human walking

As already alluded to, tendons are not inextensible, but rather they exhibit important elastic and time-dependant characteristics that may influence the function of the whole MTU (for reviews see Magnusson et al., 2008). These tendon properties result in a dynamic interaction between the muscle and tendon, which can influence not only force transmission (Reeves et al., 2003), but also energy storage and return during locomotion (Ishikawa et al., 2005; Lichtwark and Wilson, 2005b), muscle fascicle geometry during contraction (Narici et al., 1996) and the control of joint position and movement accuracy (Hoffer et al., 1989; Loram et al., 2005; Rack et al., 1983). On the basis of recent data, we also suggest that patterns of muscle–tendon interaction can have an important influence on sensory afferent feedback (see following sections).

As in animals, measurements of fascicle length during overground and treadmill walking in humans have revealed that muscle fascicle length changes do not match those of the MTU during the contact phase (e.g. Fukunaga et al., 2001; Ishikawa et al., 2005, 2007), thus generally confirming the original, direct findings obtained in cats (Griffiths, 1991; Hoffer et al., 1989). During the stance phase of walking, the triceps surae muscles and the long, compliant Achilles tendon have been suggested to act like a catapult (Ishikawa et al., 2005; Sawicki et al., 2009), whereby the Achilles tendon first stores elastic energy, and then releases it to produce a rapid recoil during the push-off phase (Fukunaga et al., 2001; Ishikawa et al., 2005). One advantage of this sequence is that it allows muscle fibres to remain nearly isometric, producing force with very little mechanical work. Concurrently, the tendon takes up most of the MTU lengthening and stores elastic energy. This pattern of muscle–tendon interaction enables each tissue to perform the task to which it is best adapted, and may thus promote metabolic efficiency during walking (Sawicki et al., 2009).

Despite the relatively small number of studies incorporating US during human walking, some common features can be extracted concerning fascicle behaviour. In the triceps surae muscles, fascicles generally shorten immediately after ground contact due to rapid plantar flexion at the ankle. Throughout the rest of the stance phase, MG fascicles tend to remain approximately isometric, although moderate lengthening has been observed (Aggeloussis et al., 2010; Fukunaga et al., 2001; Ishikawa et al., 2005, 2007; Lichtwark and Wilson, 2006; Lichtwark et al., 2007). In Sol, much less data are available, but fascicles appear to lengthen throughout stance (Cronin et al., 2009b; Ishikawa et al., 2005). Similarly, in the lateral gastrocnemius (LG), the fascicles lengthen during stance in young subjects (Mian et al., 2007), suggesting a possible difference in fascicle behaviour between the medial and lateral heads of this muscle. In all triceps surae muscles, fascicles shorten towards toe off. In the tibialis anterior muscle, fascicle length has been shown to increase towards the end of the stance phase. Furthermore, in the more proximal vastus lateralis, fascicle length increased by approximately 40% between ground contact and toe off, although most of this change occurred towards the end of stance (Chleboun et al., 2007). It is noteworthy that in LG, fascicle behaviour differed between young and older subjects (27 vs. 77 years of age; Mian et al., 2007), whereby fascicles were lengthened less in the stance phase in older subjects. This finding may well be evident in other muscles. Since Achilles tendon compliance has been shown to increase with age (for a review see Narici et al., 2008), a decrease in fascicle lengthening during stance in older subjects is not unexpected, and this issue deserves further study in other muscles.

The data summarised here suggest some important differences between muscles often assumed to be synergists. For example, as is the case with cats (Maas et al., 2009), the fascicles of MG and Sol exhibit different patterns of length change during human walking (Ishikawa et al., 2005). Differences between MG and LG are also evident, despite the fact that these muscles are often considered to be functionally equivalent. In light of recent findings, it is perhaps unsurprising that such inter-muscular differences are present. For example, the triceps surae muscles occupy different proportions of the total physiological cross sectional area of the group (approximately 15% for GL; 25% for MG and 60% for Sol; Morse et al., 2005). Length changes of triceps surae muscles have also been shown to differ during isometric contractions (e.g. Kawakami et al., 1998; Maganaris et al., 1998, 2006) and drop jumps (Sousa et al., 2007). Furthermore, MG and Sol exhibit shear between their aponeuroses, indicating that they are able to move relatively independently of each other, despite being attached to a common distal tendon. This has been observed in isometric conditions (Bojsen-Moller et al., 2004; Bojsen-Moller et al., 2010 – unpublished observations), as well as during hopping and walking (Cronin et al., 2010 – unpublished observations).

In cats, Maas et al. (2009) suggested that on the basis of fascicle length changes, length feedback from muscle spindles may be greater in Sol than MG. Based on the data reported in humans, the same logic would suggest that length feedback is also more important in Sol than in MG (but may also be substantial in LG). A significant challenge in the future will be to assess the relative importance of specific afferents in different muscles during human gait.

5. Methodological considerations

Despite the relative ease and practicality of US measurements, it is important to acknowledge some of the numerous limitations of this method. Measurements of muscle fascicle architecture during walking have been shown to be quite reproducible (Aggeloussis et al., 2010), but the method is associated with several inherent limitations (e.g. Benard et al., 2009; Klimstra et al., 2007), the most pertinent of which is that the method is two-dimensional, and is used to observe three-dimensional structures. Consequently, the 2-D method may lead to errors in actual fascicle length, and the size of these errors may vary throughout the step cycle. Unfortunately, methods do not currently exist that allow this issue to be investigated systematically during movement.

Thus far, analysis of muscle fascicle length has relied on manual methods, whereby the experimenter is required to digitise several points, and to do so in numerous consecutive images for the duration of the movement in question. Consequently, the method is very time consuming and potentially prone to human error. The advent of automated techniques may help in this regard (e.g. Rana et al., 2009), by minimising the possibility of bias and allowing a larger number of trials to be analysed in a given time. To date, automated techniques have been successfully applied to the study of tendinous tissue length changes (Bojsen-Moller et al., 2004; Magnusson et al., 2003), contractile component length changes (Loram et al., 2005, 2006, 2009a,b) and muscle fascicle orientation (Rana et al., 2009). Adaptation of existing methods may thus allow automatic tracking of fascicle length in the near future.

One issue that has caused some degree of controversy is the sampling frequency of ultrasound scanning. In general, sampling frequencies of 30–50 Hz have been reported. In fast movements such as running and hopping, or when applying rapid perturbations, a high sampling frequency is essential as this provides the greatest possible time resolution, which increases the likelihood of observing small, rapid changes in fascicle length that may otherwise be unobservable (Ishikawa and Komi, 2007). However, during unperturbed walking, qualitatively similar data have been presented by different research groups for the MG muscle, despite the use of sampling frequencies ranging between 30 and 100 Hz. Consequently, the sampling frequency may not be of major importance in slower movements like walking, unless event latencies are of particular importance (af Klint et al., 2010).

Another issue of potential importance is the use of support devices to attach the ultrasound probe to the skin surface when examining muscle–tendon properties during movement. To verify that the probe does not move relative to the structure of interest, some studies have placed a visible marker within the image (Bojsen-Moller et al., 2004; Maganaris and Paul, 1999; Magnusson et al., 2001). Although this appears to be an effective method, an inverse problem is that when attaching the probe firmly to the skin to minimise probe movement, the structures being examined are compressed, so the behaviour observed with US may not be an accurate representation of unconstrained muscle–tendon behaviour. Furthermore, during high force contractions or fast movements, the structure of interest moves proximally and/or distally relative to the skin and probe. As most probes are between 4 and 8 cm long, it may be difficult to reliably observe the same structure (such as an individual fascicle) throughout a given movement or contraction.

Walking studies incorporating US have been conducted on a treadmill with and without shoes, as well as during overground walking. The walking speeds employed have also varied between ∼3 and 5 km/h. In spite of these variations, the findings in a given muscle appear to be quite consistent between different studies performed in different labs. Furthermore, despite its inherent limitations, one of the primary reasons for the popularity of US in this field is that it remains the most versatile and capable tool of studying muscle–tendon function during human locomotion.

6. Combining the study of neural and mechanical parameters during human walking

In the MTUs of the human lower limb, tendon length is often considerably longer than muscle fibre length. Although this arrangement may be beneficial for the storage and return of elastic energy, and economical force production (Biewener and Roberts, 2000; Sawicki et al., 2009), it also complicates the ability of sensory afferents to act as length, velocity and force transducers.

We have recently performed a series of experiments using ultrasound to study muscle fascicle and tendon behaviour during human walking, and to attempt to relate this to the firing behaviour of sensory afferents. These experiments have adopted two different approaches related to the different roles of afferent feedback. The following sections outline these two broad approaches to the study of neuromechanical interaction during walking.

7. Investigating corrective responses to perturbations

A great deal of research involving perturbations during human walking has focussed on the role of sensory afferents (Dietz et al., 1987; Duysens et al., 2008; Grey et al., 2001, 2002, 2004, 2007; Sinkjaer et al., 1996, 2000; Zuur et al., 2009). We have sought to extend this analysis by using a portable stretch device (Andersen and Sinkjaer, 1995) to apply rapid dorsiflexion perturbations at the ankle joint that elicit short latency stretch reflex (SLR) responses during treadmill walking (Cronin et al., 2009b). We have then combined this method with US to examine muscle fascicle behaviour in response to the perturbations (see Fig. 2). This method enables assessment of the excitability of the stretch reflex pathway throughout the step cycle, whilst systematically varying the interaction between muscle and tendinous tissues.

Subjects were required to walk at three different speeds: 3, 4 and 5 km/h. At each speed, we applied the same dorsiflexion perturbations at the ankle joint during the mid-stance phase: 6° perturbations at 170, 230 and 280°/s. As walking speed increased, the amplitude and velocity of stretch to the Sol muscle fascicles decreased. Concurrently, we observed no changes in SLR amplitudes between walking speeds. As the decreased fascicle stretch velocity would be expected to decrease the firing rates of muscle spindle Ia afferents (Rack et al., 1983), and since Ia-mediated SLR amplitudes were unchanged, it is possible that contributions from other mechanisms served to compensate for decreases in spindle Ia afferent feedback, thus preventing a decline in SLR amplitudes in response to perturbations at faster walking speeds. Possible compensatory mechanisms include a decrease in pre-synaptic inhibition with increasing walking speed, an increase in descending drive to the motoneurones leading to an increased excitability of the motoneurone pool, an increased excitatory influence from the Ib afferent pathway and/or elevated muscle spindle sensitivity via changes

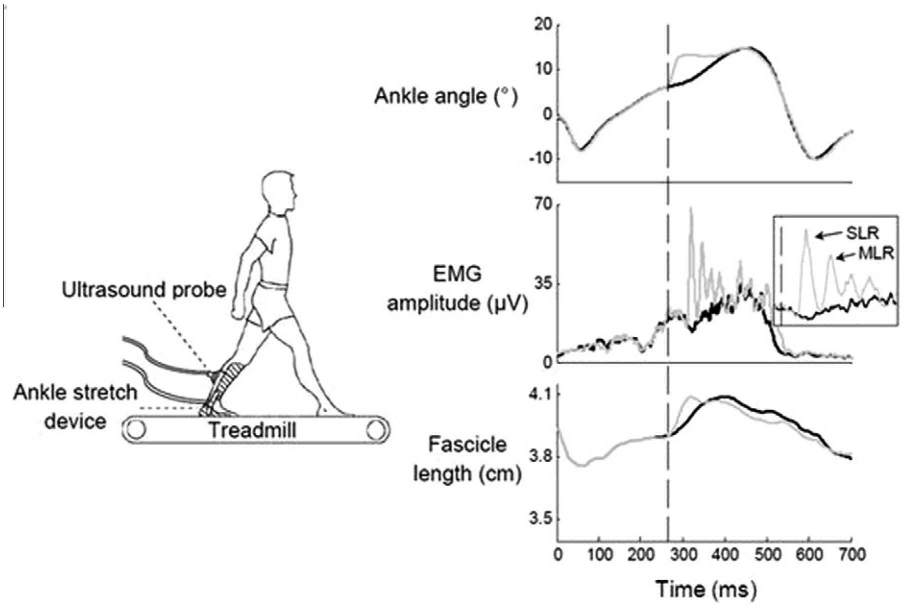

Fig. 2. Schematic of the experimental setup used to elicit fast dorsiflexion perturbations during human walking (left). An example of data obtained from a single subject is shown to the right of the figure. The dashed vertical line denotes the onset of the perturbation. EMG and ankle trajectory data were averaged from 28 trials, and fascicle length was averaged from 3 trials. Black traces represent control trials (i.e. unperturbed walking) and gray traces represent perturbed trials. Figure adapted from Sinkjaer et al. (1996) and Cronin et al. (2009b).

in gamma drive. Regardless of the mechanism(s), these changes highlight the ability of the central nervous system to regulate movement by continually compensating for changes in afferent input.

As well as examining walking speed dependence of neuromechanical responses, we have investigated time dependence using a prolonged walking protocol. After a 1 h period of repeated passive stretching in an ankle dynamometer, Avela et al. (1999) reported that SLR amplitude decreased by approximately 80% in the human Sol muscle. This was attributed to a reduction in Ia afferent activity as a result of an increase in tendinous tissue compliance (see also Avela et al., 2004; Wilson et al., 1997). After walking for approximately 75 min, we observed a similar, short-lasting effect, whereby the amplitude of SLR responses to perturbations decreased by 33% (Cronin et al., 2009a), and the amplitude of the medium latency reflex (MLR), which is thought to be predominantly mediated by length-sensitive type II afferents (e.g. Grey et al., 2001; Schieppati and Nardone, 1997), decreased by 25%. Since these changes correlated with decreases in fascicle stretch velocity and amplitude, respectively, it was concluded that the decreased reflex responses could be explained by an increase in the compliance of tendinous tissues in Sol, which decreases fascicle stretch, and presumably leads to a decrease in muscle spindle afferent output (see also Avela et al., 1999, 2004).

8. Afferent contribution to background locomotor activity in walking

Studying how the sensory afferent information is integrated in the spinal cord and contributes to the locomotor activity during normal "unperturbed" gait poses some intriguing difficulties. For example, even the relatively simple monosynaptic group Ia pathway, giving rise to the SLR response after rapid soleus stretches, is under the influence of descending pre-synaptic inhibition. Morita et al. (1998) showed that the temporal characteristics of the afferent volleys are key factors affecting neural integration. The effect of pre-synaptic inhibition on the monosynaptic group Ia pathway is significantly altered depending on the temporal dispersion of the afferent activity. Therefore, the rapid stretch of a muscle or the electrical analogue, the H-reflex, can only provide evidence for spinal integration of temporally similar responses. Hence, the stretch reflex data are valid for corrective responses to unexpected perturbations but may not give an estimate of the afferent contribution to the ongoing EMG activation in unperturbed steps (Nielsen and Sinkjaer, 2002; Sinkjaer et al., 2000). This limitation can be overcome by at least two experimental paradigms that our group has explored: removing the afferent feedback or investigating the afferent feedback in a natural setting.

8.1. Investigating afferent feedback using natural perturbations

Our group initially attempted to mimic natural variations in the ground surface by imposing small-amplitude, low-velocity perturbations on the ankle joint (Mazzaro et al., 2005). The imposed enhancements and reductions in dorsiflexion velocity produced concomitant changes in soleus locomotor activity that were clearly dependent on muscle spindle afferent input (Mazzaro et al., 2005, 2006). However, as the use of dorsiflexion perturbations would relate to stepping on an unstable moving surface, and since current microneurography techniques do not permit recording of the afferent activity during human walking, we could not ignore the risk that the afferent activity was integrated differently at the spinal level during these perturbations. Hence, a more commonly encountered walking environment was mimicked, i.e. walking over a stable, uneven ground surface. This condition was achieved by asking the subject to walk over small, unpredictable inclinations in the ground surface (af Klint et al., 2008).

A hydraulically actuated platform rotated ⩽± 3° in the parasagittal plane before the subject touched down, creating a small incline or decline in the supporting surface for one step (Fig. 3a and b). The graded contribution to locomotor activity was assessed by monitoring plantar flexor muscle activity. To estimate the effect on the muscle spindle afferents and Golgi tendon organs, US was used to assess muscle fascicle and Achilles tendon lengths as the subject stepped on the inclined surface. The small changes in the supporting surface were enough to elicit a graded change in the

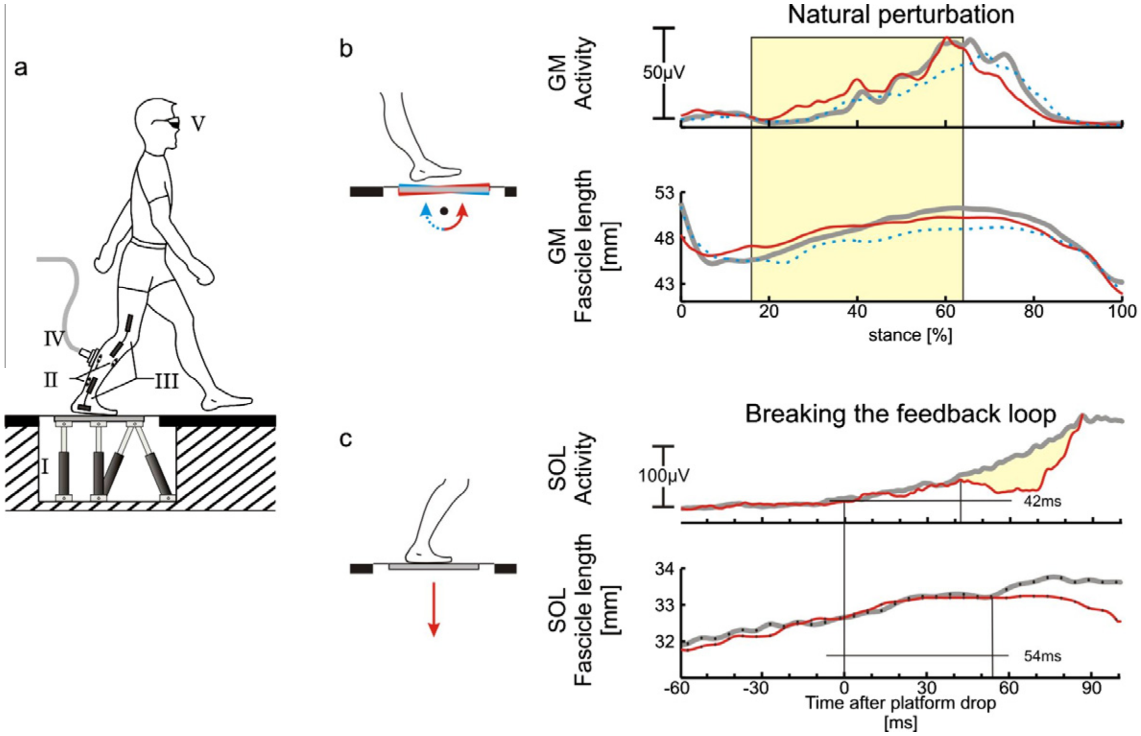

Fig. 3. Experimental setup (a): the subjects walked over a hydraulically actuated platform (I) that could rotate in the parasagittal plane or accelerate downwards. Triceps surae muscle activity (II), knee and ankle goniometry (III) were acquired in synchrony with muscle fascicle behaviour of gastrocnemius medialis (GM) and soleus (Sol) acquired using ultrasonography (IV). Natural perturbation (b): the platform was moved into a rotated configuration prior to the subjects' heel contact. The subjects wore taped glasses preventing any compensatory movement (a, V). The rotations in the parasagittal plane were very small, ⩽±3° (−3°: dotted blue; 0°: thick gray; +3°: thin red). An example of the ensemble averages of three representative trials are shown for each rotation of the platform for a single subject. The changes in muscle activity and muscle fascicle behaviour were analysed in a window from 15% to 60% of the stance phase (shaded area). Across all subjects the muscle activity, estimated Achilles tendon force and muscle fascicle length increased for the uphill condition and decreased for the downhill condition. However, no significant changes were found for muscle fascicle lengthening velocity. Breaking the feedback loop (c): The platform was accelerated downwards at 0.9 g in mid/late stance, drastically decreasing the load on the ankle joint. An example is shown of ensemble averages of soleus muscle activity and fascicle length from a representative subject as the platform was dropped in late stance. At a latency of 42 ms (48 ± 7 ms for all subjects) after the perturbation, i.e. after the ground reaction force decreased (0 ms), the muscle activity of soleus was disfacilitated (shaded area). However, the change at the muscle fascicle level was significantly delayed (54 ms; af Klint et al., 2010). Based on the latency between the changes in muscle fascicle length and the changes in soleus activity, it is concluded that a decrease in length-sensitive group II afferent activity is unlikely to contribute to the disfacilitation of the soleus. Figure adapted from af Klint et al., 2010. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

locomotor activity of the triceps surae muscles. This serves to compensate for the slope of the supporting surface, and is most likely proprioceptive in nature (af Klint et al., 2008). The lengthening velocity of the muscle fascicles did not show a clear relationship to either the inclination of the platform or the changes in locomotor activity, indicating that the modulation of locomotor activity is unlikely to be caused by a contribution from the group Ia afferents. However, a positive correlation was found between the muscle activity changes and the changes in muscle fascicle and Achilles tendon length, indicating that both group II and group Ib afferents could contribute in a graded manner to the locomotor activity during walking (af Klint et al., 2010).

These experiments showed that muscle activity is modulated by proprioceptive afferent input. Using US to monitor muscle fascicle behaviour and Achilles tendon length throughout the stance phase gave a better understanding of the length changes that the spindles undergo, and the changes in force level sensed by the Golgi tendon organs. This was essential to give a better understanding of the influence that these small, natural perturbations have on the muscle structures, as well as the influence on afferent activity.

8.2. Breaking the feedback loop

Transiently removing the proprioceptive afferent feedback, or breaking the feedback loop, has been accomplished by eliciting rapid plantar flexion perturbations in late stance during treadmill walking (Grey et al., 2004, 2007; Sinkjaer et al., 2000). The rapid plantar flexion would drastically decrease the afferent firing rates of muscle spindles and GTOs in the triceps surae muscles as muscle–tendon length is drastically shortened and the load on the Achilles tendon is removed. The afferent contribution to locomotor activity can then be estimated based on the amount of disfacilitation in the muscle activity following the unloading perturbation. This depression in soleus activity was termed the "unload response" by Sinkjaer et al. (2000), and was not influenced by an anaesthetic block of muscle and cutaneous afferents from the foot and ankle (Grey et al., 2004), ischemic depression of the largest group I afferents, or a common peroneal nerve block (Sinkjaer et al., 2000). This implies that autogenic group Ia activity, cutaneous and muscle afferents from the foot and ankle, and reciprocal inhibition from the tibialis anterior (TA) do not contribute to the reduced soleus EMG following the plantar flexion perturbations. Therefore, the unload response may arise from decreasing activity in either the excitatory force-sensitive group Ib afferent pathway, or the excitatory length-sensitive group II afferent pathway.

Though at first the group II afferent pathway was favoured, the later evidence suggests a group Ib afferent contribution to the unload response (Grey, 2004, 2007). However, these studies were based on muscle–tendon length changes that may not be representative of the sensory modalities to which muscle spindles and GTOs are most sensitive (Maas and Lichtwark, 2009). In order to separate the two proprioceptive sensory modalities, the unload experiment evolved from a single joint rotation of the ankle to a drastic decrease in ground reaction force in stance whilst monitoring the muscle fascicle behaviour (see Fig. 3a and c). Subjects stepped on a platform that was rapidly accelerated downwards to decrease the ground reaction force in mid and late stance. At a latency of 48 ± 7 ms after the perturbation, i.e. after ground reaction force decreased, the muscle activity of soleus was disfacilitated (af Klint et al., 2010). The shortening of the MTU and muscle fascicles were significantly delayed with respect to the perturbation. Based on the observed event latencies, it was concluded that the excitatory group Ib pathway was the most likely proprioceptive afferent pathway contributing to the unload response, as the latency between muscle fascicle shortening and the change in muscle activity was too short for group II afferents to contribute (af Klint et al., 2010).

This experiment showed that positive force feedback contributes to soleus muscle activity during walking. In combination with data obtained from investigations using natural perturbations, it seems likely that the regulation of muscle activity during gait is at least partly driven by force feedback. These conclusions are not new, and others have previously postulated the presence of a similar connectivity during walking (Dietz et al., 1992; Faist et al., 2006; Grey et al., 2007). However, when using mechanical perturbations, a better understanding of the influence on the muscle fascicles is essential, even when these drastic perturbations are used. Basing the conclusions solely on muscle–tendon behaviour would have over-estimated the latency of the length changes, and thus the latency of the change in muscle spindle output. Hence, high frame rate ultrasonography was instrumental in determining which afferents contribute to the locomotor activity.

9. Conclusions

In human walking, afferent feedback makes an important contribution to muscle activity, both in response to sudden perturbations (via stretch reflex activation) and during unconstrained walking. Ultrasound data recorded during human walking suggest that fascicle and tendon length changes are altered with changes in experimental parameters (e.g. walking speed). These changes are likely to influence the firing behaviour of sensory afferents in the muscle and tendinous tissues, and may thus affect motor control.

It is important to note that muscle fascicle length changes may not be a direct representation of changes in muscle spindle afferent activity (e.g. Hoffer and Andreassen, 1981). However, in response to rapid perturbations, we have observed a clear relationship between fascicle stretch velocity and velocity-sensitive SLR amplitude, and between fascicle stretch amplitude and predominantly length-sensitive MLR responses (Cronin et al., 2009a). Furthermore, in overground walking, stepping onto a surface that is inclined or declined by 3° reveals discernible lengthening or shortening of the fascicles (af Klint et al., 2010). Consequently, although ultrasound-derived fascicle lengths are unlikely to be a perfect indicator of muscle spindle output, they are likely to be a much better indicator of this parameter than changes in MTU length (see also Maas and Lichtwark, 2009).

In addition to the data already obtained, there are numerous fields of study that may benefit from the use of US to examine neuromechanical responses, one of the most prominent of which is ageing. Since changes in tendon compliance and symptoms of sarcopenia have been reported in old age (Magnusson et al., 2008; Narici and Maganaris, 2006; Reeves et al., 2006), as well as differences in fascicle behaviour between young and old subjects in the lateral gastrocnemius muscle (Mian et al., 2007), it is logical to assume that age-related differences may be present in other muscles. The behaviour of synergistic muscles should also be thoroughly investigated. The majority of studies using US have only reported data from one muscle, and there is a growing body of evidence suggesting that muscles assumed to be synergists exhibit differences in behaviour during walking, and that these differences may be functionally relevant for motor control.

This review has focussed on the effects of muscle–tendon mechanics on sensory afferent feedback, which itself represents just one sub-section of the broader issue of motor control during walking. Clearly, significant gaps are still evident in our knowledge. Until relatively recently, the study of neuromechanics during walking was limited to animal experiments. Although this work has markedly improved our conceptual understanding, the use of US imaging to study muscle–tendon behaviour in humans has helped to further our understanding of the complex control of bipedal walking. Nonetheless, there is clearly still a need for more direct methods to study both neural and mechanical parameters during human movement.

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