CAUSES OF VARIATION IN INTRINSIC ANKLE STIFFNESS AND THE CONSEQUENCES FOR STANDING

by

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Somewhere, something incredible is waiting to be known.

Carl Sagan

Abstract

Previous studies have shown that the passive intrinsic mechanical stiffness of the ankles is less than necessary to fully stabilize the body in the upright position (Loram and Lakie, 2002*a*; Morasso and Schieppati, 1999; Morasso and Sanguineti, 2002; Casadio et al., 2005). Following these studies, research about the controlling mechanisms of standing (the maintenance of an upright posture by a combination of passive and active mechanisms) has developed considerably (Lakie et al., 2003; Loram et al., 2005*a*,*b*, 2011; Masani et al., 2006; Maurer and Peterka, 2005; Peterka, 2002). However, very little attention was given to the passive mechanisms themselves. Here I tackled this issue by manipulating the ankle (and its surrounding tissues) in various ways. The objective was to investigate ankle stiffness dependency on mechanical properties that are particular to muscles and tendons. Within-individual differences were confirmed in various conditions. I have shown that in standing, passive ankle stiffness is affected by movement amplitude and history of movement, as well as active ankle torque and passive tendon stretch. I have found no dependency of ankle stiffness on localized cooling. With regards to the effect that differences in passive ankle stiffness may cause to standing sway, a between-individual analysis showed an inverse correlation between ankle stiffness and sway magnitude.

Dedicated to my parents, Paulo and Muriel Sakanaka. You were the first to show me in vivo *that it is possible to be sublimely generous, intelligent, compassionate, diligent, funny and happy, all at the same time with the same intensity. Simply by choosing to love unconditionally and enjoy life. I.e., you are awesome and I love you millions of times more than the many words of this thesis can express.*

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LIST OF ABBREVIATIONS

B	Viscosity
CNS	Central nervous system
COM	Centre of mass
COP	Centre of pressure
EMG	Electromyogram
FRT	Filamentary resting tension
I	Moment of inertia
intrinsic K	Intrinsic ankle stiffness
K	Stiffness
mgh	Mass above the ankles × gravitational acceleration × height of the COM above the ankles
MTU	Muscle-tendon unit
MVC	Maximal voluntary contraction

RMS	Root mean square
RT	Relaxation time
SD	Standard deviation
SEM	Standard error of the mean
SREC	Short-range elastic component
τ	Torque
TPT	Twitch peak tension

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Human standing

1.1.1 Human body structure: a compromise between balance and locomotion

The most remarkable advantage of vertebrates over invertebrates is the possibility of having larger, malleable structural bodies holding a more complex nervous system. This helps them improve their chances of survival in a larger repertoire of environments.

Some vertebrates migrated to dry terrain, a change of environment that came with an adaptation cost: the problem of moving and supporting their body against gravity. Water has density significantly higher than air, therefore the support given by its buoyancy and drag forces help the locomotion of aquatic animals. Without the support given by it, terrestrial vertebrates had to find methods to sustain their large, malleable bodies as an effective structure that could either stand at ease or move really fast, in air, as the situation required. They have developed limbs to be able to walk on uneven ground. However, this improvement in locomotion compromised balance. The centre of mass (COM) of these bodies had to be stabilized on top of a few flexible, long and slender structures, sparsely protruding from different locations of the body. In other words, the gravitational forces

acting on the COM could now make the animal lose balance, leading to possible fall and injury. He would only be stable as long as the COM was kept within the base of support provided by the disposition of the limbs. This danger would increase with size. In larger animals with longer limbs and even higher COM, the consequence of falling is even more severe (Biewener, 1990; Hooper, 2012). In other words, as soon as vertebrates became terrestrial and developed limbs, apart from the challenges of creating effective means of locomotion to find shelter and food, the very basic task of maintaining balance also became a problem. As a result, the whole body structure of terrestrial vertebrates was built to deal with the everlasting compromise between balance and locomotion.

Like other terrestrial vertebrates, humans also have a large and flexible body structure that can rely on the fast reactions of the nervous system. Contrary to the quadrupeds, though, they have developed a bipedal standing position with a narrow base of support that is even more difficult to stabilize.

1.1.2 Standing is precariously stable

Humans can stand for prolonged periods of time without becoming fatigued as long as they are allowed to repeatedly change their body position every couple of minutes, shifting their weight between their legs (Bridger, 1991; Duarte and Zatsiorsky, 1999; Duarte et al., 2000; Duarte and Zatsiorsky, 2001). If constrained to stay as still as possible, though, they can only stand for a few minutes before feeling discomfort. Although this may be due to multiple causes, it has been suggested that venous pooling or muscle fatigue are primary causes of this constraint (Duarte et al., 2000; Kim et al., 1994; Zhang et al., 1991). Either way, both reasons reflect limitations of human standing.

For many years researchers have been intrigued by the challenges of the bipedal human standing position, which is particularly unstable when compared to other vertebrates. This is true even when they are compared to birds, the only truly bipedal vertebrates

apart from humans. Kangaroos and other marsupials use their tail to help balance their bipedal standing posture; apes occasionally stand on two feet, but only for exhibitionism (Alexander, 2004). Birds are very stable when standing on two limbs and can even sleep while in this position. They stand on their toes and bend their knees so that the femur is locked in a nearly horizontal orientation (Herzog, 1968; Hertel and Campbell Jr, 2007) (Figure 1.1, A). This makes the alignment of their biped body disposition zigzag-oriented. Their COM is located close to the knee joint, thus their body is supported at a height which is submitted to less joint fluctuation. For birds, standing may therefore be biomechanically more stable.

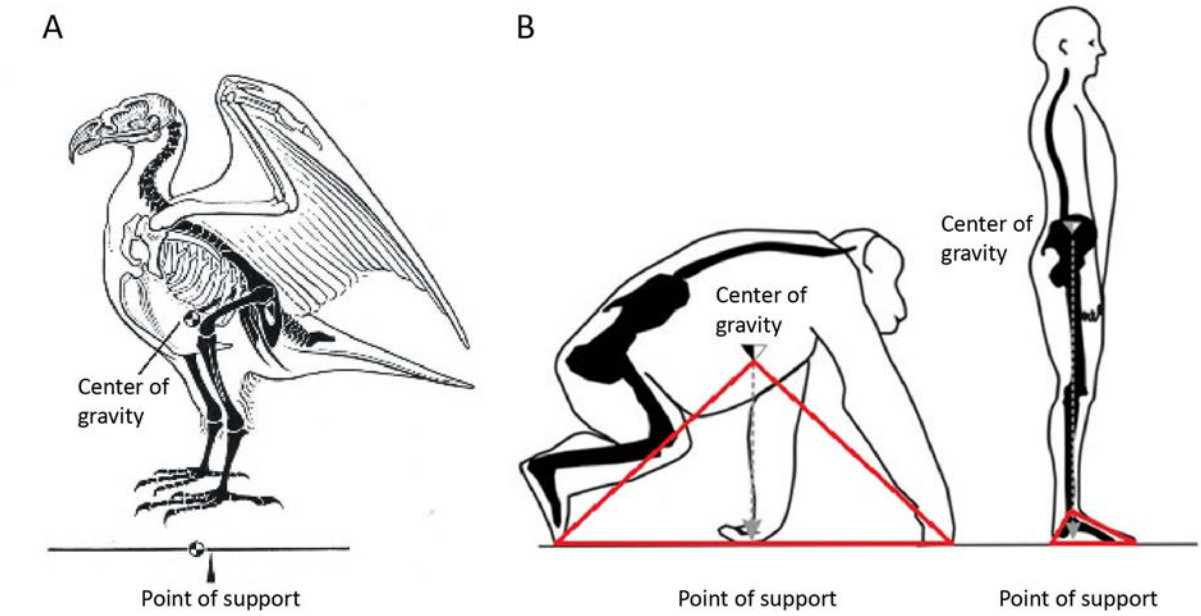


Figure 1.1: (A) Centre of gravity and point of support of a bird, in an outlined lateral view. After Herzog, 1968. (B) Outline of human and gorilla centre of gravity and base of support. After Jean, 2011.

By contrast, human standing position, apart from the two horizontally oriented feet, is basically vertically oriented. As opposed to all other animal species, human knees and hips are highly extended during this posture. All the joints are aligned to keep the whole body above the ankles as one straight long vertical mass. Long structures with high COM are more susceptible to the destabilizing forces of gravity. Added to the fact that the human COM is located above the hip joints, as a result the malleable hips, knees and

ankles are all submitted to the body gravitational forces, thus they all strongly interfere with the control of standing. The stability of the posture is dependent on these numerous joints' position, impedance and the control of the nervous system over them. This leads to an inherently unstable standing posture (Figure 1.1, B).

1.1.3 Stability through joint torque

One of the most common approaches to understand stability of a free standing system is to analyze the destabilizing gravitational forces acting on its COM and to identify the strategies applied by this system to counteract them. Regardless of which method is chosen, a free standing structure can only counteract these destabilizing forces by producing suitable ground reaction forces.

The more flexible a structure is, the more difficult it is to counteract all the divergent forces acting on it. Various force vectors at different locations have to be produced to stabilize the system. On the contrary, a rigid structure can easily be controlled by simply applying the appropriate amount of force through a single point of contact. A force vector applied perpendicularly to a rigid beam fixed at one end by a hinge will produce rotation of the beam about its axis of rotation (hinge), a tendency named torque or moment. Torque is dependent on two factors: the vector force direction and amplitude, and the distance between this vector force and the axis of rotation (moment arm). For a longer moment arm, less force will be needed to produce the same amount of torque. If the beam is not rigid, if for example it is a flexible chain of sections of small beams connected via hinges, then one torque vector applied at one point will affect each section differently. Each will move according to the stiffness level of their connecting hinge. If a hinge is compliant, then with less force the connected section will move more. This will reduce the amount of movement transferred to the subsequent sections. On the contrary, if a hinge is stiff, then with the same amount of force its connected section will move less,

but the subsequent section will move almost the same amount. In other words, the stiffer the hinges are, the easier it is to move the most distant hinge. If a beam is fixed to the ground by a hinge and put at the vertical position, it will stand upright as long as the hinge is stiff enough to counteract the gravitational forces acting on it at the COM. If this beam is composed of multiple sections of beams connected through hinges, then keeping it upright will only be possible if all the connecting hinges have the appropriate amount of stiffness. The same principles can be seen in the particular case of walking locomotion in vertebrates with long flexible limbs. When standing upright, their COM is located at a certain height from the ground. As they start to move, the gravitational forces pull the COM out of its equilibrium zone, sending the body forward to fall. Instead of counteracting it, the animals benefit from this pull by stepping forward and generating a ground reaction force to propel the body even further. This mechanism of transferring forces is only possible because the limbs act as beams mediating the gravitational and the ground reaction forces, transforming them into torques (Cavagna and Margaria, 1966; Cavagna et al., 1976). While stepping forward, once the limbs touch the ground, they are straightened and act like rigid beams that are rotated around the axis of whichever part of the body (foot and ankle, hoof, paw, toes) that is touching the ground (Lyon and Day, 1997; Mochon and McMahon, 1980). The more aligned the limbs are, the more efficient is the transmission of the COM gravitational torque applied through the beam to the ground. As it moves the body mostly in the antero-posterior direction, it is easier for the aligned body to produce an effective counteractive reaction torque to propel the body forward. If executed with relatively aligned limbs, and added to the elasticity of the Achilles tendon, this mechanism can significantly reduce the amount of energy consumption used for locomotion (Anderson and Pandy, 2001; Todorov, 2004). By using the gravity generated forces acting on the body, the most energy consuming activities that the nervous system has to be engaged with are aligning the limbs and swinging them forward while they are not touching the ground. This mechanism is identifiable with increasing animal size. The longer the limbs, the straighter they are kept. With a

longer moment arm, the gravitational torque about the COM becomes stronger as well as the generated ground reaction torque to propel the movement forward (Biewener, 1989; Hooper, 2012).

This strategy is beneficial for walking and is seen in most large vertebrates, regardless of their body structure being quadruped or biped. Likewise, in humans the most cost-effective strategy is to straighten hips and knees during single stance, and rely on the ankles and feet to generate the ground reaction torque to walk (Mochon and McMahon, 1980; Fukunaga et al., 2001; Geyer et al., 2006).

The same strategy is used for control of balance. Horses are known for locking their knee joints to be able to sleep while standing (Waring, 1983). Birds lock their hip joints (Herzog, 1968; Hertel and Campbell Jr, 2007). Contrary to other animals, though, humans present a more complex pattern of standing position. They also use moment to control their unusually unstable upright stance, but only at a certain range of movement.

1.1.4 Standing as stabilization of an inverted pendulum

For ease of standardization and understanding of basic mechanisms, although humans regularly shift their weight while standing for prolonged periods of time, research on the topic has been focused on the quiet standing position. For this task, participants are normally asked to stand still for a couple of minutes with feet either together or apart and with the body weight equally distributed between both legs. Data are recorded during these short periods of time.

There is consensus amongst different studies that, even during quiet standing, all the joints of the body are constantly moving, thus the body is not completely static (Day et al., 1993; Hsu et al., 2007). Although the system is flexible and dependent on the coordination of joints and muscles of the body, often the literature has emphasized the study of how

the body controls the single point of the estimated whole body COM as means to achieve balance. As long as the COM is stable, the body will not fall. Previous studies have shown that during quiet stance the COM is constantly moving in a pseudo-random manner, swaying in both antero-posterior and medio-lateral axes. When standing in normal stance (parallel feet at approximately shoulder-width apart), humans are comparatively more stable, especially in the medio-lateral direction. This stable position is not completely static; it presents an unpredictable and irregular sway mostly at the antero-posterior direction (Day et al., 1993; Gatev et al., 1999; McCollum and Leen, 1989; Nashner and McCollum, 1985; Smith, 1957; Winter, 1995).

Various models were proposed to explain these particularities of human balance. Widely accepted is to interpret it as a simplified task of balancing an inverted pendulum (first proposed by Smith 1957, followed by Fitzpatrick, Taylor and Mccloskey 1992; Gatev et al. 1999; Gurfinkel and Osevets 1972; Nashner 1976; Winter et al. 1998). This model proposes that, through a suitable combination of compliance and stiffness of joints, the whole body works mainly as two major blocks, the long vertical block composed of everything above the ankles and the small horizontal block composed of two feet. These blocks are rotated around a common axis of rotation, the ankles. This type of control has been named the 'ankle strategy' and is the basis of various postural control models (Jeka et al., 1998; Loram and Lakie, 2002*a*; Masani et al., 2006; McCollum and Leen, 1989; Nashner and McCollum, 1985). The body inverted pendulum is dependent on three variables: the mass above the ankles (m), the height of the COM above the ankles (h) and the gravitational forces acting on it (g). As the vertical block is supported and rotated around the ankles, it exerts a gravitational torque that is dependent on both mass and height of the COM. In terms of potential and kinetic energy dynamics, it can be said that the long vertical body has a positive potential to topple forward rotating around the ankles. This potential energy is at its maximum at the vertical equilibrium point, when the COM is located directly above the ankles. Any slight deviation from this vertically aligned position will lead to the COM generating a gravitational torque pulling the body away from the equilibrium

point, thus transforming the potential energy into kinetic energy to fall over (Figure 1.2)

During normal standing, this system is in equilibrium, which means that the destabilizing torque is counteracted by a reaction torque applied by the horizontal block of the feet against the ground (the sum of these opposing force vectors equals zero). In other words, the malleable feet, flat on the ground, act as a single base of support for the long vertical body. They constantly alter the position of the body COM through the action of the ankles. The longer are the feet, the wider the base of support, meaning that greater excursion of the COM is allowed.

As the feet protrude forward of the ankle joint, though, the most stable standing position is not when the COM is located directly above the ankles. The chances of falling backwards are high in this position. Therefore, while standing, the COM is located slightly forward of the ankle joint (Basmajian and De Luca, 1985; Gatev et al., 1999; Loram and Lakie, 2002*a*). This position is bearable as long as the ankle joint torque is enough to hold the body against the low, continuous COM gravitational torque acting on it. This action is performed by the calf muscles in combination with the viscoelastic structures of the joints. Hence, both active and passive mechanisms contribute to the ankle torque response. Due to the large moment of inertia of the vertical body, for a small level of torque imbalance the body inverted pendulum moves only a little. It is still possible to control this position mainly through the ankle strategy. Within this range of less than 6 deg deviation (Hellebrandt and Braun, 1939), the gravitational torque exerted by the body COM to topple forward is considered to have an approximately linear relationship with the COM rotation around the ankle joint (Smith, 1957; Gurfinkel and Osevets, 1972; Fitzpatrick, Taylor and Mccloskey, 1992). It is suggested that it represents the minimal ankle stiffness required to stabilize the body at the vertical equilibrium point (Gurfinkel and Osevets, 1972), and is defined as mass $\times$ gravitational acceleration $\times$ COM height ('mgh'). This is the toppling torque per unit angle. It is sometimes known as load stiffness, despite not strictly being actual stiffness.

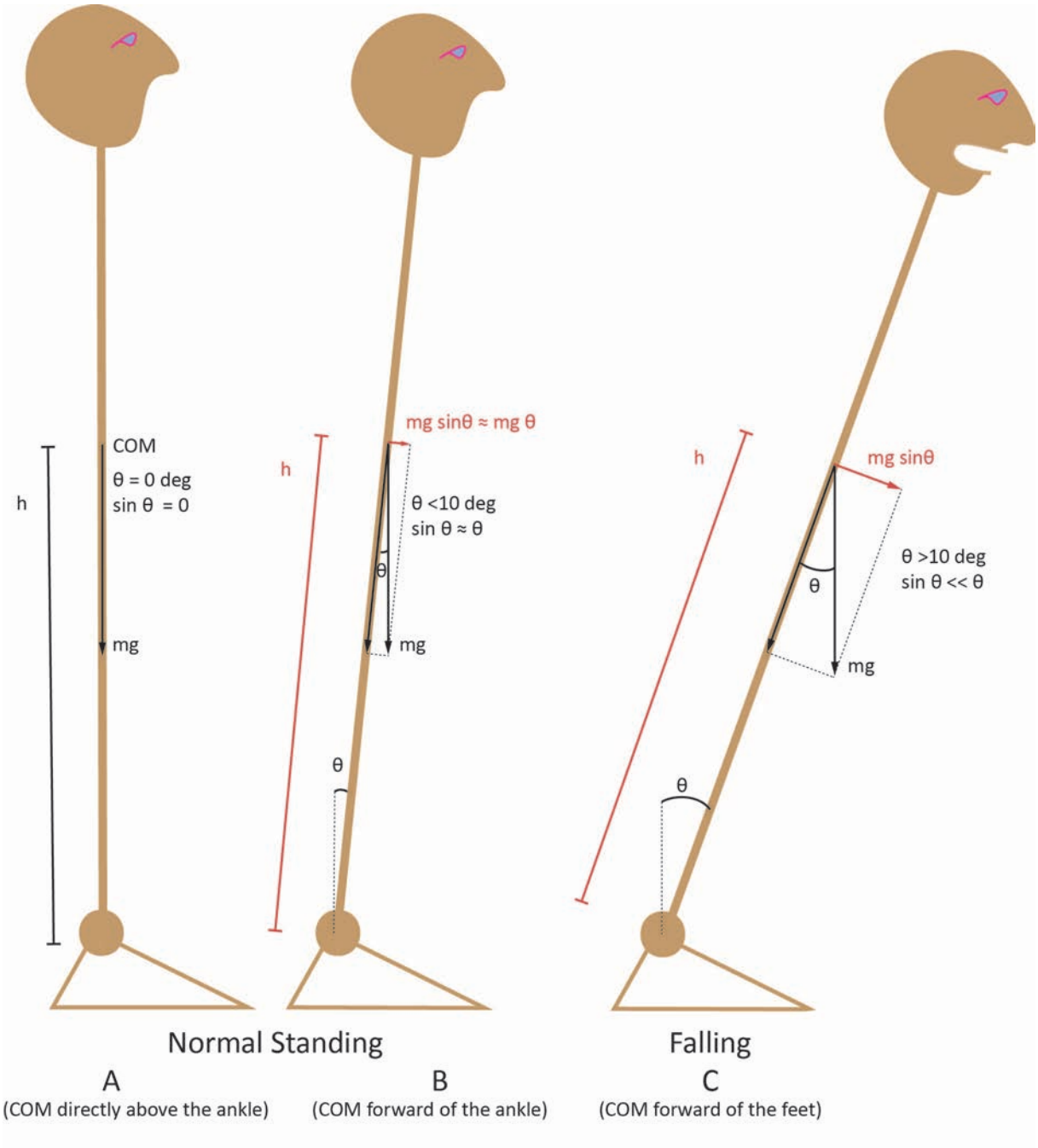


Figure 1.2: Forces acting on the standing body. Three different standing postures are presented, standing at the vertical equilibrium point (A), standing at a spontaneously chosen position (B) and falling (C). In the first position, the body COM does not apply any deviating torque and the potential energy is at its maximum. In the second position, the body COM is located slightly forward of the ankle joint, therefore it exerts a gravitational torque that has to be counteracted by a reaction torque applied by the feet against the ground. In the third position, the COM is located forward of the feet. As in this case the feet cannot exert enough amount of reaction torque, the body will fall.

The body inverted pendulum model implies that the stiffness of the ankle joint is the main source of ground reaction torque modulation in standing humans (Fitzpatrick, Taylor and Mccloskey, 1992; Winter et al., 1998). It also assumes that the two feet are rigid and fixed in one position and act as one unmovable single axis of rotation. Additionally, it considers the movement of knees, hips and all vertebral joints as well as the resultant change in COM height to be negligible. Although it is often considered as an oversimplification of the various complex mechanisms involved in standing and does not take into consideration the relationship between changes in body COM and other joints (Aramaki et al., 2001; Day et al., 1993; Hsu et al., 2007; Keshner et al., 1988; Pinter et al., 2008; Thomas and Whitney, 1959), the body inverted pendulum model simplification is adequate for the purposes of this thesis. I am mainly concerned about changes in standing ankle stiffness due to passive properties of the joints, and this model is appropriate for facilitating the distinction between different controlling mechanisms. Furthermore, here it is used mainly as a reference to normalize data from all participants, regardless of their body mass and height.

Beyond the level of torque imbalance seen during quiet stance, the exponentially increasing gravitational torque acting on the body requires more robust control of balance (Loram, 2002) (Figure 1.3).

At a certain level of instability, the ankle strategy is not appropriate and other strategies are used for an effective control of COM. For example, when the support surface is short in relation to forward and backward sway perturbations, the foot area contact with the floor is reduced thus limiting the amount of ankle torque that can be produced. As shown by (Horak and Nashner, 1986), in this situation hip strategy seems to be more attractive (Figure 1.4). While exerting hip motions, corrective horizontal shear forces are exerted against the surface and little ankle torque is needed. At this point, the body inverted pendulum model is no longer suitable to describe the event. Furthermore, if the distance and velocity of body movement exceed even the boundaries of an effective hip strategy,

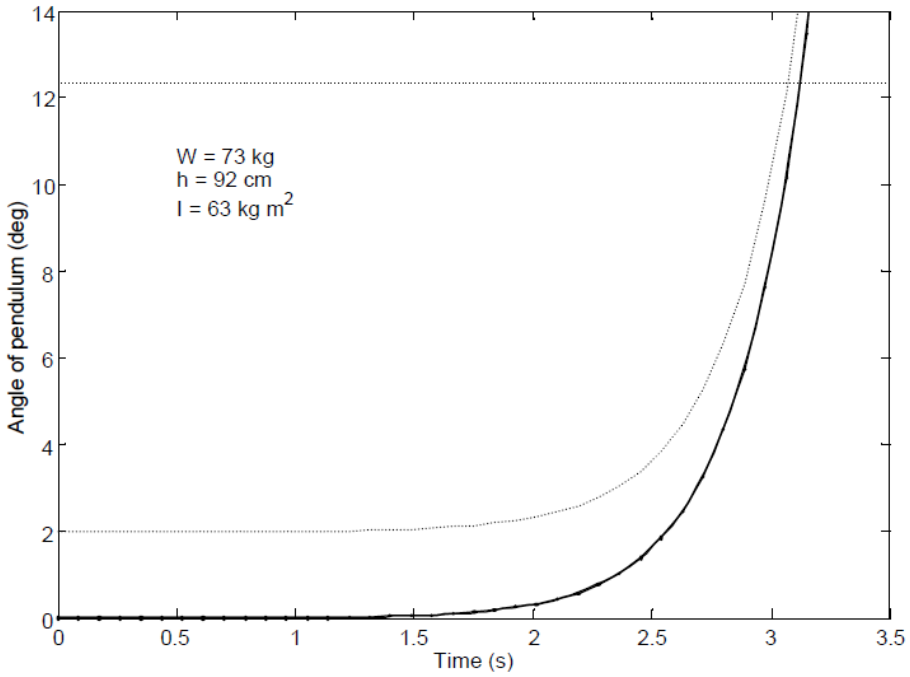


Figure 1.3: Loram (2002), representation of inverted pendulum free fall. The curves show trajectories of a pendulum released from resting position of 0.001 deg (solid line, zero Nm ankle torque) and 2.001 deg (dashed line, constant ankle torque which is appropriate to hold position). The horizontal dashed line represents the angle at which the COM is located forward of the foot (with length 0.2 m, from ankle to toe). Weight (W), COM height (h) and moment of inertia (I) of the pendulum are shown.

stepping or tumbling becomes more appropriate. If its not possible to return the COM to a comfortable position over the base of support, the base of support moves towards the COM to restore balance (Winter, 1995).

1.2 Ankle stiffness in standing humans

1.2.1 Different pathways are used to stabilize upright posture

In all terrestrial animals, the strategies to produce the counteraction forces to control their body structure can be either innate, from the intrinsic properties of their tissue, or neurally modulated. For small animals (body mass less than 0.1 kg), the forces of

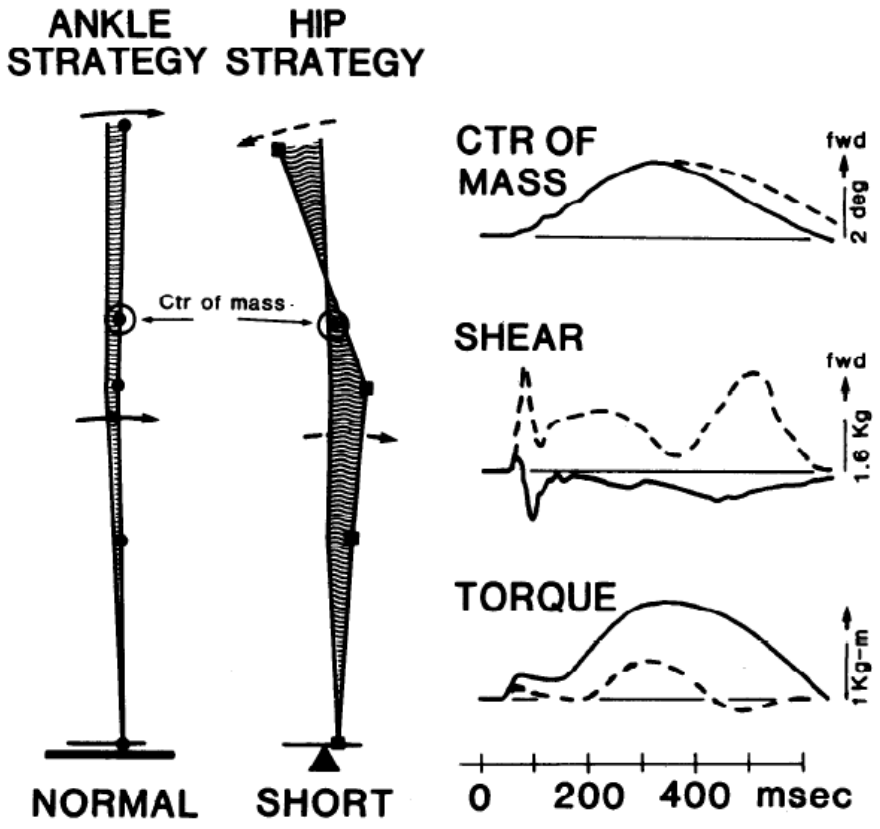


Figure 1.4: Horak and Nashner (1986). Biomechanical compensation for forward sway, change from ankle (solid lines) to hip (dashed lines) strategy when participant is standing either on normal or short surface.

gravity are not strong enough to destabilize their COM. They can rely mostly on intrinsic stiffness of joints to maintain posture. By contrast, larger animals (between 0.1 and 300 kg) cannot afford to rely only on intrinsic stiffness of the joints to support their large structure. The bone, muscle and tendon composition, across a diverse range of species, is very similar. Thus to keep the whole body structure within mechanically safe margins, these large animals would need an increase in bone diameter and stiffness of tissues that would compromise their agility and chances of survival. For them it is rather more advantageous to use neurological mechanisms and benefit from gravity to produce momentum with their limbs acting as pendulums. Consequently their bone structures can be relatively smaller and their joints can be reasonably flexible. If the body mass is even larger than approximately 300 kg, then the only resource to maintain an acceptable tissue

safety margin is to massively increase the bone and tissue shape, heavily compromising speed and mobility (Schmidt-Nielsen, 1984; Biewener, 1990). Hippopotami cannot divert!

How does the central nervous system balance the interaction between passive and active mechanisms for the control of posture and movement? As a simplification of natural phenomena, three parallel pathways ('inner', 'middle' and 'outer') can be identified as responsible for the control of posture and movement that generates force in vertebrates (Loram et al., 2009). The inner loop corresponds to the intrinsic passive properties of tissues, for example the joint stiffness and damping or limb moment of inertia. As it is a simple mechanical factor, its response is immediate (zero delay) and its range of movement is limited to the innate properties of the passive tissues. It cannot be controlled. The middle pathways are the lower-level peripheral feedback loops, represented by muscle stretch or vestibular reflexes etc. They are neurologically controlled with a certain level of precision, and their latency is very low, varying between 40 to 180 ms (Horak et al., 1997). The outer loop concerns the higher level control of movement, in which centrally mediated voluntary actions are performed with high levels of precision and at much longer latency. These three different pathways interact with each other to produce movement and stabilization. As the situation requires, the weighting of each loop differs, and if one or two of them are not necessary for completing a task, they are inhibited by the nervous system (Fitzpatrick et al., 1994; Nashner, 1976).

The interaction between the different loops can be identified when we relate to the mechanisms involved in skeletal muscle activation. The middle and outer loops act through the skeletal muscles by transforming chemical energy triggered by neurological stimuli into mechanical work, via the contraction of muscle fibers. This contraction increases the fiber stiffness and makes it possible for them to move the joints and skeletal structures (bones) underneath, generating forces to produce a coordinated motor task. This system is complex due in part to the active and passive muscle properties, and also the fact that muscles connect with the skeleton through tendons and aponeuroses, relying

on their passive properties as well. Thus for the ankle joint during a standing task, the passive inner loop is determined by the intrinsic mechanical properties of the calf muscles, Achilles tendon, foot and aponeurosis which produce an instantaneous resistance to movement. The middle and outer loops are determined by the reflexive or higher level controlled activity of the muscles around the ankles, mainly the calf muscles (the upright body stands a little forward of the ankle joint, hence the tibialis anterior muscle activity is mostly quiet). The muscle tissue can only be controlled by contraction of its fibers. This contraction is effective as long as it takes into consideration the viscous, elastic and inertial reaction forces of the load that it is targeting to move. If, while standing upright, the body COM velocity and acceleration are low, then the elastic properties of the load are predominant (Fitzpatrick, Taylor and Mccloskey, 1992). Thus the stiffness component of the ankles is more relevant than viscosity or inertia for the control of posture (walking individuals, on the other hand, predominantly rely on inertial properties of the load). The muscles surrounding the ankles can directly modulate the position of the aligned vertical body by levering it against the foot, and all three loops are responsible for producing the combined stiffness that is required to generate the counteractive torque response used to stabilize position. To achieve stable standing, this combined stiffness of the ankle joint must theoretically be at least 100% of the body gravitational toppling torque.

However, at any instant when the COM projection on the ground is not immediately above the ankle joints, the body position is diverted from the vertical equilibrium point and this value will need to be much greater. The standing body presents a characteristic forward lean of some degrees (Hellebrandt and Braun, 1939). Yet even more destabilizing is the fact that the standing body presents a characteristic standing sway of 0.5 Hz in the antero-posterior direction (Collins and De Luca, 1993). For the body to be able to enduringly stand forward of the ankle joint by <6 deg *while* swaying, the required ankle stiffness has been estimated to be ∼200% of mgh (first proposed by Winter et al. 1998, then followed by Morasso et al. 1999; Morasso and Schieppati 1999; Lakie et al. 2003; Loram and Lakie 2002*a*). However, the proportion of neural and non-neural mechanisms involved in the

production of this stiffness (or the means of production of the ground reaction torque in standing humans) has been a matter of controversy amongst researchers for the last five decades. This is highly relevant because it determines the automaticity of the task. Ultimately whenever there is reliance on the nervous system, more challenging is the task. The information has to travel through long loops of chemical signals transformed into action and there is always a chance for complications due to age-related or pathological degenerative conditions.

1.2.2 Ankle stiffness as basis for human postural control

Studies that experimentally investigate human standing analyze either unperturbed or perturbed quiet stance. The advantage of analyzing unperturbed stance is the possibility of studying the actual event without any artificial interference. From the analysis of unperturbed stances, the main outcomes are centre of mass (COM), centre of pressure (COP), joint angles and EMG activity from the muscles. From these studies, researchers have shown how quiet stance is actually a dynamic process, with the ankle joint constantly modulating torque against the ground (detected as changes in COP, or the point location of the ground reaction force vector exerted by the feet against the floor) to counteract the COM displacement. With these data, though, it is not possible to distinguish between the different controlling mechanisms involved in generating ankle torque. The advantage of applying perturbations to the standing body is the possibility of finding, through the analysis of the torque response exerted by the ankles to counteract the disturbance, answers to how do the 3 different loops interchange for the control of balance (van der Kooij et al., 2005). This responsive torque arises from two sources: active and passive. Throughout this whole thesis, I am solely studying human standing posture. As opposed to a seated position, during this task the activity of the calf muscles is not completely absent. Therefore, my definition of passive and active mechanisms implies that the passive mechanism refers to the natural visco-elastic resistance of the ankle joint to forward

body motion, assuming a fixed level of muscle activity. It does not imply that the musculature is relaxed, but that the level of activity is not altered by the nervous system. The passive mechanism has zero delay. It is predetermined either by the intrinsic mechanical properties of the tissues or by the level of pre-set 'muscle tone'. Conversely, the active mechanism is the modulation of the calf muscle activity by the nervous system. It has a certain time delay to be effected due to the time required for the neural transmission to act on the muscle (Mirbagheri et al., 2001; Peterka, 2002; van der Helm et al., 2002; van der Kooij et al., 2005).

Typical data obtained from stance perturbation studies include body motion, ground reaction force, joint torques, and muscle activity detected by EMG electrodes. In this case, most of the studies use linear regression of the ankle torque response in relation to ankle angular position, velocity and acceleration to obtain stiffness, viscosity and moment of inertia (covered here in detail in Chapter 2). The distinction between active and passive mechanisms is related to time delay response. Passive mechanisms can be estimated by restricting the analysis of torque to a time window prior to muscle stretch initiation effect on the torque response (<70 ms for the reflex and control loops to reach the ankle joint musculature) (Horak and Nashner, 1986; Nashner, 1976, 1977; Nashner et al., 1979; Stein and Kearney, 1995). Active mechanisms can be indirectly estimated with the analysis of the EMG recordings (Lloyd and Besier, 2003). In this case, distinction between middle and outer loops can only be approximately evaluated by activity modulation differences in time delays.

For many years, the majority of studies have been using abrupt and large disturbances (0.5 to 8 deg) to perturb individuals in upright stance, with the purpose of investigating latencies of visual, vestibular and proprioceptive reflex responses (Diener et al., 1984; Gollhofer et al., 1989; Nardone, Giordano, Corra and Schieppati, 1990; Nashner, 1976, 1977; van der Kooij and de Vlugt, 2007). Criticism of these methodologies, though, rely on the argument that the large neural discharge provoked by the large perturbations are

mechanisms used to prevent a fall, and are not necessarily applicable for the control of quiet stance. They largely exceed perceptible thresholds of visual and proprioceptive sensory control (Fitzpatrick et al., 1994). Thus to investigate quiet stance, Fitzpatrick and colleagues (Fitzpatrick, Taylor and Mccloskey, 1992; Fitzpatrick, Gorman, Burke and Gandevia, 1992) built an innovative apparatus of a human proportioned inverted pendulum which could be rotated around the ankles. They proposed that, by mimicking normal standing conditions, they could measure the actual ankle stiffness during this task and assess the nervous system response to it. The participants were either disturbed by slow, imperceptible perturbations of 1.5 s duration at waist level with eyes open or shut (to exclude changes in visual input as a contributor to responses seen at the ankles) (Figure 1.5, left figure), or strapped to a vertical support connected to a pendulum of similar COM weight and height of their own and instructed to rotate the ankles to control its position (to exclude changes in visual and vestibular inputs as contributors to responses seen at the ankles) (Figure 1.5, right figure). The authors claimed that because these perturbations were imperceptible, it would necessarily mean that the response was reflexive. They have found increase in torque with angle at reduced visual and vestibular sensory conditions and have concluded that the reflexes alone are sufficient to stand. They have not considered, though, that the obtained change in load could have been due to unconscious but anticipatory and predictive processes (Loram, 2002). Also, their measurement of stiffness was not only not distinctive of passive and active mechanisms but also dependent on the modulated increase in stiffness necessary to counteract perturbations applied to the COM. Lakie et al. (2003) designated it the 'effective stiffness'. The novelty of Fitzpatrick et al.'s work was the nature of the perturbations, which mimicked the slow natural sway of standing individuals.

Following it, in a highly relevant study of unperturbed stance, Winter et al. (1998) proposed that they could build a simple model of stiffness that would explain the CNS control of quiet standing simply by correlating the difference between the horizontal projections of COP and COM against the horizontal COM acceleration. This work was really im-

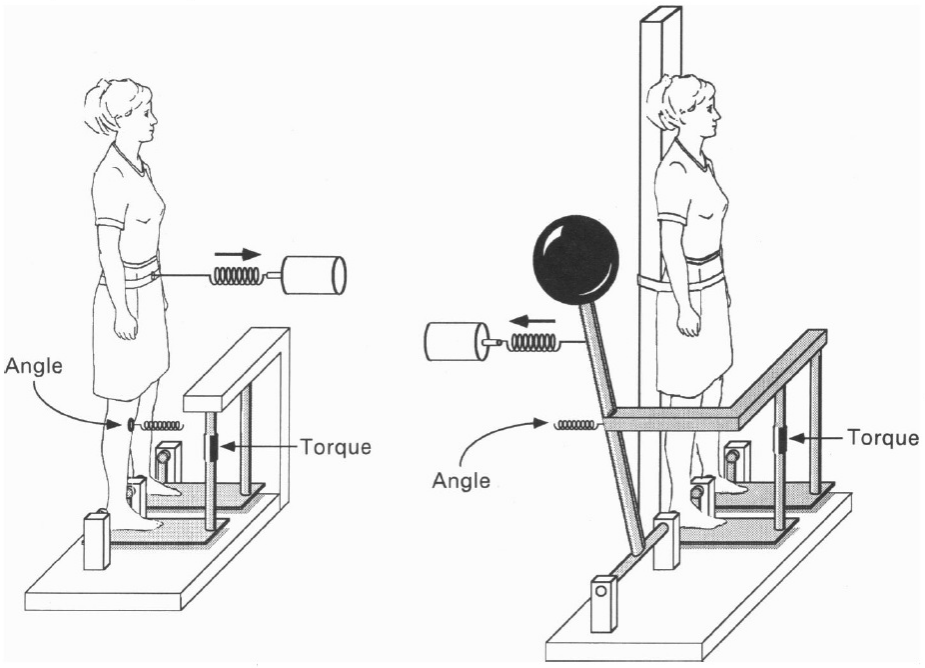


Figure 1.5: Fitzpatrick et al. (1992a). Representation of the experimental setup, applied perturbations at COM height (left figure) and stabilization of an inverted pendulum of similar COM height and weight (right figure).

portant to corroborate the use of the body inverted pendulum model to study human standing. But they went further with their speculations. They have argued that during this task the joint oscillations are lower than perceptible sensory thresholds (Fitzpatrick et al., 1994) and that a neural reaction or reflexive activity would only be justified if there was a delay between COM-COP of around 150-250 ms. As they have found a small delay of only 4 ms, they concluded that no reflex activity was involved during this task, and that passive mechanisms alone controlled position.

This argument was promptly refuted by Morasso and Schieppati (1999). In their view, the central nervous system (CNS) is capable of compensating the sensory time delays through prediction, justifying the 4 ms delay between COM and COP found by Winter et al. (1998). This line of research has developed the belief that the standing system could be described as a closed loop system. The passive mechanism is insufficient and cannot be controlled. Thus a continuous active mechanism controlled by the higher levels of the CNS is needed for the control of balance (Morasso and Sanguineti, 2002; Peterka, 2002;

van der Kooij et al., 2005).

If it was clear, theoretically, that the passive mechanism was not enough to control balance, then the next step was to experimentally determine its relevance during this task. The first group of researchers to confirm this theory was Loram and Lakie (2002*a*)'s. They were the first to directly measure the intrinsic mechanical component of standing ankle stiffness. To mimic standing conditions, tiny and brief perturbations (0.05 deg, 140 ms squared-sine type) were applied to the ankle joint, and stiffness was estimated from the torque response recorded before any intervention from active mechanisms could occur. They found that the intrinsic stiffness was ∼91% mgh, much lower than the theoretical ∼200% necessary to control balance. Three years later, Casadio et al. (2005) also performed a direct measurement of ankle stiffness with a similar approach, this time with larger perturbations (1 deg, 150 ms ramps). They have found even lower stiffness estimates, and have therefore also experimentally confirmed that the intrinsic stiffness of the ankle joint alone is insufficient to stabilize the body and must be supplemented by the active mechanism (Morasso and Schieppati, 1999; Loram and Lakie, 2002*a*; Morasso and Sanguineti, 2002).

1.3 Intrinsic mechanisms of standing ankle stiffness

In the following sections I will discourse more specifically about the themes related to the research questions proposed by this thesis. First I will comment about the factors that previous literature has found to alter standing intrinsic ankle stiffness. Then I will discuss about how the intrinsic properties of muscles and tendons might have affected these results, as well as examine other possible effects caused by these properties. The previous studies mentioned above have demonstrated that the relative importance of active and passive stiffness of standing individuals differs considerably, both between and within individuals. *Between*-subject differences are evidenced by the previously identified

considerable variation in intrinsic ankle stiffness measured using rotary perturbations (Loram and Lakie, 2002*a*; Casadio et al., 2005). This has important implications for the neural control of balance, because people who have inherently stiffer ankle joints (e.g. due to a stiffer Achilles tendon) may be able to rely more upon the passive mechanism and less upon active modulation. *Within*-subject differences were identified in studies in which human joints were perturbed at different amplitudes (Halaki et al., 2006; Loram et al., 2007*a*). Two of the main contributory structures to intrinsic ankle stiffness during upright stance, the Achilles tendon and the triceps surae muscles, have different properties and functions and are composed of different materials. Thus they react differently to contraction forces applied on them. In standing tasks, it was proposed that the low intrinsic stiffness found by the above studies is largely dependent on the high compliance of the long Achilles tendon exposed to the relatively low ankle torque involved in quiet stance (Loram and Lakie, 2002*a*). But how relevant is the tendon stiffness as opposed to the muscle stiffness in determining the overall intrinsic intrinsic ankle stiffness?

To further investigate these passive mechanisms, throughout this thesis I present various experiments conducted to apply a series of interventions aimed to alter specific intrinsic properties of the ankle joint. The estimation of ankle stiffness is dependent on two variables: ankle torque and ankle movement (position, velocity and acceleration). I therefore induce changes in these variables to verify how intrinsic stiffness would be dependent on them and if the underlying mechanisms are related to either muscle or tendon properties, or both. For example, I estimate intrinsic stiffness while altering the COM position and its projection on the ground relative to the ankle joint, thus assessing the effect of altering the level of ankle torque on intrinsic stiffness. Then I focus my analysis on emphasizing the differences found either between or within the different participants as a response to the different interventions.

1.3.1 Is intrinsic standing ankle stiffness dependent on ankle torque?

It is assumed that overall ankle stiffness increases with increased ankle torque generated by increased muscle activity. Fundamentally it must - we would not be able to jump otherwise. As muscle generates progressively more torque, more cross-bridges are formed, increasing muscle stiffness and the resistance to an imposed perturbation. Hence, estimates of stiffness will depend upon the contractile state of the muscle ('muscle tone').

Contradictory results were found in standing tasks. Loram and Lakie (2002*a*) have applied brief and small (0.05 deg amplitude, 140 ms duration, squared-sine shaped) perturbations to individuals strapped to a vertical support while standing on footplates. At this fixed ankle position, the participants were asked to maintain a constant mean level of bias ankle torque for 40 s. The researchers have found little variation of ankle stiffness (5–6 Nm deg^{-1}) with large increase in ankle torque (5–25 Nm). Their conclusion was that this reflected the predominance of the aponeurosis, tendon and foot stiffness in determining the standing ankle stiffness, resulting in the minimal effect of muscle activation.

Three years later, Casadio et al. (2005), while applying larger perturbations (1 deg, 150 ms, ramps) to freely standing individuals, have found large variation of stiffness with increased ankle torque. Their sample size (n=2), though, was very small. For a male participant, they have estimated change in ankle stiffness of 7–10 Nm deg^{-1} due to change in ankle torque of 35–74 Nm. For a female participant, a change of 4–8 Nm deg^{-1} in ankle stiffness was correlated to 27–53 Nm change in ankle torque. As the participants were standing freely on top of the footplates, increase in bias ankle torque was achieved by asking them to voluntarily lean forward by some degrees and keep this position stable until the completion of the trial. As a consequence, there was a certain level of dorsiflexion during this task.

Like Loram and Lakie, Casadio et al. also attributed the discrepancies with the first

group's results as a consequence of the use of tiny perturbations, which resulted in stiffness values close to the critical level. Within this range, both research groups believe that maybe stiffness is independent of the bias mean ankle torque because the stretches are not enough to overcome foot compliance. As the size of the perturbation was larger in Casadio et al.'s experiment, the bias torque would affect the resultant ankle stiffness. However, when comparing the two experiments, one can additionally argue that the position of the standing body may also be relevant. The positive results found by Casadio et al. with participants leaning forward could be due to the tendon itself, which gets stiffer with stretch. Stiffness would increase, then, partly due to increase in muscle tone, partly due to tendon lengthening. The results from Loram and Lakie could potentially support this assumption. They show that in humans standing in conditions without forward leaning, the isolated increase in muscle activity does not necessarily lead to increase in intrinsic ankle stiffness. While standing at a fixed normal standing position, the tendon stiffness is the weakest link and determines the overall stiffness (Loram et al., 2007*b*). When leaning forward, the tendon might be stiffer thus increasing the overall ankle stiffness.

The speculations above propose that the highly variable estimates of standing ankle stiffness with increased ankle torque found by the studies of Loram and Lakie and Casadio et al. might reflect different properties of muscles and tendons which are still unclear to us. In this thesis, I attempt to further the understanding of the relative contributions of muscle and tendon to changes in intrinsic ankle stiffness.

1.3.2 Is intrinsic standing ankle stiffness dependent on muscle properties?

Tension can be generated at specific sites within the skeletal muscle fibers. The most accepted theory used to explain this basic mechanism of muscle contraction is the sliding filament theory. It postulates that the functional unit of the muscle fiber, the sarcomere,

is composed of the actin filament sliding past the myosin filament, which results in fiber shortening (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). This action is controlled by neural input, thus it can be rapidly accomplished (<70 ms to reach the calf muscles) but requires a lot of energy. Less commonly discussed, though, are the passive transient characteristics of these filaments.

Short-range elastic stiffness and muscle thixotropy

The stiffness of the resting muscle is changeable. When a relaxed muscle fiber is stretched or shortened, there is an initial period of relatively high resistance, termed the short-range elastic component (SREC) (Hill, 1968). This phenomenon is dependent on two factors: displacement amplitude and history of movement. After a position threshold is reached, resistance to movement drops markedly and the initial high stiffness of the SREC disappears. This effect is greatly reduced when the muscle is stretched immediately after a prior stretch, with the initial SREC becoming much smaller. The stiffness of the SREC gradually recovers, but only if the muscle is left still over a period of seconds. This temporary reduction in muscle stiffness caused by movement, with recovery at rest, is known as muscle thixotropy (Denny-Brown, 1929; Hill, 1968; Lakie and Robson, 1990; Warner and Wiegner, 1990; Whitehead et al., 2001). These two effects are thought to be due to the forced detachment and spontaneous reattachment of some muscle cross-bridges over time in relaxed muscle (Hill, 1968; Campbell and Lakie, 1998; Altman et al., 2015). Various in-vivo experiments also detected these patterns at the initial stages of movement in muscle where at least part of it is tonically active. This is the manifestation of Hill (1968)'s observations from amphibian muscle fibers. Large limb movements encounter less stiffness than small ones over a range of background muscle activations (Rack and Westbury, 1974; Halaki et al., 2006). Moreover, after large joint limb movements this reduction in stiffness persists for a short time, recovering rapidly if the system is left still (Lakie et al., 1984; Proske et al., 1993; Axelson and Hagbarth, 2001; Reynolds and Lakie,

2010).

This raises the likelihood that the intrinsic ankle stiffness in standing individuals, highly dependent on the muscle properties, is also affected by the transient characteristics of its short-range stiffness. By verifying if the intrinsic stiffness of the ankles is simultaneously dependent on these two independent factors (sway size and prior history of movement), I can then confirm that the changes within subjects, in quiet standing, are due to the intrinsic mechanical properties specific to the short-range stiffness of the muscle.

Localized cooling

Most of the terrestrial large animals have found reasonable solutions to the problem of maintaining a functional internal body temperature even during harsh environmental conditions. To keep the chemical body reactions actively working throughout the whole body and also at the hardly reachable far end of their long limbs, they have either developed long body hair (monkeys, lions, horses etc) or thick skin (elephants and rhinoceros). Humans, on the contrary, have developed a thin skin with short body hair that is not enough to keep their body temperature stable in relatively hot or cold conditions for long periods of time.

There are studies looking into the effects of harsh environments upon physiological functions of the human body. Temperature changes inside the body, though, are rather difficult to control experimentally. The easiest way of assessment is to verify skin temperature, but even if the local skin surface temperature has changed, it does not necessarily mean that the temperature at the deep tissues of the body has also changed (Barcroft and Edholm, 1943). In the specific field of control of balance and locomotion, the crucial fact is that the core body temperature is very different from the skeletal muscle temperature. When people stand on a cold environment for a certain amount of time, the core temperature does not change much, but the temperature of the skeletal muscles, especially the ones located

at the extremities of the body, easily decreases. For this reason, there are a lot of studies focused mainly on the effect of localized cooling on the production of movement. Only a few studies have focused on the passive components related to the control of movement that could have been affected by temperature changes (Hunter et al., 1952; Lakie et al., 1986; Lakie and Robson, 1988*b*,*a*). For example, Hunter et al. (1952) suggested that cold could cause the synovial fluids of the joints to become more viscous, reducing the speed of joint movement. In their experimental setup, though, they have only measured the skin temperature over the joints, and definite conclusions cannot be taken from their results.

Particularly interesting to this thesis was the experiment conducted by Lakie et al. (1986) investigating the effects of cooling on the thixotropic property of the relaxed forearm muscles. They applied rhythmic sinusoidal torques of various frequencies and measured the resultant wrist/hand movement amplitude. They have found that localized cooling (in this setup, 40 min at 8 deg C) can increase the range of stiffness of relaxed muscles, increasing for large forces but not for small forces. As it was not accompanied by increase in EMG activity, they concluded that this effect was produced by passive properties of the muscles. To explain the increase in stiffness, Lakie et al. refer to Denny-Brown (1929) and Edwards et al. (1972)'s suggestion that at low temperatures the bonding of actin and myosin becomes greater. The non-linearity found between small and large forces could be explained by the in series connection of the muscles with the tendons. At small amplitude changes, the stiffness that prevails is the tendon stiffness (the weakest link) (Loram et al., 2007*b*). Therefore, even if the muscle stiffness increases, the prevailing tendon stiffness will determine the overall joint stiffness. As the amplitude of movement increases, then the muscle stiffness that in normal temperatures would decrease, will still be relatively stiff for lower temperatures. Thus the weakest link will still be the tendon and the overall stiffness of the joint will not decrease.

Surprisingly, in a series of experiments performed two years later, Lakie and Robson (1988*b*,*a*) have found that cooling of isolated muscle fibers of the frog did not affect the

stiffness of the passive muscle. The stretch size after perturbation was not altered at 3 different bathing temperatures (3 deg C, 10 deg C and 17 deg C). There are many possible reasons for the authors of this study to obtain different results from the previous one: (1) use of amphibian muscle, as opposed to human forearm muscles in the first study; (2) use of in vitro isolated muscle, as opposed to the use of the whole wrist joint (which is controlled by various muscles and is affected by tendon and aponeurosis); (3) imperfection of either study design.

With these questions still unanswered, the effect of localized cooling on the intrinsic mechanisms of the joints is yet to be confirmed. To my knowledge, only the 3 studies mentioned above have investigated the intrinsic mechanisms of localized cooled muscles. As this thesis is focused on standing individuals, the interest here was in assessing the effect of localized cooling on the standing intrinsic ankle stiffness. To my knowledge, this has not been investigated before.

1.3.3 Is intrinsic standing ankle stiffness dependent on tendon properties?

For ease of understanding, often within different areas of research, mathematical representations are used to characterize the mechanical behavior of different materials. The value of each material is measured by their interaction with others, whether they are able to resist external forces (determined by the forces of their internal molecular bonds) with the least deformation (determined by the distribution of the internal bonding forces throughout the cross sectional area of the tissue) (Butler et al., 1978). If the material is not appropriate for its task, or if it is not stiff or compliant enough, it will rupture and become useless. The human physiology discipline has a long history of constructing models to describe the properties of all the different tissues found in the human body. The common characteristic is that tissues are all built with properties that are relevant

to their specific function. This is also true for muscles and tendons.

Tendons are dense bundles of connective tissues mainly composed of collagen fibrils. Their main function is to connect muscles to bones and mediate the transmission of tensile forces between them (Kenedi et al., 1975). To be able to withstand the intermediate role between the rigid bones being moved by the flexible contractile muscles, they are built to resist high loads of tension. Some tendons are highly elastic and function as springs, which allows them to store and release elastic strain energy to produce more economic locomotion (Ker et al., 1988, 2000; Pollock and Shadwick, 1994; Shadwick, 1990). For human locomotion in particular, the Achilles tendon is the most relevant. It is the largest in the human body and can sustain forces of multiple body weights, thus allowing humans to walk, run and jump.

Often in the literature tendon stiffness is measured as a mathematical model of a ratio of stress and strain (namely Young's modulus). Stress is equivalent to the internal force per unit of cross-sectional area. It is defined as the total force supported by the tissue divided by the original cross-sectional area. Strain is the measurement of the change in shape resulted from the action of external forces on the tissue. It is calculated as the elongation of the fiber divided by its length (Butler et al., 1978). One can experimentally measure the stress of an in vivo tendon with load cells. Appropriate use of ultrasound gives the tendon strain (Maganaris and Paul, 1999; Maganaris, 2003; Peixinho et al., 2008). While being a sturdy bunch of collagenous fibers, tendons present a limited range of elasticity which can be clearly divided into 3 different levels (according to the amount of deformation). The most interesting to this thesis are the toe and linear regions because, during normal standing, the Achilles tendon is most likely kept within these more relaxed ranges. At the slack (toe) region, the fibers are not extended and present a wavy shape. Beyond this range, as the tendon is further stretched, the fibers reach a region in which they are relatively aligned and the stress-strain slope increases linearly. This is named the linear region. As the fibers are extended even more, they start to snap, until an unbearable

region is reached and the whole tissue collapses and ruptures (Figure 1.6).

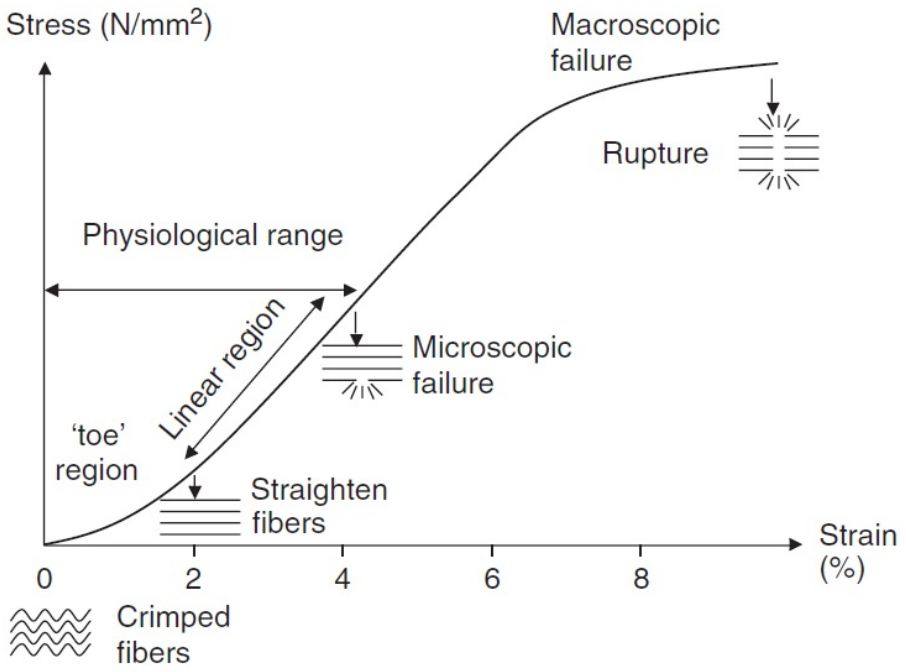


Figure 1.6: Tendon stress-strain curve (Wang, 2006, adapted from Butler et al., 1978).

The tendon properties are relatively different from the muscle properties. Its elasticity is not dependent on the properties of sliding filaments that can be actively contracted, but rather dependent on the linkage between the collagen molecules, cross-linked end-to-end within a fibril (Silver et al., 2003). This 'netted' distribution makes it possible for the tendon to be lengthened and to support high loads of tension without rupturing.

Tendon elasticity, together with muscle elasticity, is also crucial for control of locomotion and balance. Particularly during locomotion, tendon recoil, or the store and release of elastic energy when tensile force is applied against it (Alexander and Bennet-Clark, 1977; Kawakami and Lieber, 2000; Ker, 1981; Morgan et al., 1978), is often referred to as a great energy saving mechanism. Morgan et al. (1978) measured the length and tension changes of the medial gastrocnemius muscles of wallabies and determined the relative change in movement of muscles and tendons. They have found that the tendon length change was up to 8 times longer than the muscle length change when contracting close to its maximum isometric tension. The elastic energy stored in tendons is thus dependent

on whether the muscle connected to it is stiff enough to resist it. Roberts et al. (1997) have shown from their study with running turkeys that the elastic energy recovery from the tendon, after rebounding against the ground, accounts for more than 60% of the work during shortening of the lateral gastrocnemius muscle. Bennet-Clark (1976) estimated the quantity of elastic strain energy that the muscle can store per unit mass as 5 J kg^{-1}, much less than the tendon collagen, insect apodeme and resilin, which can store up to 2,000-9,000 J kg^{-1}. Many of the studies on the topic of tendon elasticity refer to this property and its effect on various tasks like walking, running and jumping. Therefore, in most of the experimental procedures the tendon is tested at the various regions of stress-strain level (Figure 1.6), either lengthened or shortened and with the participants (for in vivo experiments) applying different levels of maximum voluntary contraction against strain gauges (Alexander and Bennet-Clark, 1977; Cavagna et al., 1968).

During standing tasks, though, it is not necessary or even possible to activate the tendon's recoil mechanism. The swaying movement of the body is never abrupt and its amplitude is small. Furthermore, the calf muscles are relatively relaxed. Then how does the tendon elasticity affect the standing human?

There are many unanswered questions regarding the properties of the tendon in quiet standing. In individuals lying prone on a surface with their foot hanging off of its edge, the resting ankle angle is approximately 116 deg (DeWall et al., 2014). When the ankle is relaxed the Achilles tendon is slack (toe region of the stress-strain curve, Figure 1.6). In quiet standing, the leg is approximately perpendicular to the foot and it is assumed that the tendon is still within this more relaxed region. During this task, the stretch sizes are normally very small (as opposed to running or jumping), and the muscle is typically ∼15 times stiffer than the tendon (Loram et al., 2007*b*). As the skeletal muscles are connected to the bones through the tendons, these two structures are then considered to be springs arranged in series (Fitzpatrick, 2003; Loram and Lakie, 2002*a*) (Figure 1.7). The overall ankle stiffness is therefore determined by the weakest spring (the limiting factor), which

in this task is normally the tendon. However, while tendon stiffness changes only through lengthening variations, the muscle changes either passively (SREC, FRT and muscle tone) or actively (neural modulation). As a result, even though it is more easily changeable, the muscle stiffness will only be more relevant in the overall ankle stiffness when it is less than the tendon stiffness or when the tendon gets stiffer (e.g. during dorsiflexion or forward leaning), increasing the relative importance of the muscle.

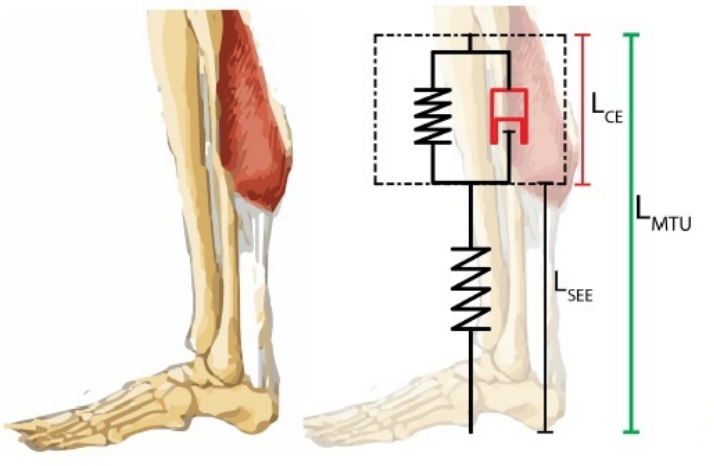


Figure 1.7: Khan (2013). Schematic design of muscle tendon unit (MTU) components. The MTU is comprised of muscle contractile element (CE, triceps surae muscles) and series elastic elements (SEE, tendon, foot and aponeurosis), with springs arranged in series.

Tendon property changes with stretch

As mentioned previously (subsection 1.1.4), the typical standing position is with the COM slightly forward of the ankle joints, thus the tendon fibres should be less crimped than when the angle between leg and foot is 116 deg. Besides that, the muscle activity used to generate muscle tone to control posture shortens the muscle fibres, possibly also straightening the tendon fibres by a certain amount. If this is enough for the fibres to reach the linear region, then the tendon stiffness is constant within the slow sway of standing humans. If it is not enough, then the tendon is still within the toe region, and the change in stiffness due to tendon properties is still non linear. It would be beneficial

for the control of standing if the tendon actually reached the linear region during normal standing. Two objects linked through a very slack elastic band cannot move dependently unless the elastic band is further stretched. Equally, it is easier for the CNS to predict the position of the body if the calf muscles and Achilles tendon offer a minimal amount of resistance.

The results from Loram and Lakie (2002*a*) and Casadio et al. (2005) cannot answer this question. In the former, participants were attached to a board in the vertical position and asked to modulate the ankle torque. Their standing position was fixed at a position slightly forward of the vertical, thus the tendon was kept within a relaxed range. The researchers have found that the increase in torque did not have a significant effect in the overall ankle stiffness. In the latter, the stiffness of participants intentionally leaning forward of their normal standing position was compared with normal stance stiffness. They have found an increase in stiffness, but it is not clear whether this was due to changes in the tendon or muscle, or both.

Change in the Achilles tendon stiffness alone with ankle position was measured previously in numerous studies with the use of ultrasound technique. Of particular interest to us are the studies with relaxed muscles in participants either sitting or lying in prone position (Fukunaga and Roy, 1992; Herbert et al., 2002; Morse et al., 2008). Morse et al. (2008) have shown that with passive ankle dorsiflexion part of the stretch is taken by the Achilles tendon, which presents a gradual non-linear decrease in stiffness until the end of range of motion. The authors suggested that this non-linearity indicated that the tendon was still within the toe region. In other more recent studies with another ultrasound technique called supersonic shear image, increase in tendon stiffness with passive dorsiflexion was also confirmed (Aubry et al., 2013; Chernak et al., 2013; DeWall et al., 2014; Hug et al., 2013). However, Hug et al. (2013) have found that the slack length of the Achilles tendon was limited to a range within the plantarflexed position of the foot. As the foot position changed from plantarflexed to dorsiflexed, the stress-strain curve of the tendon became

linear.

As seen from the above, there are still many uncertainties about the effect of stretch on tendon stiffness and the subsequent change in the overall ankle stiffness. This becomes even more relevant when postural tasks have to be performed, because it is another aspect that the CNS has to take into consideration. To my knowledge, there were no studies investigating the effects of ankle stretch on the intrinsic ankle stiffness of standing individuals. This was then proposed as one of the studies of this thesis.

1.4 Aims

Control of movement through the manipulation of bones that compose the structure of the body is dependent on the CNS modulating joint stiffness. However, joint stiffness is in turn dependent on the tissues enwrapping it, like tendon, muscles and aponeurosis. Interestingly enough, despite the fact that these tissues have really diverse mechanical properties, the CNS somehow knows how to deal with the differences and act accordingly to produce fine movements.

Previous research has shown that the human standing posture is not only maintained by intrinsic mechanisms, but also relies on neural modulation (Casadio et al., 2005; Loram and Lakie, 2002*a*; Morasso et al., 1999; Morasso and Schieppati, 1999; Morasso and Sanguineti, 2002). This dual interdependence implies that the CNS has to understand how the passive system works to be able to accurately control upright standing. Modulation of joint stiffness, in particular the ankle stiffness, is therefore dependent on the behavior of the intrinsic mechanisms of the tissues enwrapping the joint. Even though the idea of an insufficient standing intrinsic stiffness has been generally accepted, little is known about the actual properties of the intrinsic mechanisms particular to the different tissues involved in ankle movement. The objective of this thesis was to add knowledge about

these passive components of the standing ankle stiffness.

To assess intrinsic ankle stiffness, small and brief perturbations were applied to standing individuals and their body sway and torque response was recorded. The same apparatus was used in all experiments. It consisted of motorized footplates to apply the perturbations and measure the torque response, laser-reflex sensors to measure the relative body sway and electromyography to measure the lower limb muscle activity (Chapters 3–6). Throughout the duration of my studies, a series of tests to verify the accuracy of the apparatus and the robustness of the experimental designs were performed (Chapter 2).

I attempted to distinguish between the contractile (muscles) and the non-contractile (tendon and aponeurosis) components of stiffness, and how would each affect the overall intrinsic standing ankle stiffness. As opposed to completely relaxed conditions, the standing posture is only possible because the calf muscles are engaged. The extent of the contribution of the contracting muscle fibres in the standing stiffness is yet to be understood.

I performed different experiments in which distinct properties of muscles and tendons were confronted. Within-subject differences were investigated with a series of 4 experiments, followed by a fifth experiment in which between-subject differences were investigated to verify intrinsic stiffness effect on sway size. The questions addressed were:

1. Is the standing intrinsic ankle stiffness dependent on the history of movement and movement amplitude? (Chapter 3)

2. Is the standing intrinsic ankle stiffness dependent on localized cooling? (Chapter 4)

3. Is the standing intrinsic ankle stiffness dependent on active torque? (Chapter 5)

4. Is the standing intrinsic ankle stiffness dependent on passive stretch? (Chapter 5)

5. Is the sway size dependent on intrinsic ankle stiffness? I.e., would there be increased or decreased standing stability in people with stiffer ankles? (Chapter 6)

CHAPTER 2

METHODS FOR ASSESSING ANKLE STIFFNESS

Abstract The focus of this thesis is to better understand the intrinsic properties of standing ankle stiffness. For this reason, the apparatus and techniques used for the stiffness estimation had to be thoroughly validated. The main equipment consisted of two freely moving footplates coaxially aligned with a motor. The motor would apply small (<1.3 deg) and brief (140 ms) rotational perturbations, and stiffness, viscosity and moment of inertia of the ankles would be estimated as the parameters of a multilinear regression equation in which the ankle torque response was decomposed into position, velocity and acceleration. All the procedures implemented to obtain repeatable values of stiffness are explained in detail in this chapter. First, I finely calibrated the footplate torque, position, acceleration and EMG sensors. Then I defined two different methods to estimate toppling torque per unit angle and compared their results. This is used as a reference to normalize stiffness against the participants height and weight. Finally I have carried out various tests to verify the parameters needed to obtain consistent estimates of intrinsic stiffness, which include: (1) comparing the fit between actual and estimated torques; (2) checking if the stiffness values were positive; (3) calculating the minimum amount of perturbations necessary to obtain a repeatable estimate; (4) comparing the results of the stiffness estimation and the toppling torque models with the known stiffness of an inanimate spring; and (5) comparing results of the same individuals tested in different experiments. The results of this chapter show that I have successfully obtained a reliable and repeatable measurement of stiffness in both absolute and relative terms (as a percentage of toppling torque). Hence I was convinced that the estimates of stiffness performed throughout this thesis were sufficiently accurate and precise.

2.1 Introduction

This thesis is concerned with the measurement of intrinsic ankle stiffness. Stiffness of any system can be measured by applying forces and measuring the resulting motion, or by applying known movements and measuring the resulting force (Hooke's Law). As it is directly related to their ability to move, stiffness in vertebrates is mainly assessed as stiffness of the various cartilaginous and synovial joints linking the bony structure of the body. The bones and tissues directly linked to the targeted articulation are moved, and then the amount and resistance to movement are recorded. Due to the type of movement induced by the main articulations of the body, stiffness is normally assessed in the angular dimension. Here, ankle stiffness was estimated by applying small rotary perturbations at the ankle level to standing individuals. Furthermore, in living organisms stiffness is not only dependent on force and displacement, but also on the active stiffening of muscles surrounding the perturbed joint. As I was interested only in the intrinsic stiffness of the ankle joint, the perturbations were designed to induce the least possible active modulation of the muscles. The motor was then programmed to produce small (less than 1 deg) and brief (approx. 140 ms) perturbations in order not to threaten the participants balance. They were not continuous, but spanned by randomized 4–5 s intervals to allow the joint to restore its initial condition prior to each individual measurement. A squared-sine waveform was selected for a smoother initial acceleration of the perturbation (as opposed to a ramp type perturbation, for example). An illustrative segment of the recorded signals is shown in Figure 2.1.

This chapter presents the apparatus and procedures used to obtain a consistent estimation of stiffness. Firstly, the basic apparatus used for all the experiments described in this thesis is presented. Variations from the basic setup will be explained later in each chapter. Following this, a series of calibration methods is depicted. As the perturbations were rotational, all the recorded data had to be transformed from the linear to the angular dimension. Of particular importance was obtaining a precise measurement of torque

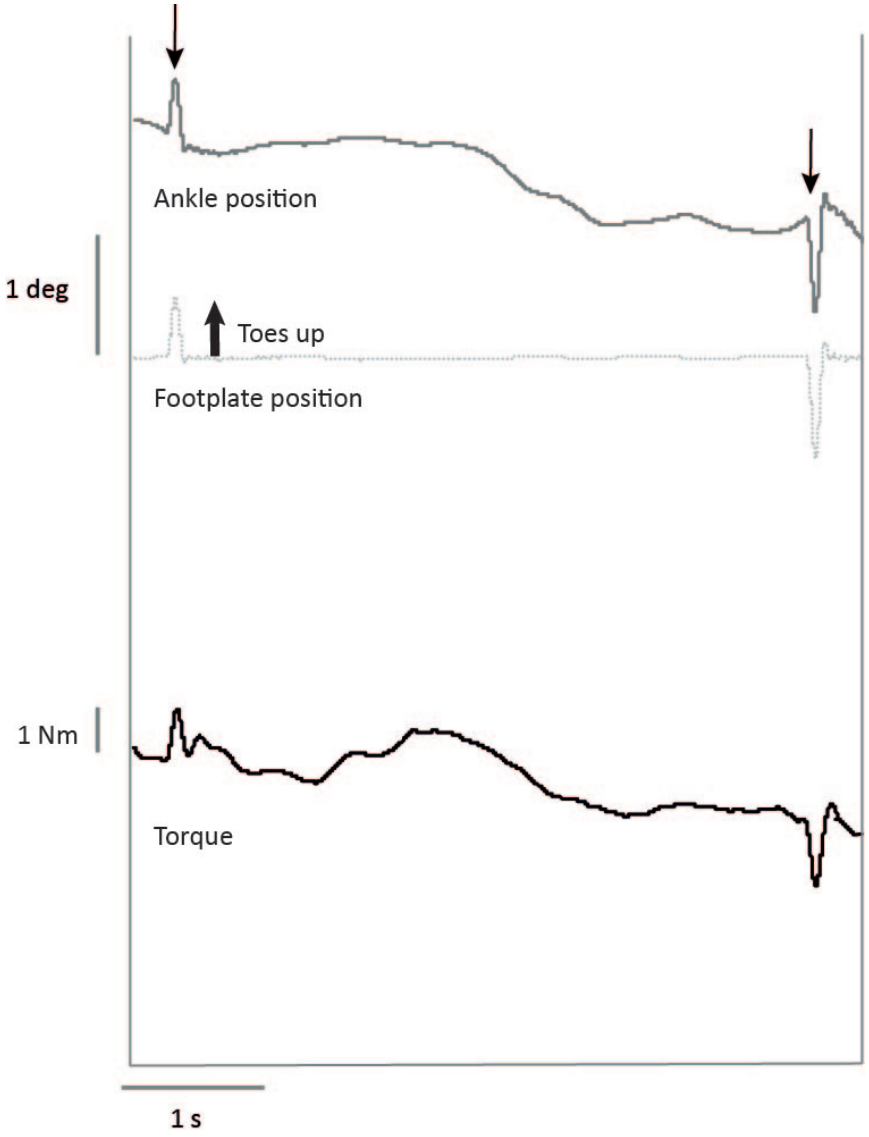


Figure 2.1: Illustrative segment of the footplate position, ankle position and ankle torque in quiet standing condition. The perturbations were randomized in amplitude, direction (toes-up or toes-down) and interstimulus interval (4–5 s). The bottom horizontal line corresponds to 9 Nm torque value.

from the linear force recorded by the load cells. These were calibrated with two different methods, and the results were compared. To account for the differences in height and weight between participants, I compared the estimated stiffness with the toppling torque per unit angle (aka 'load stiffness') of each participant. The method to calculate it is described here. Finally, a description of the calculation used to estimate stiffness and some stiffness measurement validation methods to confirm the accuracy and stability of our estimates are presented.

2.2 Apparatus

Ankle stiffness was measured with a custom-built footplate apparatus (Figure 2.2). It consisted of a motorised platform supporting two freely moving footplates which were subjected to a common rotation. A linear motor (Model XTA3810S, Copley Motion Systems LLC, GBR) was used to rotate the platform via a lever. It operated in position-servo mode; hence the motor attempted to drive the footplates to specified positions irrespective of any resistance offered by the subject.

The footplate axis was positioned 8.6 cm high to coincide approximately with the average human ankle joint height. Participants stood with each foot on separate plates and with the centre of the ankle joint aligned with the footplate axis in the frontal plane. The ankle is a very complex joint with no clear definition of its axes of rotation. Because of the nature of this joint, modelling it is very challenging. Therefore, with this setup the ankle was made to rotate around one fixed axis of rotation which I assumed was acceptably accurate. Rotation was imposed about an axis defined by the apparatus. If the vertical position of the leg is altered, there is no change in moment arm and the calculated stiffness will be unchanged. Antero-posterior shifts will change the moment arm and will therefore alter calculated stiffness. I consistently aligned the foot using the lateral malleolus as a reference as this is the landmark from which the moment arm of the calcaneneus has been established. There was inevitably some inaccuracy in this process. I estimate that the repositioning accuracy was ∼2 mm, and as the mean moment arm is ∼4.8 cm (Maganaris et al., 2000) when the ankle is in neutral position, the random error in stiffness will be of order 4%.

Platform angular displacement, velocity and acceleration, along with ankle torque, were used to estimate stiffness of the ankle joint. Torque was measured by two miniature load cells (Model Sensotec 31, Sensotec Inc., USA). These were horizontally mounted between the platform and footplates, and placed directly above their axis of rotation. To obtain

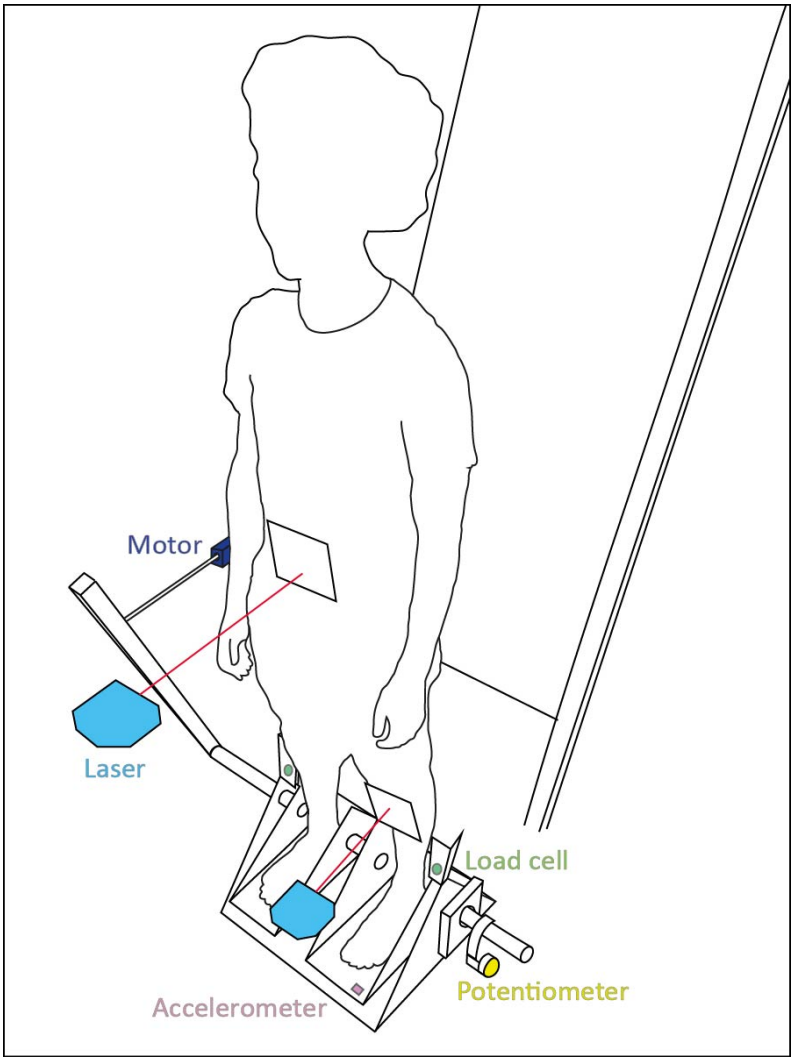


Figure 2.2: Experimental setup. The position servo motor was installed horizontally and set to apply perturbations to the crank, subsequently rotating the platform and footplates. Two load cells measured torque; a potentiometer attached to the axis of rotation measured anteroposterior rotation of the footplate; an accelerometer attached underneath the left footplate measured footplate acceleration; and two laser-reflex sensors placed at mid-tibia and umbilicus level tracked the anteroposterior shin and body tilt. The board seen in the picture behind the participant was adjusted to the vertical position during one of the conditions in Chapter 3.

the actual ankle angular rotation, foot angle was subtracted from shin angle. The foot was firmly placed on the footplate, whose angular displacement was recorded with a precision Hall effect potentiometer (Model CP-2UT, Midori Precisions Co., JPN) located on the platform axis. A laser-reflex sensor (Model YT25MGV80, Wenglor, GER), placed at the left mid-tibia level (150-250 mm away from the shin), was used to record shin linear displacement, later converted to angular rotation and subtracted from the potentiometer

data. This was used to detect shin position. A second laser (Model YT44MGV80, Wenglor, GER) was used to record an approximation of the centre of mass (COM) position and was placed around the umbilicus level (190-290 mm away from the body). Both lasers were used for the estimation of the gravitational toppling torque (see Section 4 below). A ±3 g linear accelerometer (Model ADXL335, Analog Devices Inc., USA) was used to measure vertical footplate acceleration. After calibration it was attached underneath the left footplate at a distance of 0.22 m from its axis. Hence, the signal was divided by this value to provide angular acceleration in rad s^{-2}. The three types of signals were low-pass filtered by a fourth-order Butterworth filter with a cut-off frequency at 40 Hz. It is known that muscle activity also affects joint stiffness (Mirbagheri et al., 2001; Loram and Lakie, 2002*a*; Casadio et al., 2005). Therefore I recorded surface EMG activity (Model Bagnoli-8, Delsys Inc., USA, band-pass filtered between 20-450 Hz) from the tibialis anterior and lateral and/or medial gastrocnemius muscles in both legs.

All the signals were synchronized and captured through Matlab and Simulink (v2011b).

2.3 Calibration procedures

As the perturbations were rotational, all the equipment was calibrated to transform the recorded linear analog signal into angular digital signal. The motor, potentiometer and laser-reflex sensor signals were calibrated into angular position (deg), the accelerometer signal into angular acceleration (deg s^{-2}) and the load cell signal into torque (Nm).

Motor and potentiometer calibration

The potentiometer (Rotary Position Sensor Model CP-2UT, Midori Precisions Co., JPN) was calibrated by manually rotating the footplates whilst a digital inclinometer (Smart

Tool Module, Level Developments, GBR), placed on top of it, traced the change in angle. A linear fit between the potentiometer and the inclinometer output was performed to find the calibration factor (1 V = 1.45 deg).

The same inclinometer was used to calibrate the motor (Model XTA3810S, Copley Motion Systems LLC, GBR), in terms of the input command voltage required to achieve a certain angular displacement. A step-like input of known voltage was given to the motor, and the output given by the inclinometer at each step was registered. A linear fit between both signals gave the motor calibration (1 deg = 0.69 V).

Laser-reflex sensor calibration

Two laser-reflex sensors (Model YT44MGV80, Wenglor, GER) were used to approximately record ankle and body/board angular displacement. At a fixed height H (0.26±0.03 m and 0.9±0.07 m), the lasers tracked a reflective target attached to the participants leg (shin) and body (umbilicus), measuring the horizontal change in distance (Δd). I defined H and Δd as catheti of a right-angled triangle, and assumed that the ankle angular displacement α could be calculated using the inverse tangent function (Figure 2.3):

$$ankle\ angular\ displacement\ (\alpha) = atan\frac{(\Delta d)}{H} \tag{2.1}$$

Accelerometer calibration

A ±3 g accelerometer (ADXL335, Analog Devices Inc., USA) was glued underneath the left footplate at a known distance (0.22 m from the axis) to measure the angular acceleration of the footplate.

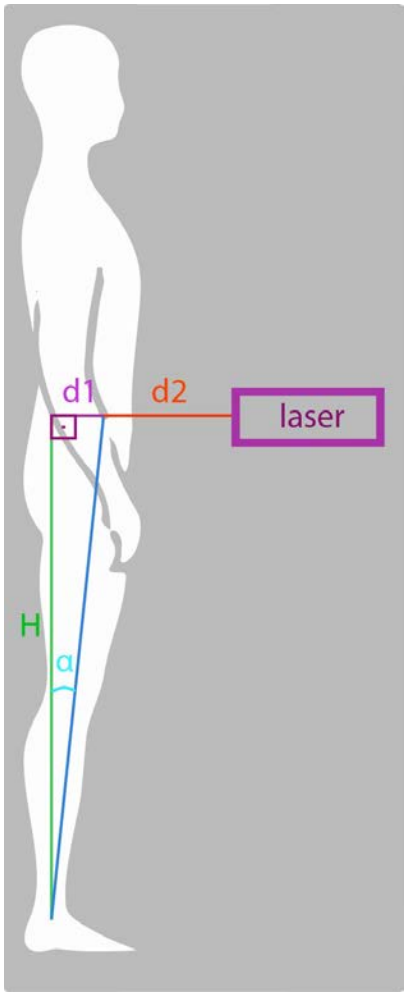


Figure 2.3: Representation of the trigonometric function used to estimate the body and ankle rotation. A simple trigonometric calculation was sufficient to find the value of angle α. H and d1 are catheti of the right triangle formed by the centre of gravity height and the body displacement in the antero-posterior direction. Calculation of the arctangent of one divided by the other gives the angle α, which corresponds to the ankle rotation. For small rotation of the ankles (less than 10 deg), a simple division will give the angular displacement. When using the inverse tangent to calculate angles, if one cathetus is a lot smaller than the other (H $\gg$ d1), then a simple d1/H can give the value of angle α.

Initially the accelerometer had to be calibrated with respect to the gravitational vector. Briefly, it was rotated between ±1 g, which corresponded to a deviation of 0.699±0.001 V (mean±SD). The calibration factor was obtained from a simple numeric calculation:

$$1\ m\ s^{-2} = \frac{\frac{0.699\ V}{2\ g}}{9.81\ m\ s^{-2}} = 0.03563\ V$$

Then:

$$1\ V = \frac{1}{0.03563} = 28.07\ m\ s^{-2}$$

The second task was to convert the signal from linear to angular acceleration. If we consider the linear acceleration of a linear system as the tangential acceleration of an angular system, we can use the following equation to calculate the angular acceleration calibration factor:

$$a\ (tangential\ acceleration) = r\ (radius) \times \alpha\ (angular\ acceleration) \qquad (2.2)$$

$$28.07\ m\ s^{-2} = 0.22\ m \times \alpha\ rad\ s^{-2} = 127.58\ rad\ s^{-2}$$

Therefore, the voltage output given by the accelerometer had to be converted to rad s^{-2} by multiplying it by 127.58.

Load cell calibration

As ankle torque was a crucial input for the stiffness calculation, two different devices were used to obtain a more precise load cell calibration factor, a separate portable strain gauge and a spring with known stiffness.

Load cell calibration with a strain gauge

Initially, the strain gauge had to be calibrated. I attached it to a horizontal support and placed known weights on top of it while recording the voltage output. With the linear

fit of these values I found the strain gauge calibration factor ($CF_{\text{strain gauge}}$). Second, I clamped the strain gauge to the footplate at a known distance (d) from its axis of rotation (Figure 2.4). With the footplate fixed at the horizontal level, I then recorded the load cell and strain gauge signal output while manually applying a slow sinusoidal force on top of the strain gauge. If the moment recorded by the load cell equals the moment recorded by the strain gauge, then:

$$M_{load\ cell} = M_{strain\ gauge}$$

$$\tau_{load\ cell}(V) \times CF_{load\ cell} = F_{strain\ gauge}(V) \times d \times CF_{strain\ gauge} \tag{2.3}$$

The load cell calibration factor ($CF_{\text{load cell}}$) was estimated with the linear fit of the recorded values of load cell torque output ($\tau_{\text{load cell}}$) against the strain gauge force output ($F_{\text{strain gauge}}$) (Figure 2.5) multiplied by the previously calculated strain gauge coefficient factor ($CF_{\text{strain gauge}}$) and its distance (d) from the axis.

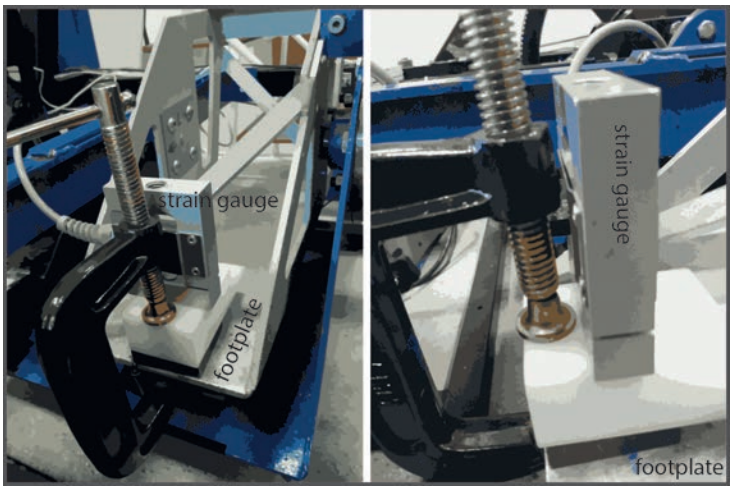


Figure 2.4: Strain gauge clamped to footplate (front and lateral view).

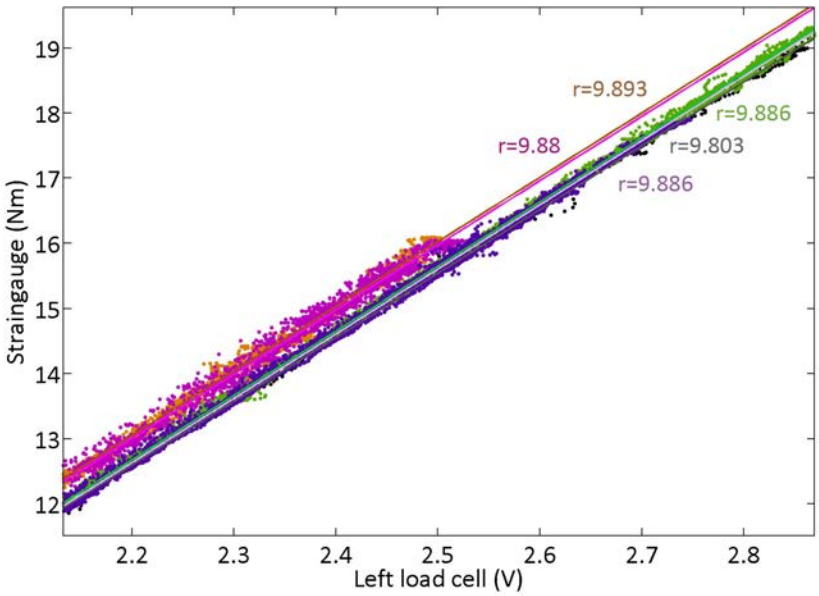


Figure 2.5: Linear fit between strain gauge and left load cell outputs. Linear fit coefficient found for each of the 5 tests is shown.

Load cell calibration with a spring with known stiffness

A second calibration method was performed with a linear spring with known stiffness (K = 10.86 N mm^{-1}) (similar setup from the spring attachment setup seen in Figure 2.18). First it was attached perpendicular to the footplate at a known distance (r) from the axis. While the top was fixed to a rigid beam, the spring was compressed at the bottom by the footplate, which was rotated sequentially at different measured angular positions (n). Data from the potentiometer, the load cell and a digital inclinometer (placed on top of the footplate) were recorded. The footplate was manually moved in steps of ∼0.2 deg and the resistance of the spring against it at different degrees of compression was recorded by the load cell. The calibration factor was calculated through the combination of recordings from the load cell, the potentiometer, the digital inclinometer and the spring stiffness constant given by the factory, as follows.

A combination of three equations was used to obtain the load cell calibration coefficient:

$$F = -K \times dx$$

Hooke's Law definition, where: F = force, dx = size of displacement and K = known stiffness of the spring.

$$\tau = r \times F$$

Torque definition, where τ = torque, r = radius or moment arm and F = force. Radius was known previously and torque was recorded in volts from the load cell signal.

$$360\ (deg) = 2\ \pi\ r\ (rad)$$
$$n\ (deg) = dx\ (rad)$$

Radians to degrees conversion, where n = position in degrees and dx = position in radians. n was recorded by the potentiometer and the inclinometer. To convert the angular displacement from degrees to radians, I relocated the elements of the equation to:

$$dx = \frac{n \times 2\ \pi\ r}{360} = n \times 0.0175 \times r$$

By interchanging the elements of the three equations described above, I got:

$$\tau = K \times n \times 0.0175 \times r \times r$$

As I knew τ in Volts from the load cell data, I found the calibration coefficient with the equation:

$$\tau\ (V) \times CF_{load\ cell} = K \times n \times r \times r \tag{2.4}$$

To my satisfaction, even though the devices and calculations were completely different, the resultant calibration factors ($CF_{load\ cell}$) found with the two different methods were relatively similar, 9.88 with the first method and 9.81 with the second method. I chose to adopt the strain gauge calibration factor because it was the most accurate (R^2=0.99, p<.001) (Figure 2.5). Due to its limitations, the use of a spring with known stiffness was used only to corroborate the strain gauge results. It was not completely identical probably due to spring deformation, either due to stiffness changes at the extreme compressed and stretched positions or due to distortions caused by the rotational compression of a vertically aligned linear spring (check Figure 2.18 and Figure 2.19 for a similar setup).

2.4 Estimation of toppling torque per unit angle ('load stiffness')

As all the studies within this thesis were used to obtain measurements of intrinsic ankle stiffness, it was important to find a method to compare these values with each other or with previous studies. A common approach is to estimate it as a percentage of the minimum ankle stiffness necessary to sustain the body at the vertical position, i.e. to resist gravitational toppling torque. I adopted this approach for all the experiments in this thesis. For this reason, the estimation of toppling torque becomes important. Here I present two procedures to estimate toppling torque, as well as the concepts upon which it is based.

As mentioned previously in Chapter 1 (section 2), the body inverted pendulum model is one of the most generally accepted mathematical models used to explain the control of the human upright stance. It postulates that, if subjected to small perturbations, the body behaves as a rigid inverted pendulum rotated around the ankle joint (Smith, 1957). The body inverted pendulum is dependent on the mass above the ankles (m), the height of the COM above the ankles (h) and the gravitational forces acting on it (g). In terms

of potential and kinetic energy dynamics, it can be said that the long vertical body has a positive potential to topple forward rotating around the ankles. This potential energy is at its maximum at the vertical equilibrium point, or when the COM is located directly above the ankles. Termed the toppling torque per unit angle, it is defined as mass × g × height, and is considered the minimum amount of ankle stiffness necessary to stabilize the body at this position (Figure 2.6). Any slight deviation from this vertically aligned position will lead to the COM generating a gravitational torque pulling the body away from the equilibrium point, thus transforming the potential energy into kinetic energy to fall over. This will be counteracted by a reaction torque applied by the foot against the floor. In a stable system, the ground reaction torque is equal to the gravitational torque. The larger the deviation from the equilibrium point, the larger the necessary torque generated by the feet and ankles to restore balance and the larger the need to generate torque that is actively controlled by neural modulation. At this point the ankle stiffness necessary to stabilize position is also dependent on the ankle angular displacement, therefore it is calculated as m × g × h × sin θ, where θ is the rotation of the ankles. As during quiet standing the forward movement of the body does not exceed 10 deg (Hellebrandt and Braun, 1939), then the small-angle approximation rule can be applied ($\sin\theta \approx \theta$) and the standing ankle stiffness necessary to stabilize position can be estimated as m × g × h × θ (Figure 2.6).

In other words, within this range, m × g × h has a linear relationship with the angular displacement θ, thus the standing ankle stiffness can be estimated as a proportion of m × g × h. This relationship stands as long as the ankle strategy is mainly used to stabilize the standing body, which is what happens during quiet standing (Figure 2.6). Therefore, throughout this thesis, the measurement of ankle stiffness is also shown as a percentage of the toppling torque per unit angle ('mgh'). Although this concept is based on the body inverted pendulum model and may underestimate the relationship between changes in body COM and other joints (Aramaki et al., 2001; Pinter et al., 2008), here it is used as a reference to normalize data from all participants, regardless of their body mass and height.

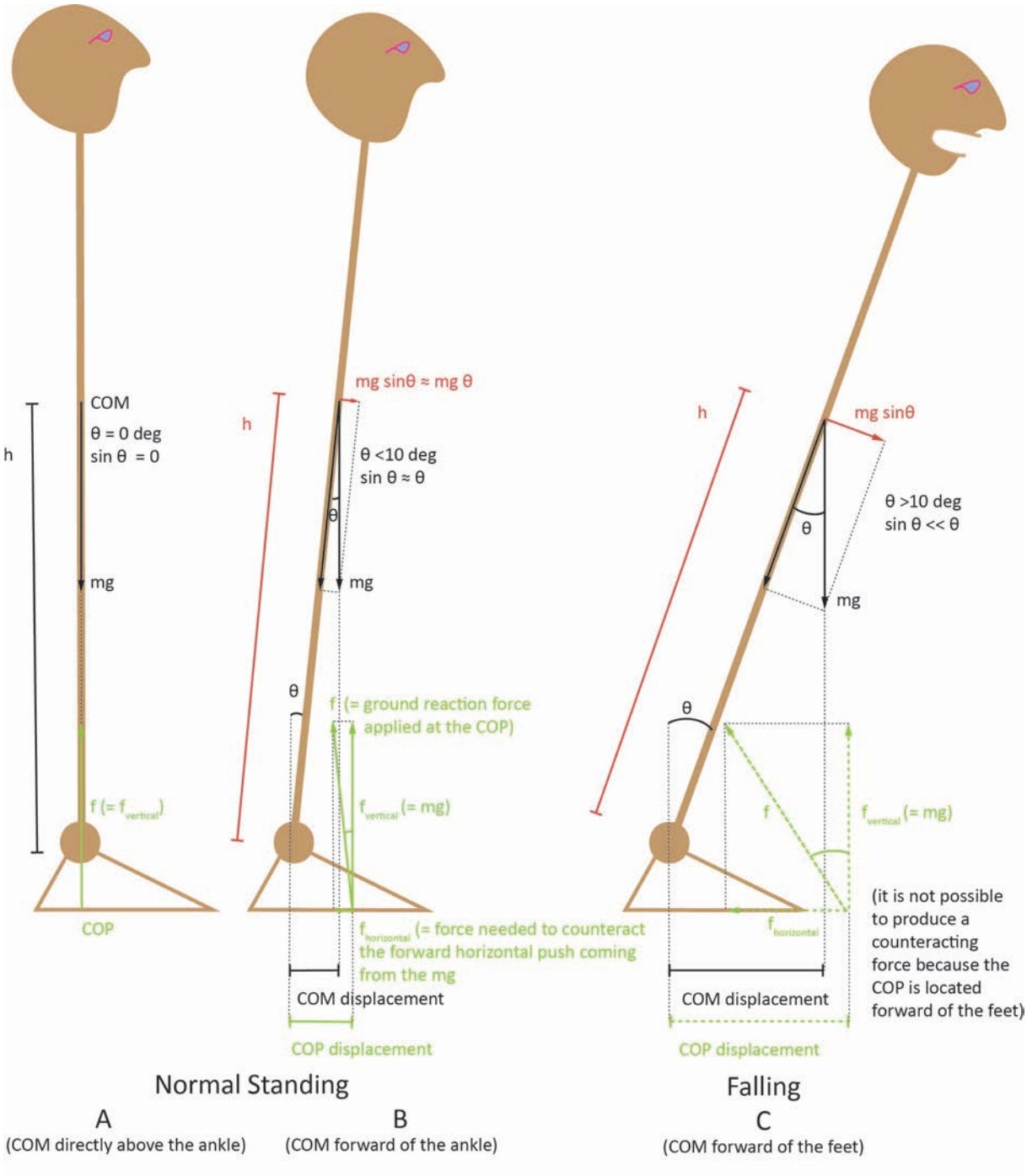


Figure 2.6: Three different standing postures are presented, standing at the vertical equilibrium point (1), standing at a spontaneously chosen position (2) and falling (3). In the first position, the body COM does not apply any deviating torque and the potential energy is at its maximum. In the second position, the body COM projection on the ground is located slightly forward of the ankle joint, therefore it exerts a gravitational torque that has to be counteracted by a reaction torque applied by the feet against the ground. In the third position, the COM projection on the ground is located forward of the feet. As in this case the feet cannot exert enough amount of reaction torque, the body will fall.

This is a crucial parameter throughout this thesis because, for intrinsic ankle stiffness to stabilize the body alone, it must be equal to, or greater than mgh. I therefore determined toppling torque per unit angle for each subject so that I could express ankle stiffness as a percentage of this value. Since I did not have precise knowledge of the height of the COM or the body mass above the ankles, mgh could not be calculated directly. I therefore used two different techniques (one with standing and another with lying individuals) to obtain toppling torque per unit angle of each participant. This was necessary to achieve a desirable level of consistency of our mgh estimates, especially relevant in experiments in which between-individual differences were correlated (Chapter 6).

2.4.1 1st method: Linear regression of standing sway versus torque

In this setup, I recorded 2–3 trials of 30–180 s during which no perturbations were applied to the footplates. The participants were asked to stand on top of the footplates and voluntarily sway around the ankle joint within a comfortable range (3–6 deg) and at a voluntarily chosen speed. I assumed that this amount of rotation forward to the vertical equilibrium point was small enough (<10 deg, small angle rule) to consider its relation with the gravitational torque as a linear relationship. The participants were instructed to maintain the upper and lower bodies as aligned and rigid as possible, minimizing the movement of the hip and knee joints. Ankle torque (load cell data) and position (laser sensor data) were recorded, and a linear regression was used to estimate the toppling torque per unit angle for each participant (Fitzpatrick, Taylor and Mccloskey, 1992). The great advantage of this method is that it expresses a direct relationship between torque and angle and does not require calculation of the COM height, which is difficult to estimate precisely. More importantly, it gives an estimation of toppling torque in standing individuals. Possible disadvantages of this method would be deviations due to hip and/or knee motion which would alter the linear relationship between ankle torque and angle.

A sample of the linear regression between body and ankle position against left and right ankle torque of one participant's data is shown in Figure 2.7. It was estimated with all the possible combinations of body and ankle laser against left and right ankle torque. This participant was successful in maintaining an aligned body position, in which hips (blue thick line) and ankles (black thick lines) moved symmetrically.

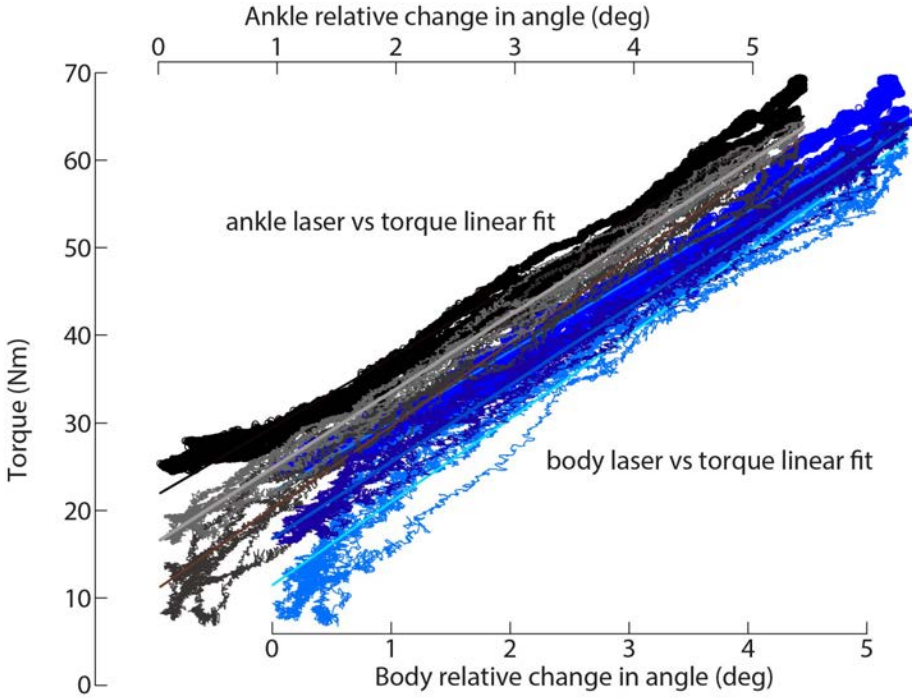


Figure 2.7: Linear regression result of 6 different types of calculation (data from one participant). It was estimated with all the possible combinations of body and ankle laser against left and right ankle torque: body laser vs left and right torque (dark blue), body laser vs left torque × 2 (cyan), body laser vs right torque × 2 (light blue), ankle laser vs left and right torque (light gray), ankle laser vs left torque × 2 (brown) and ankle laser vs right torque × 2 (black). Black and light blue torque traces are higher because this participant was standing with slightly more weight on the right foot. Body and ankle angle are not absolute in relation to Earth, but are only a display of relative change in angle. This particular participant was swaying very similarly to a real inverted pendulum as both ankle and body swayed by approximately 5.3 deg.

2.4.2 2nd method: Reaction-board

Here toppling torque was defined as the torque exerted by the body COM in a supine position. For this setup, a board placed off the ground and equally elevated by a beam at

one end and by a scale at the other was acting as a rotating object. Its moment arm was the distance between the two supports and its axis of rotation was around the support given by the scale. Participants were asked to lie parallel to the direction of the board, with the ankle medial malleolus aligned with the beam to exclude the weight of the feet, and the top of the head facing the scale. Toppling torque per unit angle (mgh) was calculated by multiplying the mass measured by the scale (m) by the length of the board moment arm (h) and the gravitational constant (g) (Figure 2.8). This procedure was performed 3 times and the average mass was used for the final calculation to avoid errors caused by misplacing the ankle position on the board. This test was done immediately prior to the main experiment to avoid effects of body weight fluctuation during and/or between days.

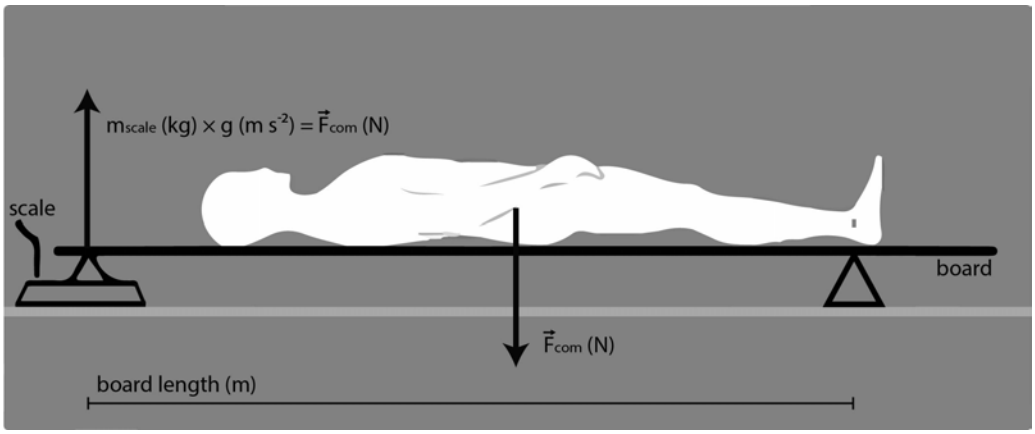


Figure 2.8: Reaction-board experimental setup.

The two different methods described above were chosen to assess toppling torque in lying and standing individuals and verify its consistency. Nevertheless, the location of the centre of mass (COM) of a lying person is slightly above a standing person's COM due to the blood and internal organs's distribution, which could have potentially increased the toppling torque value of the second method.

2.4.3 Comparison between two different methods

I measured toppling torque using the two methods on two different occasions, in this chapter and in Chapter 6, to verify the difference between the two method's results. In each experiment, toppling torque was tested with both methods in quick succession within the same day. The reaction-board method had only one outcome because it was only dependent on body weight and height and on how the body was positioned on top of the board. Hence it was considered to be the gold standard of toppling torque estimation. The linear regression method during standing was dependent on numerous factors, like alignment of the ankle with the footplate rotation, alignment of the body during sway, contraction of the muscles during sway etc. Therefore, these factors might have introduced additional variability, as compared with the reaction-board method. Furthermore, the linear regression could be estimated with all the different variables used to record ankle torque and body position: right and/or left ankle torque (from the two load cells attached to each footplate), and ankle and/or body angle (from the laser range finders tracking shin and waist position). As there were many variables, I decided to verify which combination would lead to linear regression results more similar to the ones obtained with the reaction-board method. In which case, I calculated the linear fit coefficient between the two different torque signals and the two different position signals in various combinations:

For the first experiment (Chapter 2, average of 10 participants), I performed 6 combinations:

1. Left ankle laser versus left torque $\times 2$
2. Left ankle laser versus right torque $\times 2$
3. Body laser versus left torque $\times 2$
4. Body laser versus right torque $\times 2$

5. Left ankle laser versus the sum of left and right torque

6. Body laser versus the sum of the left and right torque

For the second experiment (Chapter 6, average of 20 participants), I performed 2 combinations:

1. Left ankle laser versus the sum of left and right torque

2. Board laser versus the sum of the left and right torque

I then compared both methods in two different ways:

1. By comparing the results between each other (average and standard deviation); and

2. By correlating the results with whole body height and mass (Figure 2.10).

The results in Figure 2.9 show that in both experiments, the estimations of toppling torque with the regression technique (at all combinations) were relatively similar to the estimation obtained with the reaction-board method. The closest agreement to the reaction-board method occurred when using left torque summed with the right torque, regressed against ankle laser (average 0.2% difference in the first experiment and 19.1% difference in the second experiment).

The difference of results obtained with experiments 1 and 2, though, is more clearly shown in Figure 2.10. This time I compared toppling torque per unit angle with whole body mass and height, as it is assumed that they strongly correlate. Not surprisingly, the reaction-board results (in blue) correlate the most, in both cases. In experiment 1, $R^2 = 0.84$ was obtained when comparing toppling torque measured with the reaction-board method versus body height. In opposition, $R^2 = 0.76$ and $R^2 = 0.62$ were obtained with the linear fit of ankle laser against left plus right ankle torque and linear fit of body laser against left plus right ankle torque versus body height. The estimates for body weight were also consistently well-correlated. For the experiment 2, the estimates of toppling

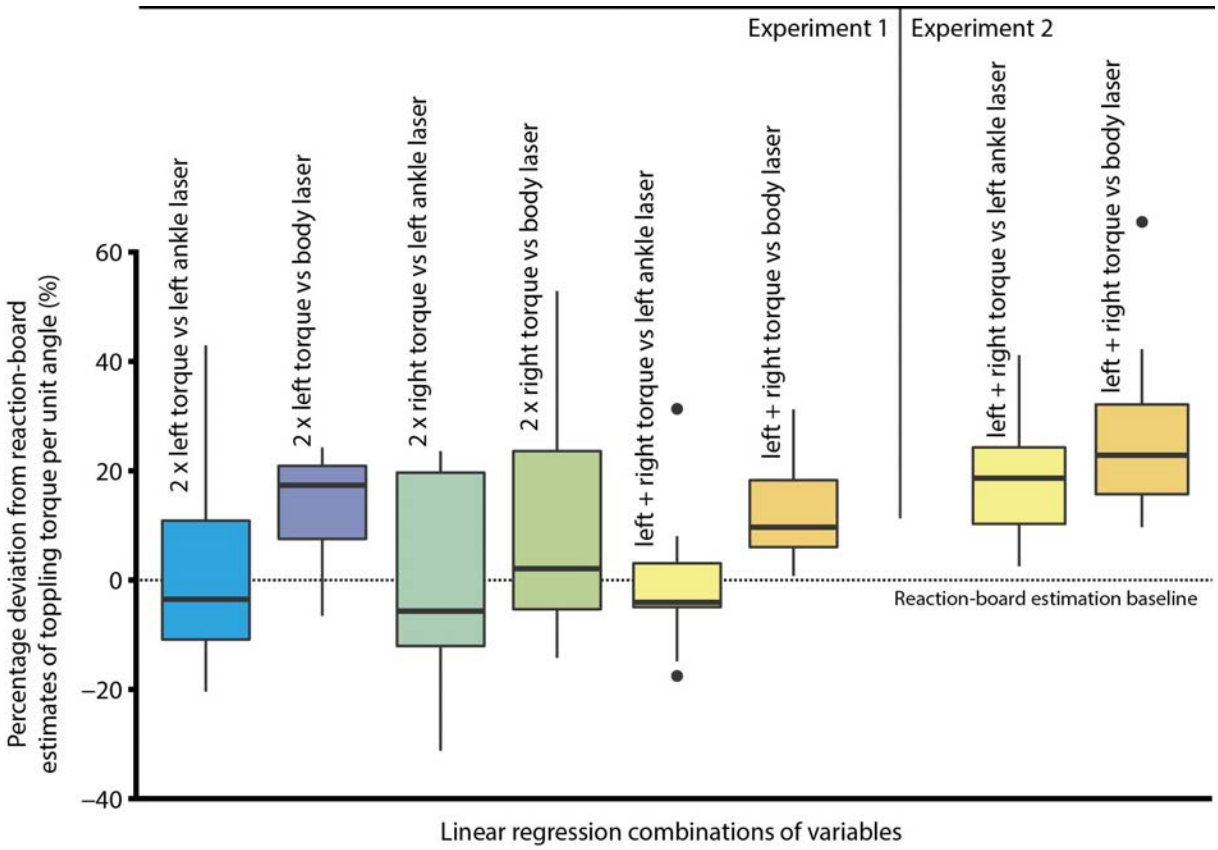


Figure 2.9: Toppling torque per unit angle estimations obtained with experiment 1 (Chapter 2, left section) and experiment 2 (Chapter 6, right section). The different linear regression measurements are shown as a percentage deviation from the reaction-board result (extended as a horizontal dashed line to facilitate visualization).

torque with linear fit against mass and height correlated similarly well ($R^2 = 0.71$ with ankle laser linear fit as opposed to 0.83 with reaction-board in height and 0.92 as opposed to 0.99 in weight).

In summary, both methods gave consistently similar results. This helps validate the use of the linear regression technique to calculate toppling torque. The reason for using both methods in this Chapter 2 and in Chapter 6 was to reassure that the values obtained with the linear fit method were consistently similar to the ones obtained with the reaction-board method, considered to be a most accurate estimation of toppling torque. Here in this chapter, the interest in using both methods was to establish the similarity between them and justify the use of the linear fit method in the subsequent experiments (Chapters 3, 4 and 5). In these 3 chapters, I was performing within-individual comparisons, therefore

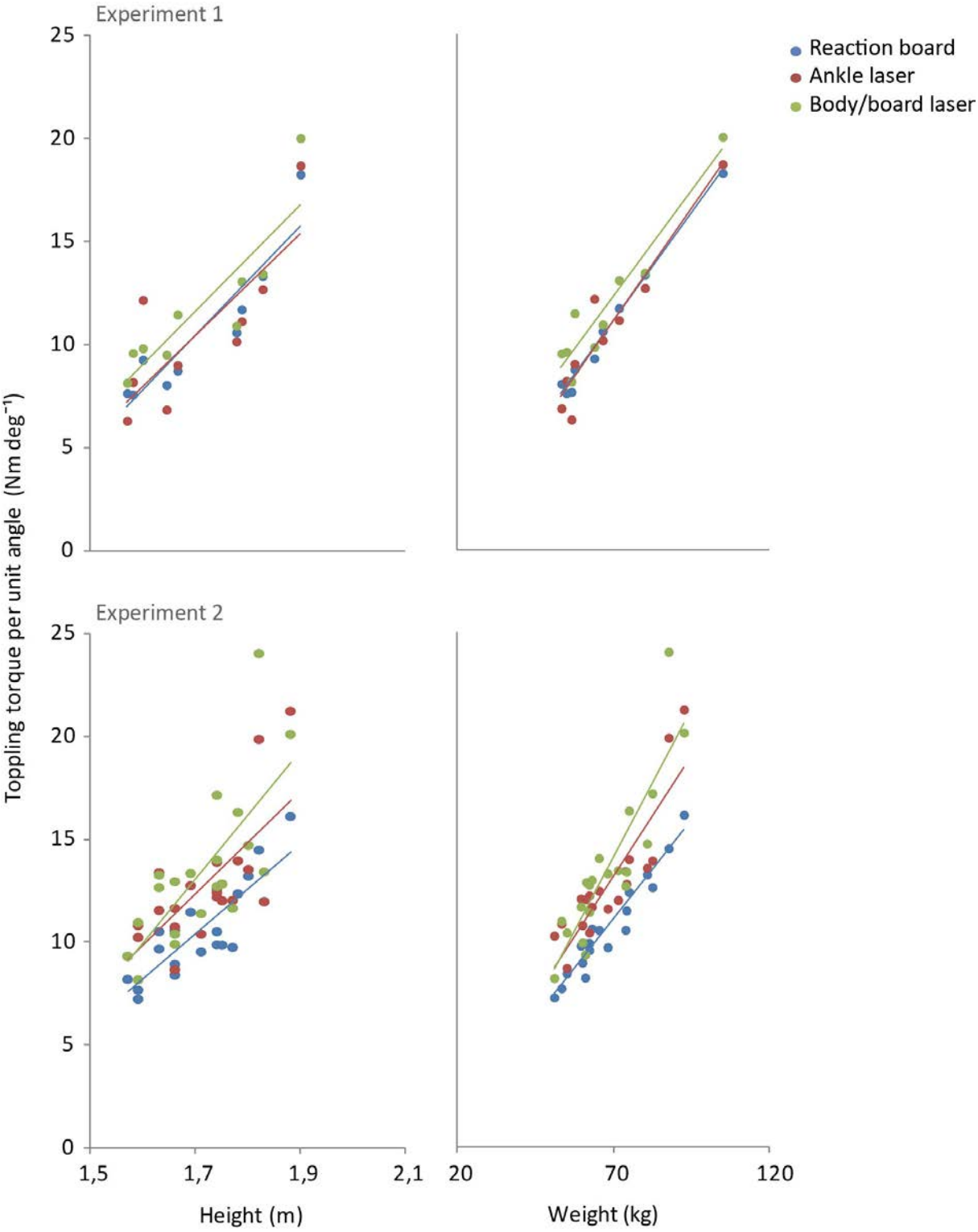


Figure 2.10: Correlation between toppling torque per unit angle and whole body mass and height. The top two graphs are results from the first experiment (Chapter 2) and the bottom graphs are from the second experiment (Chapter 6).

the differences of toppling torque values did not interfere with the final results. The advantage of using the linear fit method is the easier setup with standing individuals already prepared to perform the rest of the experiment. In Chapter 6, as I was performing between-individual comparisons, the actual intrinsic stiffness ratio in relation to toppling torque was extremely relevant. Therefore I used both methods to make sure that the toppling torque measurements were the most accurate as possible.

2.5 Stiffness measurement procedure and its validation

At the beginning of this section I present the theoretical mathematical background for the estimation of intrinsic ankle stiffness. The use of mathematical representations to describe natural phenomena is a common approach for researchers to identify some common characteristics between the different species, organs, tissues and other objects found in Nature. Here I present the reasons for adopting a mass-spring-damper system model to describe stiffness of the human ankle. Following the initial description, I then proceed to describing the various tests performed to evaluate the robustness of this approach.

First, I verify the similarity between the results obtained from the mathematical model used to estimate stiffness with the empirical data recorded from the apparatus. I correlate the recorded torque signal with the estimated torque resulting from the regression model. The results were consistent (mean R^2=0.99; $p<0.001$) to a very satisfactory level. I then proceed to establish the minimum amount of dataset needed to obtain a reliable estimate of stiffness. Stiffness is estimated with the average of a decreasing amount of perturbations, starting from 48 and decreasing to 1. After finding this value (minimum of 30 datasets), I then proceed to verify visually the plausibility of the obtained coefficients of stiffness, viscosity and moment of inertia by reconstructing the estimated torque into its K θ, B $\dot{\theta}$ and I $\ddot{\theta}$ components. Next I verify the repeatability of the apparatus and

the stiffness assessment methodology by testing it with an inanimate object, a spring with known stiffness. The spring is attached firmly at the top to a fixed metal rod and at the bottom to the footplate. I perform two different stiffness estimates with the inanimate object, a linear fit model simulating toppling torque per unit angle estimation and a multiple linear regression model simulating intrinsic ankle stiffness estimation, and compare its data with the official stiffness given by the factory. Finally, I verify the repeatability of the stiffness estimates between individuals. The estimates of participants that performed experiments within a time-scope of 1 month to 1.5 years are compared. The results are shown below.

2.5.1 Mechanical intrinsic ankle stiffness estimation model

Movement of the vertebrate joint is dependent on the properties of the bones and all the different tissues surrounding it, like skin, ligaments and muscles. Even though they all have different properties and levels of elasticity, here I consider the ankle joint as a single unit and assess stiffness as a whole. As mentioned earlier in this chapter (Introduction, section 1), the perturbations used to estimate stiffness were small (less than 1.3 deg) and brief (approx. 140 ms). This was important to ensure that they would not induce a significant disturbance of the upright stance, which could lead to unwanted active modulation of stiffness (e.g. stretch reflexes) that could affect our calculations of joint stiffness (Mirbagheri et al., 2001; Loram and Lakie, 2002*a*; Casadio et al., 2005). Using a similar setup to the one used in this thesis, Loram and Lakie (2002*a*) showed that such reflexes occurred well outside the 70 ms time window in which our analysis was restricted. The problem with faster perturbations, though, is that velocity and acceleration have a greater influence upon the resultant joint torque. At higher velocity and acceleration conditions, it is more difficult to move highly viscous or heavier objects. Thus, the resultant ankle movement and torque induced by a perturbation is not only dependent on stiffness, but also in other properties of the tissue, like viscosity and moment of inertia.

Therefore, throughout this thesis, the estimation of the intrinsic mechanical ankle stiffness was based on the assumption that the ankle joint acted as a rotating mass-spring-damper system (Agarwal and Gottlieb, 1977; Hunter and Kearney, 1982). The calf muscles (contractile element) and the tendon, aponeurosis and foot (series elastic element) act as a mass-spring-damper system responsible for generating the corrective torque applied by the feet against the ground to stabilize position (Fitzpatrick, Taylor and Mccloskey, 1992; Winter et al., 1998). The moment of inertia of the foot and moving muscle with respect to the medial malleolus acting as the axis of rotation comprises the mass component. The spring component is the combination of the muscles, tendon, aponeurosis and foot controlling stiffness of the ankles. And finally, the damper component comprises the viscosity of the joint, muscles and associated tissues.

Stiffness, viscosity and moment of inertia were estimated with a fitting equation in which the torque measured over the first 70 ms of the perturbation was compared with the torque generated by a simple second-order model. The three inputs to this model were the measured ankle angular position, angular velocity and angular acceleration (Figure 2.11 and Figure 2.12) (Agarwal and Gottlieb, 1977; Kearney and Hunter, 1982; Loram and Lakie, 2002*a*):

$$\tau = K\theta + B\dot{\theta} + I\ddot{\theta} \tag{2.5}$$

Where: τ = torque (Nm); θ = angle (deg); $\dot{\theta}$ = angular velocity (deg s^{-1}) and $\ddot{\theta}$ = angular acceleration (deg s^{-2}); K = stiffness (Nm deg^{-1}); B = viscosity (Nm s deg^{-1}) and I = moment of inertia of the foot (kg m^2).

2.5.2 Model assessment #1: fit between torque and estimated torque

The mass-spring-damper system model implies that a reliable representation of the mechanical behaviour of the joint tissue can be achieved by regressing the torque trace into its quadratic coefficient (moment of inertia), linear coefficient (viscosity) and constant term (stiffness), multiplied by the corresponded angular acceleration, velocity and position. These are mathematical conventions used for ease of interpretation of natural phenomena. To verify if this model was giving consistent results, I monitored it by correlating the estimated torque with the actual torque. If the correlation was satisfactorily similar, I would know that the constants of stiffness, viscosity and moment of inertia reflected the actual torque at a satisfactory level of accuracy.

The known inputs of the regression model were torque, angular position, angular velocity and angular acceleration. Torque and angular position were directly measured with the apparatus, therefore the signals were highly reliable. As mentioned previously, the ankle position signal was the subtraction of the foot position (measured by the potentiometer signal) from the shin position (measured with the laser pointed at shin level signal). Angular velocity could be estimated either by differentiating the position or by integrating the acceleration recordings. It is still possible to obtain reasonably consistent values from the first derivative or integration of an empirical signal. Using the second derivative to obtain acceleration, though, may be less reliable. Empirical signals tend to be noisy, affecting the final calculation. Therefore I measured angular acceleration directly (using an accelerometer) and compared this to the 2^{nd} derivate of angular position. The accelerometer measured only the foot angular acceleration. Although this ignores shin rotation, it could potentially give better estimates than the second differentiation method. I compared the estimated torques to verify which would be more suitable. The results are shown in Figure 2.11. It is clear from the graph that the estimated torque obtained when using the accelerometer signal was more robust (mean R^2=0.99; $p<0.001$) (Figure 2.11,

B). The estimation of ankle torque for the whole thesis was then calculated using these signals. A sample of the data used for torque estimation is shown in Figure 2.12.

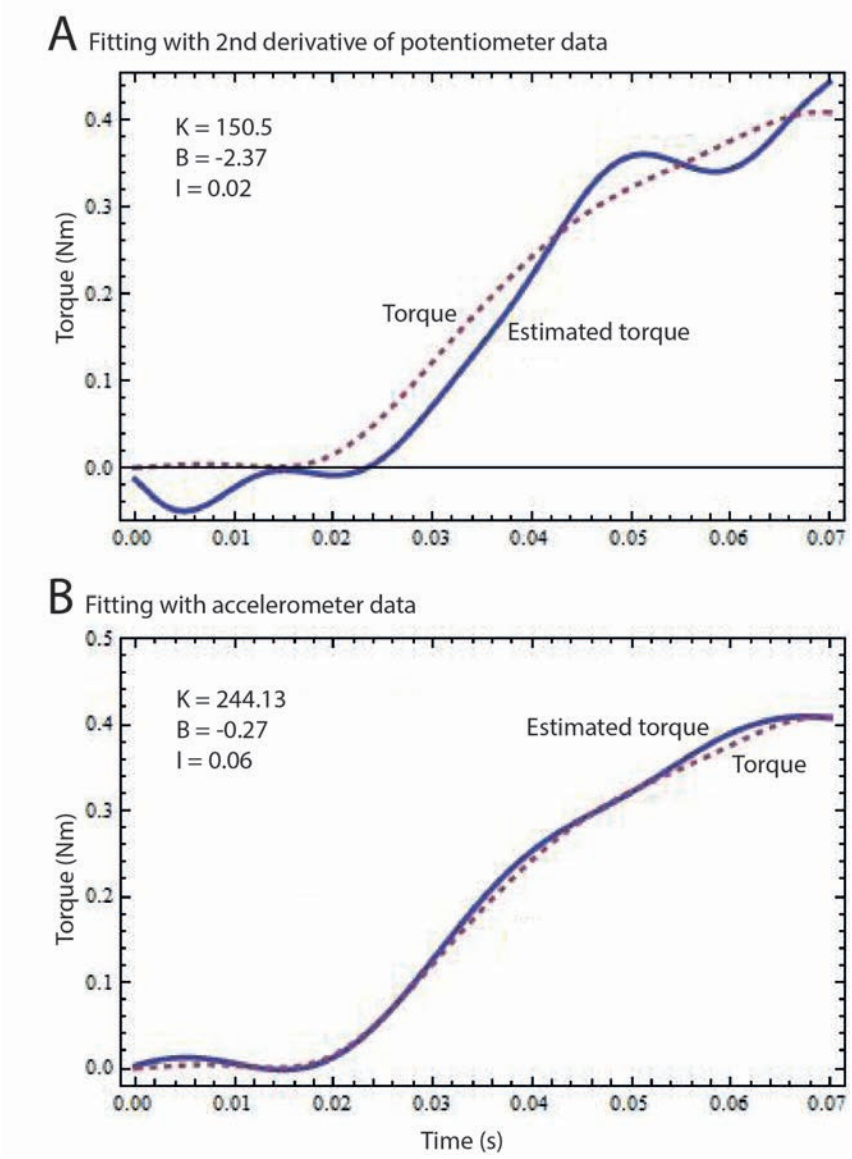


Figure 2.11: Sample of estimated torque obtained with acceleration data collected from two different sources. Red dotted line is the recorded torque trace. Blue line is the estimated torque trace. (A) Estimated torque obtained from 2nd differentiation of the angular position signal using Savitzky-Golay filtering method. (B) Estimated torque obtained from acceleration data recorded with accelerometer placed under the left footplate.

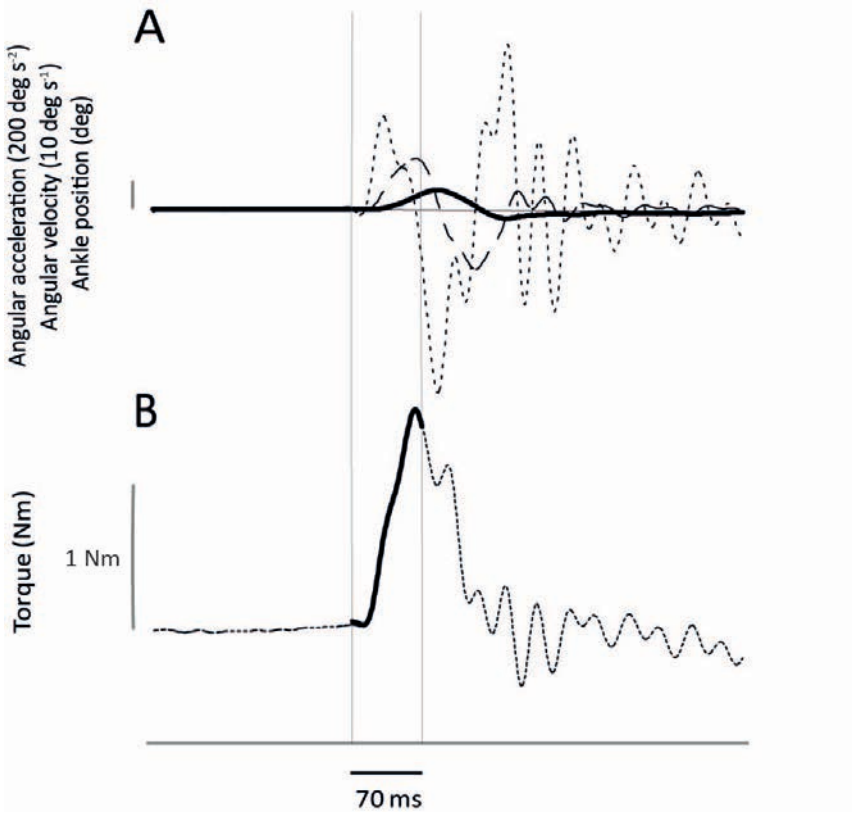


Figure 2.12: Estimating ankle stiffness. (A) Mechanical ankle stiffness was estimated by fitting the torque response with a signal generated by a second order model which utilizes the ankle angle (continuous line), angular velocity (dashed line) and angular acceleration (dotted line) as its 3 inputs. The thin vertical lines indicate the time window used for the analysis (70 ms), with the starting point coincident with the stimulus onset. (B) Ankle torque response (dotted line) and reconstructed torque (continuous line) obtained from the model.

2.5.3 Model assessment #2: minimum number of perturbations

The disadvantage of using mathematical models to describe physiological phenomena is that the latter are highly susceptible to transitory conditions, like changes in temperature, pressure, force etc. Thus to cope with the noise of unknown origin commonly found in biological tissues, it is necessary to average a large number of datasets to obtain comparable results. I then had to establish the minimum amount of data necessary to obtain a consistent estimate of the intrinsic stiffness of the ankle (K). For this, I compared and collected data of 3 different conditions from one participant and estimated stiffness with the average of a decreasing amount of datasets, starting from 48 recordings to 1

recording. The 3 conditions were: (1) 0.1 deg amplitude perturbation in normal standing condition (normal 0.1 deg), (2) 0.6 deg amplitude perturbation in normal standing condition (normal 0.6 deg) and (3) 0.1 deg amplitude perturbation in a condition with added sway applied by rotating footplates (wobble 0.1 deg). For this analysis, the main objective was to obtain consistent values within one participant during each condition. If the values were consistent for each participant in each condition, then my average estimates between participants as well as between conditions would also be consistent despite the large disparity of K values found in the literature.

The 48 available trials (including left load cell, potentiometer and accelerometer data) were randomized and divided in groups of 1 to 48 stimuli, and K was estimated with the averaged time series traces of each group. In other words, 48 different estimates were obtained with the analysis of single datasets. 24 estimates were obtained with the analysis of 2 averaged datasets, and so on. Only 2 different estimates were obtained with the average of 24 datasets. As for the average of more than 24 datasets, from 25 to 48 datasets, only one estimate was obtained. The results are shown in the graphs below (Figure 2.13).

The graphs on the left show K plotted against the number of stimuli used to estimate K (green dots). This ranges from 1 to 48. The light brown horizontal line corresponds to the estimated value obtained when data of 48 stimuli was averaged. Since it is the average of the largest range of stimuli that I have, I used it as the reference for the other calculations. The averaged values of each series of calculations (with the trendline) are shown in pink. We can see from the graphs that:

1. It appears from the left graphs that in all 3 conditions, as the quantity of trials used for each estimation of stiffness is increased, the fit with the K obtained with 48 trials (underlying brown line) becomes better and better and as more trials are added the diminishing returns reduce.

2. The estimates for 0.1 deg (normal and wobble) are less consistent than for 0.6 deg (normal).

3. For 0.1 deg estimates (normal and wobble), the average of each series (in pink) obtained from less number of stimuli is lower than the 48 stimuli estimate and increases with increasing number of stimuli. This effect is not seen for the 0.6 deg estimates, whose average value is consistently similar to the 48 stimuli estimate.

There might have been an intrinsic bias in the fitting decomposition process, hence the consistent underestimation of stiffness when less data is averaged (0.1 deg normal and 0.1 deg wobble conditions). Moment of inertia was relatively constant, therefore probably viscosity was being overestimated. Some negative stiffness values were obtained. This was probably caused by the natural sway-induced torque modulation overcoming the perturbation-induced torque spike.

To further investigate this matter, I did the analysis of the correlation between the actual torque against the estimated torque (obtained from the model used to calculate KBI, Figure 2.11 and Figure 2.12). A lower correlation would indicate a less reliable estimate of ankle stiffness. I plotted the R^2 value against the number of stimuli datasets used to calculate K. The light brown horizontal line corresponds to an ideal perfect fit, which is equal to 1. The results are shown in the second column of graphs (Figure 2.13, right column). We can see that:

1. For all three conditions, the r-squared value increases with number of stimuli.

2. For 0.1 deg estimates, with >24 stimuli for normal condition and >25 stimuli for wobble condition, the obtained correlation values reached a plateau in which they were consistently similar and closer to 1 (average 0.03% difference from 48 stimuli correlation). For 0.6 deg estimates, with >5 stimuli it was already possible to find consistently similar values (0.01% average difference from 48 stimuli correlation). By combining all these turning points and visually analyzing the graph, I estimated

that >25 stimuli would be enough to obtain a consistent estimate of K.

3. For 0.1 deg estimates (normal and wobble), the average stiffness estimation range when measured with less number of stimuli (1–10 stimuli, first section of the top and bottom left graphs from Figure 2.13) was much lower than the estimate obtained with 48 stimuli. Correlation between actual and estimated torques within this range is also more randomized and more consistently lower than 1, indicating that the lower estimates of stiffness might have been related to the estimated torque obtained from the stiffness calculation being less accurate. This inaccuracy was not as strong when the size of the perturbation applied to the ankles was 0.6 deg amplitude (normal). This indicates that the estimation of stiffness is more dependent on the number of perturbations when the perturbations are small (0.1 deg), but less relevant when the perturbations are larger (0.6 deg).

From this analysis it was concluded that, with the apparatus available, at least 25 perturbations were needed to obtain consistent stiffness estimates. This number was obtained from the correlation analysis between actual and estimated torques in 3 different conditions (0.6 deg normal, 0.1 deg normal and 0.1 deg wobble). The correlation found between actual and estimated torques with the average of 48 datasets was used as the parameter (r=0.999). This was compared with correlations found with a decreasing number of datasets, ranging from 48 to 1 perturbation. In all 3 different conditions, there was a turning point when the correlation reached a plateau and was consistently very close to 1. For 0.6 deg normal condition, more than 5 perturbations were needed to obtain consistent estimates. For 0.1 deg normal, more than 24. And for 0.1 deg wobble, more than 25 perturbation datasets were needed to obtain an average estimate of stiffnes which had a similar correlation with the estimates obtained with 48 perturbations. Therefore in all the experiments presented in this thesis, I have used a minimum of 25 perturbations to estimate stiffness, regardless of perturbation amplitude.

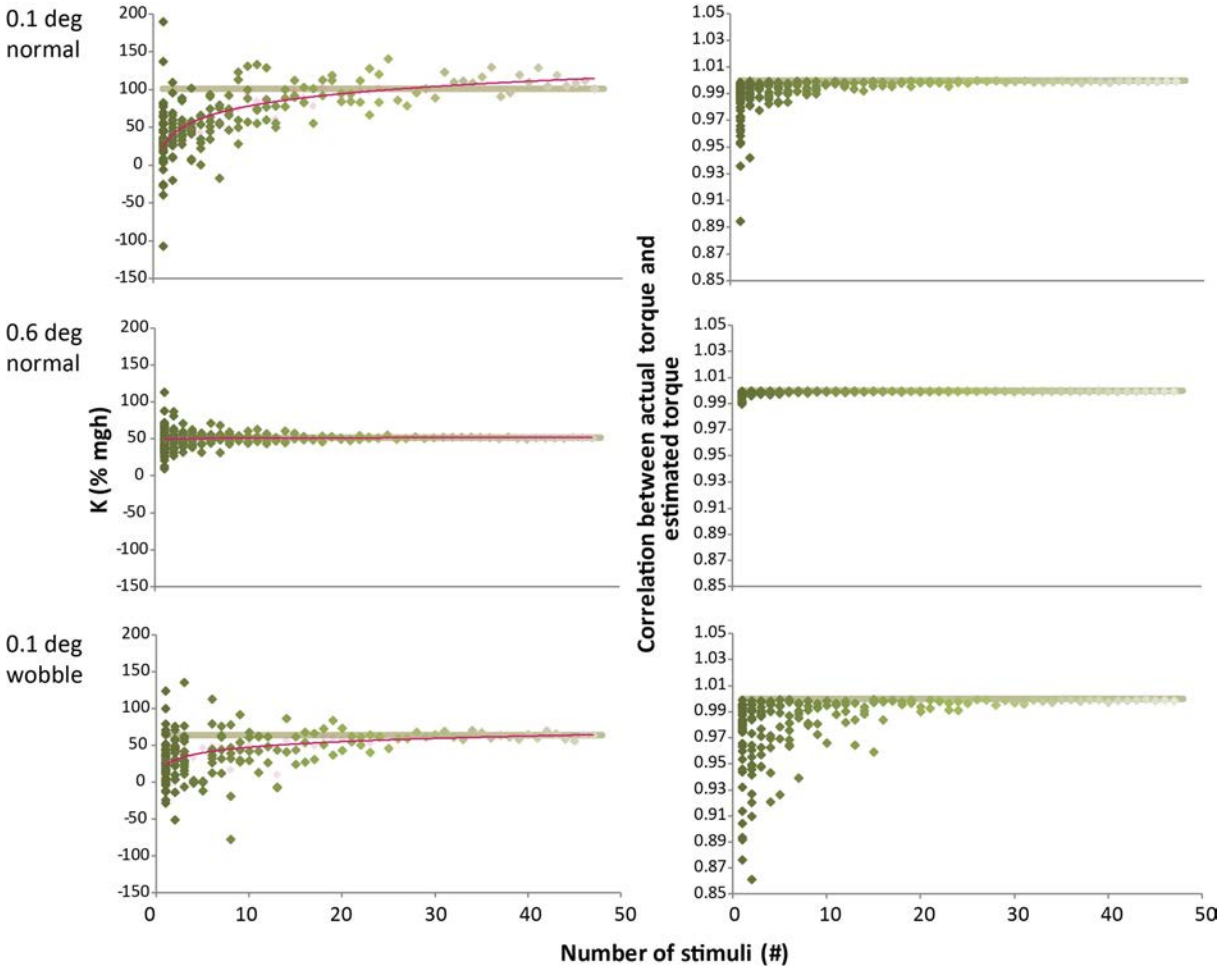


Figure 2.13: Stiffness estimation of one participant in three different conditions (0.1 and 0.6 deg normal and 0.1 deg wobble) with increasing number of perturbations (1 to 48). The horizontal brown line indicates the stiffness estimation with the maximum number of perturbations (48 perturbations, left graphs) and the optimal correlation between actual and estimated torque (=1, right graphs).

2.5.4 Model assessment #3: Contribution of KBI over the time-window used for stiffness estimation

As discussed above, one procedure to verify the validity of the stiffness model is to check the fit between torque and estimated torque ('Model assessment #1: fit between torque and estimated torque'). In this section another method to verify the soundness of the stiffness estimation is presented. For this procedure, the values obtained from the second-order regression equation (Equation 2.5, stiffness model) are monitored through the re-construction of the estimated torque into its K θ, B $\dot{\theta}$ and I $\ddot{\theta}$ components.

More specifically, I first verify if the K θ compound is positive throughout the 70 ms time-window used for the model. This component is stiffness multiplied by ankle position, the latter being the empirical average data of various perturbation trials (minimum 30 datasets). Zero position was set to be the starting point of the perturbation applied by the motor, and the squared-sine shape trajectory was positive for the whole time. Whether or not the ankle position response to the disturbance remained positive, this would depend on the type of perturbation and if its amplitude was enough to produce a significant change in ankle position. In case the perturbation would be inappropriate to induce a unidirectional change in ankle position in the same direction as the footplate, then it would be impossible to identify solely one stiffness constant for that time-window. In this test I verified if the squared-sine type perturbation was appropriate to induce a unidirectional change in ankle position. Also during the whole movement, the ankle was simply stretched and then returned to its original position. Hence the stiffness should likewise be always positive. In short, the K θ compound measured only at the first half of the perturbation should be positive at all times, and by reconstructing the estimated torque into its components I could verify if this was obtained from the estimates. This analysis was intimately related to the results from 'Model assessment #1: fit between torque and estimated torque'. The worse the fit between actual and estimated torques, the higher were the chances for the component K θ to be negative. However, even the worse fits were actually within a range of $r \geq 0.98$, which shows that the estimated torque results were very accurate.

Additionally, I investigate the contribution of the K θ, B $\dot{\theta}$ and I $\ddot{\theta}$ compounds to the estimated torque. This was used to compare two different types of perturbation (squared-sine versus ramp) and verify if the one used in this thesis was appropriate to highlight the elastic properties of the ankles over its viscous and inertial characteristics.

An example is given in Figure 2.14, which shows the instantaneous values obtained from a squared-sine perturbation of 0.6 deg amplitude over a 70 ms time-window (average

of 7 participants). I performed an analysis in which estimates of K θ, B $\dot{\theta}$ and I $\ddot{\theta}$ were integrated over the 70 ms time-window range to calculate their overall proportional relevance within the estimated torque trace. The results are shown in Table 2.1.

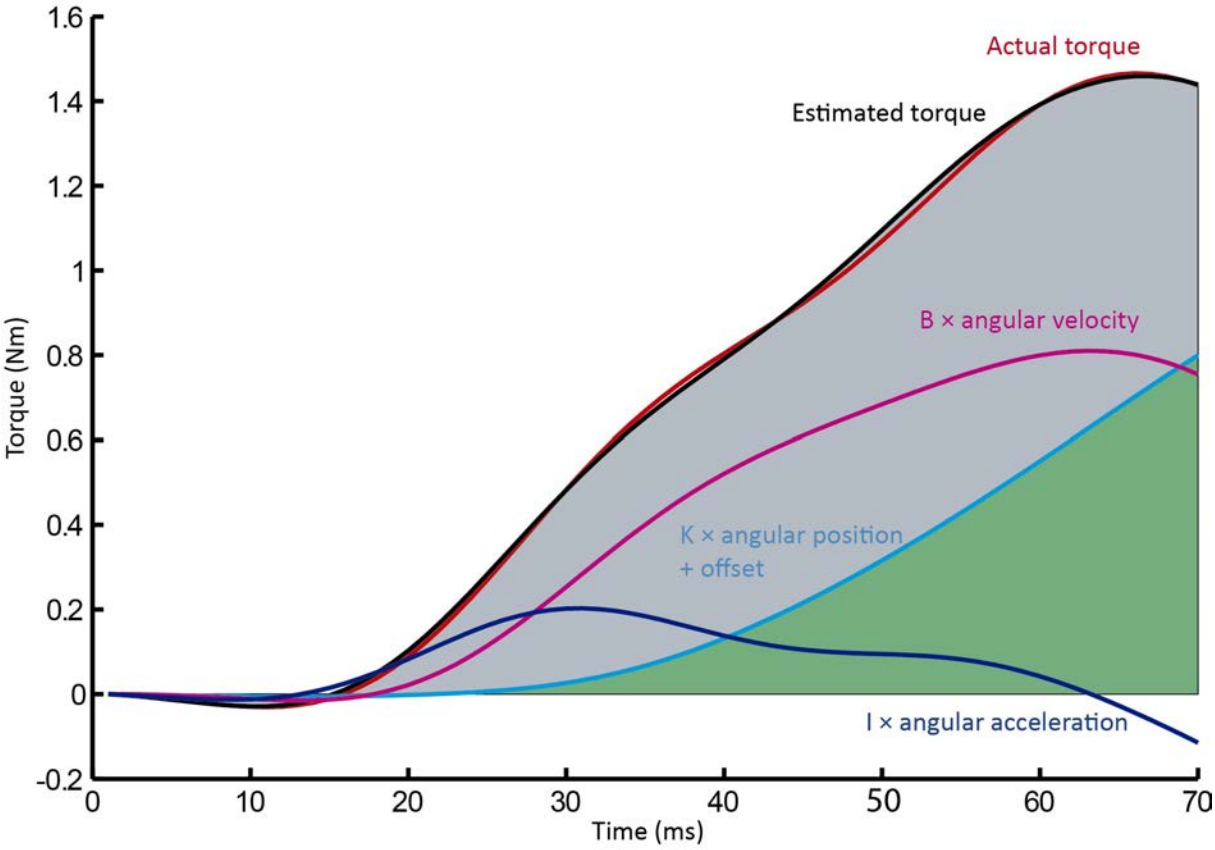


Figure 2.14: Instantaneous torque, estimated torque, K θ, B $\dot{\theta}$ and I $\ddot{\theta}$ over a 70 ms time-window (average of 7 participants for a 0.6 deg squared-sine type perturbation).

	K (% estT)		B (% estT)		I (% estT)	
	ramp	sq-sine	ramp	sq-sine	ramp	sq-sine
0.2 deg	78.7±4.3	50.3±4.7	18.6±4.7	30.2±8.0	2.7±0.8	19.5±4.4
0.3 deg	76.8±4.9	47.3±2.2	20.7±5.6	34.1±5.1	2.6±1.3	18.7±3.5
0.5/0.6 deg	75.9±2.5	42.6±3.7	20.0±3.5	38.9±6.6	4.1±1.1	18.5±3.2
0.8/0.9 deg	75.3±2.1	40.0±5.2	20.0±2.6	39.8±7.6	4.7±1.0	20.2±3.4
Mean± SD	76.6±3.7	45.0±5.6	19.8±4.1	35.7±7.6	3.5±1.4	19.2±3.5

Table 2.1: Comparison between ramp and squared-sine type perturbations.

Overall, K θ is proportionally more relevant than B $\dot{\theta}$ and I $\ddot{\theta}$ (45% against 36% and 19%, respectively, of all 70 ms of the estimated torque trace). There are differences, though, with time. At the beginning of the stimulus (up to 30 ms), most of the torque response is taken by the inertial effects of the initially stagnated foot. The K θ and B $\dot{\theta}$ effects,

on the other hand, increase with torque. B $\dot{\theta}$ becomes less relevant at the peak of the stimulus, probably due to decrease in velocity. Fortunately as desired, K θ increases in a continuous and steady fashion, closely related to the increase in torque response; more importantly, it is positive throughout the whole analysis.

A second example is given in Figure 2.15. Contrary to the example shown above (Figure 2.14), this is the result of only one participant for normal standing while 0.1 deg perturbation amplitude was applied to the footplates. The estimated/actual torque fit had a reasonably high value (r=0.997). However, here we can see a fault in the K estimate because it is negative at the range 0–35 ms and its trace is biphasic, initially decreasing then afterwards increasing. In this case, K θ, B $\dot{\theta}$ and I $\ddot{\theta}$ estimates were outside the scope of the actual torque trace, which was positive the whole time and could not have had a negative stiffness.

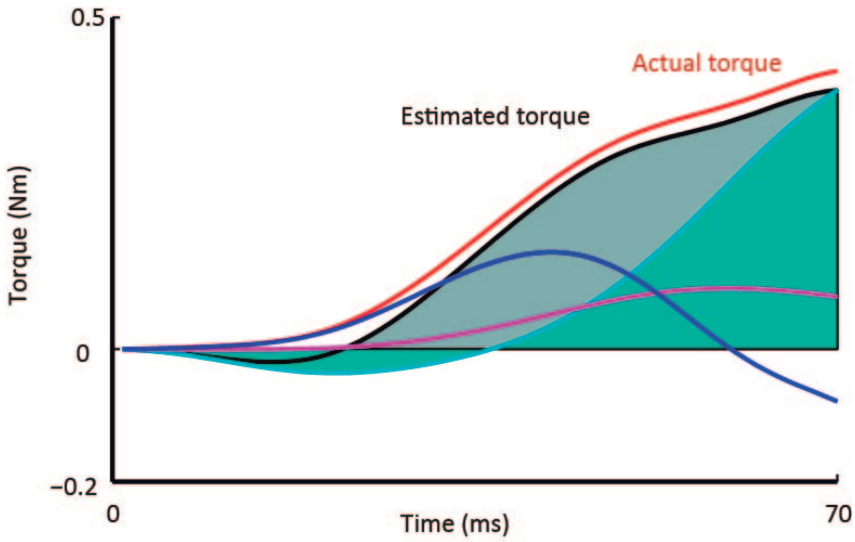


Figure 2.15: Instantaneous torque, estimated torque, K θ, B $\dot{\theta}$ and I $\ddot{\theta}$ over a 70 ms time-window (1 participant data for a 0.1 deg squared-sine type perturbation).

All the data was verified with this analysis. In general, the torque fit was less accurate for smaller perturbations. However, when averaging all the data from all the participants, stiffness was always positive.

I was also interested in verifying if the type of perturbation adopted here was appropriate

for an accurate estimation of stiffness. The concern was that the high changes in angular velocity and acceleration due to the fast perturbations could generate an undesirable high effect of viscosity and moment of inertia on the resultant torque, which could potentially reduce the overall relevance of our stiffness estimates. For example, if the perturbation was excessively abrupt, the inertial effect of the foot could offer an undesirable high resistance to movement and predominate over the stiffness effect on the estimated torque response. Similarly, the viscosity of the ankles could also offer a high resistance to movement at very fast velocity changes that could potentially predominate over the stiffness effect. Thus I conducted an experiment in which stiffness of 7 participants was tested with two different types of perturbation: the squared-sine type perturbation that I used in all the experiments presented in this thesis (<1 deg amplitude perturbation of 140 ms duration, also used by Loram and Lakie (2002*a*), but with a tiny amplitude of 0.055 deg) and a ramp type perturbation used in many studies regarding ankle stiffness (<1 deg amplitude ramp perturbation of 150 ms, comparable to the one used by Casadio et al. (2005)). The squared-sine perturbation was randomized in direction (toes-up or toes-down) and inter-stimulus interval (4–5 s). The ramp perturbation was alternating between toes-up and toes-down at randomized intervals of 2–10 s. Due to its ramp shape, this perturbation was approximately 2 times slower than the squared-sine perturbation, and the velocity was constant throughout. The results are shown in Figure 2.16.

The most remarkable difference between the two different types of perturbation is the smoothness of the torque response. Due to the initial abrupt change in position (higher initial acceleration), the ramp perturbation induces a stronger initial fluctuation of the torque response, which subsides with time. This fluctuation is particularly strong at the first 70 ms, which is the time-window used for my analysis. In general, though, the stiffness and angular position components are proportionally more relevant in the ramp perturbation (77% in ramp type perturbation against 45% in squared sine type, Table 2.1). Velocity is constant and low, therefore mostly irrelevant. The fluctuations of the acceleration response are probably due to the strong effect of the the ramp pertur-

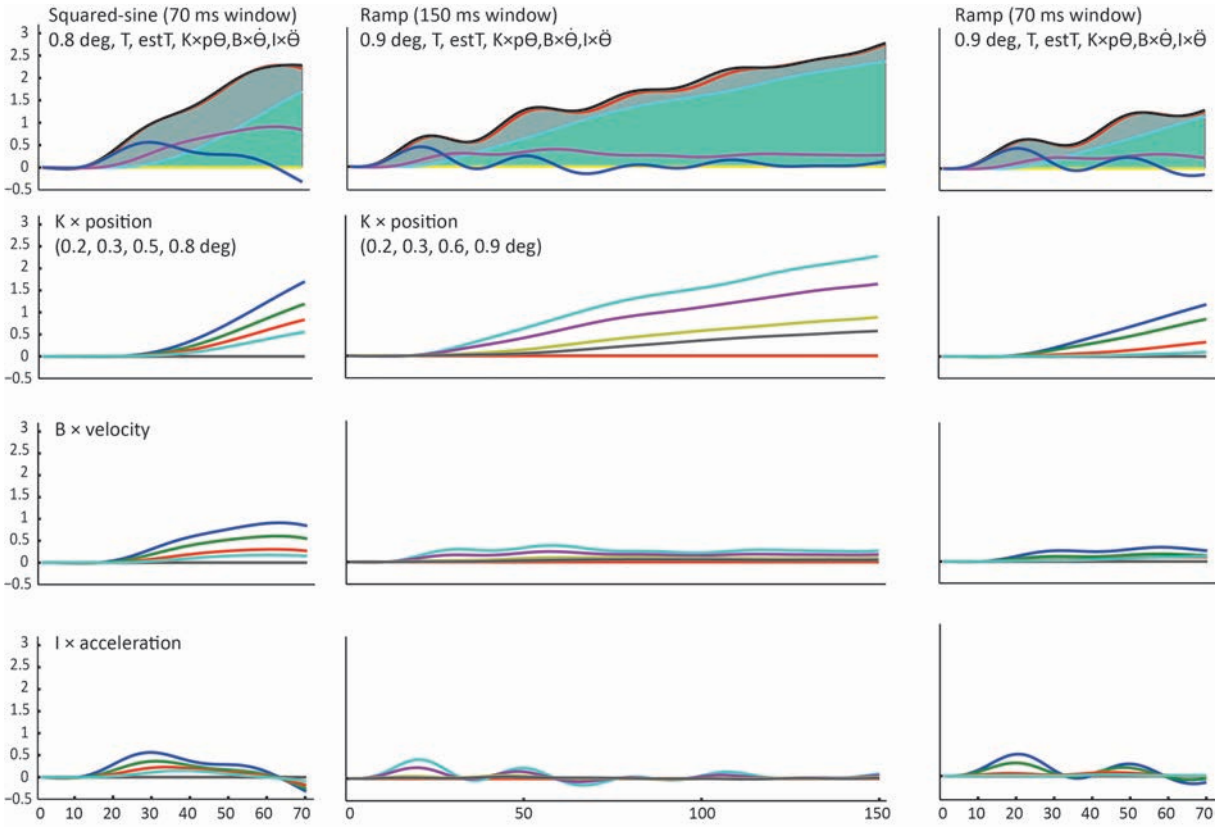


Figure 2.16: Instantaneous torque, estimated torque, K × angular position, B × angular velocity and I × angular acceleration (average data of 7 participants). Three different columns correspond to: squared-sine (over a 70 ms time-window), ramp (150 ms) and ramp (70 ms). 2nd, 3rd and 4th rows correspond to data of different amplitudes. Colours are not organized according to perturbation amplitude. Therefore, in all conditions the highest values correspond to highest stimuli amplitudes.

bation on the stagnated foot, making the body sway multiple times to restore balance. Probably this is the reason why the moment of inertia SD results are slightly higher for this type of perturbation (0.03±0.006 kg m^2, mean±SD, for ramp perturbation versus 0.04±0.003 kg m^2 for squared-sine perturbation). Nonetheless, one-way ANOVA analysis has shown that in both experiments there was no significant difference of moment of inertia within participants when measured at different amplitudes ($F_{3,24}$=1.51; p=.24 for ramp and $F_{3,24}$=0.28; p=.84 for squared-sine). This confirmed that both methods have obtained satisfactorily constant measurements of moment of inertia (Figure 2.17).

Overall, the results indicate that the squared-sine type perturbation induced a smoother initial torque response than the ramp type perturbation. The disadvantages of the ramp

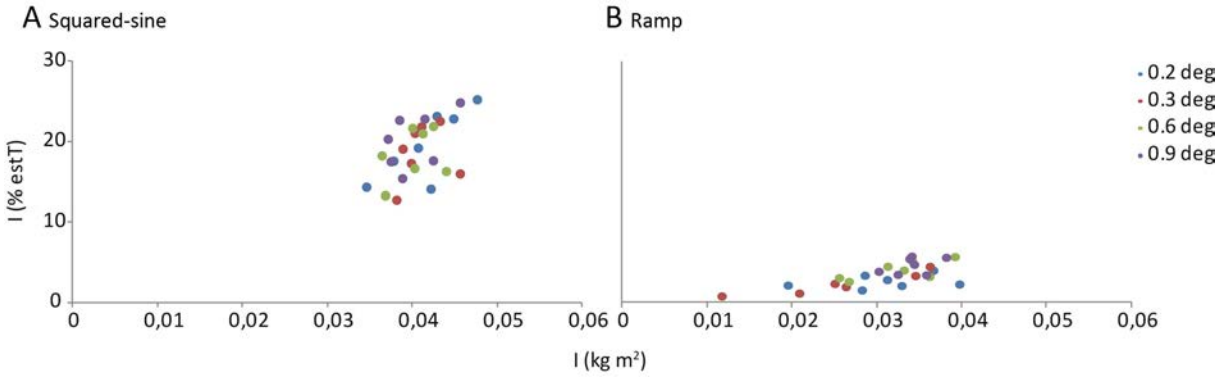


Figure 2.17: Moment of inertia comparison between squared-sine (A) and ramp (B) type perturbations.

perturbation include: a more pronounced disturbance of the body initial position (the perturbation is unidirectional, therefore the initial body position before each perturbation alternates each time) and the reduction of randomization factors (only the amount of interstimulus interval can be randomized, as the direction of the footplate rotation is constrained by the previous perturbation and cannot be randomized).

Hence to minimize the initial abruptness of the torque response and to have the option of randomizing the direction of the perturbation (toes-up or toes-down at randomized order), I have chosen to adopt a squared-sine perturbation in all the experiments performed in this thesis. As shown by the K θ slope analysis, the stiffness estimates obtained by the squared-sine perturbation were mostly positive to a satisfactory level, justifying its use.

2.5.5 Model assessment #4: stiffness measurement comparison with a known spring

The stiffness calculations presented in the previous assessments (#1-3) were all performed in human subjects. Physiological tissues are highly sensitive to different environmental conditions and it is not appropriate to use them as an ultimate reference to confirm the repeatability of an apparatus. To ensure that the mathematical model used here was appropriate to obtain accurate measurements of stiffness that were not affected by

the methodology or the apparatus, I decided to check the repeatability of the model when testing an inanimate object with known stiffness. In other words, I performed the previously described stiffness experiments on a spring with known stiffness and verified if the obtained estimates were similar to the stiffness value provided by the factory.

As described earlier in Sections 2.4.1 and 2.5.1, two methods of stiffness measurement were used for this thesis. For toppling torque per unit angle estimation, stiffness was the coefficient factor of the linear fit between torque and angular rotation (as detailed in section 2.4.1 '1st method: Linear regression of standing sway versus torque'). For the mechanical intrinsic stiffness estimation, stiffness was one of the products of the second-order linear regression of the torque signal (as detailed in section 2.5.1 'Mechanical intrinsic ankle stiffness estimation model'). Both methods were cross checked by testing the stiffness of a known spring (K = 87.56 N mm^{-1}). The spring was compressed against the footplates at different distances from the axis of rotation (Figure 2.18), and stiffness was empirically measured with torque against angular displacement, either by manually applying slow stretches on the footplate or by running a trial of 32 perturbations of 0.1, 0.2, 0.4 and 0.6 deg amplitudes. As the spring stiffness value given by the factory was linear, first I converted it to the angular dimension. This was dependent on the moment arm (r), or the spring placement distance from the footplate axis of rotation. Details of the calculation used to convert from linear to angular stiffness are shown in Figure 2.19.

When comparing the empirical data obtained from the two tests used in this thesis, the results were 99% similar. Nevertheless, similarity of both empirical data from the theoretical data - linear spring stiffness given by the factory (N mm^{-1}) transformed to rotational stiffness (Nm deg^{-1}) - was still very high, but less (91.2%) (Figure 2.20). This could be explained by the deformations occurred when measuring a linear spring with a rotational setup. Error was higher as the spring was compressed further away from the centre of the footplate.

The similarity between the 3 results indicate that both the linear fit method to assess

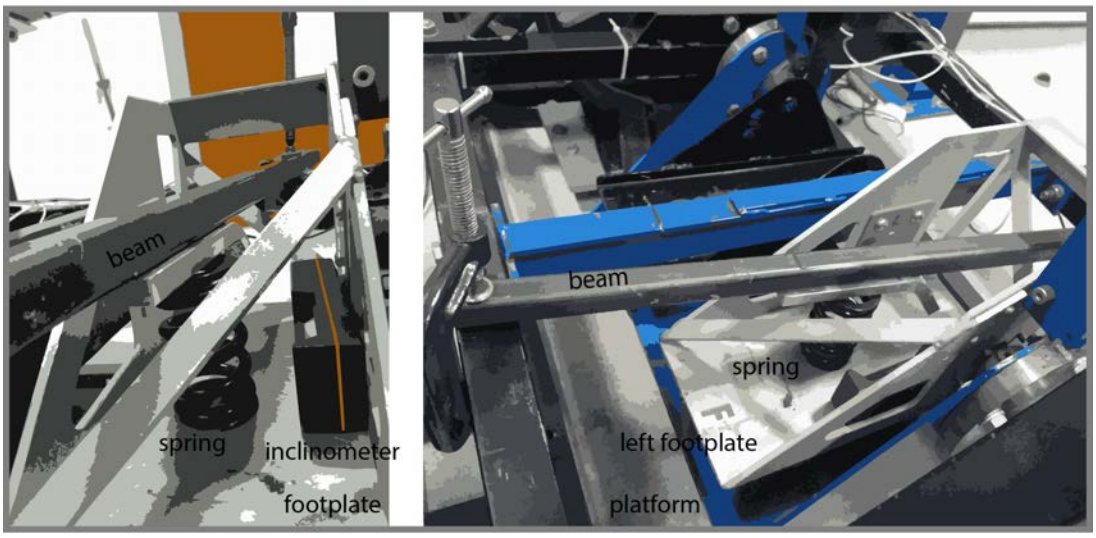


Figure 2.18: Spring with known stiffness attachment to left footplate.

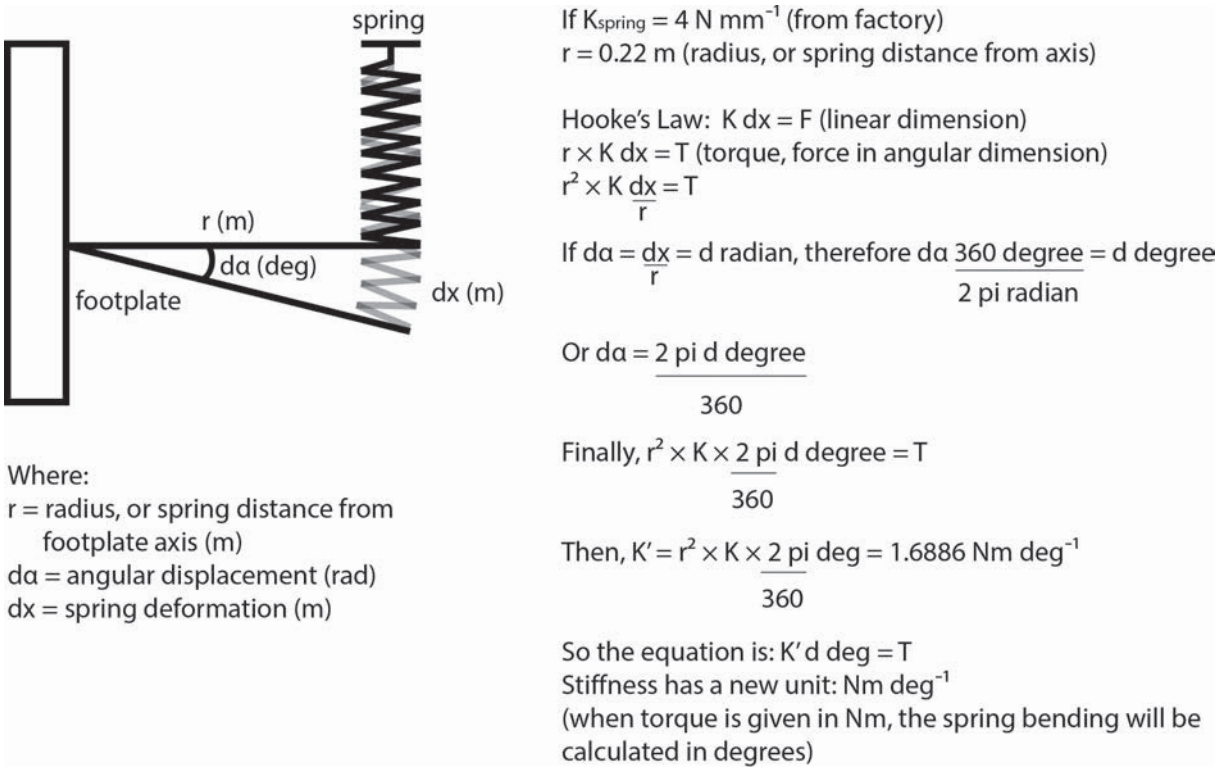


Figure 2.19: Representation of the equation used to calibrate the stiffness of the spring from linear to angular dimension.

toppling torque per unit angle and the multiple linear regression of the torque response model to assess intrinsic stiffness of the ankles were accurate in estimating the stiffness of an inanimate object. This confirms that the apparatus and the mathematical models used to estimate stiffness were appropriate and reliable.

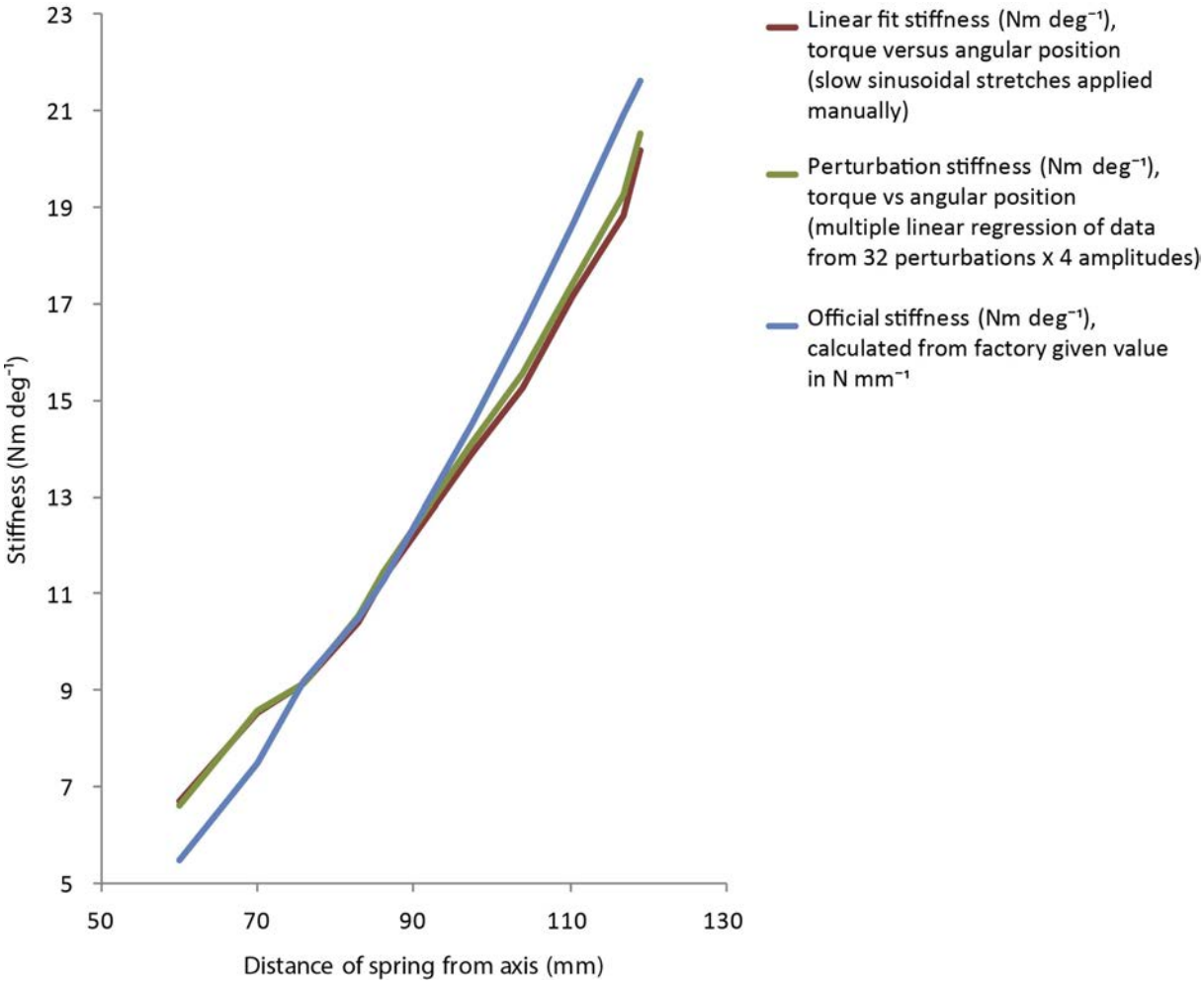


Figure 2.20: Stiffness of a spring (500 lbs in^{-1}) placed at different distances from the footplate axis of rotation. Each data set corresponds to a different stiffness estimation method: blue (official stiffness 500lbs in^{-1}, converted to Nm deg^{-1}); red (stiffness measured with slow sinusoidal sway, as in toppling torque per unit angle measurement); green (stiffness measured with small and brief perturbations - 32 in total, 4 different amplitudes). Each point of each data set represents a different distance from the axis. Stiffness increases as the distance from the axis increases, related to the increase in inertia with increased radius (I=radius (moment arm)^2 $\times$ mass). Values of load stiffness and perturbation stiffness are consistently similar, validating our measurements of stiffness.

2.5.6 Model assessment #5: Within-subject consistency of stiffness estimates

As a final assessment, I compared the intrinsic stiffness measurements of participants who performed more than one experiment. This analysis was important to verify if the estimates were repeatable across experiments, even when they had different setups and

were taken months apart.

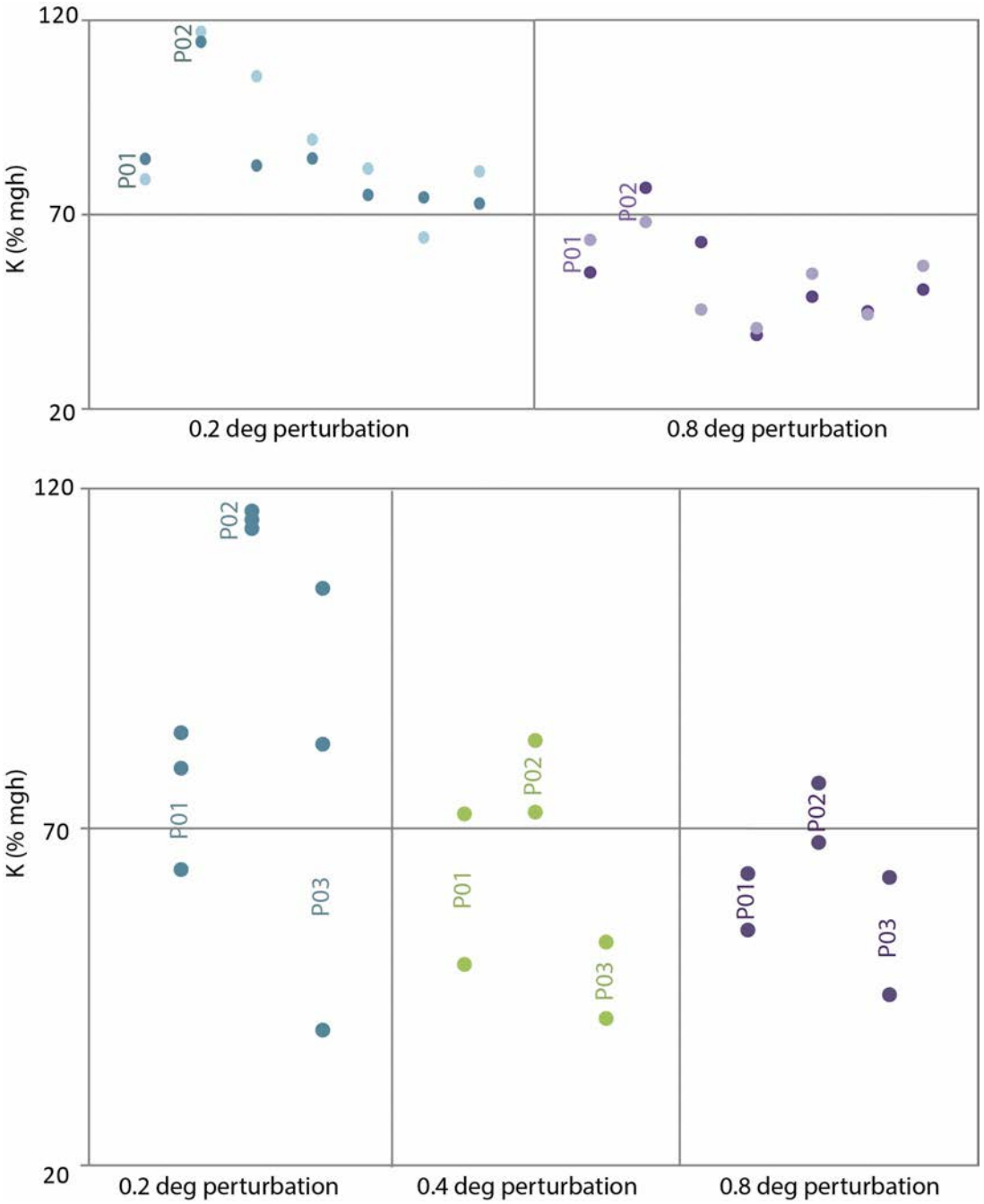


Figure 2.21: Top figures: K estimates of two different experiments with 7 overlapping participants. The time-gap between the two experiments was approximately 1 month. Bottom figure: K estimates of three different experiments with 3 overlapping participants. The time-gap between the three experiments was approximately 1.5 years between 1st and 2nd experiments and 1 month between 2nd and 3rd experiments.

The crossover of participants between the experiments from Chapter 5 (Protocol 1) and Chapter 6 was especially large, 7 people in total. The time gap between the 2 tests was

approximately 1 month. The average values of K (both stimulus amplitude assessments added together, 0.2 and 0.8 deg) in Chapter 6 (71±23 %mgh, meanSD) were only slightly higher than in Chapter 5 (Protocol 1) (69±20 %mgh) (Figure 2.21, top figures). A Pearson's r data analysis revealed a significant correlation between both experiments, r=0.7; p<0.01. This positive correlation result could not be repeated when I compared estimates of 3 participants taken 1.5 years apart from 3 distinct experiments (r=0.5; p=.23). Probably in this case the change in total body weight across the 1.5 year time gap, the setup difference (participants either strapped or not to a free moving board), the small sample size (n=3) all played a part in varying the results. But even though the correlation was not significant, from the graphs we can see that there is a reasonable amount of repeatability (Figure 2.21, bottom figures).

2.6 Summary

In summary, in this chapter I have presented the main custom-built apparatus used by all the experiments performed in this thesis. Additionally, I have shown the various calibration procedures and how the sensors' units were converted from linear to rotational. The multilinear regression model adopted to estimate stiffness was presented, as well as five assessments used to verify the validity of the results produced by it. The results have shown that the model provides highly repeatable and consistent stiffness estimates, confirming that the model is appropriate for the purposes of this thesis. To account for weight and height differences between individuals, stiffness was portrayed as a proportion of the toppling torque per unit angle, which is the minimum amount of ankle stiffness necessary to counteract the gravitational forces acting on the body when at the vertical equilibrium point. Two different methods to assess toppling torque were presented and validated, also confirming its consistency.

Appendix – List of apparatus

ID	Type	Name	Description
1	Motor	XTA3810S	Copley ServoTube Actuator (XTA3810S, Copley Motion Systems LLC, GBR)
2	Motor Controller	XTL-230-18S	Digital Servo Drive/Controller (Copley Xenus XTL-230-18S, Copley Motion Systems LLC, GBR)
3	Load Cell	Sensotec 31	Two horizontally mounted Miniature Load Cells (Sensotec 31, Sensotec Inc., USA)
4	Amplifier	Sensotec UBP	Two-Channel Bridge Amplifier and Low-Pass Filter (Sensotec UBP, Sensotec Inc., USA)
5	Potentiometer	CP-2TU	Rotary Position Sensor (Model CP-2UT, Midori Precisions Co., JPN)
6	Accelerometer	ADXL335	3-Axis±3g Accelerometer (ADXL335, Analog Devices Inc., USA)
7	Data Acquisition Rack	BNC-2090	Rack-Mounted BNC Terminal Block (BNC-2090, National Instruments Corp., USA)
8	Oscilloscope	Nicolet 310	Two-Channel Digital Oscilloscope (Nicolet 310, Nicolet Instrument Corp., USA)
9	EMG equipment	Bagnoli-8	Bagnoli Desktop EMG Systems - 8 Channel Input Module (Bagnoli-8, Delsys Inc., USA)
10	Laser-range sensor 1	YT44MGV80	Laser - Reflex Sensor for Measuring Tasks - 190-390 mm (YT44MGV80, Wenglor, GER)
11	Laser-range sensor 2	YT25MGV80	Laser - Reflex Sensor for Measuring Tasks - 150-250 mm (YT25MGV80, Wenglor, GER)

Table 2.2: List of apparatus.

CHAPTER 3

SWAY-DEPENDENT CHANGES IN STANDING INTRINSIC ANKLE STIFFNESS CAUSED BY MUSCLE THIXOTROPY

Abstract Quiet standing is achieved through a combination of active and passive mechanisms, consisting of neural control and the intrinsic mechanical stiffness of the ankle joint, respectively. The mechanical stiffness is partly determined by the calf muscles. However, the visco-elastic properties of muscle are highly labile, exhibiting strong dependence on movement history. By measuring the effect of sway history upon ankle stiffness, the present study determines whether this lability has consequences for the passive stabilization of human standing. Ten subjects stood quietly on a rotating platform whose axis was collinear with the ankle joint. Ankle sway was increased by slowly tilting this platform in a random fashion, or decreased by fixing the body to a board. Ankle stiffness was measured by using the same platform to simultaneously apply small, brief perturbations (<0.6 deg; 140 ms) at the same time as the resulting torque response was recorded. The results show that increasing sway reduces ankle stiffness by up to 43% compared to the body-fixed condition. Normal quiet stance was associated with intermediate values. The effect was most apparent when using smaller perturbation amplitudes to measure stiffness (0.1 vs. 0.6 deg). Furthermore, torque responses exhibited a biphasic pattern, consisting of an initial steep rise followed by a shallower increase. This transition occurred earlier during increased levels of ankle sway. These results are consistent with a movement-dependent change in intrinsic ankle stiffness caused by thixotropic properties of the calf muscle. The consequence is to place increased reliance upon active neural control during times when increased sway renders ankle stiffness low.

3.1 Introduction

Previous research has confirmed that the intrinsic stiffness of the ankle joint alone is insufficient to stabilize the body (Morasso et al., 1999; Morasso and Schieppati, 1999; Loram and Lakie, 2002*a*). This low stiffness is largely a result of the high compliance of the long Achilles tendon exposed to the relatively low ankle torque involved in quiet stance. Therefore, the passive mechanism must be supplemented by the active mechanism. However, their relative importance differs considerably. Within-subject differences were identified in studies in which human joints were perturbed in various ways (Halaki et al., 2006; Loram et al., 2007*a*). To our knowledge, however, the source and significance of these differences has not been clarified fully. In the present chapter we propose to investigate these within-subject differences.

How might short-term changes in intrinsic ankle stiffness occur within a person? Two of the main contributory structures to intrinsic ankle stiffness during stance are the Achilles tendon and the triceps surae muscles. In quiet standing, where the stretch sizes are normally very small, the muscle is typically ∼15 times stiffer than the tendon (Loram et al., 2007*b*, 2009). Since the two structures are arranged in series, the limiting factor in overall ankle stiffness is therefore normally the tendon. This assumes no significant changes in stiffness over time. However, although tendon stiffness changes relatively slightly and slowly, the mechanical properties of muscle tissue are highly labile. When a relaxed muscle fibre is stretched or shortened, there is an initial period of relatively high resistance, termed the short-range elastic component (SREC, described in detail in Chapter 1, Section 3.2) (Hill, 1968). It is important to emphasize that this phenomenon is dependent on two factors: displacement amplitude and history of movement. In other words, there is a temporary reduction in muscle stiffness caused by (1) movement, (2) with recovery at rest, and this latter phenomenon is known as muscle thixotropy (Denny-Brown, 1929; Hill, 1968; Lakie and Robson, 1990; Warner and Wiegner, 1990; Whitehead et al., 2001). Large limb movements encounter less stiffness than small ones over a range of

background muscle activations (Rack and Westbury, 1974; Halaki et al., 2006). Moreover, after large joint limb movements, this reduction in stiffness persists for a short time, recovering rapidly if the system is left still (Lakie et al., 1984; Proske et al., 1993; Axelson and Hagbarth, 2001; Reynolds and Lakie, 2010).

This raises the likelihood that the intrinsic ankle stiffness in standing individuals, highly dependent on the muscle properties, is also affected by the transient characteristics of its short-range stiffness. Loram et al. (2007*a*) previously investigated the effect of amplitude in standing individuals and reported that ankle stiffness is indeed less for larger movements (see also: Kearney and Hunter 1982; Vlutters et al. 2015). To our knowledge, however, the effects of thixotropy in maintaining posture are yet to be investigated. We speculated that intrinsic ankle stiffness would be greater when measured with small perturbations only when the system was moving minimally, sway size was small, and there was an opportunity for stiffness recovery. When there is an increased amount of baseline body sway there would be negligible recovery of stiffness and ankle stiffness would be less for all sizes of perturbation. In the present study, we test this hypothesis by manipulating sway size, or ankle motion, in three standing conditions. First, we study normal quiet stance. Then, we use a rotating platform, whose axis is collinear with the ankle joint, to increase sway size. Lastly, we strap the body to a stationary backboard to minimize sway. Intrinsic ankle stiffness is measured in all three situations by applying small (<0.6 degree) and brief (<140 ms) perturbations using the rotating platform. In addition to changing the history of movement to measure the thixotropic aspect, we also change the amplitude of stimuli to assess stiffness, over a range from 0.1–0.6 degree. By clarifying whether the intrinsic stiffness of the ankles is simultaneously dependent on these two independent factors, we can then confirm that the changes within subjects, in quiet standing, are a result of the intrinsic mechanical properties specific to the short-range stiffness of the muscle. The implication of an ankle stiffness that depends on the history of movement is that the demand for neural intervention to stabilize standing will not be constant but, instead, will vary continuously. It will be minor when sway size is small and intrinsic

stiffness is high. By contrast, it will be disproportionately greater when there is a history of large sway size and intrinsic stiffness is reduced. This means that the minimization of neural effort is assured by keeping sway size small. Conversely, large sways can produce a decrease in stability and will require considerable neural intervention (Sozzi et al., 2013).

3.2 Methods

Participants

Ten healthy subjects (two female, eight male; age 30.9±11.6 years (mean±SD); height 1.7±0.1 m; weight 71.6±12.0 kg) were recruited for this non-invasive experiment (Table 3.1). All provided their written informed consent to the experimental procedures, which were approved by the local human ethics committee at the University of Birmingham and conformed to the principles of the *Declaration of Helsinki*.

Participant	Sex	Age (yrs)	Weight (kg)	Height (m)	Toppling torque per unit angle (Nm deg^{-1})
P01	M	21	57.4	1.67	7.71
P02	F	35	57.9	1.57	8.28
P03	M	23	70.7	1.81	9.81
P04	M	21	71.3	1.82	12.71
P05	M	30	79.9	1.82	13.53
P06	M	28	60.8	1.75	10.27
P07	M	29	78.4	1.80	12.21
P08	F	25	64.1	1.59	11.51
P09	M	60	94.8	1.85	16.75
P10	M	37	80.7	1.84	11.76
Mean±SE	F(2),M(8)	30.9±12	71.6±12	1.75±0.1	11.4±3

Table 3.1: Participant anthropometric data.

Procedure and Apparatus

A full description of the footplate apparatus used to measure ankle stiffness as well as its estimation calculations was given in Chapter 2. In brief, the participants were asked

to stand on top of motorized footplates, coaxially aligned with their ankles, while ankle torque, ankle angular position, footplate acceleration and lower limb EMG responses were being recorded. The methodology specific to the present study is described below.

Small perturbations were applied with a variable gap of 4–5 s during trials of standing, which lasted for ∼3 minutes. Between each trial, subjects were given ∼1 min of rest, when movement was allowed. The perturbation consisted of a 7 Hz squared sine wave. Because ankle stiffness has previously been shown to depend on stimulus amplitude (Hufschmidt and Schwaller, 1987; Kearney and Hunter, 1982; Loram et al., 2007*a*), we applied four different rotation sizes of 0.1, 0.2, 0.4 and 0.6 degrees, intended to span the range of the muscle short-range elastic component (Casadio et al., 2005; Hunter and Kearney, 1982; Loram and Lakie, 2002*a*; Mirbagheri et al., 2001). The smallest perturbation (0.1 deg) was determined by the capability of our apparatus. Stimulus amplitude and direction (toes-up or toes-down) were randomized. Each subject was tested within a single session of ∼2 h, including set-up time and breaks.

To determine how baseline motion of the ankle joint would affect its stiffness we artificially manipulated the degree of ankle movement in the following three ways:

1. Normal: participants were standing freely;

2. Board: participants were strapped to a fixed vertical body support, minimizing body (and therefore ankle) movement;

3. Wobble: participants were standing freely at the same time as the footplates were continuously rotated by a randomly-varying waveform, generated by applying a 1 Hz low-pass filter to white noise. The root-mean-square amplitude of the waveform was 0.6±0.02 deg (mean±SD). This was enough to increase ankle movement without endangering balance, and no subject found this condition to be challenging in the least. The stiffness measuring perturbations were summed with this waveform.

The three conditions of baseline ankle movement (normal, board, wobble) combined with the 4 different perturbation sizes (0.1, 0.2, 0.4, 0.6 deg) resulted in a total of 12 conditions. Forty-eight perturbations were applied per condition, resulting in a total of 576 for each participant. Normal and wobble condition trials were randomized and investigated prior to board condition trials. For each standing condition, both perturbation amplitude and direction were randomized.

Data Analysis

Determination of baseline ankle sway, ankle torque and EMG activity

Our primary aim was to determine how prior ankle movement affects ankle stiffness in standing. Ankle movement was quantified as the root-mean-square ankle position over a two second time window prior to the onset of each stiffness-measuring perturbation.

Previous research also shows that ankle joint stiffness increases as a function of ankle torque (Casadio et al., 2005; Hunter and Kearney, 1982). Therefore we also measured mean ankle torque during a 70ms time window immediately prior to each perturbation.

EMG activity was rectified for analysis. A comparison of its average activity within a 70 ms time window prior to and post perturbation onset was used to verify if the time window used for the stiffness estimate was being affected by active modulation.

Statistical analysis

Repeated-measures ANOVA was used to determine effects of condition (wobble, normal, board) and stimulus amplitude (0.1, 0.2, 0.4, 0.6 degrees) upon ankle stiffness. Pearson's correlation was used to investigate the relationship between baseline ankle torque and

stiffness. P<.05 was considered statistically significant for all tests.

3.3 Results

Ankle movement

Figure 3.1 shows representative data for all three conditions: board (Figure 3.1, A), normal (Figure 3.1, B) and wobble (Figure 3.1, C). Within the 5.4 s period shown for each condition, two ankle perturbations can be identified. The traces illustrate the wide range of spontaneous ankle movement and torque observed across conditions; the average baseline results are summarized in the bar graphs (Figure 3.1, D and Figure 3.1, E). Although footplate rotation induced by the perturbation was identical between board and normal, baseline ankle movement and torque was greater for the latter. The minor fluctuations that occurred in the board condition represented the limitations of our ability to immobilize the subject. During the wobble condition, when a randomized waveform was applied to the footplates, ankle motion was inevitably and intentionally much greater, as was the intention. Mean pre-stimulus ankle movement exhibited a significant difference between conditions, approximately doubling in value between board and normal, with a much larger increase again for wobble ($F_{2,18}$ = 82.5; p<.001) (Figure 3.1, D). This confirmed that our interventions were successful in manipulating the degree of baseline ankle motion prior to each stiffness-measuring perturbation.

Intrinsic ankle stiffness, viscosity and inertia

There was no effect of perturbation direction (toes-up vs. toes-down) upon stiffness, viscosity or inertia ($F_{1,9}$=1.1; p=.32). Both directions were therefore combined for all

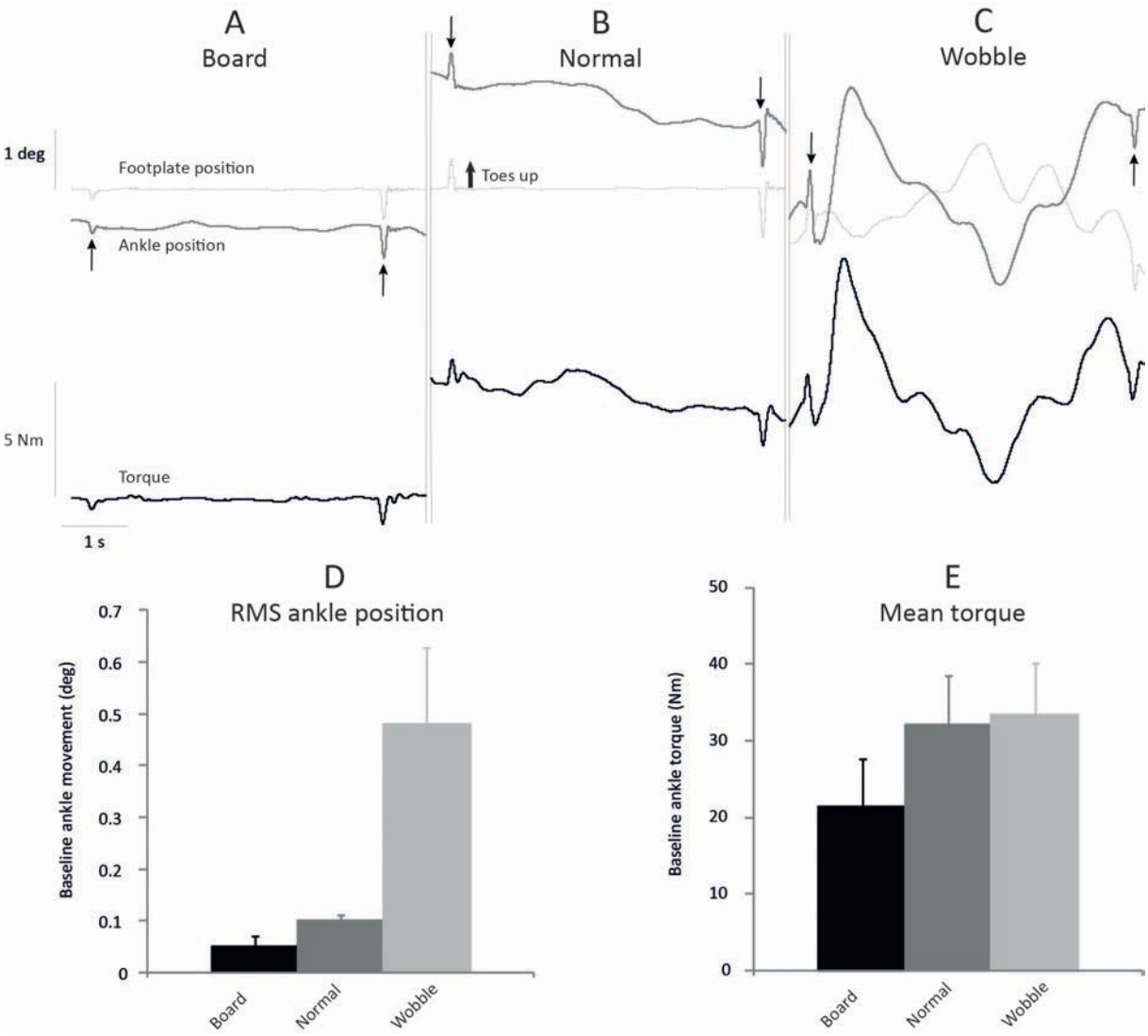


Figure 3.1: Effect of sway condition upon ankle angle and torque. Illustrative segment of the footplate position, ankle position and torque during board (A), normal (B) and wobble (C) conditions. The perturbations were randomized in amplitude, direction (toes up and toes down) and interval (4–5 s). The horizontal line at the bottom of (A) represents 9 Nm for all torque traces. Root-mean-square (RMS) ankle position during a 2 s pre-stimulus time window is shown in (D) (mean±SD). Mean ankle torque during a 70 ms pre-stimulus time window is shown in (E) (mean±SD).

further analysis. Figure 3.2 A–C depicts mean ankle stiffness, viscosity and inertia for all conditions and perturbation amplitudes. The estimated inertia of the combined foot and footplate remained similar across perturbation amplitudes ($F_{3,27}$=2.9; p=.055). However, there was a significant influence of condition upon inertia, reflecting slightly higher values with increasing baseline ankle movement ($F_{2,18}$=3.92; p=.039). Viscosity increased with

perturbation amplitude ($F_{3,27}$=23.8; p<.001), and became larger with increasing ankle movement (condition effect: $F_{2,18}$=25.5; p<.001). Crucially, neither inertia, nor viscosity exhibited an interaction between amplitude and condition (p>0.11), in contrast to ankle stiffness, which is reported below.

Because we were primarily interested in estimating intrinsic ankle stiffness, we first needed to exclude the possibility of an active contribution to the ankle torque as a result of the perturbation (e.g. stretch reflexes). We therefore compared mean EMG activity of the lateral gastrocnemius between 70 ms time windows pre– and post-stimulus but found no significant difference (pre vs. post; $F_{1,9}$=0.4; p=.54). Figure 3.2 C depicts mean ankle stiffness for all conditions and perturbation amplitudes, presented here as a percentage of toppling torque per unit angle ('% mgh'). Values ranged between 31% and 78% mgh. For both the normal and board conditions, there was a systematic nonlinear reduction in ankle stiffness with increasing perturbation amplitude. This effect was absent for the wobble condition, where stiffness was relatively low, and remained low (31-49% mgh) across all amplitudes. These observations are confirmed by a significant interaction between condition and amplitude ($F_{6,54}$=7.6; p<.001). This effect of wobble is consistent with our hypothesized effect of prior muscle movement upon joint stiffness. Contrary to our hypothesis, however, stiffness was slightly but significantly lower in board compared to normal condition, across all perturbation amplitudes. We speculated that changes in baseline torque between conditions may underlie the difference.

Intrinsic ankle stiffness normalized against baseline torque

To test this speculation, we compare baseline torque during a 70 ms pre-stimulus window (Figure 3.1, E). Values were almost identical between normal and wobble (∼33 Nm), but were ∼36% less for board ($F_{2,18}$=16.4; p<.001). We then verified the correlation between torque and stiffness. We restricted this analysis to the wobble condition because it was the

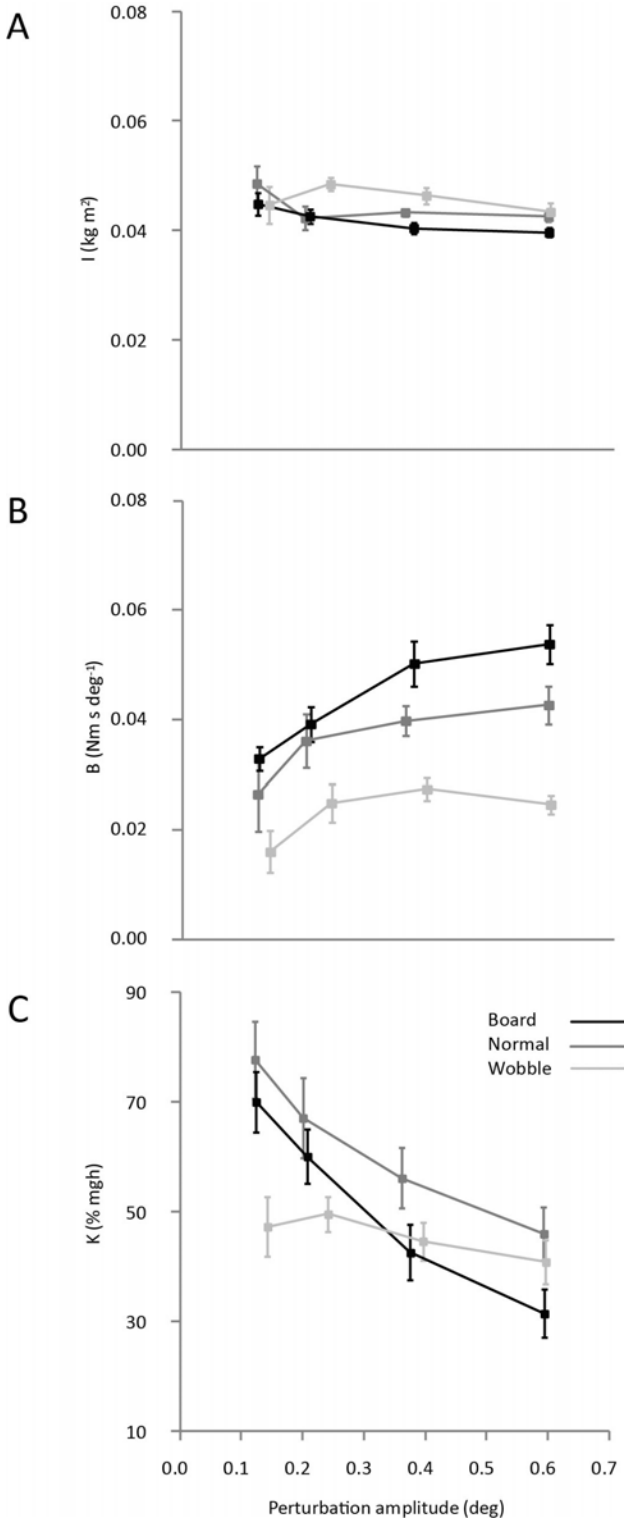


Figure 3.2: Ankle stiffness, viscosity and inertia for all conditions and perturbation amplitudes. Ankle inertia (I), viscosity (B) and stiffness (K) (mean±SEM). Values of inertia and viscosity are for one ankle only. Stiffness values have been multiplied by two to account for both legs, and are expressed as a percentage of gravitational toppling torque (mgh).

only one in which stiffness was not affected by perturbation amplitude. Figure 3.3 shows a significant positive correlation of torque (Nm) against absolute stiffness (Nm deg^{-1}) ($r=0.67$; $p<.001$). We therefore normalized stiffness values by dividing them by baseline ankle torque. The result is shown in Figure 3.4. After this normalization procedure, the non-linear qualitative shape of the board and normal results remains the same, but now stiffness is highest during the condition with least ankle movement (board). Furthermore, at the highest perturbation amplitude, stiffness converges towards the same value for all conditions. A combined view of the two factors influencing ankle stiffness is shown three-dimensionally in Figure 3.5. The cubic spline interpolation shows that stiffness decreases as a function of both prior ankle movement and perturbation amplitude.

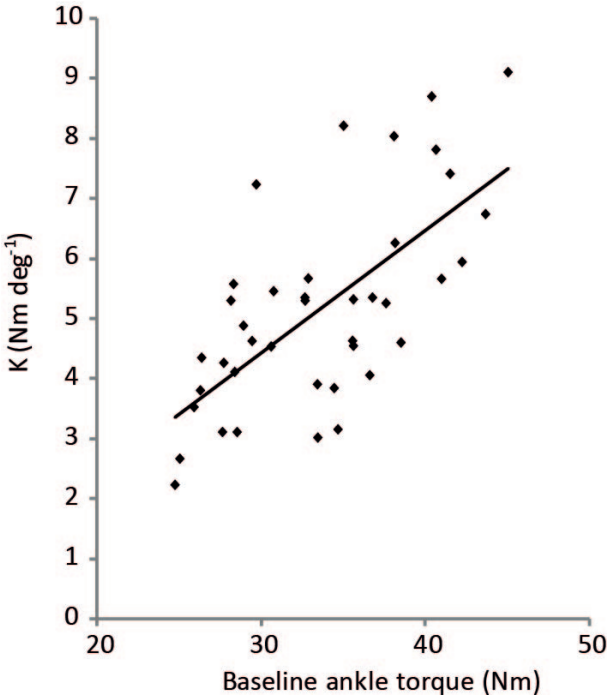


Figure 3.3: Effect of baseline ankle torque upon intrinsic stiffness. The relationship between pre-perturbation torque and intrinsic ankle stiffness is presented for all amplitudes during the wobble condition. Data from board and normal are not included because they exhibited an additional significant effect of perturbation amplitude upon stiffness.

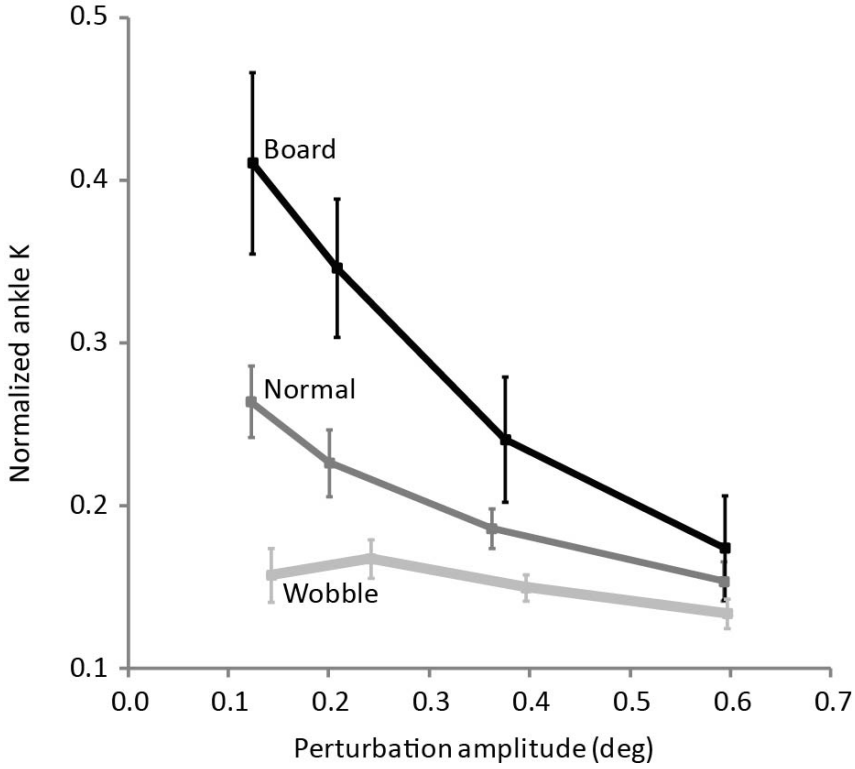


Figure 3.4: Normalized intrinsic ankle stiffness. Absolute intrinsic ankle stiffness was divided by mean torque during a 70 ms pre-stimulus time window torque to obtain normalized values (mean±SEM).

Identifying the short-range stiffness component

To identify changes in stiffness throughout the time course of each perturbation, we examined the relationship between ankle torque and position during the first 70 ms of each stimulus. Figure 3.6 shows the results for normal and wobble conditions for all perturbation amplitudes (the board condition presented similar results; however, it was not included here because the baseline ankle torque was lower, precluding direct comparison). The gradient between torque and angle is a function of stiffness. An initial steep rise in torque can be seen at the onset of ankle movement for all conditions, consistent with the short-range muscle stiffness component. This is followed by a much shallower rise in torque for the remainder of the perturbation. For all perturbation amplitudes, the transition between these two phases occurs at a lower torque and amplitude during the wobble condition.

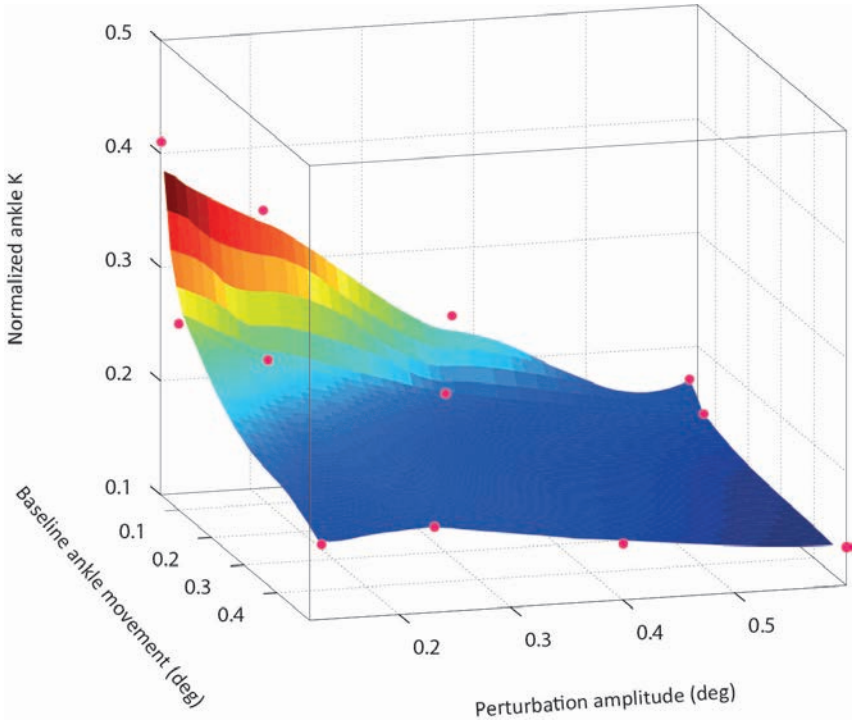


Figure 3.5: The influence of baseline movement and perturbation amplitude upon intrinsic ankle stiffness. Mean data are shown along with a cubic spline three-dimensional interpolation.

3.4 Discussion

A intrinsic ankle stiffness value equating to 100% of the body's gravitational toppling torque would be enough to stabilize the body, assuming a sway frequency of zero. However, previous research suggests that, for empirically-observed sway frequencies of ∼0.5 Hz, this value would need to be ∼200% to completely stabilize the body through passive means alone (first proposed by Winter et al. 1998, followed by Morasso et al. 1999; Morasso and Schieppati 1999; Lakie et al. 2003. In agreement with others, our estimates of K were well below this value, ranging between 31% and 78% of mgh (91% in Loram and Lakie (2002*a*) and 64% in Casadio et al. (2005). This confirms that intrinsic stiffness alone is insufficient for even minimal stabilization in standing, and suggests that active mechanisms must modulate ankle torque by changing calf muscle activity. Nevertheless, it is clear that the passive mechanism does contribute to balance, and previous results demonstrate considerable variation between people. The present study aimed to determine

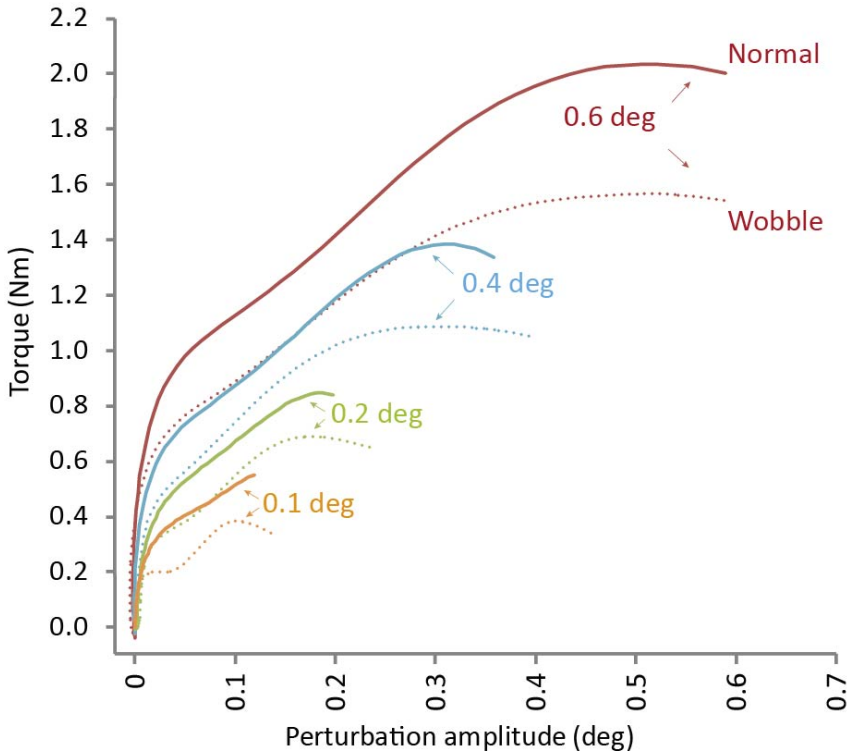


Figure 3.6: Torque-angle relationship during each perturbation. Data are shown for normal (continuous) and wobble (dotted) conditions for all perturbation amplitudes.

how it may change within a standing subject as a result of changes in ankle joint motion with baseline sway. Our results demonstrate significant changes in ankle stiffness within the same person depending upon their baseline sway. This suggests that the relative contribution of the active and passive mechanisms to balance changes over time depending on circumstances.

In addition to manipulating the level of baseline ankle sway, we also measured stiffness across a range of stimulus amplitudes. For both the normal and board conditions, there was a non-linear reduction in K with increasing stimulus amplitude (78–46% and 70–31% mgh, respectively). Such amplitude-dependence has previously been demonstrated in both the wrist and ankle joints (Halaki et al., 2006; Kearney and Hunter, 1982; Loram et al., 2007*a*,*b*; Vlutters et al., 2015), and this disproportionately high passive resistance to the initial stages of imposed stretch has been attributed to the 'short-range stiffness' of muscle tissue (Rack and Westbury, 1974). Taking into account this amplitude-dependence,

the range of K we observed in the board and normal conditions is in good agreement with previous findings. Although, when measuring K in individuals attached to a board, Loram and Lakie (2002*a*) observed a K larger than the largest estimate seen here (91% vs. 70%). Their perturbation was around half the magnitude of our smallest perturbation (0.055 vs. 0.1 deg). The results from Loram et al. (2007*a*), also obtained with participants attached to a board, are more similar, especially for short slow stretches. Even though Loram et al. (2007*a*) used repetitive contiguous triangular-shaped stimuli, which potentially diminished the thixotropic effect on stiffness, they predicted values of 67–54% for 0.15–0.4 deg perturbations, which are very similar to our estimates of 70%, 60% and 42% for 0.1, 0.2 and 0.4 deg perturbations. When calculating K using long slow continuous stretches, they estimated 30–40% for 1 deg perturbations, as opposed to our estimates of 31% for 0.6 deg. In their study, the stimulus velocity was much lower than the velocity used here ($\sim$0.35 vs. 5–22 deg s^{-1}), suggesting that amplitude is the key stimulus property affecting estimates of K.

The main objective of our study was to determine whether ankle stiffness is altered by the magnitude of baseline sway around the ankle joint. We hypothesised that K would be inversely related to ankle sway. This hypothesis is based upon the well-established thixotropic property of muscle tissue (Axelson and Hagbarth, 2001; Buchthal and Kaiser, 1951; Campbell and Lakie, 1998; Hufschmidt and Schwaller, 1987; Lakie et al., 1984; Proske et al., 1993; Reynolds and Lakie, 2010; Whitehead et al., 2001). Specifically, it has been shown that short-range stiffness is significantly reduced following muscle movement but progressively recovers if muscle movement is minimized for some time. Hence, the immediate history of calf muscle motion would also be expected to influence overall standing ankle stiffness. In the present study, we manipulated the degree of calf muscle motion by changing ankle motion across stance conditions (board, quiet, wobble). The fast brief perturbations we used to estimate stiffness might also be expected to affect stiffness by themselves. However, previous research suggests that the thixotropic time constant of the ankle joint (i.e. the time taken to recover most of the stiffness) is $\sim$4 s

(Hufschmidt and Schwaller, 1987). By adopting an inter-stimulus interval of 4–5 s, we therefore allowed sufficient time for the ankle musculature to recover the majority of its resting stiffness between perturbations. More importantly, the interstimulus interval was identical between the three stance conditions. Root-mean-square ankle movement became progressively larger from board to normal to wobble, confirming that our interventions were successful in manipulating baseline ankle sway. In confirmation of our hypothesis, the condition with the highest degree of ankle motion (wobble) exhibited the lowest stiffness, being 41–49% for all perturbation amplitudes. Board and normal exhibited higher stiffness, although this was only apparent at the lowest stimulus amplitude. As the amplitude increased, stiffness values tended to converge towards a low value for all three conditions. This caused a statistical interaction between condition and stimulus amplitude, which can be explained by taking into account the short-range stiffness of muscle described above. At the largest perturbation amplitude, the muscle is stretched beyond its short-range threshold, becoming much less stiff. The large perturbation will therefore tend to be dominated by the lowest stiffness the muscle can achieve, producing a floor effect for all conditions. This agrees with the findings of Loram et al. (2007*a*), who used ultrasound to track the origin of stiffness changes with increasing amplitude. With small perturbations, they observed minimal muscle movement for a given ankle rotation. As amplitude increased, muscle movement became disproportionately larger. Loram et al. (2007*a*) concluded that small perturbations mostly stretch the Achilles tendon because the muscle is much stiffer. As amplitude increases, the muscle is stretched beyond the short-range stiffness, producing a profound fall in overall ankle stiffness and a greater degree of muscle movement. Figure 3.6 comprises a visual representation of this phenomenon. The gradient between torque and angle varies as a function of stiffness, and the time period is the same as used for the stiffness estimation procedure (70 ms). In all conditions, the most prominent change in the steepness of the slope, which is present at a very early phase, marks the transition between short and long range stiffness. The overall stiffness is a composite of these two phases. During the initial phase, the muscle

moves less and the tendon stretches most. Torque then rises less rapidly as the muscle is stretched beyond this point and the stiffness of the contractile component is dramatically reduced. The greater the proportion of the initial steep rise in the overall torque curve, the higher the overall stiffness of the ankle joint. For all perturbation amplitudes, this initial steep rise is consistently lower in wobble when compared to normal condition, showing that the relatively low stiffness found in the wobble condition can be related to reduction in the range of the short-range stiffness. Furthermore, for small amplitudes, the initial rise in stiffness is proportionally more representative of the overall stiffness. This explains the higher stiffness values found when the ankle is moved by a small amount.

The results did not completely agree with our hypothesis, at least initially. We expected to see higher stiffness for board compared to normal, but saw the opposite across all amplitudes. This raises the issue of an additional parameter known to affect ankle stiffness, namely torque. As the muscle generates progressively more torque, more cross-bridges form, increasing muscle stiffness and the resistance to an imposed perturbation. Hence, estimates of stiffness will depend upon the contractile state of the muscle. This was demonstrated by (Hunter and Kearney, 1982; Mirbagheri et al., 2001; Weiss et al., 1986*b*) who found that the non-linearities of ankle stiffness were dependent not only upon displacement amplitude, but also in variations of ankle torque. We confirmed this effect in our data (Figure 3.2) and, furthermore, found a significant difference in baseline ankle torque between conditions. The board condition exhibited ∼36% less torque than normal and wobble conditions, which were similar to each other (mean±SD: 21.5±7.5 Nm vs. 33.4±6.7 Nm and 33.6±5.8 Nm). This would explain the consistently lower values of stiffness in board compared to normal condition, across all amplitudes. It also suggests that in leaning forward, when more torque is required, stiffness will increase, potentially increasing stability. This is a possible reason for not standing strictly at the vertical equilibrium point. However, other reasons might include minimizing the range of backward COP movement and/or involvement of the dorsal flexors. After factoring out differences in baseline torque, the data fully confirmed our hypothesis (Figure 3.4 and Figure 3.5).

The board condition exhibited the highest (normalized) stiffness, followed by normal then wobble. As stimulus amplitude increased, the difference between conditions progressively reduced, reaching a floor value at the highest amplitude (0.6 degrees). Figure 3.3 shows that, as baseline torque increased from 25 to 45 Nm, stiffness increased from 3 to 7 Nm deg^{-1}. The maximal effect of stance condition was to increase stiffness from 0.15 to 0.4 (normalized K) at the largest amplitude (Figure 3.4). Hence, the thixotropic effect upon stiffness was considerable.

The potential consequence of reduced ankle stiffness is to increase reliance upon active neural intervention to maintain balance. This would not only involve more torque modulation, but also faster modulation. Loram et al. (2007*a*) explained the importance of increased intrinsic stiffness in raising the time constant of the unstable, inverted pendulum-like, body. An increased time constant decreases the acceleration of the toppling body and in effect 'buys time' for the nervous system to act. The relevant equation for the time constant (τ) is:

$$\tau = \sqrt{\frac{I}{mgh\ (1-c)}} \tag{3.1}$$

Where c is normalized stiffness, I is moment of inertia, m is mass, h is height and g is acceleration as a result of gravity. If the moment of inertia is written as $I = kmh^2$, where k is a shape factor of value ~1.3 (Morasso and Sanguineti, 2002), the time constant becomes:

$$\tau = \sqrt{\frac{kh}{g\ (1-c)}} \tag{3.2}$$

If we assume the COM is positioned at 55% of our subjects' height (1.75m), this equates to an h of 0.96m. During normal standing, c ranged from 0.46 to 0.78, at 0.6 and 0.1 degrees of stimulus amplitude, respectively. Therefore, these stiffness values equate to time constants of 491 and 756 ms. This shows that the behaviour of the standing

subject and the size and timing of their neural response are all sensitive to the size of the perturbations applied. During the wobble condition, the time constant was always ∼490 ms. This suggests that greater control alacrity is required in situations where sway size is large, such as when standing in moving vehicles.

The results reported in the present study suggest that people with less stiffness may be less stable. To our knowledge, only (Fitzpatrick, Taylor and Mccloskey, 1992) have investigated this by showing that, in individuals who were instructed to stand at ease, physical perturbations produced larger disturbances than those attempting to stand still. However, (Fitzpatrick, Taylor and Mccloskey, 1992) did not report the size of the spontaneous sway in the two conditions and did not measure intrinsic ankle stiffness. The present study does both, and shows that the intrinsic stiffness is less in people who are (or have recently been) swaying more. Whether these stiffness changes have consequences for larger perturbations or affect postural stability in the widest sense remains to be seen.

For an ankle stiffness dependent on the history of movement, the implication is that the demand for neural intervention to stabilize standing will not be constant but, instead, will vary continuously. For control of limb movement, a reduction in stiffness as movement occurs is favorable because it allows muscles to economically control both posture and movement. For standing, it may be less beneficial because an increased sway will lead to a reduction in ankle stiffness and stability and thus, potentially, to collapse unless there is additional neural intervention.

CHAPTER 4

LOCALIZED LOWER LIMB COOLING HAS NO EFFECT ON INTRINSIC ANKLE STIFFNESS IN STANDING

Abstract Following the previous study result of a significant negative correlation between intrinsic ankle stiffness and ankle movement amplitude and history of movement due to muscle thixotropy, I proceed to investigate other properties of the calf muscle which can be relevant to the standing human. This time I determine whether localized cooling affects the intrinsic stiffness of the muscle and ankle as much as it affects the properties of the active muscle. 6 subjects were tested with alternating legs cooled in two different sessions at least 24 hours apart. In a procedure similar to the previous study, they also stood on a rotating platform which was collinear with the ankle joint. Ankle stiffness was again measured with small and brief rotations of various sizes (<1.3deg; 140 ms) and the ankle torque response to them was decomposed to calculate stiffness. The results show no dependency of intrinsic ankle stiffness on temperature. The conclusion is that 2 factors could have contributed to the null results: (1) localized cooling has no effect on the intrinsic properties of the ankle or (2) even if there was an increase in muscle stiffness, its connection in series with the tendon does not allow this increase in stiffness to be transmitted to the overall ankle stiffness because the tendon, acting as the weakest link, determines the overall stiffness.

4.1 Introduction

Following the positive results obtained in Chapter 3 about the effect of muscle thixotropy on the standing intrinsic ankle stiffness, showing significant reduction of stiffness with increased movement amplitude and more background movement, I decided to continue my investigations about the ankle mechanical properties related to the stabilization of the upright stance. I have already mentioned in previous chapters that active and passive mechanisms are both relevant to standing (Loram and Lakie, 2002*a*; Morasso and Schieppati, 1999; Morasso and Sanguineti, 2002). Changes in the active muscle by cooling or heating have been extensively studied (reviews by Heus et al., 1995; James, 2013; Racinais, 2010). There is a possibility that intrinsic muscle properties are also altered by temperature, but this has not been much studied (Lakie et al., 1986; Lakie and Robson, 1988*b*,*a*). Mixed results were found in cooled tendons (Alegre et al., 2016; Kubo et al., 2005; Muraoka et al., 2008). Therefore in this chapter I perform an experiment in which I investigate the effects of attempting to alter the standing intrinsic ankle stiffness by localized cooling.

Why is there an interest in localized cooling? People can stand for hours in cold weather without any major physiological damage, but they often get cold legs. Limb cooling and the consequent muscle weakness are probably the main reason why the swimming ability declines in cold water, not general hypothermia as most people believe (Golden and Tipton, 1987; Tipton et al., 1999; Toner et al., 1984). Both phenomena occur because in humans, as well as in other mammals and birds, the core body temperature is very different from the skeletal muscle temperature, especially when compared to the muscles located at superficial levels and at the extremities of the long arms and legs. The origin of this difference reverts to the transition to dry habitats. One of the adaptations needed for these animals to cope with the harshly changeable temperatures found in all terrestrial and aerial environments was the introduction of endothermic homeothermy. It implies that the physiological and biochemical functions of most mammalian and avian

bodies are strictly dependent on keeping a relatively high core body temperature within a daily variation of less than 3 deg C (Refinetti, 1999; Wooden and Walsberg, 2004). This demands a high rate of heat production (Bennett and Ruben, 1979), but keeping the same strictly controlled temperature at the extremities of the long limbs is highly costly and less necessary. Barcroft and Edholm (1943) have cooled the human forearm at temperatures ranging from 13 deg to 35 deg C for up to 2 hours while recording the change in blood flow (0.5–4.3 c.c./100 c.c. forearm/min). Within this large scope of water bath temperatures, the deep muscle temperature (assessed 1 inch below the skin covering the brachioradialis muscle) ranged from 18 deg to 35 deg C, a difference of 17 deg which would be unacceptable at the core level.

During standing, it is believed that the main means that the CNS has to control balance is through the combination of passive mechanisms added to the modulation of the calf muscle activity (Jeka et al., 1998; Loram and Lakie, 2002*a*; Masani et al., 2006; McCollum and Leen, 1989; Nashner and McCollum, 1985). Therefore, even if the core body temperature changes very little, the high susceptibility that the limbs have to be affected by changes in local temperature might have a role in altering the ankle active and passive mechanisms, subsequently altering the control of balance. Amphibian muscles are able to operate over a wide range of temperatures. Mammalian muscles are not. As it is uneconomical and difficult to regulate the peripheral temperature, there is a real penalty as peripheral temperature decreases: the substantial decline in mammalian muscle function at peripheral levels.

The effects of temperature on the active production of movement can be understood through the analysis of muscle response to electrical stimulation, the most direct way to measure local muscle activity. A single contractile muscle response to stimulus (muscle twitch) is characterized by a latent period followed by contraction and relaxation phases. The latent period consists of the first few milliseconds after stimulation when excitation-contraction coupling is taking place. The action potential moves through sarcolemma

causing Ca^{2+} release. Latency response time varies, amongst other variables, depending on stimulus travelling time along the nerve and its crossing time from neuromuscular junction to the target muscle, excitation-contraction coupling time, time taken by the lever to overcome the inertia of rest and time taken to overcome the viscous resistance of the muscle. During the contraction phase, calcium ions bind, cross-bridges form and tension builds to peak as muscle shortens. This is followed by a relaxation phase, in which Ca^{2+} levels fall, active sites are covered, tension drops and the muscle returns to its resting length. Since Bernstein (1902), several studies have investigated effects of temperature on the mechanical activity of nerve and muscle, finding that cooling slows down the muscle twitch response, especially the relaxation phase, and diminishes twitch response amplitude (Hill, 1970*a*,*b*, 1972; Hodgkin and Katz, 1949; Huxley, 1959; Ricker et al., 1977). Most of these studies were performed in vitro in isolated amphibian muscles, mostly frog muscles. In vivo studies in humans, whose muscles are mammalian and therefore much more sensitive to temperature, have confirmed this pattern of cooled muscle response to stretch. Tuttle (1941) has found a slight increase in latency and contraction times and doubled relaxation time in the intact gastrocnemius muscle twitch response to electrical stimulation. Lakie et al. (1986) assessed isotonic forearm muscle twitch response to stimulus, and likewise other experiments, have found a large increase in twitch duration (more remarkable in the relaxation phase), but slight increase in the latent period and contraction time; they have also found a decrease in twitch size and abolishment of overshoots. A slower rate of tension development and decelerated breaking and formation of cross-bridges are possible explanations. This delayed response to stimulus and extended relaxation time are most likely responsible for the difficulty in reaching peak torques at high speed during intense exercises (Bergh and Ekblom, 1979; Davies et al., 1982; Ranatunga et al., 1987).

As seen from the above, the effects of temperature on production of movement have been widely studied. Less investigated were its effects on the passive mechanisms of the muscle, especially in its specific role of altering joint stiffness. Particularly relevant to this thesis is the research conducted by Lakie et al. (1986). The authors have performed a

series of different experimental designs to tackle the effect of localized cooling of the forearm on the human wrist movement. Amongst other variables, they have altered cooling duration and temperature, tracked change in torque, displacement and twitch response and compared involuntary with voluntary muscle activity. In one of the protocols, they cooled the forearm of one participant for 30 min at 10 deg C, applied abrupt gradually increasing squared torque reversals at 1 Hz frequency and measured the resultant wrist passive movement amplitude. In their analysis, while correlating torque and displacement amplitude (Figure 4.1), the authors have found that the wrist was moving considerably less after cooling, but only when the movement amplitude was more than 5 deg. This phenomenon was interpreted to be passive because it was not accompanied by increase in EMG activity. The cooling effect dependency on movement amplitude was also seen in voluntary movements. When the arm was cold, subjects were unable to make rapid reciprocating movements. Slower movements were much less affected.

Lakie et al. (1986) attributed the increased range of the stiff component to an alteration in the SREC. However, the effect of localized cooling on the SREC of passive muscle is not indisputable. Lakie and Robson (1988*b*,*a*) have applied small torques to the isolated frog sartorius muscle and the resultant displacement was used to estimate stiffness. They have measured resultant stretch before and after applying a series of squared-wave torque oscillations (3 Hz, 2.5 mm) to produce 'stirring' of the relaxed muscle (Figure 4.2, top small graph). Post-stirring stretches were performed at different time-intervals to examine the history of movement effect (S3 in Figure 4.2, bottom graphs). The authors have found a non-significant effect of cooling on thixotropy. That is, frog muscle fibers cooled at different temperatures (3 deg C and 17 deg C) behaved similarly with respect to the 2 variables which are determinant of muscle thixotropy, the amplitude of stretch and the history of movement.

To my knowledge, only these 3 studies have investigated the effect of cooling on the passive muscle, either in vivo or in vitro. They have found, though, mixed results. The reason for

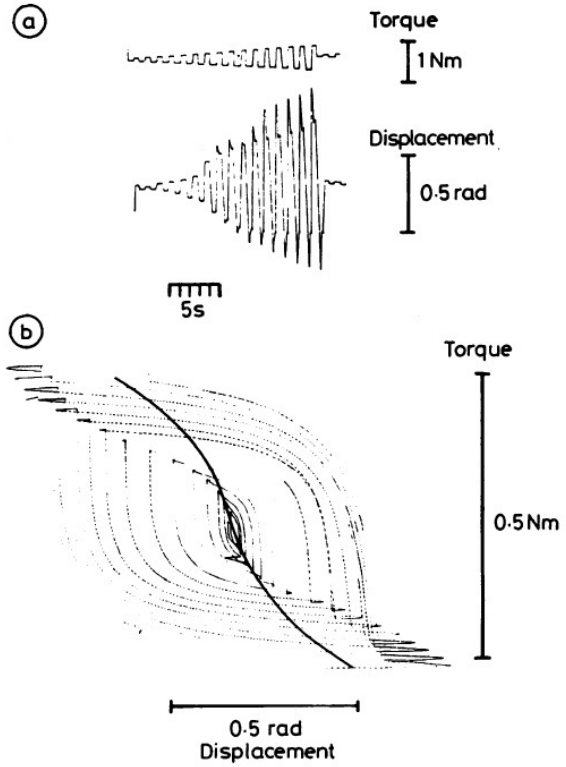


Figure 4.1: Lakie et al. (1986). (A) Driving torque and the resulting displacement of the wrist at normal temperatures. The displacement of the wrist is disproportionally small when the torque is low. (B) Relationship between displacement and torque, confirming the disproportionally high stiffness for small movement. The heavy line shows data after cooling at 10 deg C during 30 min. Stiffness for small forces was unchanged, but at large forces the loosening was less than in normal temperature.

studying standing is that it depends critically on passive and active muscle stiffness and therefore is particularly likely to be affected by cooling. The calf muscles are responsible for standing and they are peripheral muscles which are very susceptible to cooling. Studies have shown that peripheral arm cooling can greatly increase the postural stability of the hand (Lakie et al., 1995; Lakie, 2010) and it is naturally interesting to see if similar effects are observed when the stability of the body is studied when the legs are cooled. As seen in Chapter 3, the high muscle stiffness normally exists only over a short range. In the case that Lakie et al. (1986)'s results apply to the calf muscles, then cooling would make the stiffness exist over a larger range. This should produce measurable changes in the way that the ankle responds to small and large perturbations and possibly affect standing stability. In this manner, the fact that localized cooling might increase passive muscle

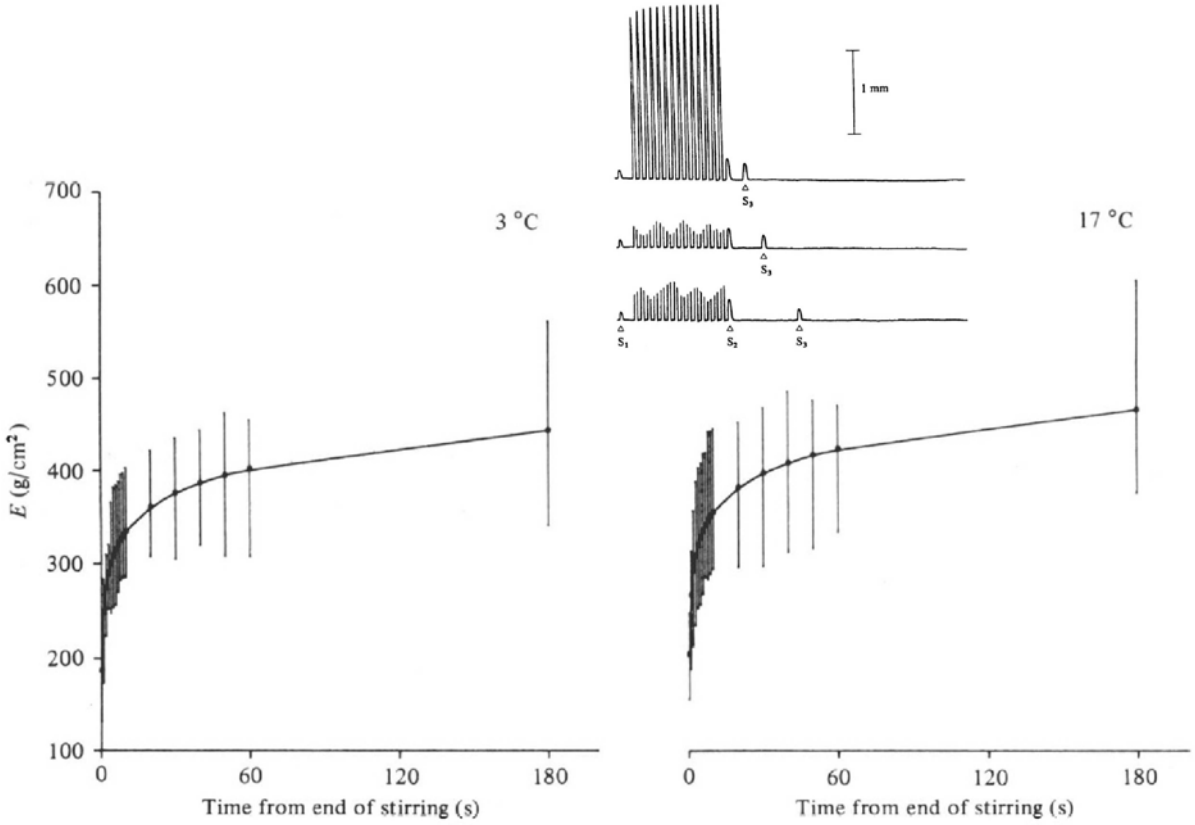


Figure 4.2: Lakie and Robson (1998b). Small top-right graph: The displacement caused by stretch is reproducible immediately before and after stirring (S1 and S2). However, there is change in stretch amplitude when it was applied with increasing intervals after stirring (in descending order, the interval between the end of stirring and S3 is 1, 2 and 4 s), which is a muscle thixotropy phenomenon. Large bottom graphs: Values of elastic modulus (E) for S3 applied at different times after stirring. The results confirm the muscle thixotropy phenomenon, but show no difference between bath temperatures (3 deg and 17 deg, left and right graphs).

stiffness was my argument to propose a study in which the impact of localized cooling on a postural task (the control of upright stance) would be investigated. For this protocol, I cooled the lower limbs of 6 people for 15 min at <5 deg C and, immediately after, measured their intrinsic standing ankle stiffness at various perturbation amplitudes.

4.2 Methods

Participants

Six healthy adult volunteers participated in this non-invasive study (1 female; age 24.3±3.1 years (mean±SD); height 1.74±0.1 m; weight 72.8±20.9 kg). All provided a written informed consent approved by the institution's local human ethics committee and conformed to the principles of the *Declaration of Helsinki*.

Participant	Sex	Age (yrs)	Weight (kg)	Height (m)	Toppling torque per unit angle (Nm deg^{-1})
P01	M	23	113.8	1.90	18.0
P02	M	21	71.9	1.82	10.2
P03	M	24	59.4	1.66	8.9
P04	F	25	64.4	1.59	11.9
P05	M	23	69.7	1.81	10.9
P06	M	30	57.4	1.66	8.3
Mean±SE	F(1),M(5)	24.3±3.1	72.8±20.9	1.74±0.1	11.4±3.5

Table 4.1: Participant anthropometric data.

Procedure and Apparatus

A full description of the footplate apparatus used to measure ankle stiffness as well as its estimation calculations were given in Chapter 2. In brief, the participants were asked to stand on top of motorized footplates, coaxially aligned with their ankles, while ankle torque, ankle angular position, footplate acceleration and lower limb EMG responses were being recorded. The methodology specific to the present study is described below.

Intrinsic ankle stiffness was assessed by cooling alternating legs in two testing sessions performed at least 24 hours apart. The right leg was cooled on the first day, while the left leg was cooled on the second day. Concerns about possible effect on balance control led to the decision of not cooling both legs simultaneously. Lakie et al. (1986) have found that cooling made the wrist stiffer at an initial range of at least 5 deg, much larger than the short-range elastic component (∼0.5 deg) of the ankle (Loram et al., 2007*a*,*b*). This meant

that the stiffness measuring perturbations could not be tiny; otherwise the cooling effect would not be detected. Therefore I decided to assess stiffness with varying perturbation amplitudes of 0.1, 0.3, 0.7 and 1.3 deg amplitudes. I decided not to apply perturbations of more than 1.3 deg because it might have been too disturbing to the standing process. The trials lasted for approximately 3 minutes, and the interstimulus interval of the randomized toes-up and toes-down perturbations lasted for variable gaps of 4–5 s. 48 perturbations were performed for each individual perturbation amplitude and condition, with a total of 384 recordings from each participant. The correct placing of electrodes for the assessment of surface EMG activity was important for the between-session comparisons. Therefore during the first session its position was carefully marked with a permanent pen, and at the next day the electrode could be reattached reasonably close to its original position.

The general cooling procedure was as follows. To avoid short term pain and also to avoid the risk of rupturing red blood cells (Marjanovic and Willis, 1992; Muldrew and McGann, 1994) during cooling of the feet and toes, the participants wore neoprene wetsuit boots. They were seated and asked to immerse their entire leg below knee height in a tank with water maintained at 3–5 deg C. The temperature of the water and skin were monitored with a multi-channel telethermometer (Yellow Springs Instrument Corp.) and a small surface thermistor probe connected to it. During the cooling procedure the thermistor probe was placed in the water to make sure its temperature was kept below 5 deg at all times. After the cooling procedure, immediately after the leg was pulled outside the water, the calf muscle skin temperature was assessed to confirm if it was effectively reduced. To avoid formation of stagnant layer of unstirred water acting as an insulator, which could potentially protect the deep muscles of the leg from being cooled, the water was periodically stirred. The cooling procedure lasted for 15 min.

One participant was also tested after cooling of 30 min of each leg. Another participant was tested in one single session in which both legs were cooled at the same time, also for 30 min. This was used to verify if the cooling period used for the main experiment was

appropriate.

Effect of cooling on muscle twitch response

I had to verify if the cooling procedure was effectively reaching the calf muscles, which are the most relevant to quiet standing. As subcutaneous temperature assessment was not available, I decided to investigate the calf muscle twitch response before and after cooling. This would confirm if our objective of changing the muscle response through cooling was successful (Ricker et al., 1977; Bolton et al., 1981). The procedure was performed in 4 participants during cooling periods of either 15 min or 30 min. First the leg was clamped to avoid movement and register isometric responses. The participant was placed on a bench in a seated position. The right thigh was pressed downwards distally by a height-adjustable clamp. This would fix the leg. Attached to the clamp was a strain gauge sensor used to detect change in ankle torque occurred while the soleus was being twitched. Then two damped custom-made electrodes (aluminum foil enclosed with Wypall L40 wipe) were placed at the origin and insertion of the soleus muscle. The stimulus intensity was adjusted to the maximal bearable by each participant (which ranged from 26 mA to 79 mA) and the impulse duration was 250 μs (Figure 4.3). The first assessment of muscle twitch response was performed while the muscle was adapted to room temperature. Next the leg below the knee was immersed in a tank with water cooled at 3–5 deg C for 15 min or 30 min, the same cooling procedure used to assess intrinsic stiffness. The leg was clamped and the muscle was stimulated at the same intensity and duration immediately after leaving the tank. After twitching, for one participant the leg was once again cooled for another 15 min. In this case, a third set of muscle stimulation took place to verify muscle responses after a combined period of 30 min of cooling. The electrodes were carefully placed at the same marked spot in all assessments.

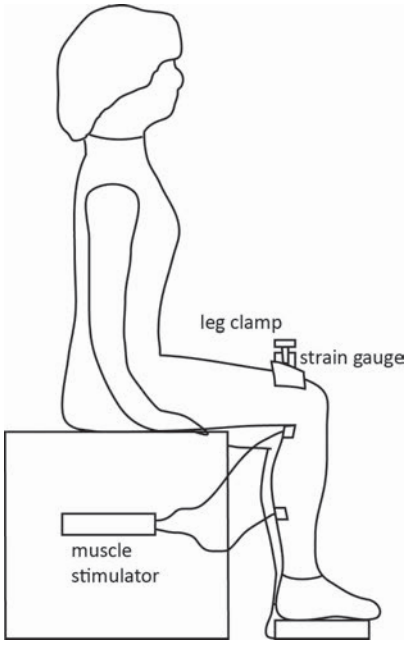


Figure 4.3: Muscle stimulation experimental setup. Participants had their right leg clamped. The muscle stimulator applied electrical stimulus through the electrodes connected to the soleus muscle while the strain gauge recorded the muscle plantarflexing torque response to stimulus.

Data Analysis

Determination of mean ankle torque and EMG activity RMS

The mean ankle torque over a 70 ms time window prior to perturbation onset was calculated to verify if baseline torque was affecting the results. In Chapter 3 I have shown that the intrinsic stiffness is dependent on ankle torque and significantly changed the outcomes of the condition in which the participants were strapped to a vertical board, when compared with normal standing. In the present study, the participants were tested in two different sessions and different legs were cooled at each time, so the chances of mean ankle torque being different were higher than in all the other experiments, which were all completed in one day and did not alter the conditions of each leg separately. Therefore the mean torque in both sessions was analyzed to verify if the sessions were comparable.

The EMG activity RMS over a 70 ms time window prior to perturbation onset was used

to verify the muscle activity related to natural sway with and without cooling. A significant change in muscle activity RMS with cooling would indicate that the effectiveness of coupling EMG to active force was altered. This might increase uncertainty about the ability of the body to maintain stability and maybe induce increased muscle tone, altering intrinsic stiffness. The relative change in baseline muscle activity was assessed as the ratio between the mean EMG activity detected during cooling condition divided by the activity detected during normal room temperature. By dividing the activity of one condition by the other, I was able to diminish the importance of other factors that influence the muscular voltage output, like electrode placement, skin cleanliness, muscle dimension etc. However, some other factors could not be eliminated, like a possible change in skin resistance with cooling.

Statistical analysis

Repeated-measures ANOVA was used to determine effects of condition (normal or cooling, left or right leg) and stimulus amplitude (0.1, 0.3, 0.7, 1.3 degrees) upon ankle stiffness. One-way ANOVA and two-tailed paired samples t-test were used to verify differences of baseline ankle torque and EMG activity between conditions. $P<.05$ was considered statistically significant for all tests.

4.3 Results

Representative data of one participant in normal (left column of datasets) and localized cooling (right column of datasets) conditions are shown in Figure 4.4. As with other participants, data are very similar between conditions, showing no significant change in ankle angle and torque with localized cooling.

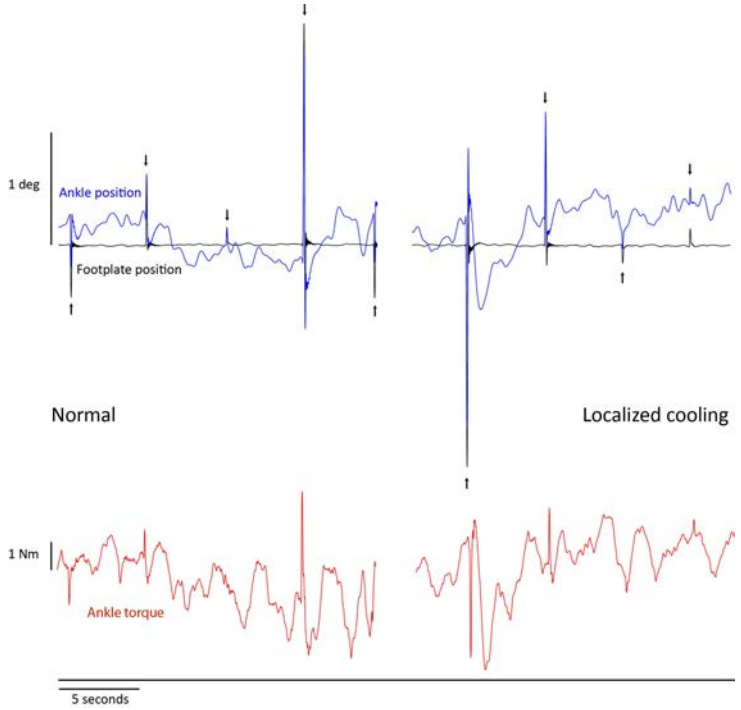


Figure 4.4: Effect of localized cooling upon ankle angle and ankle torque. Illustrative segment of footplate position, ankle position and ankle torque during a stiffness-measuring trial of one participant in normal and localized cooling conditions. The horizontal line at the bottom of torque traces represents 19 Nm.

Intrinsic ankle stiffness

The obtained results of intrinsic ankle stiffness were significantly dependent on perturbation amplitude, particularly when comparing the smallest amplitude against the 3 larger ones (repeated-measures ANOVA, $F_{3,15}$=27.6; p<.001). Stiffness was shown to be larger when the ankles were moved by smaller perturbations. However, I have found no effect of cooling upon intrinsic stiffness ($F_{1,5}$=0.2; p=.68), or no interaction between temperature and amplitude ($F_{3,15}$=2.9; p=.07) (Figure 4.5).

Ankle torque and muscle activity

I verified if ankle torque and muscle activity were affected by the leg that was being cooled. Left and right limbs were cooled alternately at different sessions taken within a minimum

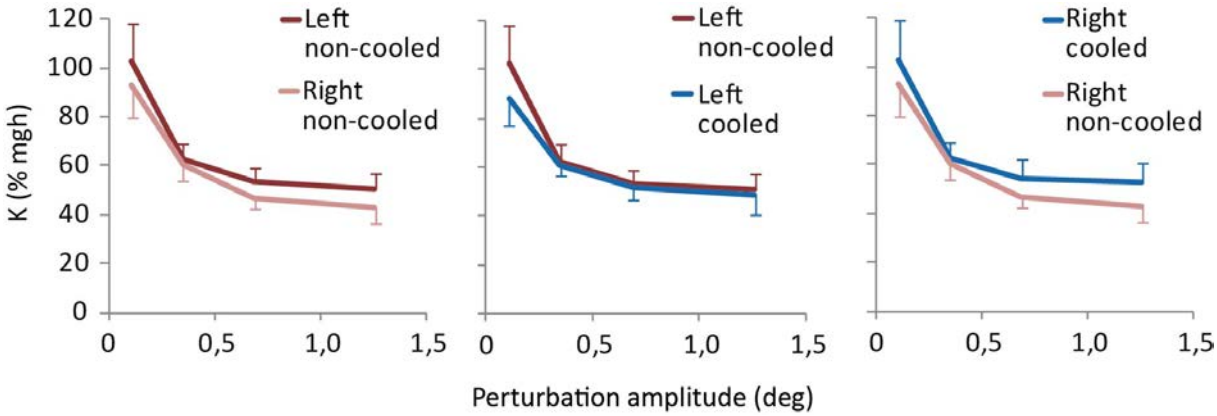


Figure 4.5: Intrinsic ankle stiffness for both conditions, both legs and 4 perturbation amplitudes (mean±SEM).

24-hour gap, and it was important to maintain the conditions as similar as possible to avoid biased results. T-test analysis has shown no effect in mean ankle torque prior to stretch between the uncooled and cooled sessions ($t_{(11)}$=0.16; p=.88) (Figure 4.6).

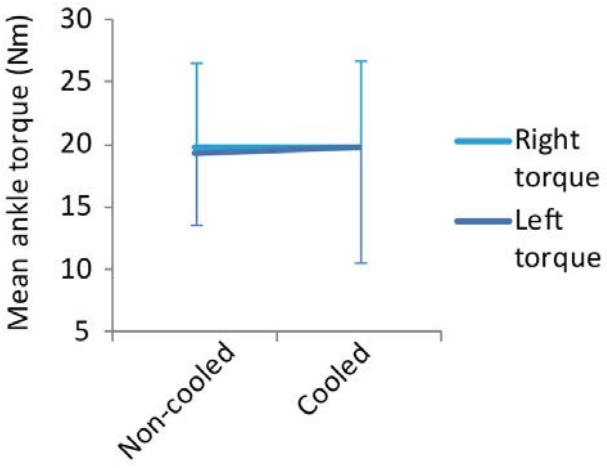


Figure 4.6: Mean ankle torque. Mean left and right ankle torque during a 70 ms pre-stimulus time window (mean±SD).

The results of baseline muscle activity are shown in Figure 4.7. No significant dependency on limb ($F_{1,5}$=0.6; p=.49) as well as no significant dependency on temperature ($F_{1,5}$=1.9; p=.22) were found. As we can see from this figure, the ratio between cooling and non-cooling consistently remains around 1.

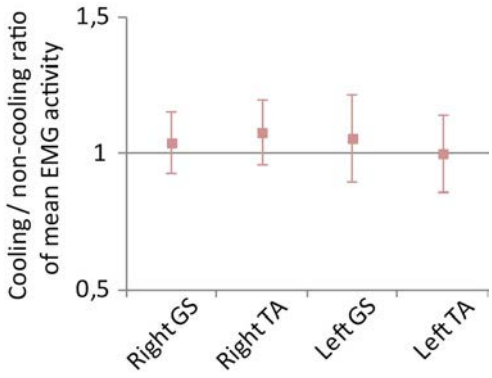


Figure 4.7: Muscle activity modulation prior to stretch, expressed as a ratio between cooling and non-cooling conditions. Surface EMG RMS cooling/non-cooling ratio during a 70 ms pre-stimulus time window (mean±SD).

Muscle twitch response under cooling conditions

In accordance with previous research, all 4 participants have shown a change in muscle response to brief electrical stimuli after cooling, the effects being more prominent as duration of intervention increased from 15 min to 30 min. Figure 4.8 shows illustrative data of one participant. After 15 min of cooling, twitch peak tension (TPT) reduced to 32% of the tension obtained before cooling. If during pre-cooling the time to TPT was 152 ms and it took 80 ms to reach half relaxation time (½ RT), after cooling there was a delay of 66 ms to reach TPT (218 ms) and ½ RT was prolonged to 107 ms, a 32% increase in relaxation time.

4.4 Discussion

The effect of localized leg cooling upon intrinsic standing ankle stiffness was investigated in this study. No significant relationship was found between temperature and intrinsic standing stiffness. There was also no relationship with ankle torque or surface EMG activity.

In accordance with the results of Chapter 3, the results for intrinsic ankle stiffness were

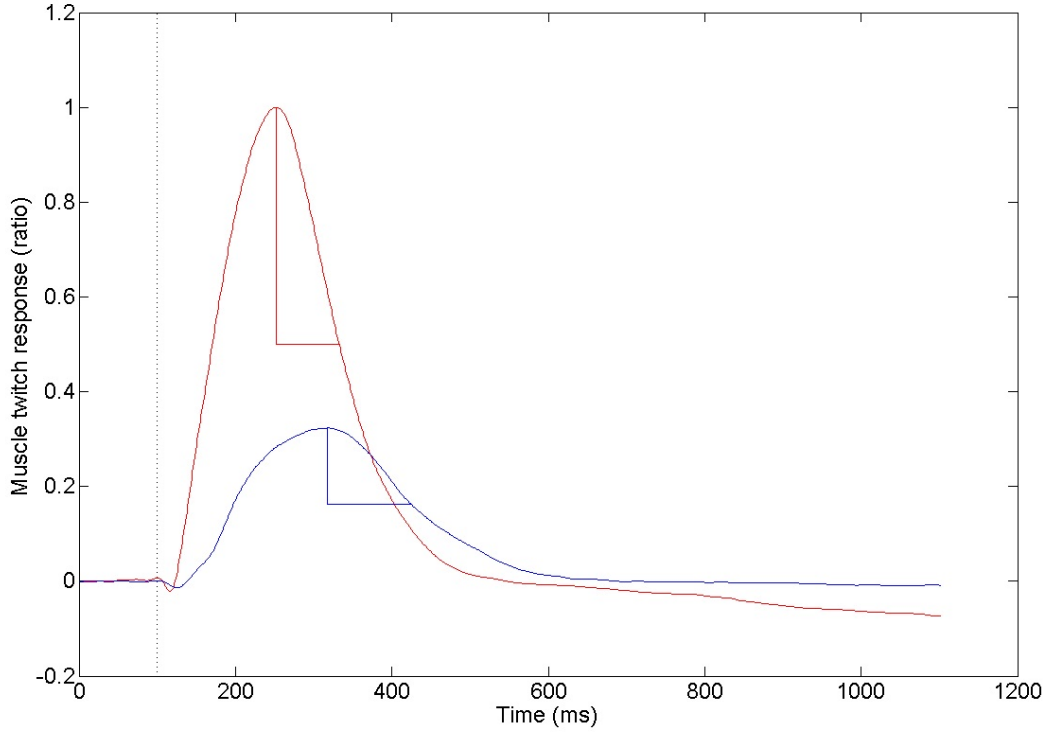


Figure 4.8: Muscle stimulation response during cooling. Illustrative data from one participant. 6 electrical impulses (250 μs duration) were applied to electrodes applied over the motor point of the soleus muscle, before and after cooling. Red color refers to muscle responses before cooling and blue after 15 min of cooling. Dashed black vertical line delimits the stimulus onset. Straight L-shaped lines indicate peak twitch tension and half relaxation time values.

significantly dependent on perturbation amplitude, showing a predicted negative relationship between these two variables. This finding confirmed that even after localized cooling, muscle thixotropy was still relevant in determining intrinsic stiffness of the ankles. Nevertheless, effect of cooling was non-significant.

I have checked various variables that could have potentially affected the final stiffness estimates, including mean ankle torque and EMG modulation prior to perturbation. Comparisons between conditions have also shown no effect of cooling in any of the variables. We have seen from the previous chapter that mean ankle torque correlates positively with stiffness. Therefore it was important for the participant to maintain the same average absolute ankle torque between the 2 different sessions taken one day apart, which the

results show was successfully maintained (Figure 4.6). Following confirmation that baseline torque was similar between sessions, I proceeded to analyze change in modulation of EMG activity with cooling (Figure 4.7). Once more, cooling had no effect either in decreasing or increasing muscle activity prior to stretch. A difference in modulation of muscle activity would indicate alteration of effective coupling between EMG and active force. If the muscle is weaker more EMG will be needed to produce the same force.

Furthermore, I have performed muscle electrical stimulation to verify if the cooling procedure was adequate to significantly decrease the temperature of the calf muscles. There was a clear and consistent decrease in twitch size, increase in twitch duration (especially in relaxation phase) and reduction of overshoot with cooling which is in agreement with findings of Lakie et al. (1986) and Davies et al. (1982). The results were more pronounced after 30 min of cooling, but still noticeable at 15 min (Figure 4.8). This confirms that there was an effect on muscle reaction to electrical stimuli with cooling, which implies that the intervention was successful in altering the muscle properties. However, even if the stimulation was effective at superficial levels, I could not guarantee if the whole muscle tissue was evenly affected. The deep tissues are difficult to reach (Barcroft and Edholm, 1943). The null effect of localized cooling on intrinsic standing ankle stiffness could either be the result of ineffective cooling procedure of the deep muscles or simply a reflection of a real phenomenon. The activation of the medial gastrocnemius muscle during standing was shown to be unevenly distributed, with the distal section being more relevant to the task (Hodson-Tole et al., 2013). However, to my knowledge there is no research neither confirming nor denying the relevance of the deep sections of the muscle to standing.

All the possible reasons for a null effect of cooling on standing passive muscle must be considered.

Lakie et al. (1986) did find increase in the stiffness of the cooled hand for large movements (possibly larger than 5 deg, as seen from Figure 4.1). However, this result was obtained from only one subject. This particularly large range of increased stiffness produced by

cooling could be explained in two different ways. Lakie et al.'s interpretation was that localized cooling of the relaxed forearm has an effect on the thixotropic properties of the muscles, increasing stiffness for large forces but not for small ones. In other words, the short range stiffness did not increase in its stiffness level but rather in its stiffness range. Another explanation would be an imperfect relaxation of the muscles antagonizing the movement, in which case muscle thixotropy would not necessarily be the cause of the phenomenon. Analysis of the rate of maximum voluntary oscillation of the hand has shown that after cooling of less than 10 deg C, it was very difficult to reach frequencies as low as 0.5 Hz. It shows that the cold wrist cannot reverse direction rapidly. This could explain why the wrist was much stiffer when induced to move at increasing torque reversals of 1 Hz frequency (Figure 4.1). Maybe in this experiment, even though the objective was to assess the passive components of the wrist, after a certain torque threshold was reached the movement became so abrupt that the participant could not help but voluntarily or by reflex action resist it. If the muscle active response to stimulus is prolonged with cooling, after a 1 s interstimulus interval the antagonist muscle relaxation might have not yet been completed. Hence the reduction in wrist movement amplitude reached at higher levels of torque imposed to a cooled forearm. At lower amplitudes of movement this effect would not be prominent because the antagonizing forces might not be induced.

Possibly the answer to this dilemma could be found in the experiments performed in isolated muscles by Lakie and Robson (1988*b*,*a*). The authors stretched clamped muscles and assessed displacement amplitude, before and after 'stirring' it. Similar to the results shown in this chapter in standing individuals, with this strictly passive procedure the authors have found no significant effect of cooling on the passive muscle, independent of stretch amplitude and recovery time. This was contrary to the results shown in vivo in the human wrist for larger amplitudes (Lakie et al., 1986). The reason for that is unknown. One possibility is that localized cooling simply has no effect on the passive mechanisms of the muscle. The isolated muscle tested by Lakie and Robson (1988*b*,*a*) was denervated and could not be activated, hence the null result. Lakie et al. (1986) have

tested torque reversals of the in vivo wrist, a movement which might have been actively performed by antagonist muscles. Maybe the effect of cooling was produced by the slower relaxation phase after the antagonist muscle was actively trying to counteract the torque reversals, therefore showing a positive effect of cooling on active mechanisms, but not on passive mechanisms. In addition, the range of movement suggested by Lakie et al. (1986) to be affected by cooling (reproduced in Figure 4.1 of this chapter) is much larger (>5 deg) than the threshold for short-range stiffness (∼0.5 deg for the ankle joint) found by latter research (Loram et al., 2007*a*,*b*). This fact supports even more the possibility that the changes observed by Lakie et al. (1986) and attributed to passive mechanisms were actually produced by active mechanisms which they did not detect and were an artifact of the technique they used.

We know that the joint tissues have particular mechanical properties that are not equally affected by different conditions. We also know that the muscle, tendon and aponeurosis are connected in series making the stiffness of the ankles dependent on the weakest spring, which during standing is normally the tendon (Loram et al., 2007*b*). What would be the consequence of this phenomenon? If controlling of the limb position requires the combined action of the muscle and the tendon connecting it to the bone, then we need to consider the effect of cooling on the in series elastic tissue complex.

To my knowledge, 3 in vivo studies used imaging techniques to assess cooling-associated changes in the human tendon stiffness. The authors of these studies tracked change in tendon force and fascicle length during knee or ankle joint passive elongation (>45 deg joint rotation). The results were mixed. While Kubo et al. (2005) found no effect of cooling on the Achilles tendon stiffness (5 deg C bath for 30 min), Muraoka et al. (2008) and Alegre et al. (2016) reported a significant increase (10% and 25%, respectively) in Achilles and patellar tendon stiffness after cooling (5-8 deg C water bath for 60 min and decrease in skin temperature to ∼7 deg C after 30 min local application of ice pack, respectively). The null effect of cooling on stiffness found here indicate that the standing

Achilles tendon stiffness was not affected by 15 min of <5 deg C water bath. These results confirm Kubo et al. (2005)'s study results.

We know that the resting muscle is very stiff. If cooling increases this stiffness, then once the joint is rotated the tendon will stretch more than in normal temperatures. Hence the muscle length will not change much and the short range stiffness will face little change, only *seeming* much bigger when measured in terms of joint rotation, as observed by Lakie et al. (1986). Also, the range of joint movement before the muscle yields will be greatly increased.

There are differences between wrist and ankle tendons in terms of stiffness that might also explain the differences between the results found in this chapter and in Lakie et al. (1986). The Achilles tendon is extremely large and compliant to be able to absorb the high loads of torque produced by walking, jumping and running. Its relative importance in its in series connection with the calf muscles is high when compared to the stiffer and smaller wrist tendon, which connects arms to fingers with the objective of not compromising their independency and dexterity. Therefore, any possible stiffening of the calf muscles would not affect the overall ankle stiffness because the Achilles tendon is very compliant and being the weakest spring of the in series stiffness model, it determines the overall stiffness. On the contrary, in a system composed of hand and arm muscles connected through taut and small tendons, the relative importance of the muscle stiffness is higher because its stiffness might not be much different from the tendon stiffness. Any increase in muscle stiffness caused by cooling would then induce a significant change in the overall wrist stiffness.

In summary, I propose that there are 2 possible reasons for the effect of cooling on standing intrinsic ankle stiffness being null:

1. Achilles tendon stiffness is not affected by cooling, as shown in vivo by Kubo et al. (2005). Calf muscle intrinsic stiffness is also not affected by cooling, as shown in

vitro for relaxed denervated amphibian muscle by Lakie and Robson (1988*b*,*a*);

2. Even if muscle intrinsic stiffness is affected by cooling as was originally suggested by Lakie et al. (1986), the tendon-muscle in series relationship during quiet standing (an Achilles tendon 15 times less stiff than the triceps surae muscles) might have been unchanged. In Chapter 3 I have shown that the overall ankle stiffness reduces with movement. In that case, the stiffness of the strongest spring (muscles) was reduced. In an in series elastic system, the stiffness is limited by the weakest link. Small changes in stiffness of the weakest link greatly affect the overall stiffness of the system. On the contrary, the strongest link will reduce the overall stiffness by a small amount only if it reduces by a large amount. This was shown in Chapter 3. Nevertheless, no matter how much stiffer the strongest link gets, it will never be able to increase the overall stiffness of the system above that of the weaker link. In this chapter, I have attempted to increase stiffness of the muscle with cooling. Hence, even if cooling had any effect whatsoever on the passive or active mechanisms of the muscle, no matter how much stiffer the muscle becomes, the weakest link will still be the tendon, and therefore the overall ankle stiffness will not be affected by it.

CHAPTER 5

STANDING INTRINSIC ANKLE STIFFNESS INCREASES DURING CONDITIONS OF INCREASED ACTIVE ANKLE TORQUE AND INCREASED PASSIVE TENDON STRETCH

Abstract In Chapters 3 and 4, I investigated the dependency of standing intrinsic ankle stiffness on mechanical properties particular to the muscle tissue (muscle thixotropy and temperature). Standing individuals may choose to balance themselves at a range of ankle angles. Furthermore, they may wear shoes that elevate or depress the heel and they may stand on a surface that is not horizontal. In this chapter I ask how these real world situations impact on ankle stiffness and balance. I perform two studies in which Achilles tendon and aponeurosis are stretched in different ways, either by means of increase in active torque or increase in passive stretch. 10 participants were tested in each experiment. They were asked to freely stand on footplates while stiffness measuring perturbations (<0.7 deg; 140 ms) were applied at intervals of 4–5 s. In the first protocol, the participants were asked to stand at different levels of body leaning forward of the ankle joint. In the second protocol, the participants were tested with the standing platform either fixed at horizontal level or rotated upwards by 15 deg so that the ankles were passively dorsiflexed. The results show a positive and significant increase in intrinsic standing ankle stiffness with increase in active torque and also with increase in passive stretch.

5.1 Introduction

In previous chapters (3 and 4), the effects of intrinsic mechanisms which are particular to muscle fibers were investigated to understand its relevance to the control of standing (positive dependency on thixotropic muscle stiffening and no dependency on limb temperature). In this chapter I decided to focus on the dependency on stretch of the calf, which is related not only to muscle activity but also to properties of tendon and aponeurosis. Two different protocols were performed, a condition in which the ankle was dorsiflexed and another in which the standing body was leaning at different levels forward of the ankle joint. The objective was to investigate how real life standing situations in which the calf is stretched in different ways can affect the stiffness of the ankles and consequently the control of standing.

Theories connecting cross-bridges to instantaneous stiffness are widely accepted (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954; Huxley, 1969; Huxley and Simmons, 1971). They imply that increase in torque is accompanied by increase in muscle stiffness. For example, during the dynamic process of generating propulsion to jump, increased ankle torque is produced by the coordinated increased activity of the lower limb muscles stiffening the ankles to push the foot against the ground. As muscle generates progressively more torque, more cross-bridges are formed, increasing muscle stiffness and the resistance to an imposed perturbation. Hence, the contractile state of the muscle ('muscle tone') will affect intrinsic ankle stiffness.

This was confirmed in human muscle by Kearney, Hunter and colleagues (Hunter and Kearney, 1982; Kearney and Hunter, 1982; Mirbagheri et al., 2001; Weiss et al., 1986*a*,*b*). In their experiments, participants lay supine on a rigid surface and had their left foot clamped to a rotary actuator operating as a position-servo and coaxially aligned with the ankle. They were asked to perform tasks in which ankle torque and ankle position were modified while ankle stiffness and reflex activity were being assessed. In a study

that is particularly interesting for this chapter, Mirbagheri et al. (2001) performed a series of experiments which elegantly summarized that group's work of previous years. In one task, the participants exerted various constant pre-determined amounts of voluntary force (<50% of plantarflexing MVC) with the ankles in neutral position. The authors have found a gradual increase in intrinsic ankle stiffness (reaching 412±64 Nm rad^{-1}, mean±SD) when the participants were exerting increasing levels of ankle torque (reaching -65±31 Nm plantarflexing torque) (Figure 5.1, top graph). This confirms the relevance of increased muscle tone and ankle torque in stiffening the ankles. However, the authors have not limited their investigation to the effects of increased muscle activity on ankle stiffness. In a second task, the participants modulated mean ankle position from near full plantarflexion (-0.48 rad, -27.5 deg) to near maximum dorsiflexion (+0.24 rad, +13.7 deg) while maintaining a constant plantarflexing contraction of -5.0 Nm. Ankle stiffness increased mostly during dorsiflexion, from ∼150 to 325 Nm rad^{-1} over the 13.7 deg (0.25 rad) range (Figure 5.1, bottom graph).

Kearney, Hunter and colleagues have shown in their experiments that intrinsic ankle stiffness depends not only on active force but also on passive stretch. If increase in ankle stiffness produced by increase in active force is understood as the increase in the number of cross-bridges, being mainly determined by the muscle tissue size, fiber type and level of contraction, how is the dependency of stiffness on passive stretch explained? Or how would the different tissues surrounding the ankle joint be involved in increasing its stiffness through passive stretch?

Most likely the reason for this significant change in intrinsic ankle stiffness during both conditions of increased active ankle torque and increased passive stretch is not only related to the contractile state of the muscle, but also related to the tendon tension. The stiffness of isolated tendon and muscle fibers has been widely studied in in vitro research (Butler et al., 1984, 1986; Ford et al., 1977; Hill, 1968; Lakie and Robson, 1988*b*,*a*). In vivo measurement of stiffness of whole bundles of tendons and muscles acting in combination

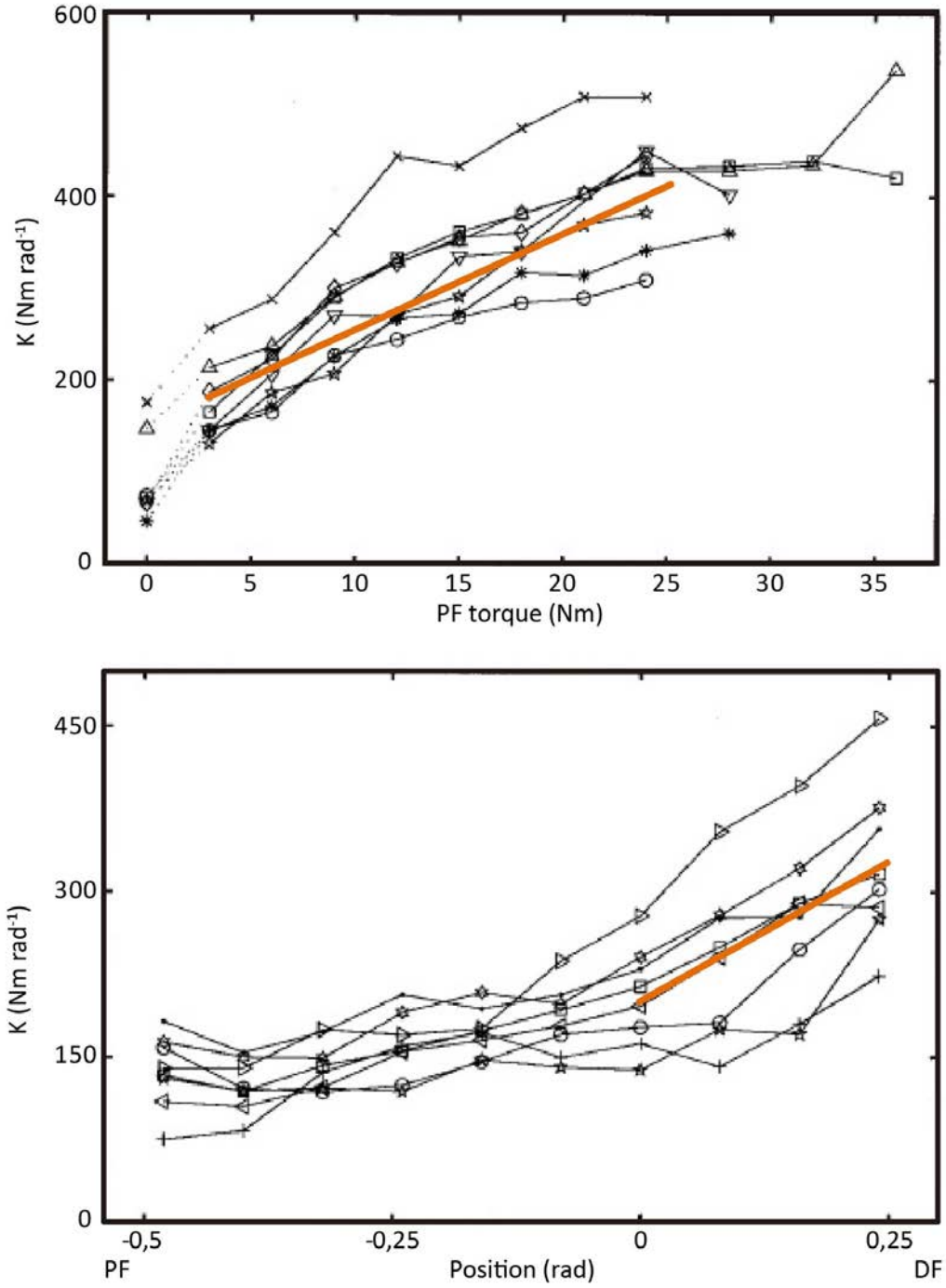


Figure 5.1: Adapted from Mirbagheri et al. (2000). Top graph: Variation in intrinsic ankle stiffness plotted against voluntary torque while the ankle was kept in neutral position. Bottom graph: Variation in intrinsic ankle stiffness plotted against ankle position (negative position=plantarflexion; positive position=dorsiflexion) while -5.0 Nm tonic contractions were maintained. All subjects (n=8) lay supine with their knee extended and foot attached to an actuator by glass-fiber boots. The orange lines represent trendlines estimated by examining the graphs provided by the authors, to be used for approximate comparison with the data obtained from the experiments in this chapter.

was much facilitated with the introduction of the ultrasound technique (Maganaris and Paul, 1999). As opposed to the muscle, the tendon is not a contractile structure, therefore its stiffness is easier to assess as it changes mostly with change in length whereas muscle stiffness depends on several active and passive factors. The tendon length is dependent on the tension passing through it. Thus the more stretched the tendon is, the more stiff it is (Chapter 1, Figure 1.6) (Alexander, 2002; Butler et al., 1978; Devkota and Weinhold, 2003; Ker, 1981; Ker et al., 2000; Pike et al., 2000; Proske and Morgan, 1987; Wang, 2006; Wang et al., 1995). It has been compared to the behavior of a knitted sock which stiffens as it is stretched. For the measurement of the Achilles tendon stiffness, for example, ultrasound probes are used to track the change in position of the distal myotendinous junction of the muscle and the insertion point of the Achilles tendon. Tendon stiffness is then expressed as the slope between this change in tendon fiber position and tendon force (Herbert and Gandevia, 1995; Herbert et al., 2002; Maganaris and Paul, 1999; Maganaris, 2002; Maganaris and Paul, 2002; Sugisaki et al., 2011). If effects of disuse and age are disregarded (Narici and Maganaris, 2007), we can say that even though the relationship between tendon lengthening and tendon stiffness is very non-linear, it is mostly positive. The tendon is also affected by conditioning (viscous component) (Finni et al., 2013; Maganaris, 2003), but it is mostly dependent on its elastic component (Butler et al., 1978; Magnusson et al., 2008; Peltonen et al., 2013). The muscle, on the contrary, can be stiffened either by the passive lengthening of its fibers, by extending the duration of recovery time after movement, by reducing the amount of movement (thixotropy and short-range stiffness properties) (Halaki et al., 2006; Hill, 1968; Lakie et al., 1984, 1986; Lakie and Robson, 1990; Loram et al., 2007*a*; Proske et al., 1993; Rack and Westbury, 1974) or by the active contraction of its fibers. Local temperature changes or fatigue levels may also contribute to stiffness modulation (Howell et al., 1993; Komi, 2000; Lakie et al., 1986; Lakie and Robson, 1988*b*,*a*). As these tissues are connected in series, it becomes rather difficult to accurately know the behavior and contribution of each to the overall ankle stiffness during the various tasks performed by the joints. We can say that the mus-

cle stiffness increases either with active contraction or passive lengthening of its fibers, whereas the tendon stiffness is mostly dependent on fiber passive lengthening. The tendon intermediates the connection of the muscles to the bones. Thus it makes no difference whether it is lengthened by being pulled by the muscle shortening at one end or if it is being pulled through the change in bone position at the other end. The Achilles tendon function is to connect the triceps surae muscles to the calcaneous bone. Therefore, during in vivo conditions it can be elongated in two different ways: (1) by shortening of the calf muscle fibers connected to it through muscle contraction or (2) by dorsiflexion of the ankles.

This change in tendon stiffness was quantified in two different studies in which different methods to elongate the gastrocnemius tendon to its maximum levels, caused either by passive stretch or by muscle activation, were investigated (Maganaris, 2002; Morse et al., 2008). Consequently, increase in tendon stiffness was achieved through very distinct approaches that isolate very well the different tissues that compose the ankles. In both experiments the participants were tested lying in prone position and with the right ankle attached to a torque transducer. In one study, the tendon was elongated through the active shortening of calf muscle fibers. Isometric muscle contraction was gradually increased by up to 100% of maximum voluntary contraction (MVC) (162±11 Nm ankle torque) (Maganaris, 2002). In another study, the tendon was elongated through passive dorsiflexion until it reached the end of range of motion (ROM) (28.1±2.3 deg max dorsiflexion; passive ankle torque at end ROM 45.6±7.0 Nm) (Morse et al., 2008). Increase in tendon elongation (1.11±0.3 cm in Maganaris (2002), and 1.15±0.09 cm in Morse et al. (2008), all values mean±SD) was very similar in both conditions. Increase in tendon stiffness (18 GPa and 34.2 Nm cm^{-1}, respectively) was also confirmed in both conditions.

The studies described above confirm that tendon stiffness tension increases as long as the tendon is stretched, regardless of it being pulled by the muscle or by the bone which it is attached to. Possibly, then, the increase in overall intrinsic ankle stiffness caused by

increase in active ankle torque found by Mirbagheri et al. (2001) (Figure 5.1, top graph) is not only related to increase in muscle stiffness, but also related to increase in tendon tension (and therefore its stiffness).

If ankle dorsiflexion also increases tendon stiffness, how would this affect the overall ankle stiffness? It is intriguing that even when maintaining a constant plantarflexing torque at -5 Nm, Mirbagheri et al. (2001) found that by changing the ankle from neutral to dorsiflexed position, ankle stiffness significantly increased. Slight reduction in stiffness was found during plantarflexing rotation of the ankles. As a general rule, regardless of how tendon and muscle tissues are affected, ankle stiffness should not change when there is no change in ankle torque because torque is a measurement of tension. A stiffer ankle will exert more resistance to movement and this should be reflected in the torque applied by the foot against the supporting surface. The authors suggested that this increase in stiffness strictly dependent on ankle dorsiflexion might not have been related to purely mechanical properties of the muscle. There are various other reasons that could explain this observation: (1) other structures in parallel (aponeurosis etc) might have stiffen the dorsiflexed ankle without affecting ankle torque, which is mainly generated by the muscles and tendon acting together to pressure the foot; (2) co-contraction of antagonist muscles, which would increase stiffness of the muscles without necessarily increasing the generated net torque; (3) change in moment arm. Moment arm decreases with dorsiflexion (An et al., 1984; Maganaris et al., 2000; Spoor et al., 1990). This was quantified in vivo with magnetic resonance imaging (MRI) and real-time ultrasonography by (Maganaris et al., 2000), who found that ankle dorsiflexion of 15 deg produces ∼0.5 cm decrease in moment arm (Figure 5.2). If dorsiflexion decreases the moment arm of the calf muscles, a given rotation of the ankle will cause a smaller linear displacement of the tendon and muscle and, consequently, measured angular stiffness will decrease. As pointed out by Mirbagheri et al. (2001) in their second experiment (Figure 5.1, bottom graph), this is the opposite of what they have found, as their results show increase in stiffness with dorsiflexion.

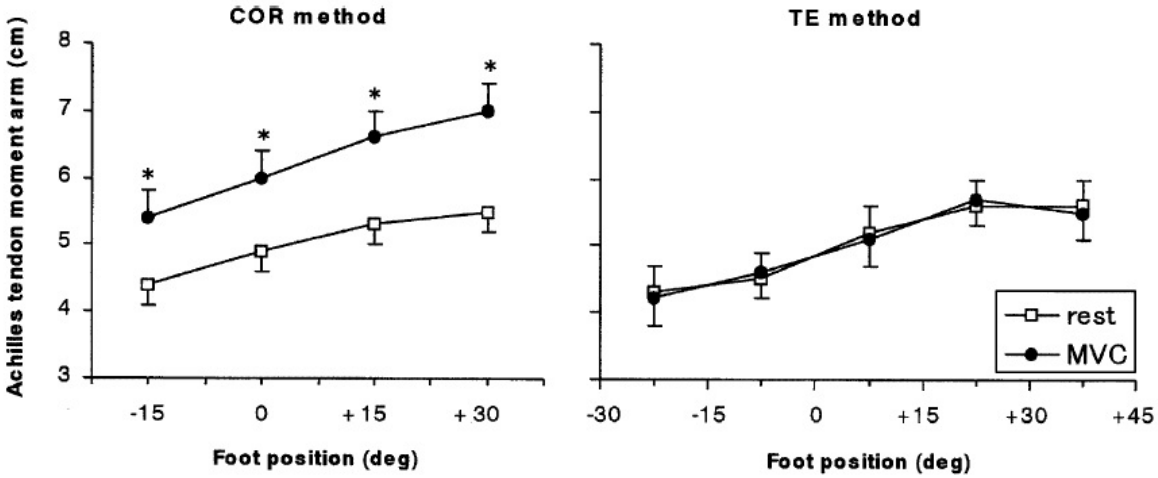


Figure 5.2: Maganaris et al. (2000). Achilles tendon moment arm estimations at rest and during MVC. Centre-of-rotation (COR) method, which measures the perpendicular distance between the moving centre of rotation in the tibio-talar joint and the Achilles tendon action line, was compared with the tendon-excursion (TE) method, based on bone kinematics during ankle plantarflexion-dorsiflexion. COR method has shown significant difference between rest and MVC which the TE method has not shown, but the results at rest are very similar between both methods. They both confirm a decrease in moment arm with dorsiflexion.

If this dependency of intrinsic ankle stiffness on tendon stiffness is as strong as these experiments with seated individuals have shown, then it should also be true during real world standing situations in which the tendon is somehow stretched. Loram et al. (2007*b*) have suggested that the triceps surae muscle stiffness is 15 times higher than the Achilles tendon stiffness during normal standing conditions, i.e. when the body is freely standing with the COM slightly forward of the ankle joint (body leaning forward by 1.5 to 4 deg from the vertical equilibrium position) and sway size is small. The ratio of stiffness between muscle and tendon is very relevant because they are linked as springs in an in series system and are both determinant of the overall ankle stiffness. If the muscle is 15 times stiffer than the tendon then the overall maximal stiffness dependency on tendon, the weakest spring, prevails. How would this relationship change with increased tendon elongation? Furthermore, how would this affect the overall intrinsic ankle stiffness and eventually the control of standing balance in humans?

Two contradictory results for ankle stiffness were found in standing tasks. Loram and Lakie (2002*a*) have applied brief and very small (0.05 deg amplitude, 140 ms duration, squared-sine shaped) perturbations to individuals strapped to a vertical support while standing on footplates. At this fixed ankle position, the participants were asked to maintain a constant mean level of bias ankle torque for 40 s. During this task there was no ankle dorsiflexion or plantarflexion that could have influenced the tendon stiffness because the participants were attached to a fixed vertical support. Therefore, during this position the tendon stiffness could only be altered by the action of the contracted muscles pulling the tendon. However, the researchers did not find a significant increase in stiffness, but instead only a little variation of ankle stiffness (5–6 Nm deg^{-1}) even within a relatively large range in ankle torque (5–25 Nm in one leg only), a 500% increase in tension. Their conclusion was that this reflected the predominance of the aponeurosis, tendon and foot stiffness in determining the standing ankle stiffness, resulting in the minimal effect of muscle activation. Possibly the tiny perturbation mainly measured the final link in the in series chain, the stiffness of the foot tissues.

Three years later, Casadio et al. (2005), while applying larger perturbations (1 deg, 150 ms, ramps) to freely standing individuals, have found large variation of stiffness with increased ankle torque. Their sample size (n=2), though, was very small. For a male participant, they have estimated rise in ankle stiffness from 7 to 10 Nm deg^{-1} due to change in ankle torque from 35 to 74 Nm. For a female participant, a rise of 4 to 8 Nm deg^{-1} in ankle stiffness was correlated to 27 to 53 Nm increase in ankle torque. As the participants were standing freely on top of the footplates, increase in bias ankle torque was achieved by asking them to voluntarily lean forward by some degrees and keep this position stable until the completion of the trial so the rise in active torque was accompanied by a slight amount of dorsiflexion.

In both experiments, there was increase in ankle torque, and one can infer that in both conditions the tendon was elongated and the muscle was shortened because of increase

in muscle contraction. However, the results were different. Loram and Lakie (2002*a*) have found only a slight and non significant increase in intrinsic standing ankle stiffness, whereas Casadio et al. (2005) have found a significant increase. What was the difference between them?

Following the results of Loram and Lakie (2002*a*) and Casadio et al. (2005), I proposed two different experiments to further investigate how standing intrinsic ankle stiffness can be affected by tendon stretch. In the first study, intrinsic stiffness was measured at various levels of forward leaning (including a position in which the body was more vertically aligned than in normal stance) with various perturbation amplitudes (Protocol 1). This was compared with a second condition in which intrinsic stiffness was estimated while standing at a very increased ankle dorsiflexion position (Protocol 2).

The idea behind this was to compare the effects of two conditions in which tendon tension was varied. In one it was varied mainly by muscle activation (forward body leaning) and in the other it was varied mainly by passive stretch (ankle dorsiflexion). We could also test an additional hypothesis. If tendon stiffens because of forward lean or dorsiflexion it suggests that an applied ankle perturbation will be more effectively coupled to the muscle. Consequently the size of perturbation required to reduce muscle stiffness will become smaller.

5.2 Methods

Participants

For each protocol, 10 healthy volunteers (Protocol 1: six female; age 28.1±4.4 years (mean±SD); height 1.68±0.1 m; weight 65.9±8.3 kg) (Protocol 2: six female; age 29.1±10.5 years) gave written informed consent and participated in this study, which was approved

by the local human ethics committee at the University of Birmingham (Table 5.1).

Protocol 1 Participant	Sex	Age (yrs)	Height (m)	Weight (kg)	Toppling torque per unit angle (Nm deg^{-1})
P01	M	31	1.67	56.8	8.47
P02	F	26	1.63	73.8	10.58
P03	F	37	1.57	60.9	8.26
P04	F	30	1.66	60.0	8.99
P05	F	22	1.63	61.2	8.92
P06	F	24	1.66	61.9	9.15
P07	M	31	1.80	80.0	13.27
P08	M	24	1.81	77.3	12.58
P09	M	29	1.77	59.5	9.81
P10	F	27	1.63	68.1	9.74
Mean±SE	F(6),M(4)	28.19±4.4	1.68±0.1	65.9±8.3	9.98±1.7
Protocol 2 Participant	Sex	Age (yrs)	Height (m)	Weight (kg)	Toppling torque per unit angle (Nm deg^{-1})
P01	M	39	1.84	80.7	12.42
P02	F	25	1.63	73.8	12.25
P03	F	37	1.57	60.9	7.44
P04	F	23	*	*	10.13
P05	F	23	*	*	14.76
P06	F	22	1.63	61.2	8.84
P07	M	25	*	*	9.92
P08	F	23	1.66	61.9	8.06
P09	M	53	*	*	14.97
P10	M	21	*	*	17.31
Mean±SE	F(6),M(4)	29.1±10.5			11.6±3.3

Table 5.1: Participant anthropometric data. (*) Some of the participants from study 2 have missing values of height and weight because the data collection report was not completed appropriately.

Procedure and Apparatus

A full description of the footplate apparatus used to measure ankle stiffness as well as its estimation calculations was given elsewhere (Chapter 2). In brief, the participants were asked to stand on top of motorized footplates, coaxially aligned with their ankles, while ankle torque, ankle angular position, footplate acceleration and lower limb EMG responses were being recorded. The methodology specific to this chapter's two different studies is described below.

Protocol 1

The main objective of this experiment was to verify intrinsic ankle stiffness changes with increase in ankle torque caused by COM displacement forward of the ankle joint. The subjects performed standing trials of approximately 3 minutes, in which small and brief perturbations were applied at a variable gap of 4–5 seconds. The perturbations were shaped as toes-up or toes-down squared-sine curves of 140 ms duration and a variable amplitude of 0.1, 0.3 or 0.7 deg to assess the effect of leaning within and beyond the short-range stiffness. The 3 different rotation sizes were chosen to verify if ankle stiffness dependency on stimulus amplitude (Kearney and Hunter, 1982; Hufschmidt and Schwaller, 1987; Loram et al., 2007*a*) would be affected by different levels of forward leaning of the vertical body. It was difficult for the participants to maintain an aligned body position while freely standing in a forward leaning posture without using hip strategy. Therefore, to reduce movement of the hips and knees, a detached light wooden board (1.2 m length, 0.5 m width and total weight 1.2 kg) was strapped to the participant's back with Terylene webbing at shoulder, waist and calf levels. The participants could then practically only use the ankle strategy to control position. The whole experimental procedure consisted of one session of approximately 1 ½ hours.

The amount of body leaning can be controlled by shifting the location of the COP under the feet. I asked the subjects to manipulate the amount of forward shift of the COP by monitoring the average baseline torque applied by the feet against the footplate, displayed on a screen located at eye level. They did 3–6 training trials of varying leaning positions. After a stable position was found for each condition, I averaged the torque trace and established this value as a guideline trace they had to follow. It was displayed on the screen during the actual trials. They performed three different levels of forward leaning:

1. Normal: standing at their spontaneously chosen position;

2. Vertical: standing with the COP shifted backwards in relation to their normal

condition. Participants were asked to reduce torque applied against the footplate, as much as possible without compromising their free standing balance control;

3. Lean: standing with the COP shifted forwards in relation to their normal condition. Participants were asked to increase torque applied against the footplate to a level that was still comfortable and sustainable for the duration of the 3 min trials.

There were 3 conditions of COP shift (normal, vertical and lean) and 3 different perturbation amplitudes (0.1, 0.3 and 0.7 deg), resulting in a total of 6 conditions. Given that 30 perturbations were applied per condition, altogether 180 events were recorded for each participant. Different standing condition trials and perturbation amplitude and direction were all randomized.

Protocol 2

For this protocol, the main objective was to increase ankle dorsiflexion with reduced change in baseline ankle torque. Participants performed standing trials with perturbations of the same shape and time-window intervals as in the previous experiment, but only with amplitudes of 0.1 or 0.7 deg. There were two different conditions:

1. Normal: standing at their spontaneously chosen position;

2. Dorsiflexion: standing with the footplate rotated upwards by 15 deg.

As it was relatively easy for the participants to maintain an aligned upright stance while the ankles were dorsiflexed, during this experiment I did not attach a wooden board to the participant's body. The whole experimental procedure consisted of one session of approximately 1 hour. Two different ankle position conditions and 2 different perturbation amplitudes summed up to 4 different conditions. 32 perturbations were recorded per condition, resulting in a total amount of 128 events from each participant. Normal standing

was investigated prior to dorsiflexion condition trials. For each standing condition, both perturbation amplitude and direction were randomized.

Data analysis

Determination of baseline ankle torque, ankle and body position, body sway and EMG activity

Here I assessed the amount of leaning as the forward shift of the COP, which is expressed as the increase in ankle torque. This was calculated as the average absolute ankle torque during a 70 ms time window prior to each perturbation onset. The amount of ankle dorsiflexion and body inclination were calculated as the mean ankle (laser signal reflecting movement of the shin subtracted by footplate position) and body position (laser signal reflecting movement of the board or waist) over a 2 s time window prior to each perturbation onset. The laser reflex sensors measured the relative change in ankle and body positions, not the absolute change in relation to Earth. Therefore, with the available data it was not possible to verify the precise position of the body in space. To verify within-individual differences between conditions, I normalized each participant's data to their normal condition, described here as 0 deg for body angle and 90 deg for ankle angle. Thus I discounted their actual elected standing position, which corresponds to a variable 1.5–4 deg forward leaning in relation to Earth (Loram and Lakie, 2002*a*).

The effect of different conditions on stability and control of movement was assessed with measurements of body sway and muscle activity. Body sway was quantified as the average root-mean-square (RMS) ankle position and velocity over a 2 s time window prior to each perturbation onset. Muscle activity was calculated as the integral of the rectified EMG activity of the medial gastrocnemius and tibialis anterior muscles over a 70 ms time window envelope prior to each perturbation onset. To compare changes within conditions,

I normalized all participants' EMG data as a ratio of normal standing data.

Statistical analysis

Repeated-measures ANOVA was used to determine effects of condition (normal, vertical and lean or normal and dorsiflexion, for 1st and 2nd protocols) and stimulus amplitude (0.1, 0.3 and 0.7 deg or 0.1 and 0.7 deg, respectively) upon ankle stiffness. Two-tailed paired samples t-test was used to verify differences of baseline ankle and body position, ankle torque, body sway and EMG activity between conditions. Pearson's correlation was used to investigate the relationship between baseline ankle torque and stiffness. $P<.05$ was considered statistically significant for all tests.

5.3 Results

Representative data of one participant during two different protocols is shown in Figure 5.3. The first 4 rows are data from Protocol 1, while the last 4 rows are data from Protocol 2.

Absolute ankle torque and relative ankle and body position

The objective of the two different experiments was to better understand the increase in intrinsic ankle stiffness caused either by passive tendon stretch or increase in active ankle torque in standing individuals. Therefore, it was important to assess if the conditions imposed by both experiments were appropriate to induce significant differences within these two variables. As was my intention, there was a significant increase in baseline mean ankle torque between each of the conditions within Protocol 1, ranging from 4.9±2.4 Nm

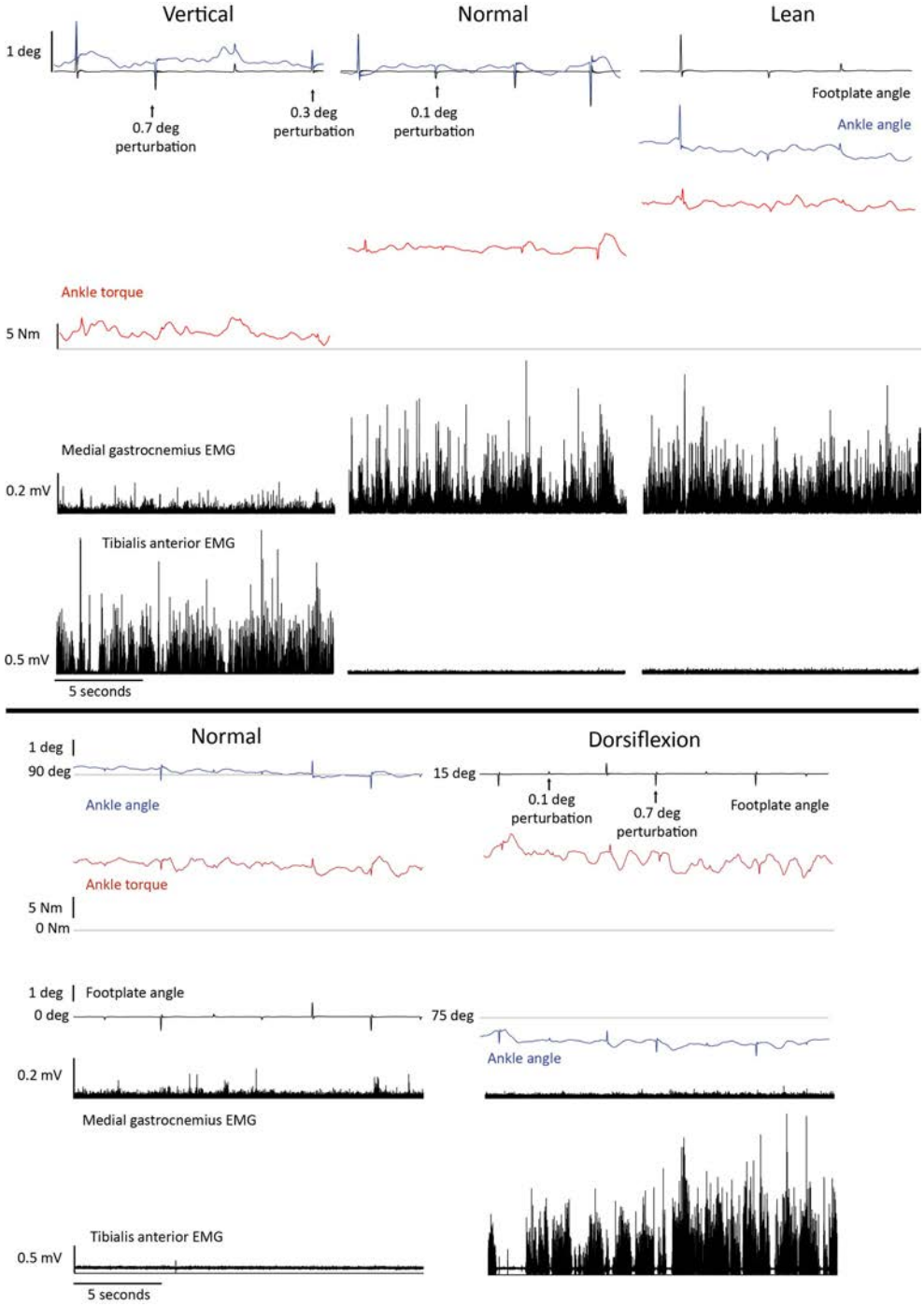


Figure 5.3: Representative segment of datasets. Effect of active ankle torque and passive tendon stretch on ankle angle (footplate minus shin angle), left ankle torque, left medial gastrocnemius EMG and tibialis anterior EMG. Top panel are data from study 1 and bottom panel are data from study 2, all taken from one participant. The horizontal line beneath the torque traces represents 0 Nm. For study 2, ankle angle equals 90 deg when the footplate is levelled; it decreases (in this case to $\sim$ 73 deg) when the footplate rotates upwards from 0 deg to 15 deg. The difference (from 75 deg) is due to body and leg movement associated with the toes up stance.

(vertical, mean±SD), 16.4±4.7 Nm (normal) and 31±5.7 Nm (lean). A two-tailed paired samples t test revealed a significant difference between vertical and normal conditions ($t_{(9)}$=-9.3; p<.001) and lean and normal conditions ($t_{(9)}$=-9.2; p<.001) (Figure 5.4, left graph). For the second experiment, my main concern was to change the amount of ankle dorsiflexion by means of tilting the standing platform by 15 deg. There was a slight and significant torque increase from 17.6±4.7 to 22.4±5.7 Nm ($t_{(9)}$=-2.4; p<.05) (Figure 5.4, right graph), a result of the participants standing with the ankles in a dorsiflexed position leaning slightly forwards compared to normal condition.

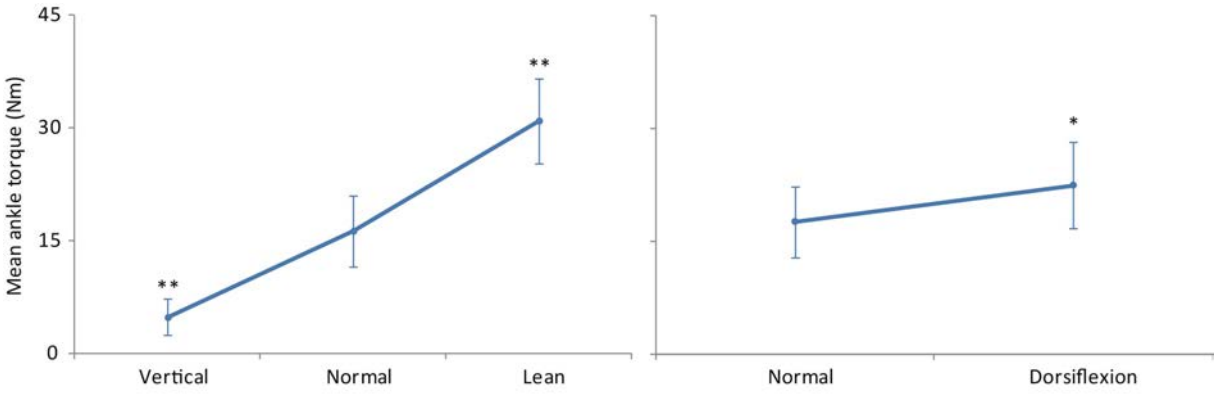


Figure 5.4: Mean ankle torque (Nm, mean±SD)

Following the analysis of ankle torque, I identified the increase in ankle dorsiflexion (measured with laser signal reflecting movement of the shin) and body mean position (measured with laser signal reflecting movement of the board or waist) in each experiment. The difference between normal and vertical was significant when comparing body ($t_{(9)}$=-3.4; p<.01), but not ankle position. The non significant result in ankle measurements probably reflect imperfect alignment of the body when the participants were leaning backwards. Nevertheless, comparison between normal and lean was significant when both body ($t_{(9)}$=-7.4; p<.001) and ankle ($t_{(9)}$=-4.5; p≤.001) mean positions were compared (Figure 5.5, left graphs).

As expected, rotating the platform by 15 deg was enough to achieve a successul difference in ankle dorsiflexion (measured with laser signal reflecting movement of the shin minus the footplate upward tilt of 15 deg) for the second experiment ($t_{(9)}$=-17.6; p<.001) (Figure 5.5,

top right graph). With the analysis of the body average position, I verified that even though instructed otherwise, the participants leaned forward by a slight amount (0.4±0.7 deg), but this was not significant ($t_{(9)}$=-1.7; p=.12) (Figure 5.5, bottom right graph).

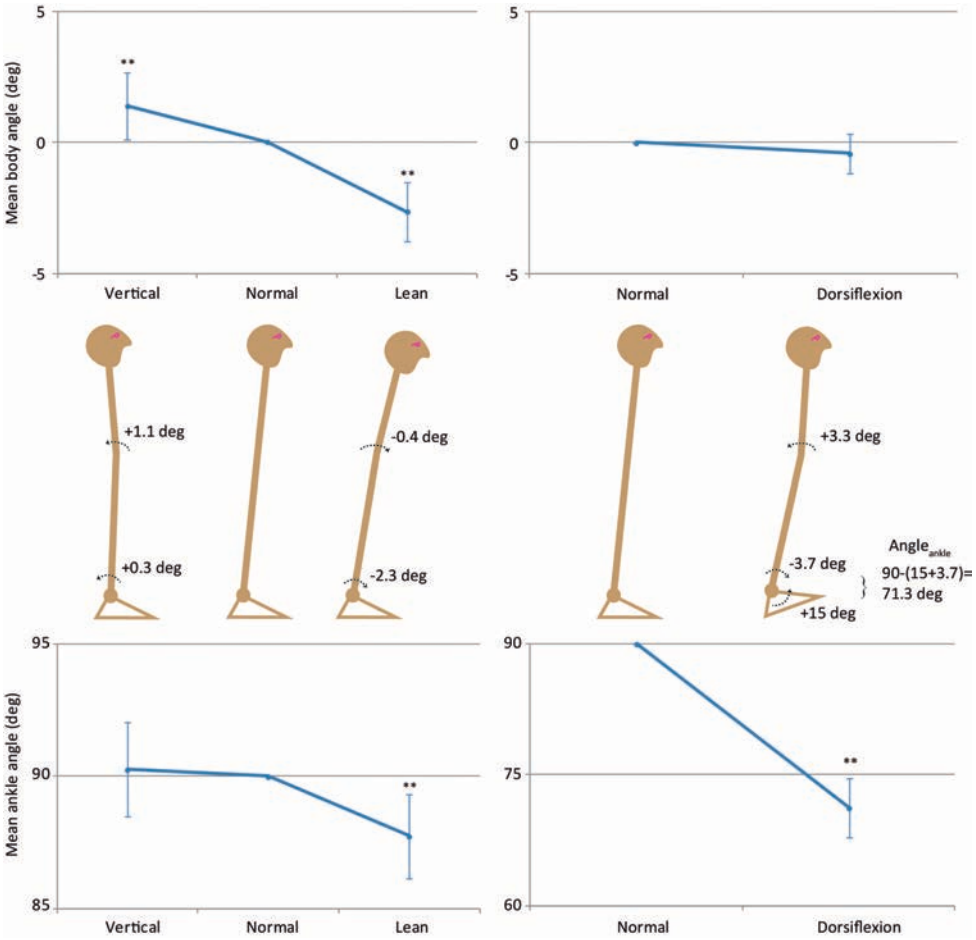


Figure 5.5: Mean body (top) and ankle (bottom) angle (deg, mean±SD) relative to normal condition (=0 for body angle, =90 for ankle angle). Schematic representation of the relative change in body and ankle angle is shown in the middle panel. In all conditions (particularly the toes-up condition) the body changes its postural configuration.

Intrinsic ankle stiffness

The main results of this study, the average intrinsic ankle stiffness results presented as a percentage of toppling torque per unit ankle (% mgh), are shown in Figure 5.6. For the first experiment (left graph), values ranged from 37% to 97% mgh. There is a systematic increase in stiffness (14%, on average) as condition moved from vertical to normal and from

normal to lean, independent of perturbation size (condition: $F_{2,18}$=18.5; p<.001; amplitude: $F_{2,18}$=170.2; p<.001). This shows no interaction between condition and amplitude ($F_{4,36}$=0.63; p=.64). Ankle dorsiflexion also produced significant increase in stiffness of on average 29% (condition: $F_{1,9}$=18.4; p=.002; amplitude: $F_{1,9}$=40.2; p<.001), also with no interaction between condition and amplitude ($F_{1,9}$=0.31; p=.59) (right graph). From the data presented in Figure 5.4 and Figure 5.5, one can assume that the verified increase in intrinsic ankle stiffness found in the second experiment was not dependent only on increased passive stretch, but also on increased forward leaning of ∼0.4 deg. Fortunately, the results obtained from normal condition in both experiments were very similar (51%-50% for 0.7 deg perturbation and 82%-77% for 0.1 deg perturbation, respectively), making comparisons between both experiments easier to interpret.

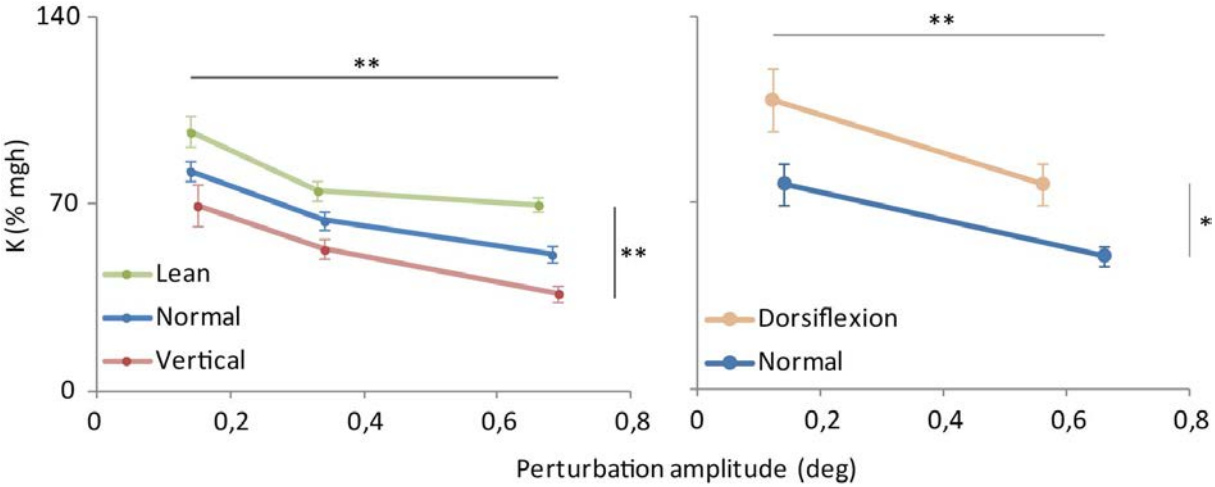


Figure 5.6: Intrinsic standing ankle stiffness (% mgh, mean±SEM) against perturbation amplitude (deg).

Relationship between intrinsic ankle stiffness and ankle torque

Both experiments have shown a significant and consistent stiffness variation. To confirm if increase in intrinsic ankle stiffness was dependent on the significant increase in ankle torque, I then proceeded to correlate these two variables' complete dataset (all participants, all conditions). The results are shown in Figure 5.7.

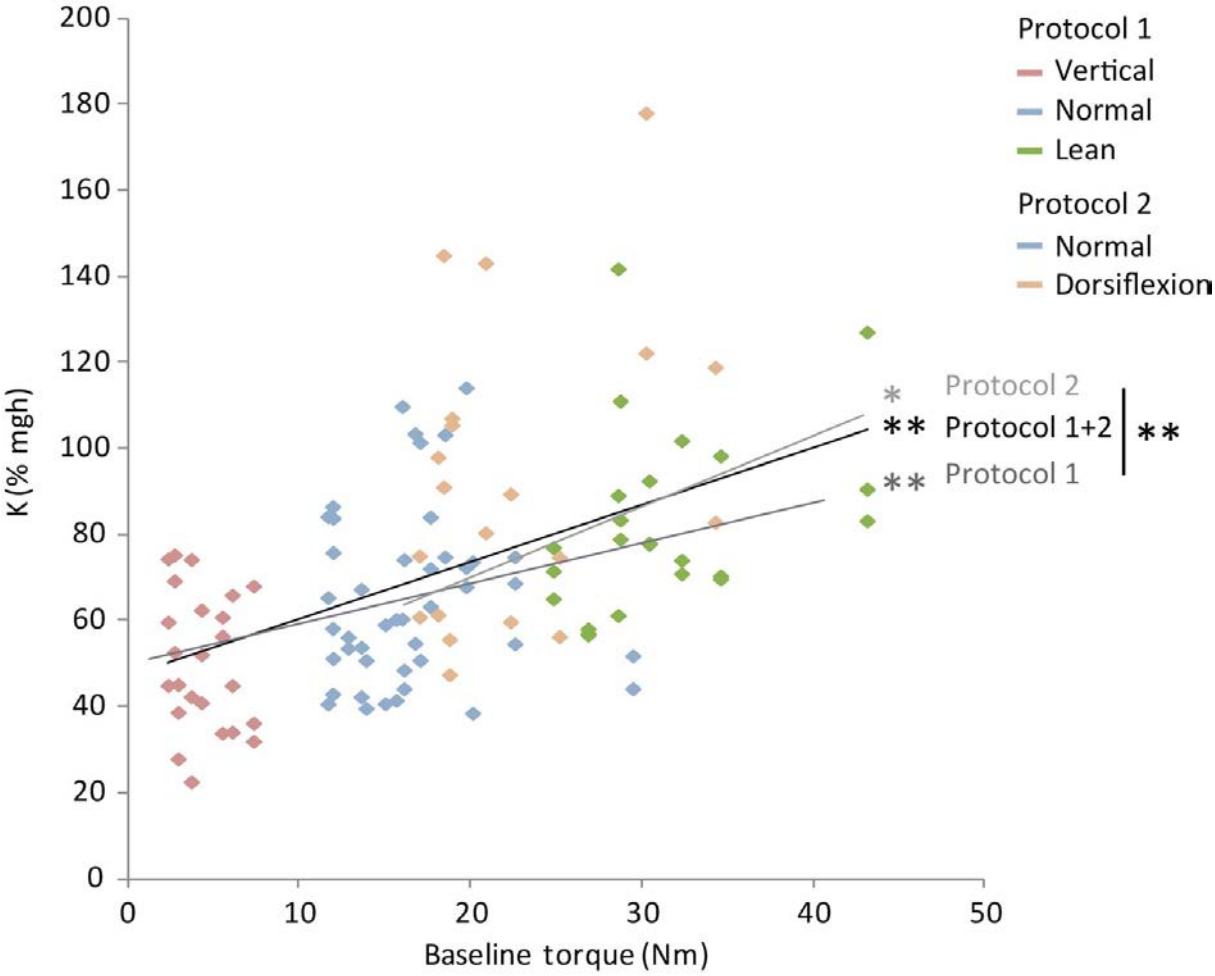


Figure 5.7: Intrinsic ankle stiffness (% mgh) against baseline ankle torque (Nm). Data from each participant for each perturbation amplitude at each condition, vertical (red), normal (blue), lean (green) and dorsiflexion (orange). Results are shown from 1st protocol, 2nd protocol and both protocols combined.

In the top left figure, each dataset corresponds to a particular condition (normal, vertical or lean, all amplitudes displayed) of the 1st experiment. The aggregation of all datasets shows a strong and significant relationship between variables (Pearson's correlation, $r_{(100)}=0.51$; $p<.001$). This confirms that there is a direct relationship between increase in standing intrinsic ankle stiffness and increase in mean ankle torque caused by change in body COM position forward of the ankle joint. In the top right figure, dataset from normal and dorsiflexion conditions of the 2nd experiment are shown, also with a significant relationship between variables (Pearson's correlation, $r_{(40)}=0.35$; $p=.02$). The bottom figure represents the data from both protocols added together, confirm-

ing the strong relationship between intrinsic K and ankle torque (Pearson's correlation, $r_{(140)}$=0.51; p<.001).

Following the confirmation that intrinsic K was significantly dependent on ankle torque, I have plotted the data from Protocol 1 (from each participant at all conditions), mainly focused on changes in ankle torque, against an estimate of the average data from the first experiment of Mirbagheri et al. (2001) (Figure 5.8, top left graph). The average relationship between intrinsic ankle stiffness and ankle torque found in Protocol 1 with standing individuals (light blue line) is very similar to their data with seated individuals generating prescribed different levels of mean ankle torque (orange line). When the data from Protocol 2 is compared, the similarities with Mirbagheri et al. (2001)'s results are less clear. Similarity of averaged data is not present when comparing with ankle torque data from the authors' first experiment (Figure 5.8, bottom left graph), but the general increasing trend is concordant when comparing body position data with the authors' second experiment (Figure 5.8, bottom right graph). It is important to emphasize that Mirbagheri et al. (2001)'s results are estimated values obtained by visual analysis of the authors' graphs (Figure 5.1 from this chapter, Figures 5A and 7A from the authors' paper).

Body sway and muscle activity

Sway size is described in Figure 5.9. Assessment of sway (body position root-mean-square) shows, apart from a significant difference between vertical and normal ($t_{(9)}$=2.9; p<0.05), mainly no significant difference between conditions. Interesting to mention, though, is that the results from experiment 1 show a slight reduction in sway size in the normal position when compared to vertical and lean (top left graph). Sway velocity (body velocity root-mean-square) is significantly different between conditions ($t_{(9)}$=6.5; p<.001 between vertical and normal; $t_{(9)}$=2.5; p<.05 between lean and normal) (bottom left graph). The

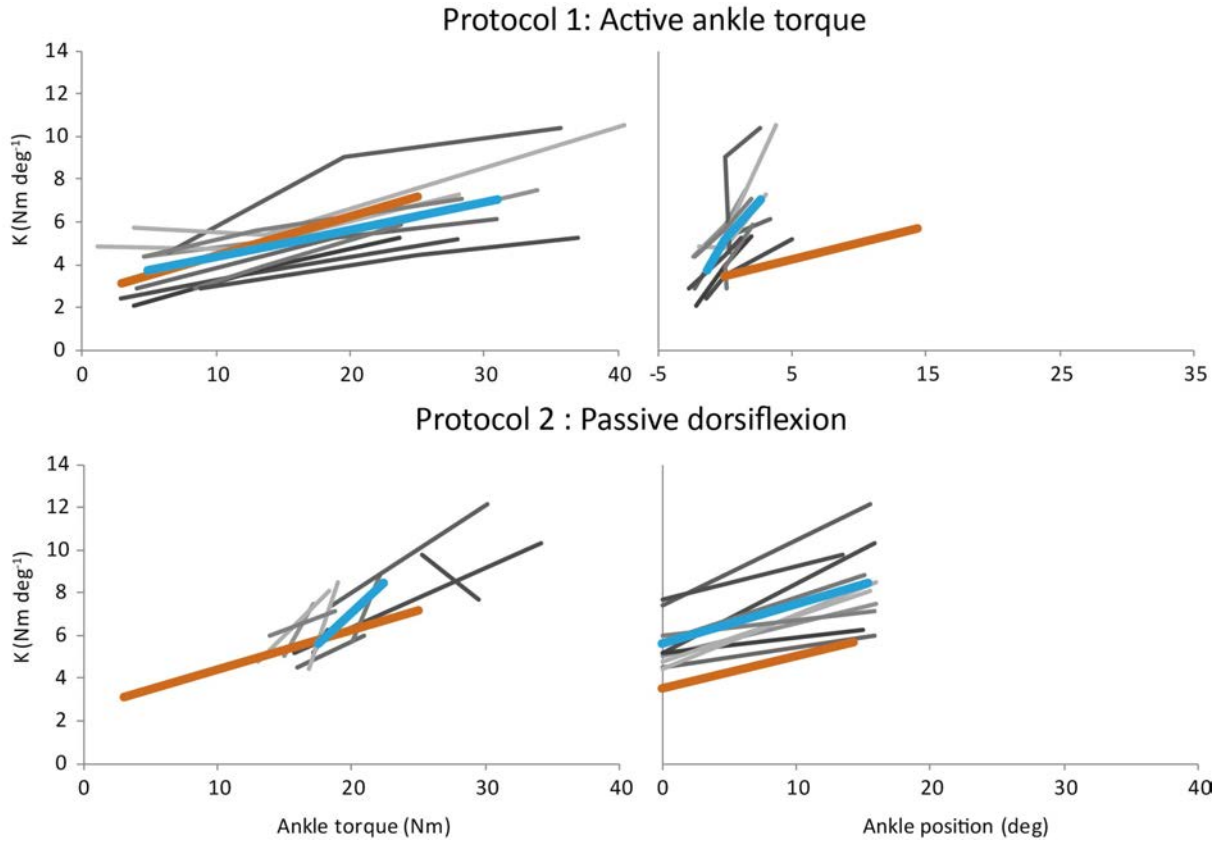


Figure 5.8: Intrinsic ankle stiffness (Nm deg^{-1}) against mean ankle torque (Nm) and mean ankle position (deg), compared with Mirbagheri et al. (2000) data. Top graphs: Ankle torque and ankle position data from each participant at all conditions of Protocol 1 (1st point=vertical, 2nd point=normal, 3rd point=lean) are plotted. Bottom graphs: Ankle torque and ankle position data from each participant at all conditions of Protocol 2 (1st point=normal, 2nd point=dorsiflexion) are plotted. The average values from Protocols 1 and 2 are shown in light blue, while the results from Mirbagheri et al. (2000) from seated participants are shown in orange.

combined data from body sway size and velocity might indicate the increased instability found when participants were either leaning forwards or backwards from the normally chosen quiet standing position. In experiment 2, the dorsiflexion condition has shown no significant difference with normal either in sway size (top right graph) or sway velocity (bottom right graph). The difference in sway velocity between experiments, even in normal condition, might be due to the use of a wooden board in the first experiment. As the participants were firmly attached to it, hip and knee joint motion was restricted, probably resulting in larger sway instability.

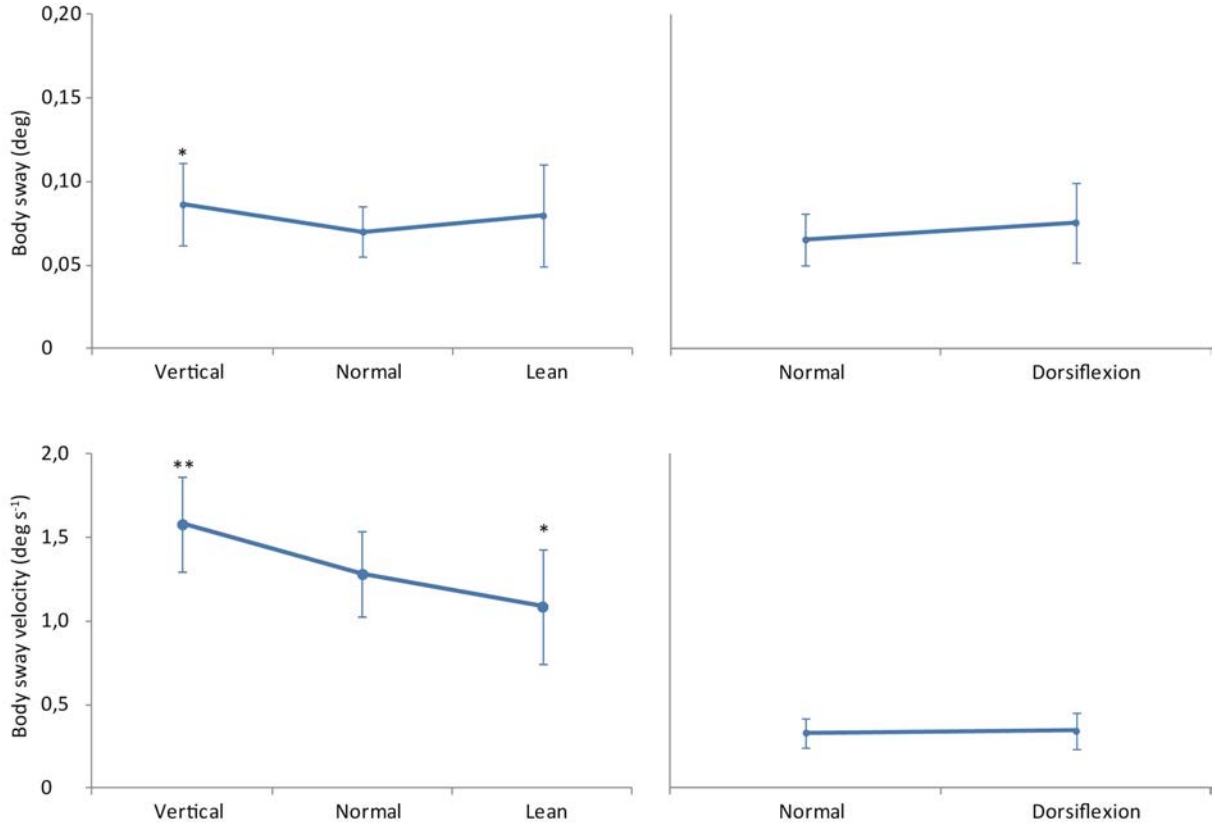


Figure 5.9: Baseline body sway (deg) and sway velocity (deg s^{-1}) (mean±SD).

The analysis of EMG activity confirms that changing the level of body leaning either backwards or forwards from normal stance will generate a greater need for neural control. Figure 5.10 shows data normalized against normal condition (normal = 1). As expected, by leaning backwards (vertical condition), the medial gastrocnemius (GM) activity remains low, but there is a high increase in tibialis anterior (TA) activity. The opposite is true when leaning forwards, there is a high increase in GM activity with only a slight increase in TA activity (one-way ANOVA, GM $F_{2,27}$=11.74; p<.001; TA $F_{2,27}$=12.6; p<.001) (left graph). Also as expected from dorsiflexion condition, the data shows a significant increase in TA activity and decrease in GM activity (one-way ANOVA, GM $F_{1,18}$=5.61; p=.029; TA $F_{1,18}$=5.7; p=.028).

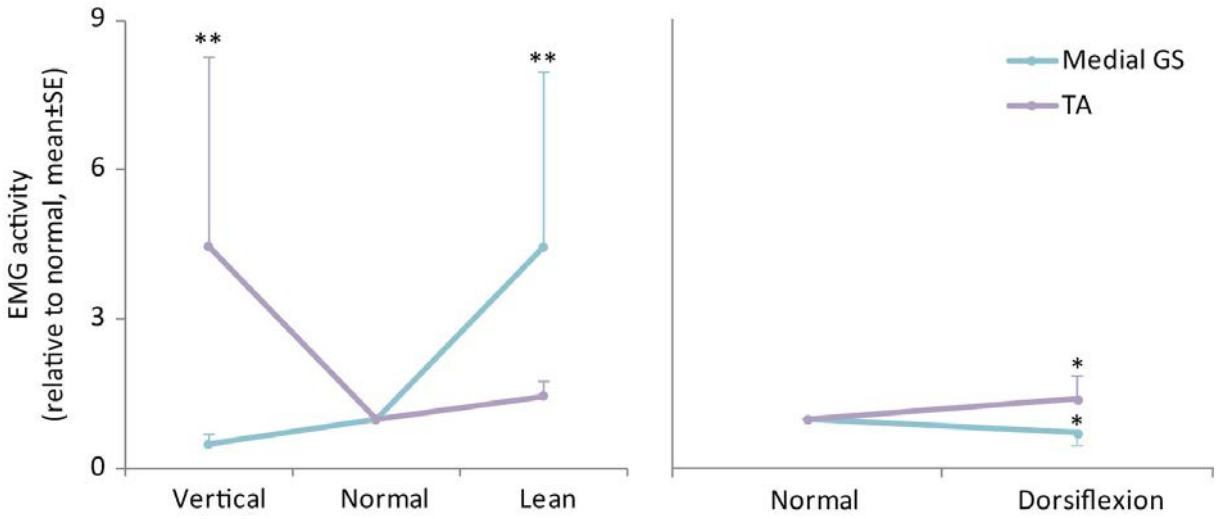


Figure 5.10: Baseline EMG activity ratio (relative to normal condition, mean±SD).

5.4 Discussion

In this chapter I attempt to better understand how intrinsic ankle stiffness changes during real life standing conditions in which there is increased tendon tension. The objective was to manipulate in various ways the tendon contribution to the overall intrinsic ankle stiffness and discover how this affects stability. To add information to previous research on standing individuals (Casadio et al., 2005; Loram and Lakie, 2002*a*), two different studies were performed. First, the active ankle torque was altered by asking the participants to lean the body at different angles to keep the COM position at various distances forward of the ankle joint while intrinsic stiffness was being assessed. Intrinsic stiffness was shown to positively correlate with increasing levels of forward leaning which produced increasing levels of active ankle torque. Second, the ankle angle was altered by rotating upwards the standing surface by 15 deg while intrinsic stiffness was being assessed. I have also found a positive intrinsic stiffness dependency on passive stretch. These results confirm Casadio et al. (2005)'s claim that stiffness increases with active torque. This effect remained similar as I reduced the perturbation size from 0.7 to 0.1 deg. In opposition to the results presented here, for much smaller perturbations (0.05 deg), Loram and Lakie (2002*a*) have found little alteration in stiffness with increase in load. The results have shown that:

(1) there was the expected greater stiffness for smaller stretches, regardless of which condition was being tested; (2) however, I expected that the results from the altered conditions (change in active ankle torque and passive stretch) would be less different for the smallest perturbation sizes, but I did not see this interaction. In the following sections I will discuss these results and the possible implications for the control of standing.

In the experiments with standing individuals presented here, as the objective was to assess stiffness in real world situations, I could not completely isolate change in active ankle torque from change in passive stretch. During Protocol 1, the participants were largely increasing active ankle torque by leaning forward (increase of ∼26.1 Nm from vertical to lean) because this is the only way that active ankle torque can be altered in freely standing individuals (unless they wear a heavy backpack). Hence there was a small level of ankle stretch added to it (2.5±1.7 deg increase in ankle dorsiflexion from vertical to lean). During Protocol 2, the participants were standing on a largely tilted surface to increase passive stretch (increase of 18.7±3.4 deg ankle dorsiflexion from normal), but they did not maintain the active ankle torque at the same level as in normal standing. They slightly increased it by leaning forward by a small amount in order to maintain balance (increase of ∼4.8 Nm from normal to dorsiflexion).

In standing, intrinsic ankle stiffness has a positive dependency on active ankle torque

The results of Protocol 1 were comparable to the results of seated individuals (Mirbagheri et al. (2001), Figure 5.8 top graph), which also found significant increase in intrinsic ankle stiffness with increase in active ankle torque. The protocols were very different, but the results were still congruent. It was interesting to identify a satisfactory match between both experiments performed under very different conditions with 2 different research groups.

However, in a task similar to Mirbagheri et al. (2001) but with individuals in upright posi-

tion, Loram and Lakie (2002*a*) found no relationship between intrinsic ankle stiffness and ankle torque. In their setup, the participants were instructed to modulate plantarflexing ankle torque from a fixed ankle position, while the participants were standing upright and strapped to a fixed board. Casadio et al. (2005), on the contrary, have found significant increase in intrinsic stiffness with increase in ankle torque when having freely standing individuals changing the position of their body COM by leaning forward to a sustainable level that would not misalign the body.

What was the difference between these 2 studies? Controlled large increase in ankle torque was possible in Loram and Lakie (2002*a*) because the participants could use the fixed board to support the body against the increased plantarflexing ankle torque, whereas in Casadio et al. (2005) increase in ankle torque was strictly dependent on the amount of COM displacement forward of the ankle joint because the participants were freely standing. In Loram and Lakie (2002*a*) there was a 500% increase in ankle torque whereas in Casadio et al. (2005) only a 100% increase in ankle torque was induced. This indicates that the tendon was actually stretched more in the first experiment. Then why would there be a significant difference in the second rather than in the first experiment's results? The reason given by Loram and Lakie (2002*a*) was that the perturbations in their experiment were tiny (0.05 deg amplitude), so although the overall stiffness may have become great this was 'hidden' by the compliance of the tissues of the foot.

The results shown in this chapter (Protocol 1), also with freely standing individuals, confirm Casadio et al. (2005)'s significant positive correlation between active ankle torque and standing intrinsic ankle stiffness. This relationship is clearly shown in Figure 5.7. For an average stiffness of 53–66–81% mgh for each condition (vertical-normal-lean), the average active ankle torque immediately before perturbation ranged from 5–16–31 Nm (sixfold increase).

Despite the fact that Casadio et al. (2005) have only tested two people, their results were still comparable to mine. With an average increase in ankle torque of 13.9 Nm from

normal to leaning condition, Casadio et al. have found increase in intrinsic stiffness of 33.5% mgh. In Protocol 1, I have obtained an average increase in ankle torque of 26 Nm from vertical to lean conditions (Figure 5.4, left graph). This corresponded to an intrinsic stiffness increase of 33.1% mgh when measured with 0.7 deg perturbation (Figure 5.6, left graph), more similar to the 1 deg perturbation used by Casadio et al. (2005). Therefore, with less increase in ankle torque Casadio et al. have found higher increase in intrinsic stiffness. In their experiment, the participants shifted the COP forwards by 4.9 cm. For the average adult height, one degree equals to about 1.7 cm so the participants might have leaned forward by ∼3 deg. Nevertheless, both studies confirm a positive correlation between increase in forward leaning and increase in standing intrinsic stiffness of the ankles.

Because the different results found by Loram and Lakie (2002*a*) and Casadio et al. (2005) were possibly related to the different perturbation size used to assess stiffness, I expected to find less difference of stiffness for the smallest perturbation sizes with increase in active ankle torque. However, I did not see this interaction. The perturbation size (0.1–0.3–0.7 deg) used in Protocol 1 was lower than in Casadio et al. (2005) (1 deg), but larger than the perturbation size used by Loram and Lakie (2002*a*) (0.05 deg). Between the lowest (vertical) and highest (lean) estimated values, difference of stiffness was 27.5% mgh for 0.1 deg perturbation, 22.0% for 0.3 deg and 33.1% for 0.7 deg. Probably in the Protocol 1 case, as in Casadio et al. (2005), 0.1 deg perturbation was large enough for the foot compliance not to affect the final estimate as it might have in Loram and Lakie (2002*a*).

Interestingly, even when the participants were leaning backwards and applying an average ankle torque of as little as 2.2 Nm against the ground, the intrinsic standing intrinsic stiffness remained relatively high, at 70% mgh when perturbed by 0.1 stimulus amplitude and down to 36% mgh when perturbed by 0.7 stimulus amplitude (Figure 5.6, left graph). It shows that even at conditions close to the vertical equilibrium position when there is less ankle torque and the ankle is closer to neutral position, standing intrinsic stiffness is

still relatively high.

In standing, intrinsic ankle stiffness has a positive dependency on passive ankle position

To my knowledge, there has not been any previous study about the effects of passive stretch on the intrinsic ankle stiffness of standing individuals. In this chapter I have shown that this relationship is significantly positive. With a large increase in passive stretch by 18.7 deg ankle dorsiflexion and a small increase in active ankle torque of ∼4.8 Nm, stiffness ranged from 50–77% mgh to 77–109% mgh (0.7 and 0.1 deg perturbations, respectively). The change in ankle torque is probably because in standing people become accustomed to generating a certain amount of active force. When passive force is generated by ankle dorsiflexion, the only way active force can be kept at the same level is by leaning forward. Nevertheless, even with the mixed type of intervention during standing condition, it was possible to approximately compare mine with the data from Mirbagheri et al. (2001). In that study, the authors have also shown in seated individuals that intrinsic ankle stiffness depends on passive stretch (from maximum plantarflexion to maximum dorsiflexion) while the ankle was exerting constant -5 Nm plantarflexion torque against the footplate (Figure 5.1, bottom graph). In an estimated comparison based on averaging of the authors' data, the results are apparently similar to the results from Protocol 2 (Figure 5.8, bottom right graph).

This strong relationship between ankle stiffness and passive stretch was previously demonstrated in another very relevant paper written by Kearney, Hunter and colleagues (Weiss et al., 1986*b*). The authors have shown that when the ankle is passively stretched from neutral until end of ROM, there is very little change in ankle torque (ranging from -6 to -12.4 Nm plantarflexing torque), but intrinsic stiffness increases considerably (1.3 to 3.6 Nm deg^{-1} Nm^{-1}). The authors compared these with the results of another set of trials in

which the participants were asked to actively modulate ankle torque. They have found similar values for contraction levels ranging from 10 to 20% MVC, when ankle intrinsic stiffness ranged from 1.5 to 3.6 Nm deg^{-1} Nm^{-1} and active plantarflexor torques ranged from -5.7 to -14.4 Nm. Although contraction levels of 10-20% MVC can be maintained for long periods without apparent fatigue, it is interesting to have the option of increasing joint stiffness with solely intrinsic mechanisms for energy saving. This shows the importance of the passive structures and emphasizes the relevant effect of passive stretch on stiffness and how it has to be taken into consideration when analyzing the dependency of intrinsic stiffness on ankle torque.

As the results of Protocol 1 have shown that different conditions of increased active ankle torque did not have any dependency on perturbation amplitude, for Protocol 2 I have used only two different perturbation sizes, 0.1 and 0.7 deg. Once again, I have found no dependency on perturbation amplitude in a between-condition analysis. The decrease in intrinsic stiffness with increase in perturbation amplitude was similar between conditions, with a difference of 31.5% at 0.1 deg and 26.9% mgh at 0.7 deg.

This significant change in ankle intrinsic stiffness with little change in ankle torque was explained previously as probably a consequence of either co-contraction of antagonist muscles, resistance of other tissues enwrapping the ankle or change in moment arm. Analysis of muscle activity shows that, although significant, the degree of coactivation during dorsiflexion is very slight (Figure 5.10, right graph). Thus the degree of coactivation seems inadequate to produce the large increase in intrinsic stiffness observed here. A reduction in moment arm, caused by dorsiflexion, should produce a decrease in stiffness rather than the increase that was observed in the second experiment.

Therefore the rise in stiffness is most likely caused by the stretching of other tissues enwrapping the ankle joint. Most muscles connected to a long free tendon like the Achilles tendon are partially covered by sheet-like aponeuroses that, extending contiguous to the tendon and attaching to the bone, also act as a tendinous muscle insertion. Recent

studies have shown that it is subject to strains that differ from tendon strain in different ways (Azizi et al., 2009; Azizi and Roberts, 2009; Herbert et al., 2014). However, due to difficulty in isolating aponeurosis from muscle and tendon, it is not clear in which way its stiffness affects the overall ankle stiffness.

When compared with the second experiment of Mirbagheri et al. (2001) which had a constant mean ankle torque of -5 Nm and a similar change in ankle position, the difference is that in Protocol 2 the mean ankle torque during normal condition was 17.6±4.7 Nm, increasing to 22.4±5.7 Nm during dorsiflexion. As a consequence, stiffness at neutral position was less in their experiment (∼3.5 Nm deg^{-1}, as opposed to 5.6±1.1 Nm deg^{-1} in Protocol 2). However, it seems likely that the intrinsic stiffness increase with dorsiflexion found by Mirbagheri et al. (2001) was very similar to the results from Protocol 2 (∼2.2 and 2.9 Nm deg^{-1} increase, respectively). This similarity with Mirbagheri et al. (2001) confirms that in Protocol 2 the increase in intrinsic stiffness was most likely affected by the increase in passive stretch rather than the increase in active ankle torque. It also indicates that mostly the main reason for this change in intrinsic stiffness was the change in tendon tension.

Simultaneous dependency of intrinsic ankle stiffness on active ankle torque and passive ankle stretch during normal standing

Weiss et al. (1986*b*) mentioned in their paper the importance of passive stretch during postural control. If during normal standing people tend to lean forward of the ankle joint from the vertical equilibrium point by a small amount, then the increase in intrinsic stiffness will necessarily be affected by these 2 variables, increase in the triceps surae muscle tone and increase in passive stretch. When I compare vertical with lean data from Protocol 1, this effect of forward leaning on the normally standing individual's intrinsic stiffness is clearly seen (average increase in stiffness of ∼27.6% mgh between 3 perturbation

amplitudes). Interestingly, results from Protocol 2 also confirm the importance of passive stretch. As shown by the analysis of body position, the participants leaned forward when dorsiflexed by 0.4 deg (Figure 5.5, bottom right graph). Because they were standing, to cope with the change in COM position, more toque would be needed. However, analysis of surface EMG of the gastrocnemius muscle has shown that there is a slight but significant reduction in activity during dorsiflexion (Figure 5.10, right graph). Most likely, then, some of the torque was produced by passive stretch of the ankle tissues. Not only tendon and aponeurosis stiffness increased with passive stretch, but muscle stiffness most likely also increased with stretching of parallel structures.

Confirming the general assumption that the disposition of the foot forward to the ankle joint shifts the most stable body COM position from the vertical equilibrium to some centimeters forward of the ankle joint, I have found that a significant 4.5 ratio of lean to normal condition increase in gastrocnemius medialis activity was necessary when the body was leaning forwards by as little as 2.6 deg. Accordingly, a similar 4.5 ratio of vertical to normal condition increase in tibialis anterior activity was necessary when the body was leaning backwards by only 1.4 deg (Figure 5.5 bottom left and Figure 5.10 left). It indicates that the chosen normal standing position is the best compromise between the activities of these antagonistic muscles. During quiet standing the TA activity is mostly quiet. Most of the modulation of torque is dependent upon the ballistic input from the CNS acting on the triceps surae muscle (Lakie et al., 2003). As the foot is located mostly forward of the ankle joint, the effective production of the counteractive torque requires that the body is kept leaning slightly forward of the vertical equilibrium point. Thus the modulation of the CNS has to be performed by the triceps surae muscle ballistically pulling the body backwards when the gravitational forces are inducing the body to lean too much forwards. However, once the body position is pulled closer to the vertical equilibrium point, then the TA activity has to be increased to counteract the gravitational forces that start to pull the body to fall backwards. Hence the increased TA activity found in our results reflects this switch of the controlling mechanism. During this

experiment, tendon and aponeurosis stiffness increased with increase in passive stretch. Even though it might have not affected the overall ankle stiffness results, in this case muscle stiffness most likely increased due to increased cross-bridge activity, either from triceps surae or from tibialis anterior muscles.

From the combination of results shown in Protocols 1 and 2 and the studies performed by Weiss et al. (1986*b*), maybe the low activity of tibialis anterior and triceps surae muscles during normal standing are caused by the increase in intrinsic stiffness. This in turn is caused by a discrete level of passive stretch that induces very little change in ankle torque but a relatively high increase in intrinsic stiffness. This is effective enough to significantly reduce the level of active modulation of muscle activity during normal standing, thus saving energy for other activities.

In summary, the results from the combination of all the studies cited above confirm that increase in standing intrinsic ankle stiffness is dependent on increase in tendon stiffness, whether it is produced by active ankle torque or by passive stretch. In this chapter I presented changes in intrinsic stiffness occuring with experimental conditions that are representative of every day life existing standing situations. Significant increase in intrinsic stiffness was found in conditions of large ankle torque change and little dorsiflexion as well as in conditions of large dorsiflexion and little ankle torque change.

CHAPTER 6

INDIVIDUAL DIFFERENCES IN INTRINSIC ANKLE STIFFNESS AND THE IMPLICATIONS FOR BODY SWAY

Abstract In previous studies (Chapters 3, 4 and 5), I examined the standing intrinsic ankle stiffness dependency on muscle thixotropy, localized cooling, active ankle torque and passive ankle stretch. These relationships are related to mechanical properties particular to muscles and tendons and most of them produced significant within-subject changes in standing intrinsic ankle stiffness. Within these studies and in other previous research (Casadio et al., 2005; Loram and Lakie, 2002*a*; Loram et al., 2007*a*,*b*), there have been reports of a consistently large range of stiffness between individuals, even with measurements taken within the same condition. In the present study I investigate if these between-subject differences are significant enough to affect body sway behavior. A general assumption is that people with stiffer ankles should sway less when standing still and also when perturbed by ultra slow tilting of the standing surface. I decided to verify if this was true, and if it was related to intrinsic stiffness of the ankles. Intrinsic ankle stiffness of 20 participants standing freely on a rotating platform was estimated with the recordings of ankle displacement and torque responses to small and brief perturbations (0.1 and 0.7 deg; 140 ms). In a block of separate trials, the participants either stood quietly on a fixed platform or stood on the same platform when it was being moved by very slow sinusoidal tilts (0.2 and 0.4 deg amplitude at 0.1 Hz). This was used to provoke slightly larger sway size so that it could be seen if the relationship between intrinsic stiffness and sway size was maintained. All results demonstrate an inverse correlation between ankle stiffness and sway magnitude.

6.1 Introduction

All previous research on standing intrinsic ankle stiffness (Casadio et al., 2005; Loram and Lakie, 2002*a*; Loram et al., 2007*a*,*b*) and the experiments previously described in this thesis (Chapters 3, 4 and 5) have reported a large range of stiffness between individuals, even with recordings taken under the same conditions. As the main concern of the authors of those studies was to identify the generalities of the standing process, disparities *between* individuals were not investigated in detail. My proposal in this chapter is to tackle these variations and verify if people with stiffer ankles would sway less or more, either when attempting to stand still or when perturbed by a small amount.

In Chapter 3 I have induced varying types of support-surface disturbances, either to alter baseline body sway or to assess stiffness. I found a wide range of intrinsic ankle stiffness values, which reduced by up to 43% mgh when baseline sway was artificially increased. This confirms the previous suggestion (Loram et al., 2007*a*,*b*) that when standing quietly, if baseline ankle sway is less than ∼0.5 deg, intrinsic ankle stiffness is dependent on the muscle short-range stiffness. But as soon as this range is exceeded, this short-range component of stiffness is reduced, gradually decreasing with increased movement until it is no longer present. At which point, only the much lower long-range stiffness prevails. This implies that active mechanisms may be required to compensate for this dramatic reduction in passive resistance to movement.

In a different setup, Fitzpatrick, Taylor and Mccloskey (1992) identified higher ankle stiffness in individuals instructed to stand still rather than at ease. However, instead of measuring responses to a tilting platform, they pulled participants at waist level using a weak spring, and assessed the body sway response to it. This approach involved perturbing the COM, rather than simply rotating the ankle joint. More importantly, contrary to my estimates which are restricted to intrinsic components, the authors estimated stiffness as the slope between ankle torque and ankle angle within a time-window (1.5 s) that did not

exclude torque increments resultant from the reflex or higher-level activity of the CNS used to maintain balance. Previously termed 'effective stiffness' (Lakie et al., 2003), this method of analysis measures a combination of active control mechanisms and intrinsic stiffness. Inevitably therefore, to cope with the body moving away from the vertical equilibrium point, this effective stiffness was higher than mgh (Loram and Lakie, 2002*b*; Morasso and Schieppati, 1999). Otherwise the effective stiffness of the ankles would not be enough to maintain balance and the body would fall. Fitzpatrick et al. have found differences between standing at ease versus standing still, confirming that active intervention to unperceived sway increases effective ankle stiffness.

In another study, Julien and Bendrups (2016) studied between-individual differences in effective stiffness when participants were also being pulled at waist level by imperceptible (<0.2 deg) (Fitzpatrick and McCloskey, 1994) perturbations. Similar to Fitzpatrick, Taylor and Mccloskey (1992)'s results, they found an inverse correlation between effective ankle stiffness and body sway, implying that people who actively increased ankle stiffness swayed less. In summary, the results of both studies cited above demonstrate that when the body COM is perturbed, the more the participant intervenes to maintain balance, the more resistant to movement their ankles become and the less they sway. When people are instructed to resist an unperceived perturbation, they can do so successfully and will limit the size of their body lean as a consequence. People can limit their response to externally applied or internally generated disequilibrium in apparently identical ways. However, this alteration in behaviour is unlikely to be in any way related to mechanical stiffness. If this is the case, then how would just the intrinsic component of standing stiffness relate to sway?

A general speculation is that two variables related to balance control are directly dependent on standing intrinsic ankle stiffness: body sway and neural modulation (of muscle stiffness). If there is no neural intervention when a body stabilized by an intrinsic stiffness less than mgh is perturbed, then the body will inevitably fall over. It is known

that the standing body presents a certain amount of natural sway, but less understood is how the nervous system actively intervenes to maintain balance. Fitzpatrick and McCloskey (1994) asked participants to report whenever they felt that their body was being moved by externally applied disturbances at waist level. The authors found consciously perceptible thresholds of body sway at 0.06 to 0.12 deg when sway velocity decreased from 0.13 to 0.043 deg s^{-1}, respectively. Osborne (2013) investigated neural intervention with both surface EMG and ultrasound techniques and found even lower thresholds. The medial gastrocnemius response to an ultra-slow 0.5 deg s^{-1} tilt of the standing platform came 290 ms after the onset of perturbation (at 0.0024 deg and 0.035 deg s^{-1}). Would between-individual differences in intrinsic stiffness relate to neural intervention and sensory thresholds, subsequently altering the need for control of balance?

I wanted to determine if stiffer ankles were associated with smaller postural sway. For this reason, I decided to correlate intrinsic stiffness against (1) spontaneous (or natural) sway and (2) sway increased by subtle tilting of a platform. I hypothesize that the outcomes of the study would be:

1. People with stiffer ankles have less spontaneous sway;

2. People with stiffer ankles have less increase in spontaneous sway when standing on tilting surface.

6.2 Methods

Participants

Twenty healthy adults, aged between 24 and 37 years (eight female; height 1.71±0.1 m (mean±SD); weight 67.9±11.5 kg), were recruited for this non-invasive experiment

(Table 6.1). The study was approved by the institution's local human ethics committee and conformed to the principles of the *Declaration of Helsinki*.

Participant	Sex	Age (yrs)	Weight (kg)	Height (m)	Toppling torque per unit angle (Nm deg^{-1})
P01	M	31	80.7	1.80	13.3
P02	M	28	73.0	1.83	12.0
P03	M	32	82.4	1.74	12.6
P04	M	29	61.2	1.75	9.9
P05	F	27	68.1	1.63	9.7
P06	F	31	62.2	1.71	9.6
P07	M	32	74.9	1.78	12.4
P08	M	26	65.3	1.74	10.6
P09	F	32	51.0	1.59	7.3
P10	M	31	55.0	1.66	8.5
P11	F	37	60.9	1.57	8.3
P12	M	29	59.5	1.77	9.8
P13	F	26	73.8	1.63	10.6
P14	M	24	92.5	1.88	16.2
P15	F	26	53.3	1.59	7.7
P16	F	28	62.2	1.74	9.9
P17	F	30	60.0	1.66	9.0
P18	M	25	74.1	1.69	11.5
P19	M	25	63.0	1.66	10.6
P20	M	31	87.6	1.82	14.5
Mean±SE	F(8),M(12)	29±3.2	67.9±11.5	1.71±0.1	10.7±2.3

Table 6.1: Participant anthropometric data.

Procedure and Apparatus

A full description of the footplate apparatus used to measure ankle stiffness as well as its estimation calculations was given in Chapter 2. In brief, the participants were asked to stand on top of motorized footplates, coaxially aligned with their ankles, while ankle torque, ankle angle, footplate acceleration and lower limb EMG responses were being recorded. The methodology specific to the present study is described below.

I wanted to determine if people with stiffer ankles sway less or more, either during spontaneous sway or when the support surface is tilted. I was focused on the properties of the ankles, and in order to reduce the participant use of hip and knee strategies, a light wooden board (1.2 m length, 0.5 m width and total weight 1.2 kg) was strapped to the participant's back with Terylene webbing at shoulder, waist and calf levels. All subjects were tested in one session of approximately 2 hours.

Since the wooden board was strapped securely to the subject, the measure of sagittal body sway was taken from a laser reflex sensor pointing directly at the board. I assumed that, as the board was a solid straight material firmly fixed to the participant's body, its position would give a better estimate of the overall body position by minimizing any hip or knee motion. The placement of the surface EMG electrodes was different from previous chapters, this time recording the electrical activity of the tibialis anterior and medial gastrocnemius muscles of both left and right legs. I chose to verify medial gastrocnemius activity rather than soleus activity because responses due to reflex or higher level activity are more prominent and easier to identify (Loram and Lakie, 2002*a*; Nardone, Giordano, Corra and Schieppati, 1990; Nashner, 1976).

For the measurement of intrinsic ankle stiffness, small and brief squared-sine shape type perturbations (140 ms duration, or 0.7 Hz) were applied to the footplates. The gap interval between them (4–5 s) and also their direction (toes-up or toes-down) and amplitude (0.1 and 0.7 deg) were all randomized. The choice of perturbation amplitude was based on previous studies (Casadio et al., 2005; Loram and Lakie, 2002*a*; Sakanaka et al., 2016), and the objective was to assess stiffness within the short- (0.1 deg perturbation amplitude) and long-range stiffness (0.7 deg).

Two different types of baseline conditions were studied, quiet standing and exaggerated sway caused by slight platform tilt. The quiet standing condition was used to assess spontaneous sway dependency on intrinsic stiffness. The two types of tilts I used were designed to be small and smooth enough to be barely detectable and minimize the possibility of neural intervention, whether voluntary or reflexive. I expected that this would induce increased spontaneous sway size. The participants were unaware of the experiment protocol and were asked to stand freely with eyes closed to eliminate visual cues. Increasing disturbance of posture was manipulated in 3 conditions:

1. Normal: unperturbed horizontal footplates;

2. Sine 0.2: footplates were continuously rotated by a 0.1 Hz sine waveform of 0.2 deg peak-to-peak amplitude;

3. Sine 0.4: footplates were continuously rotated by a 0.1 Hz sine waveform of 0.4 deg peak-too-peak amplitude.

The sinewave type of disturbance was chosen because its shape would induce an especially smooth and subtle movement with minimal acceleration and, as the rhythm was consistent, a more systematic approach could be used to analyze any responses to the perturbation. This compromised the predictability factor, as a sinewave tilting of the footplates would be more easily predicted than a randomized one. To my satisfaction, though, most of the participants reported that they did not feel any difference between normal and sine 0.2 conditions. When they could feel it, only a sporadic disturbance was detected. The sine 0.4 condition had more varied responses. Participants reported that they could either feel nothing, feel a sporadic disturbance, or feel the complete sinusoidal movement of the footplate. This is in accordance with previously reported levels of consciously perceptible sways (Fitzpatrick and McCloskey, 1994; Maurer et al., 2006). Nevertheless, Osborne (2013) has shown that, surprisingly, neural modulation (assessed with both surface EMG responses and ultrasonography of the calf muscles) was detected even when perturbations were as subtle as a 0.05 deg s^{-1} ramp perturbations of 0.5 deg over a 10 s period at the very early stages of disturbance. This confirms that neural intervention is present even at levels of perturbation which the participants could not report.

Altogether I recorded 12 trials from each participant. Each trial lasted approximately 3 minutes. 2 trials for each stability condition (normal, sine 0.2 and sine 0.4) had no added stiffness-measuring perturbation. In order to assess how stiffness was affected by each stability condition, I recorded another set of 2 trials for each stability condition, this time adding stiffness-measuring perturbations of 0.1 deg and 0.7 deg amplitude (total of 192 perturbations recorded from each participant). Different standing condition trials and

perturbation amplitude and direction were all randomized.

Data Analysis

Determination of body sway

The root-mean-square (RMS) values of body velocity were taken from the laser signal aimed at the board attached to the participant's body. At first, this was used to verify if the conditions of tilting surface (sine 0.2 and sine 0.4) were appropriate to produce increased spontaneous sway (recorded from normal standing condition). Once the study protocol was proven to generate a certain level of increased sway, then the between-individual analysis commenced.

Next, body velocity RMS was used in between-individual comparisons in which the correlation (measure of extent to which two datasets fluctuate together) between measured stiffness and amount of body sway velocity was determined. This was used to determine if stiffer people had less spontaneous sway and if they also presented less increased sway during tilting.

The relationship between platform and body movement velocity was quantified by the time lagged cross-correlation function. This provides two measures of similarity between signals: lag and peak. Lag determines the point in time where the signals are best aligned, whereas peak determines the degree of similarity between signals. In the first case, the less the time lag, the less the delay between footplate input and the resultant body movement. In the second case, a peak value between 1 and -1 represents perfect positive and inverse correlations, respectively. The lag and peak value of the cross-correlation function were taken as a measure of the degree of similarity between body and platform movement. In turn, these values were correlated with ankle stiffness to determine if stiffer people exhibited greater similarity with platform movement. The human body is

fundamentally unstable and presents a certain level of spontaneous sway. Spontaneous sway is characteristically random so not synchronised to platform motion. Therefore, less spontaneous sway results in a body movement more synchronized with the very slow platform tilt. In other words, lag and peak cross-correlation analysis was used to complement the results from body velocity RMS analysis.

Statistical analysis

Repeated-measures ANOVA was used to determine effects of condition (normal, sine 0.2 and sine 0.4) and stimulus amplitude (0.1 and 0.7 deg) upon ankle stiffness. One-way ANOVA was used for within-condition comparisons of body sway velocity. Pearson's correlation was used to investigate the relationship between stiffness and body movement. $P<.05$ was considered statistically significant for all tests.

6.3 Results

Figure 6.1 shows representative data from a participant with very stiff ankles (left column of datasets) and from a participant with very compliant ankles (right column of datasets). The first row shows the recordings of stiffness-measuring trials. The next row shows quiet standing with no perturbations. The random reversals in body position occurring at intervals of approximately every second are a result of spontaneous, or natural postural sway. The bottom two rows show the response to ultra-slow tilts. During these trials, spontaneous sway is increased and added to an evoked sway, which is the larger sway of the body at a frequency clearly synchronized to the platform. Although the body position signal is dominated by evoked sway, the velocity signal is less affected and was therefore used as a measure of increased level of spontaneous sway during tilting conditions.

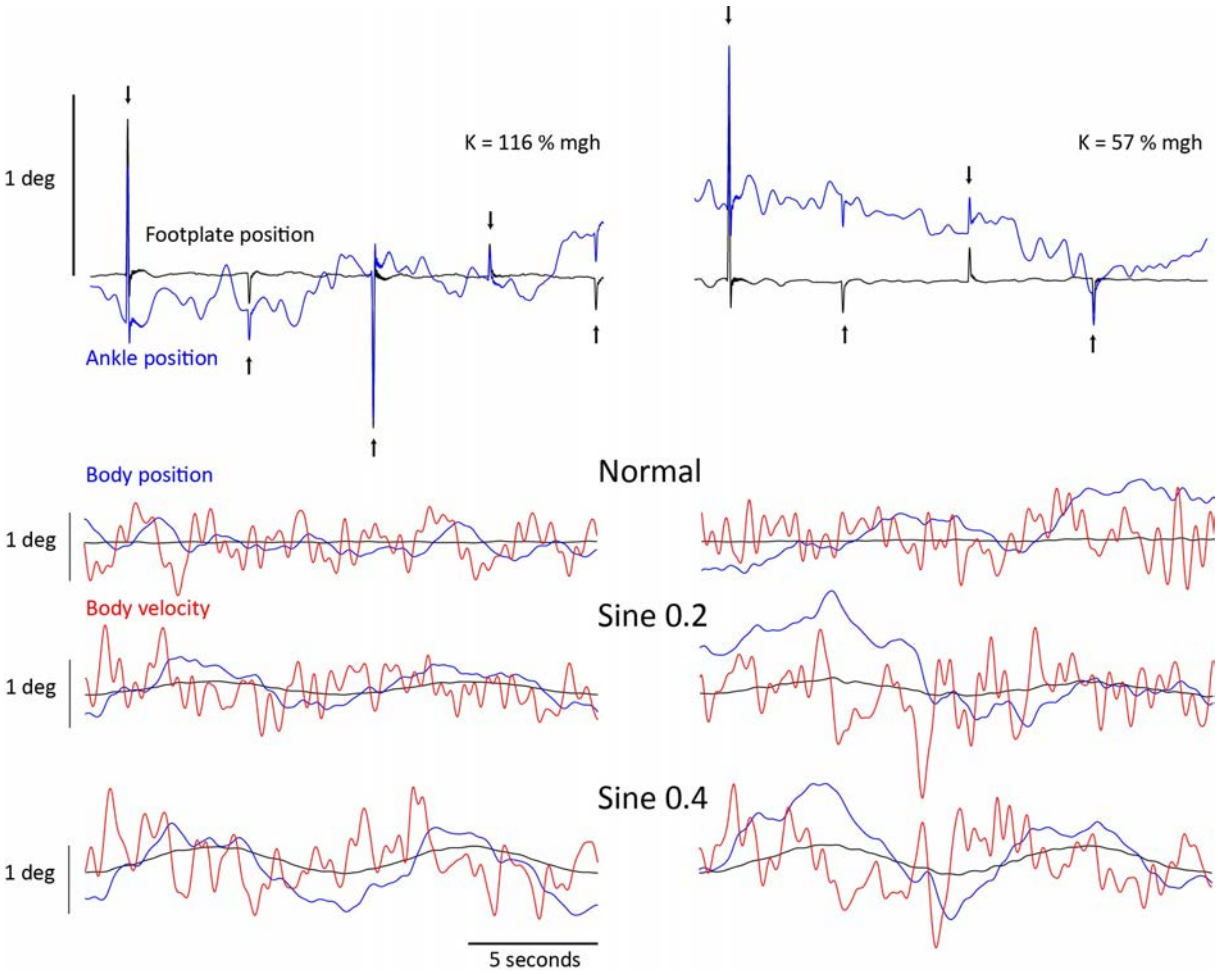


Figure 6.1: Effect of intrinsic anke stiffness on sway in stiff (left column) and compliant ankles. Illustrative segment of footplate and ankle angle during a stiffness-measuring trial of 2 different participants is shown on the top graphs. Below, segments of footplate and body angle and velocity during normal, sine 0.2 and sine 0.4 standing conditions are shown.

Intrinsic ankle stiffness and average body sway

Although the main purpose was to study individual differences in stiffness, I first examined the average stiffness to determine any effect of condition (Figure 6.2). Consistent with the results of Chapter 3, stiffness is higher for the smaller stiffness-measuring perturbation (0.1 vs. 0.7 deg; $F_{1,19} = 176.7$; $p<.001$). There is also a significant effect of condition ($F_{2,38} = 6.4$; $p=.004$), which shows significant reduced stiffness during the conditions of increasing sway. However, the interaction between perturbation size and condition was not quite significant ($F_{2,38}=3.1$; $p=.054$). Importantly, the added background disturbance

during the continuous sinusoidal conditions did not completely reduce stiffness to the floor values seen with the larger perturbations (as occurred in Chapter 3), remaining >70% mgh.

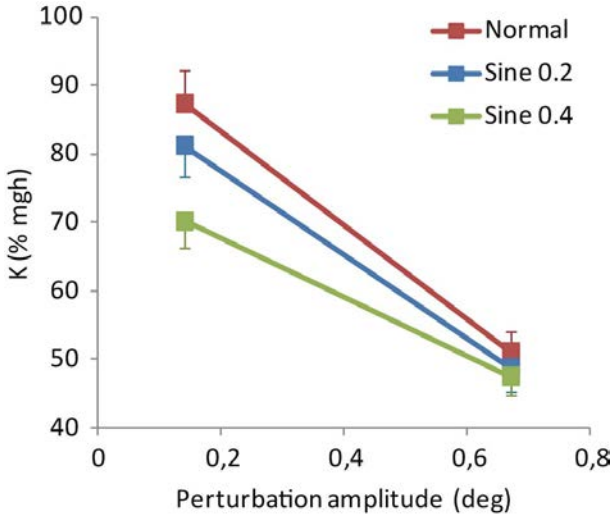


Figure 6.2: Average results of intrinsic ankle stiffness in 3 different conditions for perturbations of 2 different sizes (0.1 and 0.7 deg perturbation amplitude) (mean±SEM).

I determined body sway for each person as RMS body velocity from data without stiffness measuring perturbations (average of 2 whole trials of 160 s). This was used to determine if the increased sway induced by tilting conditions was significant enough to produce an overall sway that was larger than the spontaneous sway detected in normal condition. The average sway of all participants showed a non-significant but nevertheless gradual increase, ranging from 0.58±0.19 deg s^{-1} (mean±SD) in normal condition, to 0.65±0.16 deg s^{-1} in sine 0.2 condition and 0.72±0.19 deg s^{-1} in sine 0.4 condition ($F_{2,59}$=2.9; p=.064).

Analysis of body position RMS has shown that during quiet standing the spontaneous sway induces a rotation of the ankles that is on average less (0.40±0.15 deg, mean±SD) than that necessary to break the initial resistance of the short-range stiffness (∼0.5 deg threshold for the ankle joint) (Loram et al., 2007*a*,*b*). Because ankle stiffness is dependent on sway size (Chapter 3), the question arises on how to best characterize an individual's intrinsic stiffness. In the results that follow, I used the stiffness estimates taken from 0.1 deg perturbation applied exclusively in normal (no tilting) condition.

Relationship between intrinsic ankle stiffness and body sway

The between-individual analysis is depicted in Figure 6.3. Body sway velocity for each person was defined as the RMS body velocity from data without stiffness measuring perturbations (average of 2 whole trials of 160 s). The results show that people with stiffer ankles have lower sway velocity. The relationship was significant for all comparisons (normal: $r_{(20)}$=-0.56; p=.01, sine 0.2: $r_{(20)}$=-0.65; p=.002, sine 0.4: $r_{(20)}$=-0.60; p=.005).

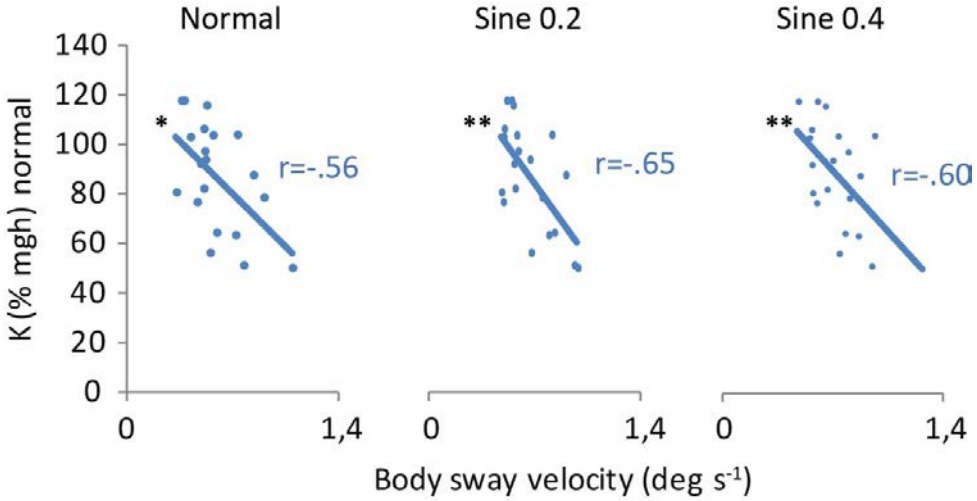


Figure 6.3: Correlation between intrinsic K sway velocity. Left graph (normal), middle (sine 0.2) and right (sine 0.4) graphs all show significant correlations. (*) indicates p<.05 significance and (**) indicates p<.01 significance.

Following the analysis of sway amplitude, I analyzed similarity between body and footplate during induced tilting conditions. This was done by performing cross-correlations between footplate and body velocity, and taking the lag and peak values. There was no significant relationship between intrinsic stiffness and lag. However, significant correlations were found between intrinsic stiffness and peak cross-correlation (sine 0.2: $r_{(20)}$=0.45; p=.05, sine 0.4: $r_{(20)}$=0.52; p=.02). (Figure 6.4)

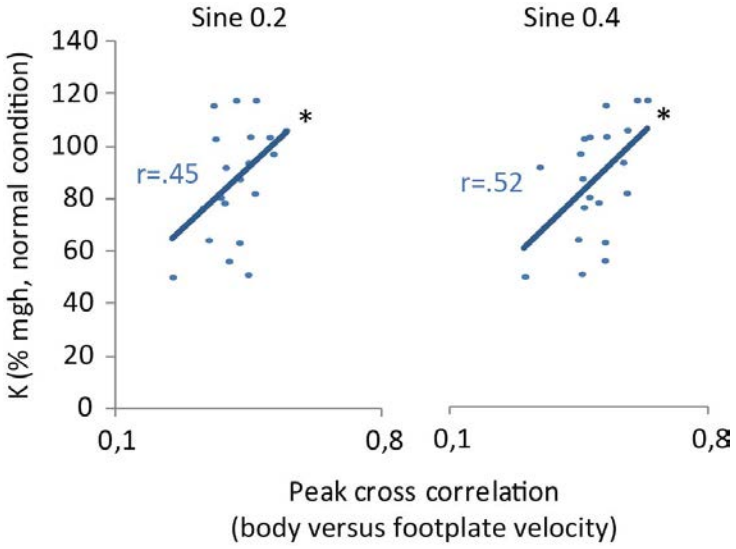


Figure 6.4: Peak cross-correlation between body and footplate velocity in sine 0.2 (left) and sine 0.4 (right) conditions, correlated with intrinsic K. (*) indicates $p<.05$ significance.

6.4 Discussion

In this chapter I have demonstrated that body sway is dependent on individual differences of intrinsic ankle stiffness. Two different parameters were used to verify body sway dependency on intrinsic stiffness: sway velocity (body velocity RMS) and peak cross-correlation between body and footplate velocity. Each parameter described a different aspect of the relationship between amount of sway and stiffness. Sway velocity shows the average amount of sway and is a useful measure of postural instability (Lafond et al., 2004; Pai and Patton, 1997). The peak cross-correlation between body and footplate would detect similarity between variables, and is an indication of how faithfully the body is synchronized to platform movement. Because spontaneous sway is random, a high cross correlation at zero lag between body and footplate indicates that the spontaneous sway is small. Theoretically a cross correlation of one would show that there is no random sway.

The results from these data demonstrated that people with stiffer ankles sway significantly less, both during unperturbed stance and also when standing on a support surface which is repetitively slightly tilted. This confirms the relationship between amount of sway and stiffness. Because their random postural sway is smaller, the sway of people with stiffer

ankles is more similar to the standing surface.

Protocol suitability for between-individual analysis

For a between-individual comparison, the first pre-requisite was obtaining a wide range of intrinsic stiffness levels. Simultaneously, the participants should have a certain degree of homogeneity to reduce the influence of factors that are not strictly related to the mechanical properties of the ankles. Thus to minimize any possible proprioceptive effect of ageing (Wiesmeier et al., 2015), I have purposely chosen young healthy adults (29±3.2, mean±SD). To my satisfaction, even within this category of people, I have found a large range of stiffness spanning by up to 98% mgh difference between maximum and minimum values.

The second pre-requisite for the between-individual comparison was creating conditions that would induce a gradual increase of spontaneous sway. Apart from the recording of quiet standing to assess level of spontaneous sway, two other conditions were created in which the standing surface was rotated by a very slow and small amount to assess increase in spontaneous sway (continuous sinewave of 0.1 Hz and either 0.2 deg or 0.4 deg amplitude). The average value of body sway (measured as body *position* RMS) obtained here for normal standing (0.4 deg) was only slightly higher than the 0.23 deg obtained by Loram et al. (2005*a*,*b*), also from individuals standing quietly with eyes closed. Perhaps in my experiment, the board attached to the participants to reduce hip rotation might have contributed to this general increase in body sway. More importantly, during the transition from normal stance to the increasing levels of sinusoidal disturbance, body sway *velocity* exhibited a very small and gradual increase of 0.58–0.65–0.72 deg s^{-1}. Hence, the 3 conditions were successful in their objective of inducing gradually increasing body sway.

The results of intrinsic stiffness are in accordance with those of Chapter 3, i.e. stiffness was higher when the smaller perturbation (0.1 deg amplitude) was applied to the ankles,

and lower for the larger perturbation (0.7 deg). More relevant to this chapter, though, was the consistently high results obtained by the small perturbation, regardless of background condition (normal, sine 0.2 or sine 0.4). In all 3 conditions, intrinsic stiffness remained >70% mgh when 0.1 deg perturbation was applied (Figure 6.2). Because ankle stiffness is dependent on sway size (Chapter 3), the question arises on how to best characterize an individual's intrinsic stiffness. In the following discussion, I used the stiffness estimates taken from 0.1 deg perturbation applied exclusively in normal (no tilting) condition. This would emphasize more the importance of the short-range stiffness effect on body sway.

Spontaneous sway velocity has a significant dependency on intrinsic ankle stiffness

Spontaneous sway velocity (body velocity RMS) was shown to have a strong, consistent and significant negative correlation with intrinsic K in all 3 conditions (Figure 6.3). This indicates that people with stiffer ankles sway less regardless of the condition of the standing surface.

To further emphasize the results of body velocity RMS, I have performed analysis of peak cross-correlation between body and footplate velocity during tilting conditions (Figure 6.4). No significant relationship was found between lag (the time at which the peak in cross correlation occurred) and intrinsic stiffness. Probably this occurred because naturally occurring sway frequency is about 0.5 Hz (Collins and De Luca, 1995), much higher than the tilting frequency applied by the footplate during this experiment (0.1 Hz). Therefore, the lag was affected by the spontaneous oscillations that occurred around the peak value, determined by the phase of naturally occurring sway matching the tilt-induced increase in body movement (Figure 6.1, 3rd and 4th rows). However, peak cross-correlation of both sine 0.2 and sine 0.4 conditions have shown a positive and significant relationship with intrinsic stiffness (measured within lag of 10 s). It shows that during discrete

increased sway conditions, people with stiffer ankles sway less, as shown by the higher similarity between the body and footplate velocity.

These results above show a significant relationship between intrinsic stiffness and body sway and confirm that the consequence of having stiffer ankles is less sway. However, the causality could not be determined positively with the data available. There are two possible interpretations of the results of this chapter. The first option would be a genuine difference in stiffness caused by intrinsic mechanisms and that this causes less sway. The second option would be the inverse causal relationship, less sway causing stiffer ankles (negative relationship between stiffness and muscle thixotropy, as shown in Chapter 3).

Summary

Fitzpatrick, Taylor and Mccloskey (1992) and Julien and Bendrups (2016) have shown how the ankles get stiffer when people are pulled at waist level. In their studies, they assessed the combined intrinsic and active components of ankle stiffness and have detected that, after being pulled, the ankle gets stiffer to reduce body sway. Conversely, in this chapter I have shown that people with intrinsically stiff ankles sway less.

However, I could not determine the causality of the higher levels of stiffness. I could not determine if the ankles were stiffer therefore people were swaying less, or if people were swaying less therefore their ankles were stiffer (as seen in Chapter 3).

For future studies, a solution for this dilemma would be having the participants strapped to a fixed board when performing the stiffness-measurement trials. If measured this way, the estimation of stiffness would be independent of body sway.

CHAPTER 7

GENERAL DISCUSSION

Human standing is precariously stable because it depends on only two limbs sustaining a disproportionately long vertical body. Ever since it was shown by previous research that the standing posture is not only maintained by intrinsic mechanisms, but also relies on neural modulation (Casadio et al., 2005; Loram and Lakie, 2002*a*; Morasso et al., 1999; Morasso and Schieppati, 1999; Morasso and Sanguineti, 2002), there has been a lot of focus on understanding the process adopted by the CNS to control posture (Lakie et al., 2003; Masani et al., 2006; Osborne, 2013; Peterka, 2002; Sasagawa et al., 2009; Zenzeri et al., 2014). However, little has been done in comprehending the intrinsic mechanisms, or how the different properties of the tissues enwrapping the ankles affect the standing intrinsic ankle stiffness (Loram et al., 2007*a*,*b*).

In this thesis some knowledge has been obtained about the intrinsic properties of the ankles that are so relevant to standing. Four different experimental chapters were presented. The main objective was to manipulate in various ways the type of disturbance applied to quiet standing. In each chapter, properties inferred to the muscle and tendon tissues were confronted to investigate the variability of the mechanisms related to the standing intrinsic ankle stiffness. Participants were asked to stand on freely moving footplates which were collinear with the ankles. Small (<1.3 deg) and brief (140 ms) perturbations were applied to the footplates, and recordings of ankle torque response, ankle angular position, footplate acceleration and lower limb EMG activity were taken to estimate stiffness of

the ankles. The ankle is a very complex joint that moves in various directions. For this reason, I had to confirm that the estimates of stiffness were not affected by the height of the footplate axis of rotation which imposed a fixed rotation to the participant's ankle regardless of individual differences. I plotted the estimated stiffness obtained from normal condition of various experiments against body height (Figure 7.1). The non-significant results (0.1 deg perturbation, $r_{(29)}$ = -0.36; p=.721; 0.7 deg perturbation, $r_{(29)}$ = -1.64; p=.112) indicate that intrinsic K estimates were not dependent on participant's body height and, by default, also not affected by ankle height, assuming that taller participants should have higher ankle height.

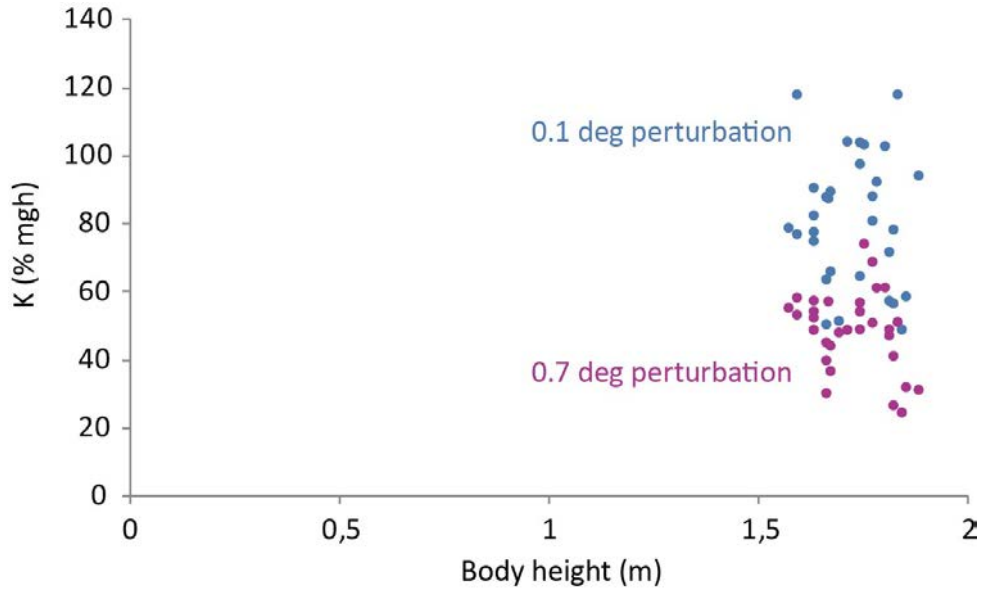


Figure 7.1: Relationship between intrinsic ankle stiffness (K %mgh) and body height (m). Data were taken from experiments from chapters 3, 5 and 6.

In Chapter 3, two variables were checked to confirm the dependency of intrinsic ankle stiffness on short-range stiffness, or muscle thixotropy: ankle movement amplitude and history of movement. This was assumed to be a property of the muscles because this paired dependency was shown previously in isolated muscle fibers (Denny-Brown, 1929; Hill, 1968; Lakie and Robson, 1988*b*,*a*; Rack and Westbury, 1974; Whitehead et al., 2001), being later also demonstrated in vivo for joint movements (Halaki et al., 2006; Warner and Wiegner, 1990). The results show that increasing sway reduces ankle stiffness by up to 43%

compared to a condition in which the body was fixed to a vertical board, consistent with a movement-dependent change in intrinsic ankle stiffness caused by thixotropic properties of the calf muscle.

In Chapter 4, I determined whether localized cooling affected intrinsic stiffness of the muscle and ankle as much as it affected the properties of the active muscle, as shown in previous research. Legs were cooled alternately and stiffness was assessed. The results show no dependency of intrinsic ankle stiffness on temperature. The conclusion was that localized cooling might have no effect on the intrinsic properties of the muscles, and even if it had, the muscle connection in series with the tendon does not allow this increase in stiffness to be transmitted to the overall ankle stiffness because the tendon, acting as the weakest link, determines the overall stiffness. With the experimental design adopted in this study, it was not possible to determine if the actual intrinsic muscle stiffness is affected by cooling or not. In a future approach, it would be interesting to verify change in stiffness in completely relaxed lower limbs of seated individuals. This would answer the question of whether localized cooling has an effect on intrinsic mechanisms when the muscles are not engaged with any task. It would also be interesting to measure stiffness as the slope of ankle torque changes which result from very slow and small sinusoidal tilts of ankle position. This would reduce the possibility of having fast reversals of the cooled limb that might affect the result (as it might have happened in Lakie et al. 1986 during assessment of increasing torque reversals of 1 Hz frequency).

In Chapter 5, I performed two different studies in which the Achilles tendon and aponeurosis were stretched in different ways, either by means of increase in active torque or increase in passive stretch. The experimental conditions tested in this chapter are representative of everyday life existing standing situations. Intrinsic ankle stiffness was shown to significantly increase in both occasions, in conditions of large ankle torque change and little dorsiflexion as well as in conditions of large dorsiflexion and little ankle torque change, confirming its dependency on tendon stiffness.

In Chapter 6, I investigated variations of stiffness between individuals and verified if people with stiffer ankles would sway less or more, either when attempting to stand still or when perturbed to a small degree. All previous research on standing intrinsic ankle stiffness (Casadio et al., 2005; Loram and Lakie, 2002*a*; Loram et al., 2007*a*,*b*) and the experiments previously described in this thesis (Chapters 3, 4 and 5) have reported a large range of stiffness between individuals, even with recordings taken under the same conditions. As the main concern of the authors of those studies was to identify the generalities of the standing process, disparities between individuals were not investigated in detail. The proposal for this chapter was to tackle this issue. The results demonstrate an inverse correlation between ankle stiffness and sway magnitude, i.e. people with stiffer ankles sway less. The main shortcoming of this experimental design was that I could not determine the causality, or the origin of the differences in stiffness levels. I could not determine if the ankles were stiffer therefore people were swaying less, or if people were swaying less therefore their ankles were stiffer (as seen in Chapter 3). For future studies, a solution for this dilemma would be having the participants strapped to a fixed board when performing the stiffness-measurement trials. If measured this way, the estimation of stiffness would be independent of body sway.

All the experiments had to be performed during standing tasks, therefore only in vivo measurements were taken. With the apparatus available, it was not possible to verify individual fibre changes. Hence, I did not have direct measurements of fibre stretch or fibre resistance to movement, which would be possible for example with ultrasound imagery (Maganaris and Paul, 1999). However, even if ultrasound imagery was used, techniques to measure stiffness of the muscles and tendon like tracking of fibre length (Maganaris, 2002; Morse et al., 2008) or shear wave elastography (Brum et al., 2014; DeWall et al., 2014; Gennisson et al., 2010) are restricted by the probe location and dimensions (not able to measure at once the entity of the muscles and tendon), synchronization of images with other apparatus and time-window necessary to capture image. Ideally, the use of ultrasound shear wave elastrography added to the techniques used in this thesis would

have been desirable, especially for the experiments with added ankle stretch (Chapter 5, protocols 1 and 2) and localized cooling (Chapter 4). This would add understanding about the distinctive behaviour of the muscle and tendon fibres, but would not affect the overall stiffness estimates, which could be well performed with the apparatus used here.

As mentioned above, the different intrinsic mechanisms could not be directly verified with the apparatus available, hence the indirect approach was to record the effect of disturbing the ankles in various ways and verifying how the tissue would react to it. I tested the overall ankle resistance to movement with the recordings of ankle torque and ankle movement response to small and brief perturbations. This method was used previously and has been shown to give repeatable estimates (Casadio et al., 2005; Loram and Lakie, 2002*a*). The results of all the experiments shown in this thesis could be related to the results from Casadio et al. (2005) and Loram and Lakie (2002*a*). Their estimates ranged from 64% (for 1 deg perturbation amplitude) to 91% (for 0.05 deg perturbation amplitude), respectively, in individuals standing normally. In all studies presented here for quiet standing, stiffness remained within the proximities of this range, fluctuating between 42% and 98% (1.3 deg to 0.1 deg perturbation amplitudes, Figure 7.2). All the experiments presented in this thesis (not only from Chapter 3, but also from Chapters 4, 5 and 6) were performed with 2 or more perturbation amplitudes and all of them, without any exception, have confirmed the inverse relationship between ankle movement and intrinsic stiffness.

The main argument used to ensure that I have estimated only the intrinsic components of the ankle stiffness was that the perturbations were small and brief, and the time-window used for the estimates (70 ms) was less than that necessary for the neural information to travel from/to the lower limbs to the CNS (Loram and Lakie, 2002*a*; Nardone, Corra and Schieppati, 1990; Nashner, 1976). However, I did not solely rely on that premise, and to make sure that active modulation of stiffness was not affecting the intrinsic stiffness estimates, in all experiments I have simultaneously assessed the surface EMG activity

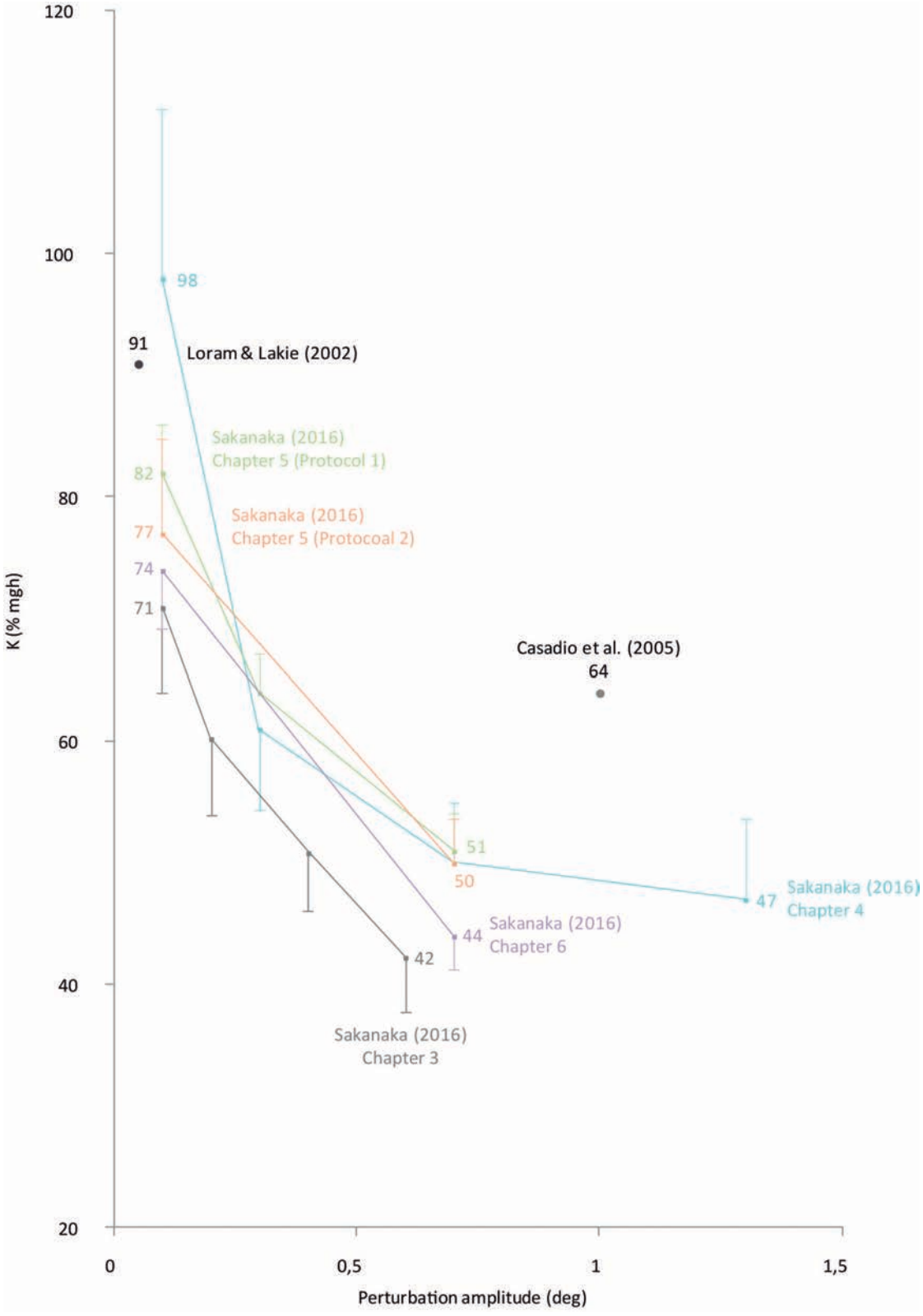


Figure 7.2: Results from all experiments during the condition when participants were standing normally (mean±SEM). Data from Loram and Lakie (2002b) and Casadio et al. (2005) are added for comparison.

of the leg muscles. The tibialis anterior activity was confirmed to be absent in most cases, except during backward leaning from the normal position (Chapter 5, protocol 1) or during dorsiflexion (Chapter 5, protocol 2). However in these cases, I have not detected modulation of muscle activity during individual perturbations, but instead an increase in baseline 'muscle tone'. For the calf muscles, I chose to verify either the medial or the lateral gastrocnemius activity rather than soleus activity because responses due to reflex or higher level activity are more prominent and easier to identify (Loram and Lakie, 2002*a*; Nardone, Corra and Schieppati, 1990; Nashner, 1976). Moreover, although I did not record directly over the soleus muscle, previous research reports considerable cross-talk between the triceps surae muscles when using surface EMG (Toft et al., 1991). In all cases, modulation of muscle activity was not seen during the 70 ms time-window used for the estimation of stiffness.

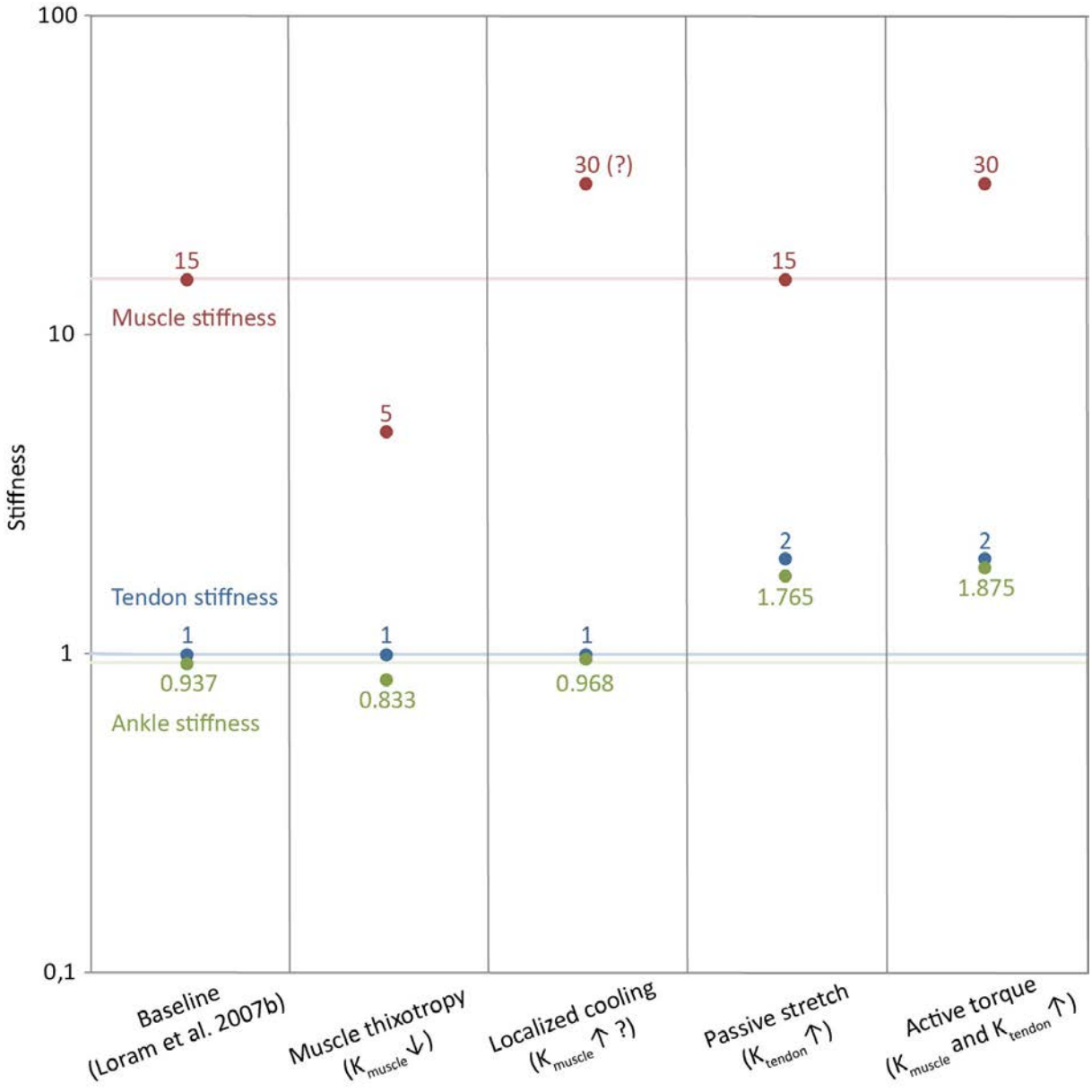
Loram et al. (2007*b*) proposed that during quiet standing the calf muscles are typically stiffer (∼15 times) than the Achilles tendon. This estimation was based on the premise that the intrinsic ankle stiffness system has a dependency on the in series coupling between muscles and tendon. As the skeletal muscles are connected to the bones through the tendons, these two structures are considered to be springs arranged in series (Fitzpatrick, 2003; Loram and Lakie, 2002*a*). The overall ankle stiffness (K_{ankle}) of a system composed of two springs (muscles and tendon) arranged in series is described as:

$$\frac{1}{K_{ankle}} = \frac{1}{K_{tendon}} + \frac{1}{K_{muscle}}$$

In a system arranged this way, the weakest spring is the limiting factor and mainly determines the overall stiffness. If it gets stiffer or more compliant, the whole system will be easily affected. On the contrary, the strongest spring will only affect the system if it gets more compliant. Regardless of how much stiffer it gets, the limiting factor will still be the weakest spring. All the results from the various studies presented in this thesis indirectly confirm this idea. A schematic representation of the effect that all the

different procedures presented in this thesis had on intrinsic ankle stiffness is shown in Figure 7.3. Additionally, the stipulated distinct effect on stiffness of muscles and tendon is also represented. The overall intrinsic stiffness ratio in relation to muscle and tendon stiffness, calculated from the equation above, is shown in each column. The estimates shown in the left column were taken from Loram et al. (2007*b*) when analyzing normal standing. This ratio is used as baseline for the subsequent interventions applied by the experiments presented in this thesis. For example, the effect of muscle thixotropy is a drop in muscle stiffness. However, even with the large reduction in muscle stiffness, the overall ankle stiffness does not reduce as much because the relative importance of the tendon, the weaker spring, is higher than the muscle's. During cooling condition, even if there is increase in muscle stiffness, the overall ankle stiffness can only increase to levels closer to tendon stiffness levels, but never surpassing it. And so on.

The large variability in intrinsic ankle stiffness found in everyday life situations, as the ones presented here, shows that the tissues surrounding the ankles are very labile and dependent on many factors that can either reduce or improve stability in various ways. If the intrinsic stiffness is not enough to completely stabilize the vertical body, then the action of the CNS will be very much affected by these transitory changes in intrinsic stiffness. In other words, while controlling the standing body, the CNS not only has to detect the position of the body in space and predict changes in this position caused by its action, but it also has to cope with intrinsic mechanical properties of the ankles that are constantly changing the baseline stiffness from which these calculations are based on. Pathological conditions, like Parkinson's disease and stroke, which are related to a reduction in the ability that the CNS has to send the contracting command to the muscle, would be very affected by an intrinsic mechanism that is constantly changing. In the future, it would be interesting to verify how this large variability in intrinsic stiffness could be related to the difficulty that many patients with pathological conditions (e.g. Parkinson's disease and stroke) have in maintaining balance.



$$\frac{1}{K_{ankle}} = \frac{1}{K_{tendon}} + \frac{1}{K_{muscle}}$$

Figure 7.3: Schematic representation of a hypothetical change in muscle and tendon stiffness during different standing tasks. The starting point is the 15:1 ratio between muscle (blue) and tendon (red) stiffness during normal standing (baseline, Loram et al. 2007b). Each task induced increase or decrease in ankle stiffness (green), probably caused by different mechanical properties of the muscles and tendon.

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