Modeling and Simulating the Neuromuscular Mechanisms regulating Ankle and Knee Joint Stiffness during Human Locomotion

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Abstract (max 250 words)

This work presents an electrophysiologically and dynamically consistent musculoskeletal model to predict stiffness in the human ankle and knee joints as derived from the joints constituent biological tissues, i.e. the spanning musculotendon units. The modeling method we propose uses electromyograms (EMG) recorded from 13 muscle groups to drive forward dynamic simulations of the human leg for five healthy subjects during over-ground walking and running. The EMG-driven musculoskeletal model estimates musculotendon and resulting joint stiffness that is consistent with experimental EMG data as well as with the experimental joint moments. This provides a framework that allows for the first time observing (1) the elastic interplay between the knee and ankle joints (2) the individual muscle contribution to joint stiffness, and (3) the underlying co-contraction strategies. It provides a theoretical description of how stiffness modulates as a function of muscle activation, fiber contraction, and interacting tendon dynamics. Furthermore, it describes how this differs from currently available stiffness definitions including quasi-stiffness and short-range stiffness. This work offers a theoretical and computational basis for describing and investigating the neuromuscular mechanisms underlying human locomotion.
Introduction

Human locomotion results from afferent and efferent neural activity being translated into mechanical output by muscles acting on skeletal joints and in the subsequent exchange of interaction forces with the external environment (Zajac et al., 2002; Enoka, 2008; Winter, 2009). Humans are remarkably capable of physically interacting across large varieties of terrains and naturally adjusting to their demand. This efficient adaptation process occurs subconsciously and normally involves little or no mental effort (Morton and Fuller, 1952). It is largely dealt by at the neuromuscular level by continuous modulation of viscoelastic characteristics in multiple musculotendon units (MTUs) simultaneously (Latash and Zatsiorsky, 1993; Todorov, 2000; Enoka, 2008; Wakeling et al., 2012). The viscoelasticity of individual MTUs spanning multiple skeletal joint degrees of freedom (DOFs) determines the resulting viscoelasticity (i.e. impedance) at the joint level (Latash and Zatsiorsky, 1993; McIntyre et al., 1996; Hu et al., 2011; Pfeifer et al., 2012). It is worth stressing that the term “viscoelasticity” traditionally refers in physics to the property of materials to mechanically respond to deformations by exhibiting both elastic and viscous characteristics (Stachurski, 2009). While viscoelasticity classical definition is naturally applicable to biological MTUs, it does not directly apply to a skeletal joint, which cannot be seen as a ‘body’ that responds to deformations with viscoelastic material properties. In this context, we will refer to “joint viscoelasticity” as the combined viscoelastic response of the joint constituent biological tissues, the spanning MTUs, to joint kinematics variations as previously proposed (Latash and Zatsiorsky, 1993).

Accessing MTU and joint viscoelasticity estimates at any particular instant in time is crucial for explaining the neurophysiology, the biomechanics and the energetics of natural movement with direct implications in the development of neurorehabilitation and augmentation technologies (Farris and Sawicki, 2012; Farris et al., 2014; Collins et al., 2015). The MTU viscoelasticity can be expressed at
the level of stiffness (i.e., resistive force due to MTU elastic characteristics) and damping (i.e., resistive force due to MTU viscous characteristics) (Winter, 2009). This study focuses on the stiffness level.

During locomotion, joint elastic properties have been investigated by means of the quasi-stiffness (QS) estimate (Stefanyshyn and Nigg, 1998; Kuitunen et al., 2002; Charalambous et al., 2012; Shamaei et al., 2013a, 2013b, 2013c). This represents the slope of the best linear fit on the joint moment-angle characteristics throughout a specific phase of the gait cycle. Therefore, QS only provides a representative stiffness estimate within a specific gait phase. System identification methods in conjunction with joint perturbation techniques have also been used to infer joint stiffness by determining the dynamic relationship between experimental recordings of joint angles and moments both in isometric and dynamic conditions (Weiss et al., 1986a, 1986b; Bennett et al., 1992; Ludvig and Perreault, 2012; Plocharski and Plocharski, 2013; Lee and Hogan, 2014; Rouse et al., 2014). Several theoretical and empirical planar spring-mass models have been used to describe the equivalent linear spring stiffness coefficient of the whole lower extremity needed to describe walking and running under different conditions including: speed, stride length, stride frequency, and duty factor (Alexander, 1992; Farley and González, 1996; Morin et al., 2005; Geyer et al., 2006; Blum et al., 2009; Dean and Kuo, 2009). Theoretical planar spring-mass models have also been used to investigate QS at the joint level (Günther and Blickhan, 2002).

Recently, musculoskeletal modeling methods have been applied to estimate “endpoint stiffness” in the human arm (Hu et al., 2011) and joint stiffness in the human knee (Pfeifer et al., 2012) in isometric conditions. In this, the term “endpoint stiffness” refers to the spring-like behavior that the human arm endpoint displays when the arm is constrained to operate in the horizontal plane (McIntyre et al., 1996). These studies (Hu et al., 2011; Pfeifer et al., 2012) showed that net isometric stiffness could be well reconstructed from muscle fibers short-range stiffness (SRS) and series elastic tendon stiffness.
However, while SRS is an appropriate model for characterizing muscle stiffness during isometric conditions, it could not be applied to characterize muscle stiffness underlying dynamic movement.

Muscle SRS describes the initial steep rise in force when contractile muscle fibers are stretched within a 3% of their length (Walmsley and Proske, 1981). This represents the intrinsic stiffness of the fibers’ myofilaments resulting from the deformation of existing cross-bridges without significant breakdown or reformation, i.e., no change in fiber length (Walmsley and Proske, 1981). From a modeling point of view, SRS is viably computed as the scaled ratio between muscle force and optimal fiber length (see the Material and Methods Section) (Cui et al., 2007, 2008; Hu et al., 2011). This substantially relaxes the modeling requirements because it provides a formulation that is invariant to changes in fiber contraction length and velocity, which do not need to be modeled and estimated. However, during dynamic movement muscle fibers are stretched beyond the limits of SRS. This results in fiber stiffness being dominated by changes in fiber length, contraction velocity and activation, with a substantial drop in the fiber resistive force with respect to what is observed within the SRS range (Walmsley and Proske, 1981; Campbell and Moss, 2000). This is referred to as the thixotropic behavior of skeletal muscles (Campbell and Moss, 2000). Therefore, during dynamic movement, SRS is not descriptive of the elastic characteristics of muscle fibers as it would overestimate the underlying stiffness, as previously discussed (Walmsley and Proske, 1981; Campbell and Moss, 2000; Cui et al., 2007, 2008; de Vlugt et al., 2011; Hu et al., 2011). The joint stiffness decrease in transitioning from an isometric pose to dynamic movement was also recently observed experimentally in humans (van Eesbeek et al., 2010; de Vlugt et al., 2011; Ludvig et al., 2012). In this context, it was also shown that SRS is a temporal-dependent feature of muscle contraction lasting approximately 30ms from the joint movement initiation (de Vlugt et al., 2011), thus not persisting during regime locomotion.

Furthermore, these previous modeling studies could not account for physiological co-contraction ratios between agonist and antagonist muscles. Dynamic locomotion is strongly characterized by
variable co-contraction ratios between agonist and antagonist muscles (Collins, 1995; Lloyd and Buchanan, 2001; Besier et al., 2003a, 2009). Physiological co-contraction plays a major role in determining the net stiffness of a joint (Hirokawa et al., 1991; Nielsen and Kagamihara, 1993; Todorov, 2000, 2004). Therefore, it needs to be properly accounted for in musculoskeletal modeling methods that aim to characterize muscle and joint stiffness during natural movement. The previous studies used static optimization methods (e.g. Crowninshield and Brand, 1981; Anderson and Pandy, 2001) to solve for muscle force patterns that track experimental net joint moments (Hu et al., 2011) or flexion/extension moments linearly derived from average flexors/extensors’ EMGs (Pfeifer et al., 2012) during isometric tasks. This results in force patterns that satisfy constraints imposed by an objective function (i.e. minimal sum of squared forces). In this, the a-priori chosen optimization criterion only characterizes muscle function within a specific regime (Todorov, 2004). However, it is known that the same joint angle and moment can underlie substantially different patterns of muscle excitation and co-contraction. These vary with the control task and demand of the external environment (Tax et al., 1990) and can include reflex-based excitation components (Sinkjær et al., 1988; Perreault et al., 2000; Geyer and Herr, 2010). This all cannot be accounted for by a single optimization criterion (Todorov, 2004).

None of the studies so far quoted provided a framework for estimating instantaneous modulations of muscle and joint stiffness during natural locomotion. This requires a musculoskeletal modeling method that accounts for physiological muscle recruitment strategies and contraction dynamics. In this study we address this challenge by using an electromyography (EMG)-driven musculoskeletal model of the human leg (Lloyd and Besier, 2003; Buchanan et al., 2004; Krishnaswamy et al., 2011; Sartori et al., 2012b; Farris et al., 2014). The term “leg” is used throughout this manuscript as per its anatomical definition. In this, experimental EMG signals are used to directly drive the individual MTUs spanning the knee and ankle joints. The underlying musculoskeletal model is scaled and calibrated to match an
individual’s anthropometry and EMG-to-force generating properties. The major benefit is the possibility of determining the subject-specific relationships between patterns of MTU excitation and the resulting MTU force without making any *a priori* assumption on how MTUs are recruited and excited (Sartori et al., 2014).

The generalizability with respect to a specific optimization criterion enabled EMG-driven modeling to predict a number of internal musculoskeletal variables that directly emerge from physiological muscle co-contraction strategies during locomotion. They included joint moments (Lloyd and Besier, 2003; Buchanan et al., 2004; Sartori et al., 2012b, 2013, 2014), *in vivo* knee joint contact forces (Winby et al., 2009; Gardinier et al., 2013; Gerus et al., 2013), and hip joint compressive forces (Fernandez et al., 2014). Furthermore, it enabled studying individuals with neuromuscular or orthopedic impairments that underlay abnormal neuromuscular control strategies. These included patients following anterior cruciate ligament rupture (Shao et al., 2011), patellofemoral pain (Besier et al., 2009), osteoarthritis (Fregly, 2009; Kumar et al., 2012), stroke (Fregly, 2009; Shao et al., 2009) and upper extremity neuromuscular injuries (Manal and Buchanan, 2005).

In this study we show, for the first time, the use of EMG-driven musculoskeletal modeling for deriving instantaneous estimates of joint stiffness from the constituent MTUs stiffness during locomotion. We illustrate how this approach differs fundamentally from alternative stiffness estimates, including the SRS and the QS. In five healthy subjects we validate our method’s ability of generating simulations of ground-level walking and running that are both electrophysiological consistent (i.e. driven by experimental EMG data) and dynamically consistent (i.e. the simulated forces match experimental joint moments). We investigate the resulting joint stiffness profiles emerging from these subjects during the performed motor tasks. We assess the major muscles contributing to the estimated joint stiffness and how muscle co-contraction contributes to its modulation. Finally, we compare and further validate our results with findings available in the literature.
Material and Methods

Data Collection

The Human Research Ethics Committee at the University of Western Australia approved all procedures and participants provided their informed, written consent. Motion capture data were recorded from five healthy male subjects (age: 26.6±1.3 years, weight: 73.9±11.8 Kg, height: 1.77±0.1 m) who performed one static anatomical pose, and repeated trials of ground level fast walking (number of trials: 7.8±0.8, speed: 1.9±0.25 m/s) and fast running (number of trials: 8.6±1.9, speed: 4.6±0.38 m/s). The locomotion speed was not controlled across trials and subjects, hence the substantial speed variability observed over the entire dataset.

Recordings of dynamic trials included the full gait stance phase of the subjects’ right leg. Each subject had 27 retro-reflective markers placed on their lower extremities, pelvis and trunk (Dempsey et al., 2009; Sartori et al., 2012b). Three-dimensional marker locations were recorded at 250 Hz using a 12-camera Vicon MX Motion Analysis System (Oxford Metrics, Oxford, UK). The ground reaction forces (GRFs) and EMG data were recorded at 2000 Hz using an in-ground force plate (AMTI, Watertown, MA) and a 16-channel acquisition system (Noraxon, Scottsdale, USA), respectively. Both GRFs and marker trajectories were low-pass filtered with the same zero-phase second-order Butterworth filter. Cut-off frequencies (between 8 and 14 Hz) were determined by a trial-specific residual analysis (Winter, 2009). The EMG data were collected from 13 leg muscles: tensor fascia latae, gracilis, lateral and medial hamstrings, rectus femoris, sartorius, vastus lateralis and medialis, gastrocnemius lateralis and medialis, soleus, peroneus group, and tibialis anterior. Raw EMGs were band-pass filtered (30–450 Hz), full-wave rectified, and low-pass filtered (6 Hz) using a zero-phase second-order Butterworth filter (Lloyd and Besier, 2003). For each subject and muscle, the resulting EMG linear envelopes were normalized with respect to the peak-processed values obtained from the entire set of recorded trials. We will refer to processed and normalized EMG signals as “excitations”.
From the dynamic trials two distinct datasets were created. One was for the EMG-driven model calibration, which consisted of two walking and two running trials per subject. The second set was for the EMG-driven model test, which consisted of 5.8±0.8 trials of walking and 6.6±1.9 trials of running per subject.

Movement Analysis

Using the open-source software OpenSim (Delp et al., 2007), a 12 segment, 19 DOF, generic rigid-body model of the human skeletal geometry was scaled to match each individual subject’s anthropometry (Sartori et al., 2012b). The scaling process used experimentally measured marker positions recorded from the static standing poses, and from the estimated location of the hip, knee and ankle joint centers using functional trials (Besier et al., 2003b). In this procedure, virtual markers were created and placed on the generic rigid-body model based on the experimental markers position. The rigid-body model anthropometric properties were linearly scaled on the basis of the relative distances between experimental and corresponding virtual markers (Delp et al., 2007). This adjusted anatomical segment length, width, depth, center of mass location, and mass moment of inertia. The OpenSim inverse kinematics (IK) algorithm solved for joint angles that minimized the least-squared error between experimental and virtual marker locations from the scaled rigid-body model (Lu and O’Connor, 1999). The IK-generated joint angles and the experimental GRFs were used to obtain joint moments via inverse dynamics (ID) and residual reduction analysis (RRA) using the scaled rigid-body model (Thelen and Anderson, 2006). This ensured the resulting joint moments and joint angles were dynamically consistent (Thelen and Anderson, 2006; Delp et al., 2007), thus addressing inverse dynamics uncertainties (Piovesan et al., 2011). The joint moments produced by this pathway were referred to as “the experimental” joint moments. The alternative pathway to estimate joint moments was by the calibrated, subject-specific, EMG-driven musculoskeletal model.
EMG-driven model-based estimation of MTU forces and joint moments

The EMG-driven model employed in this study was previously presented in details (Lloyd and Besier, 2003; Buchanan et al., 2004; Sartori et al., 2012b). The model structure (Fig. 1) is outlined below. It is composed of four main blocks, all of which are internally consistent (Lloyd and Besier, 2003; Sartori et al., 2014).

The **MTU activation block** (Fig. 1A) receives excitations recorded from 13 muscle groups (*Data Collection* Section) and uses them to drive 18 MTUs (Table 1) (Sartori et al., 2012b). Experimental excitations are processed using an infinite impulsive response filter to model the muscle fibers twitch response to the excitation onset (Lloyd and Besier, 2003). These are further processed by a non-linear transfer function to account for the non-linearity in the excitation-to-force relationship and to determine the resulting muscle activation (Lloyd and Besier, 2003; Buchanan et al., 2004).

The **MTU kinematics block** (Fig. 1B) employs a five-DOF, 18-MTU, generic model of the human muscle geometry (Sartori et al., 2012a). This is adjusted to match the dimensions of the scaled OpenSim rigid-body model used for the IK, ID and RRA calculations (*Movement Analysis* Section). In this, each MTU’s insertion, origin, and MTU-to-bone wrapping point is linearly scaled to remain in the same relative position with respect to the segment dimensions of the scaled rigid-body model. The MTUs in the muscle geometry model span five kinematic DOFs including: hip adduction-abduction, hip internal-external rotation, hip flexion-extension, knee flexion-extension, and ankle plantar-dorsi flexion. The IK-generated angles about these five DOFs directly inform the MTU kinematics block, which computes instantaneous MTU length $\ell^{mt}$, velocity $v^{mt}$, and moment arms $r$ (Sartori et al., 2012a).

In this, a multidimensional cubic B-spline function is created for each MTU and is used to represent how MTU length, velocity, and moment arms change versus IK-generated joint angles as discussed in our previous work (Sartori et al., 2012a).
The MTU dynamics block (Fig. 1C) takes MTU activation \( a(t) \) as well as \( \ell^{mt} \) and \( v^{mt} \) from the previous blocks as input. It uses a Hill-type muscle model to estimate instantaneous length, contraction velocity, and force in the muscle fibers as well as strain and force in the series-elastic tendon within each MTU (Lloyd and Besier, 2003; Buchanan et al., 2004; Winby et al., 2009; Sartori et al., 2012b).

The static properties of fibers are modeled using parallel force-length passive \( f_p(\tilde{l}^m) \) and activation-dependent \( f_a(\tilde{l}^m) \) curves (Fig. 2A) (Lloyd and Besier, 2003). The dynamic properties of fibers are modeled using an activation-dependent force-velocity \( f(\tilde{v}^m) \) curve (Fig. 2B), which includes a passive parallel damping element to prevent any singularities of the mass-less model when activation or isometric force are zero (Schutte et al., 1993; Lloyd and Besier, 2003). These curves are normalized to maximum isometric muscle force (\( F^{\text{max}} \)), while \( \tilde{l}^m \) and \( \tilde{v}^m \) represent fiber length and velocity normalized to optimal fiber length \( l^m_o \) and maximum muscle contraction velocity respectively (Zajac, 1989). The coupling between \( l^m_o \) and \( a(t) \) is incorporated as described in (Lloyd and Besier, 2003). The tendon properties are modeled using a force-strain function \( f(\varepsilon) \) with non-linear toe region normalized to \( F^{\text{max}} \) (Zajac, 1989). The total MTU force \( F^{\text{MTU}} \) is calculated as a function of \( a(t) \), normalized fiber length \( \tilde{l}^m \) and contraction velocity \( \tilde{v}^m \):
is done using a Wijngaarden-Dekker-Brent optimisation routine (Brent, 1973), which finds the root of the equilibrium between the muscle fibres and the tendon forces.

The joint dynamics block (Fig. 1D) computes joint moments about the knee flexion-extension and ankle plantar-dorsi flexion DOFs as the product of each MTU force (Equation 1) and their associated moment arms from the MTU kinematics block (Lloyd and Besier, 2003; Sartori et al., 2012b, 2013).

The model calibration block (Fig. 1E) determines subject-specific parameters for the EMG-driven model as extensively described previously (Sartori et al., 2012b, 2013). Very briefly, calibrated parameters in the MTU activation block (Fig. 1A) include: two MTU-specific excitation-to-activation filtering coefficients, which are varied between ±1 to realize a stable positive solution and a critically damped impulsive response for the EMG-excitation recursive filter (Lloyd and Besier, 2003) and one MTU-specific shape factor parameter that is altered between -3 and 0 to account for the non-linear EMG-to-force relationship (Lloyd and Besier, 2003; Buchanan et al., 2004). In the MTU dynamics block (Fig. 1C), MTU strength coefficients $\gamma$ adjust MTU-specific nominal $F_{\text{max}}$ to match the person’s strength, i.e. $F_{\text{max}} = \gamma \cdot \text{nomial } F_{\text{max}}$. Strength coefficients are varied between $0.5 \leq \gamma \leq 1.5$ and gather MTUs in seven groups according to their functional action including uniarticular knee flexors, uniarticular knee extensors, uniarticular ankle plantar flexors, uniarticular ankle dorsi flexors, biarticular quadriceps, biarticular hamstrings, and biarticular calf muscles. The nominal $F_{\text{max}}$ for MTUs within the same group are scaled by the same $\gamma$ coefficient. The nominal $F_{\text{max}}$ values are taken from (Yamaguchi et al., 1990). Muscle tendon slack length $l_s'$, and optimal fiber length $l_o^n$ are adjusted so that $l_s' = \text{initial value} \pm 5\%$ and $l_o^n = \text{initial value} \pm 2.5\%$ with initial values obtained using the methodology presented in (Winby et al., 2008) that accounts for how $l_s'$ and $l_o^n$ vary non-linearly with an individual’s anthropometry. A simulated annealing algorithm (Goffe et al., 1994) varies the parameters within the pre-defined boundaries to minimize the sum of the mean square differences between the predicted and experimental joint moments calculated over the two DOFs and the four
calibration trials (Data Collection Section) recorded for a specific subject (Sartori et al., 2012b, 2013, 2014). In this, the mean square differences relative to one specific joint DOF are normalized by the reference DOF-moments variance (Lloyd et al., 2008; Winby et al., 2009). This is done to prevent inadvertently assigning greater emphasis to longer trials with higher joint moment values and assure that error terms across all DOFs are equally minimized. Moreover, the objective function also includes a penalty factor that penalizes instances of normalized fiber length being less than 0.5 or greater than 1.5, thus operating outside physiological ranges (Lloyd and Besier, 2003). After calibration, the model is validated on its ability of blindly matching experimental ankle and knee joint moments during novel validation trials that are not used for calibration (see Data Collection Section). During the validation step, optimization is no longer used, with joint moments and stiffness being blindly predicted as a function of EMG signals and joint angles. Therefore, the calibrated model operates according to an open-loop paradigm.

EMG-driven model-based estimation of MTU and joint stiffness

The calibrated, subject-specific EMG-driven model (Fig. 1) is used to estimate instantaneous stiffness in 18 leg MTUs (Table 1). The MTU-specific stiffness is then projected to the joint level to derive the resulting stiffness about two DOFs, i.e. knee flexion-extension and ankle plantar-dorsi flexion. The net stiffness about a joint DOF, $K_{DOF}^{DOF}$, is determined as:

$$K_{DOF}^{DOF} = \sum_{i=1}^{8} K_{i}^{MTU} \cdot r_{i}^{2} + \frac{\partial r_{i}}{\partial \theta_{DOF}} \cdot F_{i}^{MTU}$$

(2)

where $K_{i}^{MTU}$ and $r_{i}$ respectively represent the stiffness and moment arm of the $i^{th}$ MTU spanning the specific DOF, whereas $\theta_{DOF}$ is the joint angle about the specific DOF, as previously described (McIntyre et al., 1996; Hu et al., 2011; Pfeifer et al., 2012). The moment arm partial derivative $\frac{\partial r_{i}}{\partial \theta_{DOF}}$ in Equation 2 is calculated by creating a cubic B-spline function per MTU (Sartori et al., 2012a). The
MTU-specific spline represents how \( r \) changes with respect to \( \theta^{\text{DOF}} \) (i.e. \( \forall \text{DOFs} \in \text{MTU} \)). The nominal data (i.e. \( \theta^{\text{DOF}} \) and \( r \), \( \forall \text{DOFs} \in \text{MTU} \)) needed to compute the spline coefficients are created using the MTU kinematics block (Fig. 1B) and by sampling each DOF range of motion in 100 equidistant values.

The \( K^{\text{DOF}} \) term represents the instantaneous torque response about a joint-DOF, for every degree of perturbation, from its current angular position \( \theta^{\text{DOF}} \).

The total stiffness \( K^{\text{MTU}} \) in each MTU is modeled as muscle fiber stiffness \( K^m \) in series with tendon stiffness \( K^t \) (Latash and Zatsiorsky, 1993; Cui et al., 2008; Pfeifer et al., 2012):

\[
K^{\text{MTU}} = \left( \frac{1}{K^m} + \frac{1}{K^t} \right)^{-1}
\]

Tendon stiffness \( K^t \) is estimated from the slope of the non-linear force-strain relationship \( f(\varepsilon) \) in the correspondence of the instantaneous tendon strain value \( \varepsilon \). Muscle fiber stiffness \( K^m \) is calculated as the partial derivative of fiber force \( F^m \) (Equation 1) with respect to the normalized fiber length \( \tilde{l}^m \):

\[
K^m = \frac{\partial F^m(a, \tilde{l}^m, \tilde{v}^m)}{\partial \tilde{l}^m}
\]

The partial derivative in Equation 4 is calculated by creating a multi-dimensional cubic spline function per muscle (Sartori et al., 2012a). The muscle-specific spline describes how \( F^m \) changes simultaneously with respects to its states, which include activation \( a \), normalized fiber length \( \tilde{l}^m \), and normalized fiber velocity \( \tilde{v}^m \) (Fig. 2C). The nominal data (i.e. \( a, \tilde{l}^m, \tilde{v}^m \), and \( F^m \)) needed to compute the spline coefficients are generated using the subject-specific EMG-driven model (Sartori et al., 2012a, 2012b).

Nominal data are chosen so that they sample the entire \( F^m \) solution space, i.e. \( 0 < a < 1, 0 < \tilde{l}^m < 2, \) and \(-1 < \tilde{v}^m < 1\), with the same sample step = 0.05 for all three variables. In this context, \( K^m \) (i.e. Equation 4) is the directional derivative computed from the force-length-velocity-activation surface.
(Fig. 2C) along the normalized fiber length directional axis, for given instantaneous states \((a, \bar{l}^m, \bar{v}^m)\) and corresponding force level \(F^m\).

This is further exemplified in Figs 3A and 3B. These depict the soleus fiber force-length profile throughout the stance phase of walking for one subject. Fig. 3B also shows the force-length profile projected on the force-length-velocity-activation surface (Equations 1-4). As the stance phase progresses, the triplet \((a, \bar{l}^m, \bar{v}^m)\) is modulated dynamically and contributes to define instantaneous \(F^m\) points that lay both on the force-length profile and on the force-length-velocity-activation surface, i.e. see the two points at 57.6% and 72% stance in Figs 3A and 3B. Note how these two points lay on the same force-length profile (Fig. 3A) but on two different surfaces (Fig. 3B), each of which is relative to a different instantaneous \(\bar{v}^m\) value. The instantaneous stiffness in a point of the gait cycle is calculated from the slope of the surface cross-section along the \(\bar{l}^m\)-axis (see dotted lines in Fig. 3A). The surface cross section is modulated as a function of \(a\), \(\bar{l}^m\), and \(\bar{v}^m\), and the force slope (i.e. stiffness) is computed in the correspondence of the current \(\bar{l}^m\) value (Figs. 3A and 3B).

This formulation properly accounts for changes in fiber force \(F^m\) that occur due to rapid, dynamic changes in fiber length for given instantaneous values of contraction velocity and activation (Figs 2 and 3). Note that this is different than simply estimating the slope on the isometric force-length relationship \(f_A(\bar{l}^m) + f_P(\bar{l}^m)\) in Fig. 2A. This would only describe the “static state” of muscle fibers, as previously discussed (Hu et al., 2011), i.e. the isometric force that muscle fibers can generate at a specific length and activation. Our proposed modeling methodology also accounts for the “dynamic state” of the muscle fibers, which also depends on the fibers contraction velocity \(\bar{v}^m\) and activation, as shown in Equations 1-4 and Figs 2B-C. Furthermore, it accounts for how fibers interact with the series-elastic tendon. In this scenario, the ability to drive each individual MTU as a function of experimental
EMG-excitations is crucial to properly account for physiological co-contraction strategies in agonist and antagonist MTUs during dynamic motor tasks.

Relation to quasi-stiffness and short-range stiffness

The quasi-stiffness, or QS, is used to estimate joint stiffness properties throughout entire gait phases during dynamic locomotion (Stefanyshyn and Nigg, 1998; Coyles et al., 2011; Charalambous et al., 2012; Shamaei et al., 2013a, 2013b, 2013c). It reflects a fundamentally different physical variable with respect to the instantaneous stiffness discussed in the present study, i.e. Equations 2-4 (Latash and Zatsiorsky, 1993; Rouse et al., 2013). At the muscle fibers level, QS can be derived from the slope of the force-length curve over time (Fig. 3A). Mathematically, this represents the ratio between the total differential of the fiber force function (i.e. \(dF^m(a,\tilde{l}^m,\tilde{v}^m)\)) and the differential of fiber length (\(d\tilde{l}^m\)) over time. The total differential of fiber force is reported below:

\[
dF^m(a,\tilde{l}^m,\tilde{v}^m) = \frac{\partial F(a,\tilde{l}^m,\tilde{v}^m)}{\partial a} da + \frac{\partial F(a,\tilde{l}^m,\tilde{v}^m)}{\partial \tilde{l}^m} d\tilde{l}^m + \frac{\partial F(a,\tilde{l}^m,\tilde{v}^m)}{\partial \tilde{v}^m} d\tilde{v}^m
\]

By dividing Equation 5 with respect to \(d\tilde{l}^m\) and by substituting Equation 4 (i.e. \(K^m(t)\)) into it, we derive a compact mathematical formalization of QS:

\[
QS(t,dt) = \frac{\partial F(a,\tilde{l}^m,\tilde{v}^m)}{\partial a} \frac{da}{d\tilde{l}^m} + K^m + \frac{\partial F(a,\tilde{l}^m,\tilde{v}^m)}{\partial \tilde{v}^m} \frac{d\tilde{v}^m}{d\tilde{l}^m} = A(t,dt) + K^m(t) + V(t,dt)
\]

showing that muscle fiber QS does not solely represent changes in muscle force due to changes in fiber length (i.e. the muscle fiber stiffness term \(K^m(t)\) in Equation 4) but also incorporates terms that vary with the activation (i.e. \(A(t,dt)\)) and contraction velocity (i.e. \(V(t,dt)\)) differentials as well as with the time interval \(dt\) used to compute the slope on the force-length curve. At the joint level, QS is biased by all these terms for all MTUs spanning that joint.
The short-range stiffness, or SRS, reflects the intrinsic stiffness of the fibers myofilaments when contractile fibers are stretched within a 3% of their length (Walmsley and Proske, 1981). This can be estimated using the model developed by (Cui et al., 2008) for muscles in the cat hindlimb:

$$\gamma = \frac{F^m}{l_o}$$

where $\gamma = 23.4$ is a dimensionless scaling constant (Cui et al., 2008), whereas $l_o$ is the optimal fiber length. This formulation was subsequently used in conjunction with human musculoskeletal models to study isometric joint stiffness in the arm and knee joint (Hu et al., 2011; Pfeifer et al., 2012). In Equation 7, SRS is proportional to muscle fiber force and is invariant to changes in fiber length and contraction velocity. SRS well describes the isometric characteristics of muscle stiffness but would overestimate them in dynamic conditions, as previously discussed (Walmsley and Proske, 1981; Campbell and Moss, 2000; Cui et al., 2007, 2008; de Vlugt et al., 2011; Hu et al., 2011).

**Validation Procedures and Analyses**

After scaling and calibrating the EMG-driven model to each individual subject, it was examined in a series of validation tests. The first test assessed the subject-specific EMG-driven model’s ability to blindly predict dynamically consistent joint moments, from experimental excitations, about the knee flexion-extension and ankle plantar-dorsi flexion DOFs and across the 12.4±2.6 validation trials performed by each of the five subjects, i.e. 62 validation trials across all subjects. Therefore, across all validation trials the model was validated on 124 estimations, i.e. 62 trials and two degrees of freedom.

The second test analyzed the muscle and joint stiffness underlying the dynamically consistent EMG-driven simulations of walking and running for all subjects. The contribution of each MTU to the net stiffness about a specific joint DOF was determined by means of the variability accounted for (VAF) index (d’Avella et al., 2003; Ivanenko et al., 2006; Sartori et al., 2013):

Validation Procedures and Analyses
\[ VAF_{DOF}^{MTU} = 1 - \frac{\sum_{s=0\%}^{100\%} (K(s)^{DOF} - K(s)^{MTU})^2}{\sum_{s=0\%}^{100\%} (K(s)^{DOF})^2} \]  

(8)

where \( K_{DOF}^{MTU} \) is the single MTU stiffness projected on the specific DOF using Equation 2, whereas \( s \) represents a stance phase point.

The third test assessed the co-contraction ratio (CCR) between agonist and antagonist muscles spanning a joint DOF (Lloyd and Buchanan, 2001):

\[
\begin{align*}
\text{CCR}(t) & = \frac{EMG(t)_{\text{agonist}}}{EMG(t)_{\text{antagonist}}} - 1, \quad \text{if } EMG(t)_{\text{agonist}} < EMG(t)_{\text{antagonist}} \\
\text{CCR}(t) &= 1 - \frac{EMG(t)_{\text{antagonist}}}{EMG(t)_{\text{agonist}}}, \quad \text{otherwise}
\end{align*}
\]  

(9)

where \( EMG(t)_{\text{agonist}} \) and \( EMG(t)_{\text{antagonist}} \) respectively represent the average across all agonist and antagonist muscle excitations at time instant \( t \). The CCR index varies between -1 (i.e. reflecting solely agonist excitation) and 1 (i.e. reflecting solely antagonist excitation), with 0 reflecting a balanced contribution between agonist and antagonist muscle excitation. The MTUs spanning a specific joint DOF were gathered between agonist and antagonist groups based on the moment arm sign throughout specific joint DOF range of motion observed during walking and running (Table 1).

Across the validation tests and analyses, all EMG-driven model estimates were time-normalized to 100 samples using cubic splines. Experimental and predicted joint moments were further normalized to subject’s body mass. Similarity between predicted and experimental quantities (i.e. joint moments and stiffness) was calculated using the coefficient of determination (\( R^2 \), square of the Pearson product moment correlation coefficient) and the root mean squared error normalized with respect to the root mean squared sum of the corresponding experimental quantity (NRMSE). The 90% confidence interval, was estimated for \( R^2 \) and NRMSE using the Chebyshev’s theorem, i.e. expected interval =
mean ± 3.16·std. This could be applied with no assumption on the normality of R² and NMSEs distributions (Fig. 6).

Results

We first provide representative comparisons between our proposed instantaneous stiffness estimates (Equations 2-4) and those derived from QS (Equations 5-6) and SRS (Equation 7) respectively. These representative comparisons are used to further illustrate the conceptual differences between stiffness estimation methods. Then, we report quantitative results across the tests described in the Validation Procedures and Analyses Section. Finally, we compare our results with those available from the literature.

Fig. 3A shows how the soleus force slope (i.e. stiffness) in the correspondence of a specific stance point of walking (i.e. 57.6% and 72% stance respectively) can substantially differ when estimated directly from the force-length profile (i.e. see the continuous workloop profile, QS) or from the force-length-velocity-activation surface cross section (i.e. see the dotted gray line, $K^m$). Instantaneous stiffness throughout the stance phase is reported in Figs 3C (i.e. $K^m$) and 3D (i.e. QS). This further illustrates how differences between instantaneous estimates of $K^m$ and QS can be of several orders of magnitude even at the single muscle level, i.e. also see Video 1 in supplementary material. The force-length profile (Fig. 3A) from which QS is derived, directly relates changes in $F^m$ with changes in $\tilde{r}^m$ without accounting for the fact that $F^m$ may be instead varying because of $a$ and $\tilde{v}^m$. In this, the chosen time interval $dt$ from which to compute QS, impacts the final stiffness estimate (Equation 6). Short intervals are most affected by the instantaneous peaks depicted in Fig. 3D. These peaks directly relate to the activation and contraction velocity differentials (Equation 6), which make fiber force vary with no underlying changes in fiber length, hence the vertical tangent on the force-length profile as depicted
in Figs 3A-B in the correspondence of the point at 57.6% stance. In these conditions, QS estimates do not reflect changes in muscle force due to changes in fiber length, thus biasing the final stiffness result.

Fig. 4 shows the gastrocnemius medialis MTU stiffness as well as how this brakes down into individual tendon and fibers stiffness, i.e. using our proposed $K^m$ and SRS respectively. Reported stiffness curves are averaged across all walking trials performed by all subjects. Fig. 4A shows that our proposed method ($K^m$, Equation 4) results in fibers that are more compliant than the series elastic tendon, thus dominating on the resulting MTU stiffness as previously reported in experimental and theoretical studies (Walmsley and Proske, 1981; Zajac, 1989; Latash and Zatsiorsky, 1993; Cook and McDonagh, 1996; Endo and Herr, 2014). Notably, the peaks in the gastrocnemious medialis’ tendon stiffness predicted by our proposed method (i.e. 160±69N/mm, Fig. 4A) are within literature values available for this muscle (Lichtwark and Wilson, 2005, 2008; Krishnaswamy et al., 2011). In this context, the tendon stiffness in the linear region of the force-strain relationship was investigated for the gastrocnemius medialis in relation to the MTU efficiency during the stance phase of walking. Optimal tendon stiffness for the gastrocnemius medialis was found to be 144N/mm in (Lichtwark and Wilson, 2008) and ranged between 103N/mm and 150N/mm in (Krishnaswamy et al., 2011). Experimental studies found in vivo gastrocnemius tendon stiffness in the range of 174±38N/mm (Maganaris and Paul, 2002; Lichtwark and Wilson, 2005). Conversely, SRS (Equation 7) resulted in gastrocnemius medialis fibers substantially stiffer than the series elastic tendon (Fig. 4B), which does not represent physiological MTU behavior (Walmsley and Proske, 1981; Latash and Zatsiorsky, 1993; Lichtwark and Wilson, 2008; Endo and Herr, 2014).

In the first test described in the Validation Procedures and Analyses Section, the subject-specific EMG-driven model predicted joint moments that matched the experimental moments across all 124 performed estimations, i.e. 62 validation trials of walking and running (i.e. from all five subjects) from which joint moments were estimated about two DOFs: the knee flexion extension and the ankle
plantar-dorsi flexion (Fig. 5). The similarity indexes ranged between $0.1 \leq \text{NRMSE} \leq 0.7$ and $0.57 \leq R^2 \leq 0.99$ (Fig. 6). The 77% of all 124 performed estimations had $R^2 > 0.9$ and NRMSEs $< 0.3$, across all tasks, subjects and DOFs (Fig. 6). The average values were distributed towards low NRMSEs (i.e. 0.29±0.14) and high $R^2$ (i.e. 0.9±0.08) values (Fig. 6).

The second test revealed distinct stiffness mechanisms across joints and motor tasks, which were consistent across subjects (Figs 7-9). Across all performed trials and subjects, walking was characterized by a knee stiffness peak (i.e. 4.8±1.1 Nm/deg) occurring at 19.3±6.5% stance, followed by a higher and minimally overlapped ankle stiffness peak (i.e. 5.9±1.8 Nm/deg) at 83.9±5.4% stance (Fig. 7). Running displayed different stiffness mechanisms with respect to walking (Fig. 8). It was characterized by double-peak stiffness curves both at the ankle and knee, with levels of synchronization across joints. The ankle stiffness first peak (i.e. 5.5±1.3 Nm/deg) occurred at 27.8±10.7% stance, whereas the second peak (i.e. 6.5±1.6 Nm/deg) occurred at 89.4±3.1% stance. For subjects 2 and 5 the knee stiffness had two pronounced peaks. The first peak (8.7±3.8 Nm/deg) occurred at 20.3±8.1% stance and a second peak (i.e. 7.9±3.9 Nm/deg) at 74.1±11.7% stance. Subjects 1, 3 and 4 generated knee stiffness with a single pronounced peak. Subject 1 had a peak of 8.9±1.3 Nm/deg occurring at 84.7±5.1%. Subjects 3 and 4 had peaks of 10.2±1.3 Nm/deg occurring at 32±7.4% stance.

The joint moment and stiffness predicted by our proposed method underlay muscles operating on the ascending portion of the force-length-velocity-activation surface (Table 2, Fig. 3, and supplementary video 1) across all trials and subjects. The muscles that accounted for most of the ankle stiffness variability (i.e. VAF index in Equation 8, Fig. 9) were the plantar flexors including the soleus ($\text{VAF}_{\text{walking}} = 0.79±0.2$ and $\text{VAF}_{\text{running}} = 0.84±0.1$), the gastrocnemius medialis ($\text{VAF}_{\text{walking}} = 0.34±0.3$ and $\text{VAF}_{\text{running}} = 0.31±0.2$) and the gastrocnemius lateralis ($\text{VAF}_{\text{walking}} = 0.17±0.1$ and $\text{VAF}_{\text{running}} = 0.19±0.1$) with peroneus brevis and longus displaying negligible values, i.e. $\text{VAF}_{\text{walking/running}} \leq 0.05$. Small VAFs were observed in the dorsi flexors including tibialis anterior (i.e. $\text{VAF}_{\text{walking/running}} = \ldots$
0.09±0.05) and peroneus tertius (i.e. VAF_{walking/running} ≤ 0.03). Unlike for the ankle, the muscles that accounted for most of the knee stiffness variability were apportioned between the extensors and the antagonist flexors groups (Fig. 9). Extensor muscles included the vastus medialis (i.e. VAF_{walking} = 0.19±0.2 and VAF_{running} = 0.36±0.1), intermedius (i.e. VAF_{walking} = 0.28±0.2 and VAF_{running} = 0.35±0.1), lateralis (i.e. VAF_{walking} = 0.47±0.3 and VAF_{running} = 0.4±0.2), and the rectus femoris (i.e. VAF_{walking} = 0.26±0.2 and VAF_{running} = 0.2±0.2) with negligible contribution coming from the tensor fasciae latae (i.e. VAF_{walking/running} = 0.01±0.01). Flexor muscles included the medial hamstrings (i.e. cumulative VAF_{walking} = 0.14±0.2 and VAF_{running} = 0.04±0.04) and lateral hamstrings (i.e. cumulative VAF_{walking} = 0.19±0.1 and VAF_{running} = 0.3±0.1) with negligible contributions from the gastrocnemii (i.e. cumulative VAF_{walking/running} = 0.05±0.04), sartorius (i.e. VAF_{walking/running} = 0.01±0.01) and gracilis (i.e. VAF_{walking/running} = 0.01±0.01).

The third test revealed that joint stiffness emerged from substantial modulation of the underlying muscles co-contraction ratios (CCRs, Equation 9) across all performed trials and subjects (Fig. 10). In the early stance of walking (i.e. 0% ≤ stance ≤ 25%), the knee stiffness peak underlay muscle activity that shifted from dominant knee flexors (i.e. CCR = 0.62±0.2 at 0% stance) to lesser dominant knee extensors (i.e. CCR = -0.4±0.3 at 25% stance). This enabled shock absorption and weight acceptance functions predominantly at the knee joint with low ankle joint stiffness corresponding to dominant dorsi flexors activity (i.e. CCR = 0.86±0.01 between 0% ≤ stance ≤ 25%). Throughout the midstance (i.e. 25% ≤ stance ≤ 85%), ankle stiffness increased as a function of dominant plantar flexors activity (i.e. CCR = -0.63±0.2 at 85% stance) while knee stiffness decreased as a function of dominant knee flexors activity (i.e. CCR = 0.6±0.1 between 45% ≤ stance ≤ 85%). This corresponded to foot push-off and body propulsion with production of joint moment (Fig. 5) and stiffness (Fig. 10) mainly at the ankle joint. In the terminal stance (i.e. stance ≥ 85%), both ankle and knee stiffness reached their
lowest values in the correspondence of dominant dorsi flexors (CCR =0.6±0.2 at 100% stance) and knee flexors activity (CCR =0.65±0.2 at 100% stance), in preparation for the swing phase.

In the early stance of running (i.e. 0% ≤ stance ≤ 30%) the synchronized stiffness peaks in the ankle and knee joints, indicated highly balanced (i.e. CCR ≈ 0) co-contraction ratios between agonist and antagonist muscles, i.e. CCR\textsubscript{knee} = 0.02±0.2 and CCR\textsubscript{ankle} = -0.15±0.1 at 30% stance (Fig. 10). This enabled shock absorption and weight acceptance functions both at the knee and ankle joints.

Throughout the midstance (30% ≤ stance ≤ 65%), muscle activity transitioned towards dominant ankle plantar flexors (CCR = -0.6±0.1), with a resulting sharp drop in ankle stiffness, whereas co-contraction remained balanced at the knee joint (CCR = 0.14±0.1) with no substantial drop in joint stiffness. In the terminal stance (i.e. stance ≥ 65%), the second ankle stiffness peak corresponded to dominant plantar-flexor activity transitioning towards ankle dorsi flexors in the terminal stance (CCR = -0.18±0.5 at 100% stance). In this phase, knee joint stiffness substantially dropped as a result of dominant knee flexors activity (CCR = 0.66±0.1) in preparation for the swing phase.

**Discussion**

This study explored the theoretical and experimental aspects of a subject-specific EMG-driven model of the human leg to predict electrophysiologically and dynamically consistent estimates of ankle and knee joint stiffness directly from the joint constituent MTUs during dynamic locomotion. In this scenario, we aimed to highlight the hierarchical nature of how stiffness emerges at the joint. That is, how net joint stiffness (Figs 7, 8, and 10) emerges from the contribution of individual MTUs (Figs 9) as well as how individual MTU stiffness emerges from the contribution of muscle fibers in series with elastic tendons (Figs 2-4). This is further highlighted in Fig. 11. This study highlighted how our proposed stiffness estimates fundamentally differs with respect to short-range stiffness and quasi-stiffness estimates.
Previous work based on musculoskeletal modeling focused on isometric tasks only and did not use experimental EMG-excitations to calibrate and drive forward dynamic subject-specific musculoskeletal models (Hu et al., 2011; Pfeifer et al., 2012). Furthermore, previous work was based on the short-range stiffness component of muscle fibers, or SRS. The SRS mathematical formulation (Cui et al., 2008) provides estimates that are directly proportional to muscle force and invariant to changes in fiber contraction length and velocity. Therefore, MTU forces could be determined, in these studies, directly from external isometric joint moments with no need for explicitly solving for the underlying fiber contraction and tendon dynamics (Hu et al., 2011; Pfeifer et al., 2012). That is, by balancing external (i.e. measured) and internal moments (i.e. unknown MTU force by the known moment arms) while solving for the MTU force distribution problem by imposing a static optimization criterion chosen *a priori* (Crowninshield and Brand, 1981; Anderson and Pandy, 2001). This predicts muscle forces and short-range stiffness that directly reflect isometric joint moments but do not account for force/stiffness modulations due to changes in the neural drive to muscles and in the underlying muscle fiber compliance, which are dominant factors in dynamic motor tasks. In this context, it is known that SRS would over-estimate muscle stiffness (and therefore joint stiffness) during dynamic locomotion (Walmsley and Proske, 1981; Hu et al., 2011; Pfeifer et al., 2012). This was illustrated in our representative comparisons (Fig. 4). Moreover, the *a priori* chosen static optimization criterion used in these previous works (Hu et al., 2011; Pfeifer et al., 2012), could not explain large repertoires of muscle co-contraction typically observed across dynamically different motor tasks such as walking and running (Tax et al., 1990; Besier et al., 2003a; Heintz and Gutierrez-Farewik, 2007; Vigouroux et al., 2007; Sartori et al., 2012b, 2013). Nevertheless, SRS is an important mechanism to be included in current modeling methods to characterize the stiffness mechanisms underlying isometric muscle function as well as postural tasks and gait initiation (Hu et al., 2011; Pfeifer et al., 2012).
Our proposed method addressed some of the above-described limitations. It provided a framework that enabled for the first time investigating estimates of MTU and joint stiffness during dynamic locomotion tasks. This could be done by (1) ensuring physiological co-contraction ratios in the simulated muscles via experimental EMG-excitations used as a direct input drive (Fig. 10), (2) modeling the non-linear transfer function between EMG-excitation and MTU force, and (3) modeling the *dynamic contraction* of muscle fibers (due to activation, contraction length and velocity) and their interaction with the series elastic tendon (Figs 2-4) (Lloyd and Besier, 2003; Sartori et al., 2012b, 2013, 2014). This generated joint stiffness estimates that resulted from experimental excitations and physiological muscle operation, and matched experimental joint moments about multiple DOFs, subjects, and across different motor tasks (Figs 2-6).

This work revealed for the first time the elastic interplay between the knee and ankle joints. Results showed regularities in the production of muscle and joint stiffness with distinctive strategies for walking (Fig., 7) and running (Fig. 8). Observed regularities included stiffness peak timings (Result Section, Figs 7, 8, and 10), muscle fibers consistently operating on the ascending portion of the force-length-velocity-activation surface (Table 2, Fig. 3, supplementary Video 1), and consistent co-contraction ratio modulations (Result Section, Fig. 10). Specifically, results indicated that locomotion is characterized by joint stiffness strategies of impulsive nature, i.e. having burst-like transients (Figs 7, 8 and 10). During walking, the knee and ankle joints displayed minimally overlapped burst-like transients, thus reflecting inter-joint low elastic coupling. This was characterized by an initial stiffness burst in the knee joint in the early stance followed by a minimally overlapped stiffness burst in the ankle joint during the terminal stance (Figs 7 and 10). Running displayed higher elastic coupling between the two joints (Figs 8 and 10). This was characterized by double-burst stiffness curves both at the ankle and knee joints, which emerged in the early (i.e. weight-acceptance phase) and terminal (i.e. push-off phase) stance phases (Latash, 2010). Stiffness peaks underlay co-contraction ratios that were
always more balanced (i.e. CCR closer to zero) in the knee joint than in the ankle joint (Fig. 10). This
was also reflected by the fact that net knee joint stiffness was contributed both by knee extensors and
flexors (Fig. 9) whereas ankle stiffness was predominantly contributed by plantar-flexors (Fig. 9) as
previous related work confirmed (Farris and Sawicki, 2012). This suggested that the ankle joint
modulates stiffness for propelling the body while knee joint stiffness appeared to be modulated for
body weight acceptance and joint stability functions.

There are limitations to this study that should be discussed. This work was based on five subjects.
Therefore, results may not be completely generalizable. However, this study aimed at developing the
theoretical and computational modelling framework for investigating the neuro-musculo-skeletal
mechanisms regulating dynamic stiffness on a subject-specific basis. This step needs to be necessarily
taken before the developed framework can be applied systematically to large subject populations. In
this context, the proposed EMG-driven musculoskeletal model was first scaled and then calibrated to
each subject to account for subject-specific anthropometry, EMG-to-activation mapping, and MTU
force-generating properties. This enabled our methods to be specifically applied across individuals,
while accounting for the subject’s actual muscle activation patterns. This represents an improvement in
current state of the art modeling methodologies where the recruited subjects are chosen to be of similar
build of the anatomical model (Lloyd and Besier, 2003; Martelli et al., 2011, 2015) or where only
model anthropometric properties are scaled with no identification of subject-specific EMG-to-force
parameters (Hamner and Delp, 2013; Hicks et al., 2014). Future work will couple our proposed
modeling methods with dimensionality reduction techniques (Lee and Seung, 1999; Sartori et al.,
2013), thus enabling characterizing robust regularities in the neuromuscular control of joint stiffness
across large subject populations, something this study could only partially address due to the reduced
subject population size.
Results were generated during the walking and running stance phases only. This was done because the model calibration included running trials for which the swing phase occurred partially or totally out of the motion capture volume. Therefore there was an incomplete swing phase data available for calibration and validation across trials. However, contrary to the stance phase, the human leg during swing undergoes a ballistic movement largely supported by the hip with no considerable muscular effort in knee and ankle joints (Collins et al., 2005; Hamner et al., 2010; Hamner and Delp, 2013; Shamaei et al., 2013a, 2013c). Therefore, our proposed analysis allowed observing the major phases underlying stiffness modulation at the knee and ankle. Future work will however include the analysis of the swing phase and stiffness estimation from the hip joint.

Our methods employed Hill-type muscle models that did not characterize a number of important muscle-tendon force generating mechanisms. Future work will address this limitation by (1) including more detailed models of the triceps surae mechanical contribution to the Achilles tendon strain and stiffness (Gerus et al., 2012; Shim et al., 2014), (2) modeling history-dependent muscle force generating mechanisms including stretch-induced force enhancement and shortening-induced force depression (McGowan et al., 2012), (3) incorporating more realistic damping and force-velocity characteristics (Haeufle et al., 2014) as well as (4) muscle energetics characteristics resulting from MTU stiffness modulation (Umberger and Rubenson, 2011; Endo and Herr, 2014).

Surface electromyography only provides a surrogate measurement of the neural drive received by muscles, from which it is currently not possible to discern the underlying afferent and efferent components as well their contribution to muscle and skeletal joint stiffness regulation (Farina and Negro, 2012). Future work will employ high-density EMG in conjunction with decomposition techniques to experimentally decode single motor unit action potential and spiking events (Farina et al., 2014b). In combination with our proposed musculoskeletal modeling framework, this will enable identifying different neural components in the drive to muscles (Farina et al., 2014a). This will open
new avenues for investigating the roles of central and peripheral circuitries in the modulation of stiffness (Piovesan et al., 2013). Furthermore, it will enable estimating the effective muscle excitation level as a direct function of motor unit spikes. This will address limitations in current EMG amplitude estimation methods, which heavily rely on EMG linear envelope maxima and filtering parameters identification (Sartori et al., 2014).

The joint stiffness predicted during walking and running could not be directly validated with experimental stiffness recordings. While joint stiffness can be viably recorded in isometric or postural tasks (Perreault et al., 2001, 2004; Pfeifer et al., 2012), the reliable measurement of stiffness during natural locomotion still remains an open challenge and currently relies on non-commercial instrumentation and non-standard identification procedures. However, in this current study we have compared our results directly with experimental stiffness recordings available from the literature (Plocharski and Plocharski, 2013; Lee and Hogan, 2014; Rouse et al., 2014). Please, refer to the “Comparison with experimental stiffness estimates from the literature” Section. It is worth stressing that comparing results with perturbation-based studies in the literature needs to be done with caution due to inherent differences including: subject population size and type, locomotion speed, system identification method, as well as perturbation properties. These factors introduce variability across perturbation-based studies as well as with respect to the results derived from our study, which involved different locomotion speeds and unperturbed locomotion conditions. In this context, it was previously shown that experimentally recorded joint stiffness varies with the perturbation amplitude and speed as well as a function of muscle stretch reflexes elicited by the perturbation system (Bennett, 1994; Loram and Lakie, 2002; Vlutters et al., 2015). Finally, our results only reflect joint stiffness contributed from muscles for which surface EMG data are available (Table 1). This may further contribute to the differences observed in experimental studies where the experimentally measured stiffness encompasses all muscles spanning the joint (Plocharski and Plocharski, 2013; Lee and Hogan, 2014; Rouse et al.,
Future work will seek to employ wearable joint perturbation systems (Andersen and Sinkjaer, 1995; Plocharski and Plocharski, 2013) in combination with system identification methodologies (Ludvig and Perreault, 2012) for a more direct validation of the modeling methods presented in our study.

It is worth stressing that the aim of our proposed work was not that of addressing, in a single study, all limitations existing in the fields of model-based estimation and system identification of musculoskeletal stiffness. Our aim was to provide a theoretical and computational framework to calculate joint stiffness that explained both measured electrophysiological and mechanical data. Furthermore, our aim was to investigate how the predicted stiffness modulates during locomotion and indicate how this differs to current stiffness estimates including QS and SRS. Notably, our results demonstrated that our proposed method could use EMG signals recorded from 13 muscles to blindly predict dynamically consistent estimates of ankle plantar-dorsi flexion and knee flexion-extension moments and resulting stiffness during walking and running and across five subjects by means of 124 evaluations, i.e. see Validation Analyses Section. In this, validation was performed according to an open-loop paradigm where joint moments were predicted with no knowledge of the corresponding experimental values (Sartori et al., 2012b). Although this does not validate directly the stiffness estimates, it does prove that the predicted joint stiffness is consistent with the subjects’ actual electrophysiological activity and resulting multi-joint moments.

Future work will employ our recently proposed closed-loop EMG-informed modeling paradigm (Sartori et al., 2014). This will ensure precise and consistent tracking of reference joint moments by minimally adjusting the input experimental EMGs and by synthetizing excitations for muscles with no experimental EMG data available (Sartori et al., 2014). This will open to possibilities including: (1) estimating stiffness at the hip joint, for which the major flexor muscles (i.e. iliacus and psoas) are deeply located, (2) computing the stiffness contribution of intrinsic ankle muscles for which surface
EMG is not accessible including: tibialis posterior, flexor digitorum/hallucis muscles, and extensor digitorum/hallucis muscles (3) assessing differences in joint stiffness estimation with respect to the fully predictive open-loop paradigm of this study, which may not preserve prediction accuracy across validation trials (Fig. 6), and (4) coupling our modeling methods with numerical forward integrators and postural controllers (Leroux et al., 2002; Wang et al., 2007; Latash, 2010) for computing segmental/articular kinematics emerging from the predicted joint moments and stiffness.

Remarkably, our validation results also showed that our methodology estimated gastrocnemius tendon stiffness that was in line with literature values available for this muscle, i.e. see Results Section (Maganaris and Paul, 2002; Lichtwark and Wilson, 2005, 2008; Krishnaswamy et al., 2011). Moreover, our simulations underlay muscle fibers predominantly operating along the ascending portion of the force-length-velocity-activation surface (Table 2, Fig. 3, and Video 1 in supplementary material). This may underlie a metabolic advantage since, on the ascending portion, muscle activation, length and contraction velocity have the most influence on the mechanical force output. This enables muscle force production with minimally required activation and contraction modulations, thus saving on the metabolic cost necessary to actively recruit muscle fibers (Collins et al., 2015). This is also in agreement with experimental findings that reported normal muscle fiber operation in the ascending portion of the force-length relationship (Maganaris, 2001; Rubenson et al., 2012), with contractions within confined ranges of the sarcomere length (Burkholder and Lieber, 2001). Future work will also employ ultrasound imaging and in vivo measurements to further validate our methods at the MTU level (Farris et al., 2014).

These observations provide some confidence on the physiological validity of our proposed EMG-driven simulations. Furthermore, they put further emphasis on the importance of using experimental EMG data for driving the underlying musculoskeletal model. Because normalized fiber length varies around rather confined ranges (Table 2), as opposed to EMG-derived muscle activation that largely
varies between 0 and 1 (Fig 3), abnormal muscle activation patterns (i.e. from static optimization-based methods) would have the most impact on the predicted muscle and joint stiffness and would adversely bias their final estimations (Burkholder and Lieber, 2001).

Comparison with QS estimates from the literature

The estimates of joint QS available in the literature were always greater than the instantaneous stiffness estimates computed by our method (Figs 7-10) when averaged across the respective gait phases (Stefanyshyn and Nigg, 1998; Günther and Blickhan, 2002; Coyles et al., 2011; Charalambous et al., 2012; Plocharski and Plocharski, 2013; Shamaei et al., 2013a, 2013c). This may be a direct consequence of the time interval $dt$ needed to compute QS (Equation 6). As previously discussed (i.e. Relation to quasi-stiffness and short-range stiffness Section), small $dt$ would make QS more sensitive to vertical tangents on the torque-angle relationship similarly to what shown in our results at the muscle level, i.e. see Fig. 3D and Result Section. However, QS estimates were generally in agreement with instantaneous stiffness peaks predicted by our method in specific points within the same gait phases.

A modeling study that used a 2D spring-mass model reported QS estimates at the ankle and knee over the stance phase of running (i.e. 4.8±0.5 m/s) in the order of 7.1±1.9 Nm/deg and 10.8±3.0 Nm/deg, respectively (Günther and Blickhan, 2002). These were 1-3Nm/deg higher than the stiffness peaks predicted by our method at the ankle (i.e. 5.9±1.8 Nm/deg, at 88.9±3.2% stance) and knee (8.2±3.3 Nm/deg, at 29.8±8.0% stance) joints (Fig. 10).

Experimental studies reported knee QS, throughout the weight-acceptance phase of walking, in the order of 4.9±1.3 Nm/deg (Shamaei et al., 2013c). This reflected the knee stiffness early peak (i.e. 4.3±1.1 Nm/deg, at 20.7±6.5% stance) predicted using our EMG-driven modeling method (see second test and Figs 7 and 10). Throughout the push-off phase of walking, the ankle QS was reported to be in the order of 17 Nm/deg (Shamaei et al., 2013a). This was substantially higher than the highest ankle
stiffness values (i.e. 7.8±0.5 Nm/deg, at 85.3±7.2% stance) predicted using our EMG-driven modeling methods (see second test and Figs 7, subject 3). Throughout the push-off phase of sprint running, the ankle QS was reported to be in the order of 5.93±0.75Nm/deg (Stefanyshyn and Nigg, 1998; Coyles et al., 2011; Charalambous et al., 2012). This reflected ankle stiffness peak values (i.e. 5.8±1.8 Nm/deg, at 88.9±3.2% stance) predicted using our EMG-driven modeling method (see second test and Figs 7 and 10). Throughout the first 100 ms of the stance phase of running (4.5±0.2 m/s), the knee QS was reported to be in the order of 8.74±2.73 Nm/deg (Coyles et al., 2011), which was in agreement with knee stiffness peaks (8.2±3.3 Nm/deg, at 29.8±8.0% stance) predicted using our EMG-driven modeling method.

Comparison with experimental stiffness estimates from the literature

Experimental recordings of ankle stiffness were reported (Plocharski and Plocharski, 2013) from 11 subjects walking on a treadmill (1.1±0.04 m/s) and wearing an ankle joint perturbation system (Andersen and Sinkjaer, 1995). Ankle joint stiffness was derived from the perturbation system recorded data (i.e. joint angle and moment) using a multi-segment system identification algorithm (Ludvig and Perreault, 2012). This provided quasi-instantaneous recordings of ankle joint stiffness at 20%, 50% and 90% gait cycle, resulting from fast perturbations, i.e. perturbation window: 100ms, angular velocity: 300 deg/s, amplitude: 8 deg, hold-window: 200ms. At 20% gait cycle (i.e. ≈ 33% stance), the experimentally measured ankle stiffness was in the order of 4.7±1.9 Nm/deg over the 100ms-perturbation window and was 2.0±1.8 Nm/deg over the 200ms-hold phase (Plocharski and Plocharski, 2013). At the same stance point our EMG-driven modeling method predicted instantaneous peaks of ankle stiffness (i.e. 1.4±0.8 Nm/deg) in line with values extracted from the 100ms-perturbation window. At 50% gait cycle (i.e. ≈ 83% stance), the experimentally measured ankle stiffness was in the order of 5.3±2.6 Nm/deg over the 100ms-perturbation window and was 2.9±3.0 Nm/deg over the
200ms-hold phase (Plocharski and Plocharski, 2013). At the same gait cycle point our EMG-driven modeling method predicted instantaneous peaks of ankle stiffness (i.e. 5.3±1.7 Nm/deg) in line with values extracted from the 100ms-perturbation window. The 90% gait cycle corresponded to the swing phase of walking, which was not recorded in our proposed study.

Rouse and colleagues reported experimental ankle stiffness estimates during the locomotion stance phase from 10 subjects walking over-ground (85-90 steps/min) on a perturbation platform (Rouse et al., 2014). A second-order parametric model was established based on recorded ankle torque and kinematics to characterize ankle impedance during perturbation (Rouse et al., 2012). This provided recordings of ankle joint stiffness at about 20%, 35%, 55% and 70% stance, resulting from mild perturbations, i.e. perturbation window: 75ms, angular velocity: 45 deg/s, amplitude: 2 deg. Stiffness increased by a factor of four from 20% to 70% of stance phase, starting at approximately 1.5 Nm/rad/kg (i.e. ≈ 20% stance phase) and increasing to 6.5 Nm/rad/kg (i.e. ≈ 70% stance phase). Rouse et al.’s results are within one to two standard deviations from our reported values, i.e. ankle stiffness increasing throughout the stance phase starting at 1.01±0.6 Nm/rad/kg (i.e. ≈ 20% stance phase) and increasing to 3.8±2.6 Nm/rad/kg (i.e. ≈ 80% stance phase).

Lee and Hogan reported experimental ankle stiffness estimates during the locomotion initial stance phase from 13 subjects walking on a treadmill (0.7±0.1 m/s) and wearing a perturbation robot (Khanna et al., 2010; Lee and Hogan, 2014). A time-varying ensemble-based system identification method was used to extract ankle stiffness during perturbation (MacNeil et al., 1992). This provided recordings of ankle joint stiffness in the order of 1.1±0.1 Nm/deg at about 5% stance, resulting from random torque perturbations, i.e. peak-to-peak perturbation torque amplitude: ±7.7Nm/rad, stop frequency 100 Hz (Lee and Hogan, 2014). Results are in line with our reported values, i.e. 1.2±1.5 Nm/deg at 5% stance.

Conclusions
The proposed method is an internally consistent, subject-specific musculoskeletal model for estimating MTU and joint stiffness that results from experimental EMG-excitations and that underlie MTU forces that match experimental joint moments measured from dynamic locomotion. This fundamentally differs from current stiffness estimates, including QS and SRS. In this context, our methods and results show that:

1) Quasi-stiffness (QS, Equation 6) and stiffness ($K_m$, Equations 3-4) are two distinct concepts and are only equivalent in a passive system (Rouse et al., 2013). This is mathematically formulated in Equation 6, which relates QS to $K_m$ in the context of an active muscle. In this, QS = $K_m$ only if the muscle activation and contraction velocity components (i.e. $A(dt,t)$ and $V(dt,t)$ respectively) were disregarded. Our results showed that in the context of a muscle (i.e. active system), QS would directly relate changes in muscle force to changes in muscle fiber length without accounting for the fact that muscle force may be instead varying because of other factors including muscle activation or contraction velocity (Equation 6 and Fig. 3). Therefore, QS provides biased estimates of the mechanical stiffness of an active system, such as a muscle or a human joint, i.e. estimates that do not strictly describe resistive force responses due to MTU or joint elastic characteristics (Rouse et al., 2013).

2) While SRS is an important model for describing isometric muscle stiffness, it would predict non-physiological MTU stiffness when applied to dynamic locomotion (Equation 7 and Fig. 4). In contrast, this study proposed a modeling formulation of how muscle and joint stiffness modulate as a function of muscle activation, fiber contraction, and interacting tendon dynamics. As a result, this formulation can be applied to dynamic locomotion (i.e. unlike SRS) and can generalize across passive and active systems (i.e. unlike QS).

This methodology could be used, in the future, in conjunction with predictive models of muscle recruitment and modularity (Sartori et al., 2013) for synthetizing the neuromuscular mechanisms
underlying human-like locomotion and adaptation capacity to different terrains (Wang et al., 2007; Song and Geyer, 2015) in artificial systems such as bipedal robots or prosthetic limbs (Farahat and Herr, 2010; Beyl et al., 2011; Markowitz et al., 2011; Karavas et al., 2013).

The ability of our proposed methodology to characterize subject-specific and motor task-specific MTU and joint stiffness is critical for sizing wearable robots actuator power and spring stiffness to individual users' joint properties and external mechanical demands (Shamaei et al., 2013a).

The ability of estimating net joint stiffness in real-time from EMG signals could be used in scenarios involving patients with uni-lateral impairments (Pfeifer et al., 2012, 2014). In this context, stiffness could be estimated from healthy side joints and used as a control signal for variable-stiffness prostheses and orthoses acting on the contralateral affected leg so to mimic (i.e. restores) healthy leg behavior across different locomotion tasks and terrains.

The ability of estimating in real-time the individual muscle fibers and tendon stiffness could be used to modulate the metabolic cost of locomotion in individuals wearing orthoses. In this context, the orthosis could be controlled so that the user’s muscle fibers always operate on the most favorable portion of the force-length-velocity-activation surface (Farris and Sawicki, 2012; Collins et al., 2015).

Finally, the ability of translating an individual’s electromyographic activity into muscle and joint stiffness estimates could help understand abnormal stiffness strategies in patients with neurological and orthopedic conditions and deliver personalized rehabilitation treatments that restore physiological properties (Wolbrecht et al., 2008; Ogawa et al., 2014).

In conclusion, the proposed method is a theoretical and computational framework for investigating the neuromuscular basis of locomotion and motor control. This may open up new avenues for examining human neuromuscular control and musculoskeletal mechanics, with implications in neurorehabilitation technologies.
Acknowledgments

The presented methodology of muscle and joint stiffness estimation was developed as part of the Calibrated EMG-Informed Neuromusculoskeletal Modelling Toolbox (CEINMS, https://simtk.org/home/ceinms).

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

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Figure 1: The schematic structure of the electromyography (EMG)-driven musculoskeletal model. It comprises five components: (A) Musculotendon Activation, (B) Musculotendon Kinematics, (C) Musculotendon Dynamics, (D) Joint Dynamics, and (E) Model Calibration. The EMG-driven model is initially calibrated (E) using experimental EMG-excitations, experimental three-dimensional (3D) joint angles, and matching experimental joint moments from a set of calibration trials. After calibration, the model is operated in open-loop, i.e. it does not require experimental joint moments to track. The calibrated model is validated on its ability to blindly match experimental joint moments from a set of novel trials that were not used for calibration. The Musculotendon Activation component (A) maps initial EMG-excitations recorded from 13 muscle groups to activations for 18 musculotendon units (MTUs, Table 1). Subsequently, MTU force and stiffness are determined (C) as a function of muscle activation (A) and experimental 3D musculotendon kinematics derived from experimental 3D joint kinematics (B). The resulting MTU forces and stiffness are simultaneously projected (D) on two degrees of freedom including the knee flexion-extension (KneeFE) and the ankle plantar-dorsi flexion (AnkleFE).

Figure 2: (A) Nominal static properties of muscle tissues. These are determined by the active and passive isometric force-length curves. Fiber force and length values are normalized by the maximal isometric force $F_{\text{max}}$ and optimal fiber length $l_{\text{opt}}$ respectively. (B) Nominal dynamic properties of muscle tissues. These are determined by the active force-velocity curve. Fiber force and contraction velocity values are normalized by the $F_{\text{max}}$ and maximal contraction velocity $v_{\text{max}}$ respectively. Both active force-length and force-velocity curves are depicted for different levels of muscle activation. (C) Solution space of the fiber force. This results from combining together passive and active force-length and force-velocity curves. Note how muscle force is modulated simultaneously by activation, fiber length and contraction velocity.
**Figure 3:** (A) Soleus’ fiber force-length profile over the stance phase of walking (i.e. see continuous colored workloop) with 0% being heel-strike and 100% toe-off events. Two cross-sections of the force-length-velocity-activation solution space along the fiber length axis are depicted in the correspondence of 57.6% stance (red dot) and 72% (black dot) respectively (i.e. see dotted lines). In these two stance points, the respective cross-sections differ substantially to that derived from the force-length-velocity-activation cross section. (B) Soleus’ force solution surfaces in the correspondence of 57.6% stance (red 3D surface and red dot) and 72% (black 3D surface and black dot). The soleus’ fiber force-length profile (i.e. green work loop) throughout the stance phase of walking (as in A) is also projected on the muscle force solution spaces. (C) Instantaneous soleus’ stiffness ($K_m$, Equation 4) throughout the stance phase of walking. This is predicted from the slope of the fiber force-length-velocity-activation surface in B as the slope along the normalized fiber length directional axis in the correspondence of the instant normalized fiber length predicted by our EMG-driven method (also see Materials and Methods Section). (D) Instantaneous soleus’ quasi-stiffness ($Q_S$, Equation 6) throughout the stance phase of walking. This is predicted from the tangent of the fiber force-length profile in A. Note how stiffness and quasi-stiffness can predict substantially different slopes in the correspondence of the same stance point. Quasi-stiffness relates changes in fiber force with changes in fiber length without accounting for the fact that force may be instead varying because of the activation or contraction velocity components, hence the peaks in (B) relative to the steep tangents in (A). The reported data are relative to Subject 1 and are calculated as a function of normalized fiber length and contraction velocity values. Also see Video 1 in supplementary material.

**Figure 4:** (A) Stiffness predicted by our proposed method (Equation 4) for the gastrocnemius medialis’ fibers, tendon and musculotendon unit. (B) Short-range stiffness (Equation 5) predicted for the gastrocnemius medialis’ fibers, as well as tendon stiffness and resulting musculotendon unit stiffness. Values are averaged across all walking trials performed by all subjects and are reported throughout the stance phase of walking with 0% being heel-strike and 100% toe-off events.

**Figure 5:** Predicted and experimental joint moments from all validation trials and subjects. The ensemble average (continuous lines) curves are depicted for experimental and predicted joint moments. The standard deviations (shaded area) are also depicted for the experimental joint moments. Experimental and predicted joint moments are reported about about two degrees of freedom including: knee flexion-extension (KneeFE) and ankle plantar-dorsi flexion (AnkleFE). The reported data are from the stance phase with 0% being heel-strike and 100% toe-off events.

**Figure 6:** Distribution of the similarity indexes computed between experimental and predicted joint moments from all validation trials and subjects. Similarity indexes include the coefficient of determination ($R^2$) and the root mean square error normalized to the root mean square sum of the experimental joint moments (NRMSE). Histograms gather the $R^2$ and NRMSE values in intervals with a 0.1-fixed-width in the [0-1] range. In this range the most unfavorable values include $R^2 = 0.58$ and NRMSE = 0.704, i.e. see vertical red lines.
Figure 7: The ensemble average curves (continuous line) and standard deviation (shaded area) for the stiffness predicted about two degrees of freedom including ankle plantar-dorsi flexion (AnkleFE) and knee flexion-extension (KneeFE) during walking across all validation trials and subjects. Data are reported for the stance phase of walking with 0% being heel-strike and 100% toe-off events.

Figure 8: The ensemble average curves (continuous line) and standard deviation (shaded area) for the stiffness predicted about two degrees of freedom including ankle plantar-dorsi flexion (AnkleFE) and knee flexion-extension (KneeFE) during running across all validation trials and subjects. Data are reported for the stance phase of running with 0% being heel-strike and 100% toe-off events.

Figure 9: Variability accounted for (VAF) by all 18 musculotendon units in the model in the net joint stiffness about two degrees of freedom including: ankle plantar-dorsi flexion (AnkleFE) and knee flexion-extension (KneeFE). VAF values are averaged from all performed trials and subjects (black bar) and are reported together with the standard deviation (vertical line).

Figure 10: Ensemble average joint stiffness (shaded area) and co-contraction ratio (continuous green line). Data are averaged across all trials performed by all subjects across walking and running respectively. Standard deviation curves are also reported relative to the co-contraction ratio (dotted green lines). Data are reported about the ankle plantar-dorsi flexion (AnkleFE) and knee flexion-extension (KneeFE) degrees of freedom and throughout the stance phase with 0% being heel-strike and 100% toe-off events.

Figure 11: Hierarchical structure of how net joint stiffness (A) emerges, in our framework, from the stiffness contribution of the joint constituent MTUs (B and C) as well as how MTU stiffness (D) results from the constituent fibers stiffness in series with tendon stiffness (E). Data are averaged across all trials performed by all subjects across walking. Data are reported about the knee flexion-extension (KneeFE) degrees of freedom, for the associated MTUs with names as defined in Table 1 and throughout the stance phase with 0% being heel-strike and 100% toe-off events. Results show that the early stance of walking (i.e. 0% ≤ stance ≤ 25%) underlay a progressive decrease of knee flexors joint stiffness (B) and a simultaneous increase of net joint stiffness (A) mainly contributed by knee extensors (B). Throughout the midstance (i.e. 25% ≤ stance ≤ 85%), net knee stiffness (A) decreased due to gradual decrease in knee extensors joint stiffness (B). In the terminal stance (i.e. stance ≥ 85%), net knee stiffness (A) reached its lowest value in the correspondence of knee flexors activity in preparation for the swing phase.
Table 1. Electromyograms, musculotendon units, and moment arm sign. Muscle groups from which experimental electromyograms (EMG) were recorded and the associated musculotendon units (MTUs) that were driven by these EMGs. In this, the vastus intermedius EMG activity was derived as the mean between the vastus lateralis and vastus medialis EMGs (Lloyd and Besier, 2003). The biceps femoris long head and short head were driven by the same EMG signal. The same applied to the semimembranosus and semitendinosus as well as to the peroneus longus, brevis and tertius (Sartori et al., 2012b). The MTUs spanning a specific joint DOF were gathered into agonist and antagonist groups based on the moment arm sign observed during walking and running and using the moment arm sign convention from (Sartori et al., 2012).

<table>
<thead>
<tr>
<th>Experimental Muscle EMG</th>
<th>Musculotendon Units</th>
<th>Knee Flexion-Extension Moment Arm</th>
<th>Ankle Plantar-Dorsi Flexion Moment Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensor Fasciae Latae</td>
<td>tfl</td>
<td>Knee Extensor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Gracilis</td>
<td>gra</td>
<td>Knee Extensor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Lateral Hamstring</td>
<td>biceps femoris long head (bicfemlh)</td>
<td>Knee Flexor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td></td>
<td>biceps femoris short head (bicfemsh)</td>
<td>Knee Flexor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Medial Hamstring</td>
<td>semimembranosus</td>
<td>Knee Flexor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td></td>
<td>(semimem)</td>
<td>Knee Flexor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td></td>
<td>semitendinosus</td>
<td>Knee Flexor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td></td>
<td>(semiten)</td>
<td>Knee Flexor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Rectus Femoris</td>
<td>recfem</td>
<td>Knee Extensor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Sartorius</td>
<td>sar</td>
<td>Knee Extensor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Vastus Lateralis</td>
<td>vaslat</td>
<td>Knee Extensor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Vastus Medialis</td>
<td>vasmed</td>
<td>Knee Extensor</td>
<td>Knee Extensor</td>
</tr>
<tr>
<td>(Vastus Lateralis + Vastus Medialis)/2</td>
<td>vastus intermedius (vasint)</td>
<td>Knee Flexor</td>
<td>Knee Flexor</td>
</tr>
<tr>
<td>Gastrocnemius Lateralis</td>
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<td>Knee Flexor</td>
<td>Plantar Flexor</td>
</tr>
<tr>
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<td>gasmed</td>
<td>Knee Flexor</td>
<td>Plantar Flexor</td>
</tr>
<tr>
<td>Soleus</td>
<td>sol</td>
<td>Knee Flexor</td>
<td>Plantar Flexor</td>
</tr>
<tr>
<td>Peroneus Group</td>
<td>peroneus longus (perlong)</td>
<td>Plantar Flexor</td>
<td>Dorsi Flexor</td>
</tr>
<tr>
<td></td>
<td>peroneus brevis (perbrev)</td>
<td>Plantar Flexor</td>
<td>Dorsi Flexor</td>
</tr>
<tr>
<td></td>
<td>peroneus tertius (pertert)</td>
<td>Plantar Flexor</td>
<td>Dorsi Flexor</td>
</tr>
<tr>
<td>Tibialis Anterior</td>
<td>tibant</td>
<td>Dorsi Flexor</td>
<td>Dorsi Flexor</td>
</tr>
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</table>
Table 2. Normalized muscle fiber lengths. Normalized fiber length for all 18 musculotendon units in the model. Data reported in this table are mean (standard deviation) values averaged across all trials and subjects within each motor task, which included walking (WK) and running (RN). Musculotendon unit names are defined in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>bicfemh</th>
<th>bicfemsh</th>
<th>gaslat</th>
<th>gasmed</th>
<th>gra</th>
<th>perbrev</th>
<th>perlong</th>
<th>perter</th>
<th>recfem</th>
<th>sar</th>
<th>semimem</th>
<th>semiten</th>
<th>sol</th>
<th>tfl</th>
<th>tibant</th>
<th>vasint</th>
<th>vaslat</th>
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<tbody>
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<td>0.9(0.1)</td>
<td>0.89(0.2)</td>
<td>0.61(0.1)</td>
<td>0.66(0.1)</td>
<td>1.12(0.1)</td>
<td>0.69(0.1)</td>
<td>0.85(0.1)</td>
<td>0.38(0.1)</td>
<td>0.57(0.1)</td>
<td>0.62(0.1)</td>
<td>1.23(0.1)</td>
<td>0.9(0.1)</td>
<td>0.43(0.1)</td>
<td>0.51(0.1)</td>
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</tr>
<tr>
<td>RN</td>
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<td>0.71(0.1)</td>
<td>0.88(0.1)</td>
<td>0.79(0.2)</td>
<td>0.59(0.1)</td>
<td>0.7(0.1)</td>
<td>0.65(0.1)</td>
<td>1.1(0.1)</td>
<td>0.76(0.1)</td>
<td>0.84(0.1)</td>
<td>0.39(0.1)</td>
<td>0.58(0.1)</td>
<td>0.7(0.1)</td>
<td>1.22(0.1)</td>
<td>0.84(0.1)</td>
<td>0.63(0.1)</td>
<td>0.72(0.1)</td>
</tr>
</tbody>
</table>
A

Knee FE stiffness (Nm/deg)

B

\[
\begin{align*}
\text{knee extensor MTUs} & \quad \text{knee flexor MTUs}
\end{align*}
\]

C

MTU stiffness projection on joint DOF (Eq. 2)

D

MTU stiffness (N)

E

Fiber–tendon series stiffness (Eq. 3)