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Series elasticity facilitates safe plantar flexor muscle–tendon shock absorption during perturbed human hopping

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In our everyday lives, we negotiate complex and unpredictable environments. Yet, much of our knowledge regarding locomotion has come from studies conducted under steady-state conditions. We have previously shown that humans rely on the ankle joint to absorb energy and recover from perturbations; however, the muscle–tendon unit (MTU) behaviour and motor control strategies that accompany these joint-level responses are not yet understood. In this study, we determined how neuromuscular control and plantar flexor MTU dynamics are modulated to maintain stability during unexpected vertical perturbations. Participants performed steady-state hopping and, at an unknown time, we elicited an unexpected perturbation via rapid removal of a platform. In addition to kinematics and kinetics, we measured gastrocnemius and soleus muscle activations using electromyography and *in vivo* fascicle dynamics using B-mode ultrasound. Here, we show that an unexpected drop in ground height introduces an automatic phase shift in the timing of plantar flexor muscle activity relative to MTU length changes. This altered timing initiates a cascade of responses including increased MTU and fascicle length changes and increased muscle forces which, when taken together, enables the plantar flexors to effectively dissipate energy. Our results also show another mechanism, whereby increased co-activation of the plantar- and dorsiflexors enables shortening of the plantar flexor fascicles prior to ground contact. This co-activation improves the capacity of the plantar flexors to rapidly absorb energy upon ground contact, and may also aid in the avoidance of potentially damaging muscle strains. Our study provides novel insight into how humans alter their neural control to modulate *in vivo* muscle–tendon interaction dynamics in response to unexpected perturbations. These data provide essential insight to help guide design of lower-limb assistive devices that can perform within varied and unpredictable environments.

1. Introduction

Animals display a fascinating ability to stay upright during movement in the face of uneven or unpredictable environments. Yet, we still know very little about how this is achieved. By contrast, the mechanisms that enable steady-state locomotion on level surfaces are more familiar. The dynamics of steady-state walking, running and hopping can be described using simple spring-mass models [1–3], and stable running over a broad range of conditions can be explained by adjusting the compliance of the 'leg spring' (e.g. [4,5]). An important component of economical, steady-state locomotion in both bipedal [6] and quadrupedal [7] animals is the highly tuned neuromechanical interaction between active muscle fibres and passive elastic structures in the ankle plantar flexors. This 'tuned' interaction refers to muscles that are activated with a

timing and magnitude that enables elastic tissues to stretch and recoil to cycle the energy required for steady gait. The aim of this study was to provide fundamental insights into the mechanisms for locomotor stability during unexpected perturbations and to reveal the interplay between neuromuscular control and muscle–tendon dynamics.

Maintaining stability in the face of variable and unpredictable locomotor environments requires adaptive changes in the neural control strategy. For example, when humans encounter an unexpected loss of ground support during walking, they initially re-establish balance via reflexive muscle activity in both the ankle plantar flexors and dorsiflexors upon ground contact [8,9]. This strategy has been suggested as a potential mechanism to stiffen the ankle joint to cope with the perturbation [9] and to prevent forward propulsion of the body [8]. Similarly, Panizzolo *et al*. [10] have shown that when humans encounter an unanticipated bump during walking, they recover via increased soleus (SOL) and tibialis anterior (TA) co-activation. This suggests that the nervous system appears to react to different unexpected perturbation tasks (i.e. loss in ground support versus bump in ground) in a similar manner. However, the underlying *in vivo* muscle–tendon dynamics that accompany these alterations in neural control are far less studied. This information is critical, given that the relationship between the timing of muscle activation and muscle length change determines whether a muscle produces energy to act as a motor, absorbs energy to act as a brake, or remains energy neutral to act like a spring [11,12]. Recent muscle-level studies highlight that compliant muscle–tendon units (MTUs) are able to shift from energy producers (net positive work) to energy absorbers (net negative work) during cyclical tasks via one simple neural mechanism: adjusting the onset timing of muscle activation [13]. However, our current understanding of the *in vivo* patterns of neural control necessary for muscle–tendon interactions that effectively dissipate energy remains limited.

In addition to altered neural control strategies, animals achieve stability when moving in unpredictable terrains via alterations in muscle and tendon force–length dynamics. A series of studies in bipedal birds have shown that the distal leg extensors play a key role in stabilization and perturbation recovery. Specifically, *in vivo* recordings of the underlying neural and mechanical behaviour of the gastrocnemius muscle in guinea fowl illustrate rapid changes in muscle fascicle length, force and work output for the perturbed stride and subsequent recovery strides [14]. In contrast with human unexpected perturbations, muscle activation increased only in the first stride following the perturbation, and not in the perturbed stride itself, suggesting that neural drive may not be the primary mechanism during recovery to steady gait, and other factors such as muscle–tendon contractile dynamics play an important role. Drop landing experiments in turkeys highlight the role of series elasticity, whereby the distal ankle extensor tendons absorb mechanical energy upon ground contact, acting as a buffer to modulate the magnitude and timing of active muscle lengthening [15,16]. Thus, the elastic properties of distal extensor MTUs lower the rate of muscular energy dissipation and potentially reduce the risk of muscle injury that can result from rapid and forceful muscle lengthening [17].

Both human [18] and animal [19] studies have shown that the mechanics of multiple lower-limb joints are altered to recover from unexpected changes in the height of the ground. We have previously quantified the ankle, knee and hip mechanical strategies that humans use to dissipate energy and stay upright when falling into a hole [18]. Our results show that humans increase the energy absorbed in the ankle across a range of perturbation heights, but at the largest perturbation heights, similar to birds, increase their reliance on more proximal lower-limb joints (i.e. the knee and the hip) to absorb mechanical energy and stabilize fall recovery. Interestingly, humans tend to overcompensate and dissipate more mechanical energy than is necessary due to the change in vertical height. This could be a safety prioritization strategy to avoid a potential fall. Thus, both human and animal studies indicate that the ankle plays a critical role in perturbation recovery. Human studies suggest that recovery from an unexpected perturbation is associated with alterations in the timing and magnitude of muscle activity, whereas animal studies highlight that alterations in fascicle length and work output, along with an increased role of series elasticity, aid in rapid energy dissipation. However, the neuromuscular control strategies and muscle–tendon dynamics that underpin joint-level responses in humans remain unclear and have rich potential to provide bio-inspiration for the design of wearable devices and robotic systems capable of performing when the going gets rough.

Here, we show how neuromuscular control and muscle–tendon dynamics are modulated in the ankle plantar flexors to maintain stability during unexpected vertical perturbations. To do this, we measured *in vivo* muscle activations and fascicle dynamics as well as joint kinematics and kinetics in humans during two-legged hopping with a rapid drop in the height (20 cm) of the ground. We hypothesized that a drop in substrate height would lead to an automatic phase shift that adjusts the timing of muscle activation relative to the MTU dynamics. Additionally, we hypothesized that this altered timing leads to effective energy dissipation through a more optimal alignment of active force and fascicle lengthening for net negative work.

2. Methods

(a) Experimental protocol

Data were recorded from 10 healthy individuals (mean ± s.d., six male and four female; 26.7 ± 4.4 years; 1.73 ± 0.09 m; 70.1 ± 10.9 kg). All participants were in good health and had no recent history of lower-limb musculoskeletal injury. Subjects provided written informed consent and all protocols were approved by the Institutional Review Board at the University of North Carolina at Chapel Hill, USA. Participants were instructed to perform bilateral hopping in place on a stationary split-belt treadmill (Bertec, Columbus, OH, USA) (figure 1). Participants wore a safety harness and hopped continuously at their preferred frequency (2.4 ± 0.02 Hz, range 2.13–2.8 Hz). An unexpected perturbation, via manual removal of two custom-built platforms (20 cm height) from directly under the left and right lower limbs, was elicited at a random time between the 10th and 20th hop. Subjects were instructed to continue hopping following the perturbation event. During each trial, we collected lower-limb joint kinematics, kinetics, fascicle dynamics and muscle activations.

(b) External kinematics and kinetics

An eight-camera motion analysis system (Vicon, Oxford, UK) was used to capture the three-dimensional (3D) positions of 32

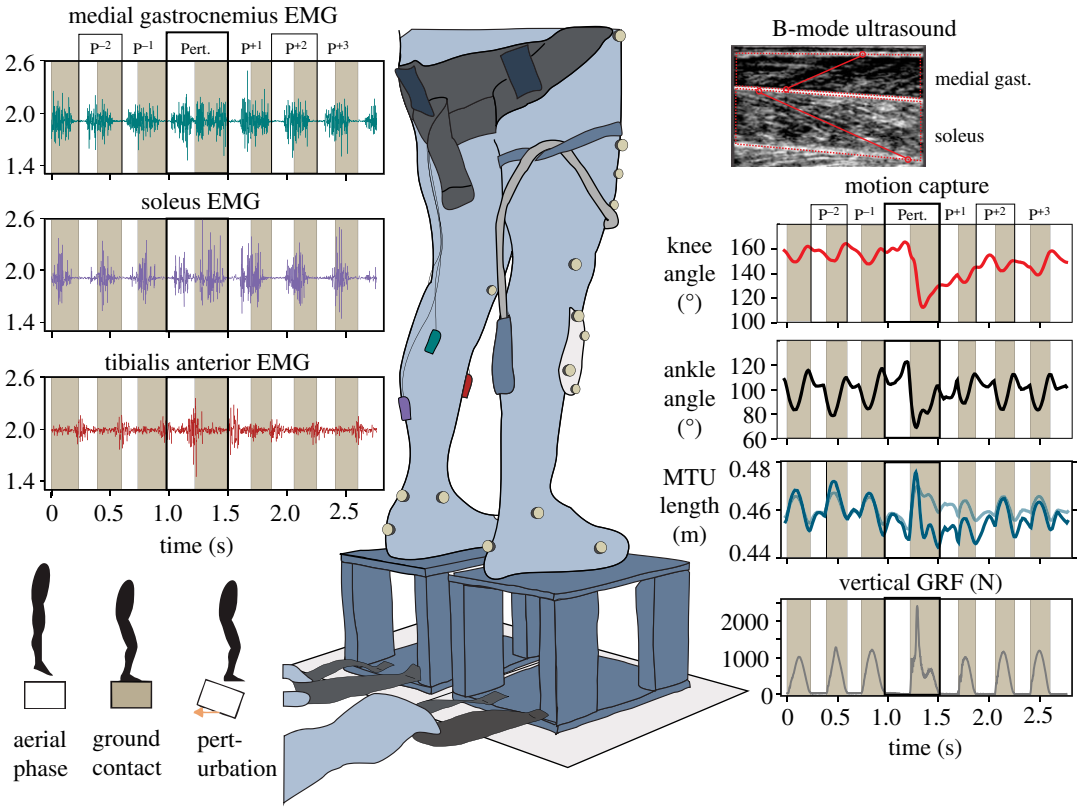

Figure 1. The drop in the height of the ground due to the unexpected perturbation introduces a time delay that alters the timing of neuromuscular control relative to muscle–tendon dynamics. An illustration of the experimental set-up for perturbation trials. Individuals hopped continuously at their preferred frequency (2.4 ± 0.02 Hz) until an unexpected vertical perturbation was elicited via manual removal of the 20 cm platforms. Muscle activation patterns and fascicle length changes were measured using surface electromyography (EMG) and B-mode ultrasound, respectively. Lower-limb kinematics were recorded using 3D motion caption and a stationary split-belt treadmill captured the ground reaction forces. Shaded regions represent the ground contact phases. (Online version in colour.)

reflective markers attached to the pelvis and lower limbs. Individual markers were placed bilaterally on lower-limb bony landmarks and rigid marker clusters of three or four markers were attached to the pelvis, thigh and shank segments to track segment motion during hopping. This marker set is consistent with previous experimental protocols (e.g. [20]). Motion capture data were collected at 120 Hz and raw marker positions were filtered using a second-order low-pass Butterworth filter with a cut-off frequency of 10 Hz. A static calibration trial was collected to scale a musculoskeletal model to each subject (*OpenSim* v. 3.3, [21,22]). The subject-specific scaled model was used together with the motion capture data in an inverse kinematics analysis to determine the time-varying joint angles for the ankle, knee and hip.

Three-dimensional ground reaction forces applied to the left and right legs were measured using a split-belt instrumented treadmill (980 Hz, Bertec, Columbus, OH, USA). Participants hopped such that each foot was on a separate belt of the treadmill, and thus the two 3D force vectors could be attributed separately to the left and right legs. The raw analogue force platform data were filtered using a second-order low-pass Butterworth filter with the cut-off frequency of 35 Hz. Kinematics were combined with measured GRFs in an inverse dynamics analysis to compute the net joint moments at the ankle, knee and hip. Kinematics and kinetics were calculated for the right leg only, and it was assumed that the left leg behaved symmetrically. We also calculated the timing of 'expected ground contact' with the platform during the perturbation (i.e. the theoretical start of ground contact if the platform had not been removed) based on the average time between the apex of the hop, determined from the motion capture data, and the timing of initial ground contact, determined from the force plate data. For each individual, the average time from the apex to ground contact was added to the timing of apex for the perturbation to calculate the timing of 'expected ground contact'.

(c) Electromyography

Surface electromyography (EMG) was used to record muscle activity from the medial gastrocnemius (MG), SOL and TA on the left leg using wired electrodes (SX230, Biometrics Ltd, Newport, UK) that were carefully placed over muscle bellies after the skin surface was prepared by light abrasion and cleaned with alcohol. To obtain an EMG envelope, we high-pass filtered the raw EMG data at 20 Hz, rectified and low-pass filtered at 10 Hz. We then normalized the EMG envelope for each muscle to the maximum amplitude recorded for that muscle in each participant during the hopping-perturbation trial. To compare alterations in the magnitude of muscle activation during normal hopping and the perturbation, peak EMG, integrated EMG (iEMG) and average EMG of each muscle's signal were calculated over two discrete phases of the hop cycle: the ground contact phase and the aerial phase. This analysis was conducted for normal hopping (averaged over three hop cycles directly prior to the perturbation) and for the perturbation hop. Peak EMG was determined as the local maxima within the aerial and ground contact phases during a hop cycle. iEMG was determined as the time integral of the EMG envelope averaged across each given time period. The average EMG was calculated by dividing the iEMG by the time period over which it was integrated.

To compare alterations in the timing of muscle activation during normal hopping versus the perturbation, we determined the timing of muscle activation (relative to ground contact) using an EMG threshold fixed at 20% of the peak EMG recorded during the normal (i.e. unperturbed) hop cycle [23].

We calculated the absolute onset timing of muscle activation prior to ground contact. All data processing following inverse dynamics was conducted using custom-written scripts in Mathematica v. 11 (Wolfram Research Inc., Champaign, IL, USA).

(d) Determination of fascicle, tendon and muscle–tendon unit parameters

We recorded B-mode ultrasound images of the MG and SOL muscles using a 60 mm linear probe ultrasound system (LV 7.5/60/96Z, Telemed, Lithuania). The probe was positioned using elastic wrap over the muscle belly of the MG on the right leg. The probe was aligned so that the MG and the underlying SOL fascicles could be visualized from deep to superficial aponeuroses (figure 1). Images were sampled at 60 Hz, and a square wave digital pulse (3–5 V) from the ultrasound system triggered the collection of all other data synchronously.

We digitized ultrasound images using semi-automated tracking software [24] to determine time-varying MG and SOL fascicle lengths and pennation angles. Specifically, we manually selected a region of interest over each muscle belly and selected a fascicle of interest, in the mid-region of the muscle belly, in an initial frame. The algorithm tracks the fascicle in sequential frames by implementing an affine flow model. The fascicle length was calculated as the distance between the points of attachment of the defined fascicle on the superficial and deep aponeuroses. The pennation angle was defined for each muscle as the angle between the digitized fascicle and the deep aponeurosis. The instantaneous length of the MG and SOL MTUs was determined using the scaled musculoskeletal models and inverse kinematics results. Similar to Farris *et al*. [25], the initial fascicle length (L_i) was defined as the length of the fascicle at the timing of initial ground contact. Following landing, the muscle fascicles lengthened and then shortened before take-off. Fascicle lengthening was calculated relative to L_i by subtracting L_i from the peak length during ground contact. Fascicle shortening was calculated as the fascicle length at take-off minus the peak length during the ground contact phase. The initial fascicle length L_i, fascicle lengthening and fascicle shortening were calculated for normal hopping and for the perturbation hop. We note that dynamic ultrasound data for the SOL were excluded for two participants due to the inability to track the ultrasound data.

(e) Estimation of muscle kinetics

Direct measurements of muscle forces are not possible in humans, and thus, forces were estimated from inverse dynamics and *in vivo* muscle parameters, similar to previous studies [25,26] for the MG and SOL, respectively. Plantar flexor forces were calculated as the ankle moment, from inverse dynamics, divided by the MG and SOL moment arms. Moment arms were calculated as the first derivative of MTU length, determined from the ankle and knee joint angles using the equations of Hawkins & Hull [27], with respect to the ankle angle for the MG and SOL [25]. To estimate the relative amount of plantar flexor force contributed by the MG and SOL, total force was multiplied by the relative physiological cross-sectional area (PCSA) of the MG and SOL within the plantar flexors (MG: 0.17; SOL: 0.59, [28]). To calculate the force generated along the axis of the muscle fascicles, force was divided by the cosine of the pennation angle to determine time-varying muscle force for the MG and the SOL.

The velocities of the MG and SOL fascicles and MTU were calculated as the first derivative of their lengths with respect to time. The power outputs of the fascicles and MTU were then calculated as the product of their respective forces and velocities. We used the trapezium method to integrate power data for the fascicle and MTU with respect to time over discrete periods of positive and negative work [29]. Positive and negative mechanical work values were divided by ground contact time to calculate the average positive and negative fascicle and MTU power for the MG and SOL during the ground contact phase of normal hopping and during the perturbation.

(f) Statistical analysis

For all analyses, a within-subject design was used, including subject as random factor using a linear mixed effects model using the lme.R function from the nlme package [30] in R (v. 3.4.3, Vienna, Austria). To examine variation in muscle–tendon dynamics and neuromuscular activation between factors (muscle; condition), we specified the model with lme.R function, including interactions. Differences were considered significant at the $p < 0.05$ level. All statistical code and output is available as electronic supplementary material.

3. Results

The unexpected change in the height of the ground: (i) introduced an automatic phase shift that extended the aerial phase of the perturbed hop and led to a time delay in ground contact and (ii) altered the timing of muscle activation relative to MTU and fascicle length changes (figures 1 and 2). Specifically, there was a significant phase advance in the timing of muscle activation relative to ground contact for the MG and SOL ($p < 0.001$) during the perturbation (figure 2*a*,*b*), with no interaction between muscle and condition. Both the MG and SOL activated prior to ground contact; however, this was shifted 0.085 ± 0.031 s earlier for the MG and 0.066 ± 0.045 s earlier for the SOL during the perturbation compared to unperturbed hopping. During normal hopping the MG and SOL activated, on average, 0.077 ± 0.0084 s and 0.034 ± 0.013 s prior to ground contact, respectively. There were no significant differences in the timing of muscle activity relative to ground contact ($p = 0.085$) between normal hopping and the 'expected ground contact' of the perturbation.

We also find alterations in the magnitude of muscle activation in the plantar- and dorsiflexor muscles in response to a rapid unexpected perturbation (figure 2*e*,*f*). We found an increase in both average muscle activation ($p < 0.001$) and peak muscle activation ($p < 0.001$) during the aerial phase prior to the perturbation, compared to the aerial phase of normal unperturbed hopping. There was no significant interaction between muscle and condition, indicating a similar increase in activation for each muscle (average: $p = 0.962$; peak $p = 0.870$). The increase in the average activation for the aerial phase of the perturbation compared to normal hopping was 11.7 ± 7.2% for the MG and 10.4 ± 3.6% for the SOL. The increase in peak activation for the aerial phase of the unexpected perturbation was 34.4 ± 4.4% for the MG and 34.6 ± 5.4% for the SOL. Similarly, average and peak muscle activation increased during the ground contact phase of the perturbation compared to normal hopping (average: $p < 0.001$; peak: $p < 0.001$). The increase in the average activation for the ground contact phase of the unexpected perturbation was 10.8 ± 7.8% for the MG and 14.9 ± 8.2% for the SOL. The increase in peak activation for the ground contact phase of the unexpected perturbation was 18.7 ± 3.9% for the MG and 16.1 ± 4.5% for the SOL. We found similar shifts in the average muscle activation for the antagonist TA when comparing normal hopping to the perturbation, which increased 11.1 ± 9.9% and 19.1 ± 16.0% for the aerial phase and the ground phase, respectively. TA peak activation increased 38.9 ± 6.2% for the aerial phase

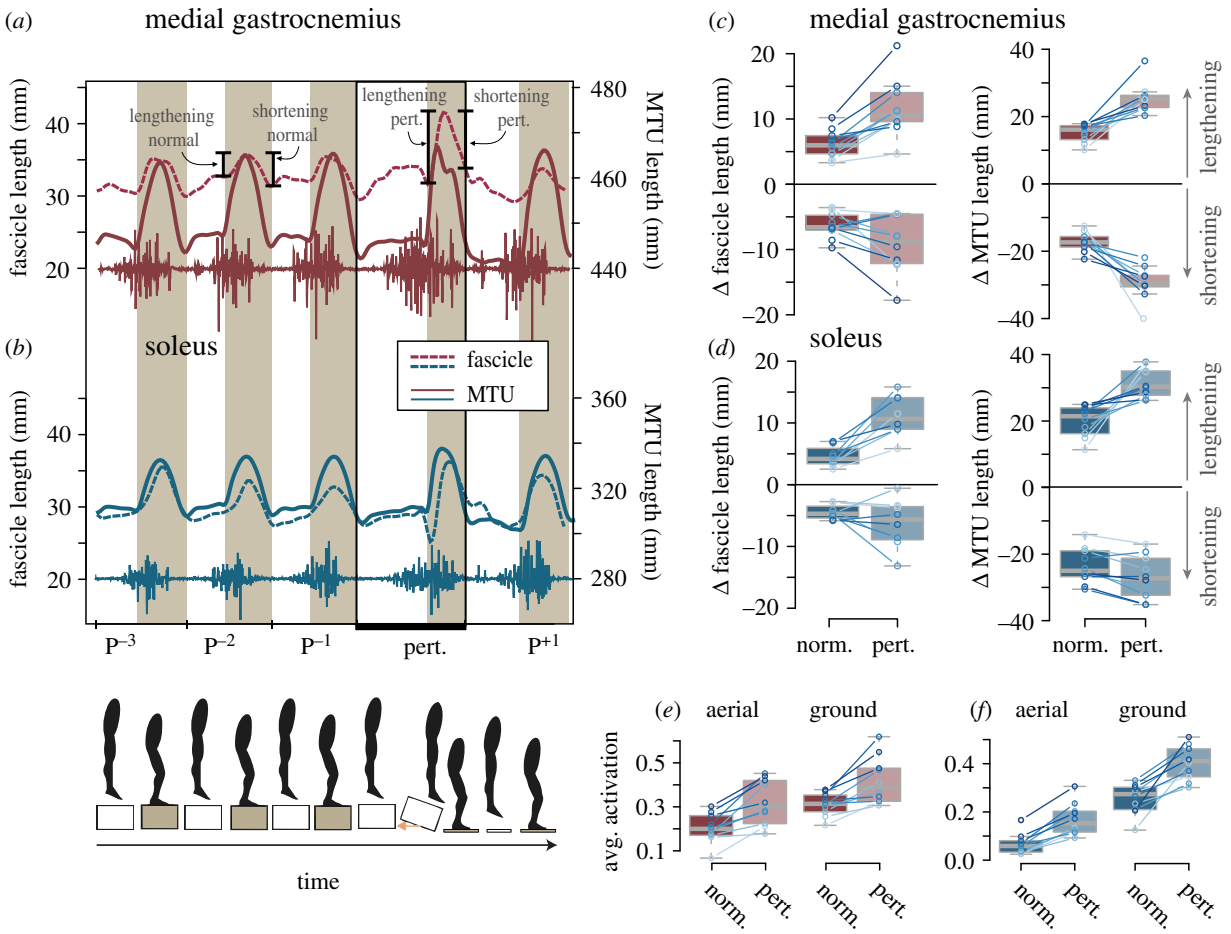

Figure 2. Time-varying muscle activation, muscle–tendon unit (MTU) length and fascicle length during normal hopping (P^{-3}, P^{-2}, P^{-1}) and the perturbation (Pert.) highlights changes in the timing and magnitude of activation relative to length changes in the MG (*a*) and SOL (*b*). The perturbation elicited significant increases ($p < 0.05$) in the total amount of fascicle and MTU lengthening and shortening during both the aerial and the ground contact phases when compared with normal (Norm.) hopping in the MG (*c*) and SOL (*d*). Average muscle activation, normalized to the maximum amplitude during the hopping-perturbation trial, significantly increased ($p < 0.05$) in the MG (*e*; red) and SOL (*f*; blue) during the aerial and ground contact phases of the perturbation compared to normal hopping. Box and whisker plots show the median, with hinges representing the first and third quartiles; whiskers represent the 95% CIs. Each individual is shown as a circle with a solid line connecting each individual across conditions. Shaded regions represent the ground contact phases. (Online version in colour.)

and by 48.1 ± 10.2% for the ground contact phase. The average muscle activation ($p = 0.052$) and peak muscle activation ($p = 0.494$) for the MG, SOL and TA during the aerial phase were not different when comparing these measures over the aerial phase of normal hopping to the aerial phase prior to the 'expected ground contact' of the perturbation.

Fascicle and MTU length change patterns in the MG and SOL varied during the perturbation compared to normal hopping (figure 2*c*,*d*). Specifically, there was a significant increase in both fascicle lengthening ($p < 0.001$) and fascicle shortening ($p = 0.029$) during the ground contact phase of the perturbation when compared with normal hopping; however, the magnitude did not vary between the MG and SOL (lengthening $p = 0.565$; shortening $p = 0.583$). During the perturbation, the MG and SOL fascicles lengthened by 5.4 ± 2.9 mm 6.6 ± 3.2 mm more than normal hopping, respectively. These increases in fascicle excursion during the perturbation were accompanied by significantly shorter ($p = 0.030$) MG (1.7 ± 3.5 mm) and SOL (2.7 ± 0.05 mm) fascicles at the initial timing of ground contact during the perturbation, when compared with normal hopping. These alterations in fascicle length upon ground contact were facilitated by a 3.4 ± 1.4 mm (MG) and 3.6 ± 1.3 mm (SOL) increase in total fascicle shortening during the aerial phase prior to the perturbation compared to normal hopping ($p < 0.001$).

For the MTU, the perturbation resulted in a significant increase in MTU lengthening ($p < 0.001$) and shortening ($p < 0.001$) during the ground contact phase, when compared with normal hopping (figure 2*c*,*d*). Further, we found that the magnitude of the change did vary between the MG and SOL for MTU shortening, but not for MTU lengthening (shortening: $p = 0.007$; lengthening: $p = 0.674$;). On average, the MG MTU lengthened 10.4 ± 5.1 mm more and the SOL MTU 11.4 ± 8.1 mm more during the perturbation, compared to normal hopping. The MG and SOL MTUs were at significantly ($p < 0.001$) shorter lengths at the timing of ground contact during the perturbation compared to the timing of ground contact during normal hopping. This was associated with the ankle joint being more plantarflexed. A full description of joint kinematics and kinetics has been previously published [18]. We found no differences when comparing MG and SOL fascicle and MTU lengths at the timing of ground contact during normal hopping to the 'expected ground contact' of the perturbation.

MG and SOL peak and average force both increased ($p < 0.001$), on average by 40–50%, during the ground contact phase of the perturbation, compared to unperturbed hopping. Duty factors were not significantly different ($p = 0.189$) between normal hopping and the perturbation hop, although the aerial phase for the hop leading into

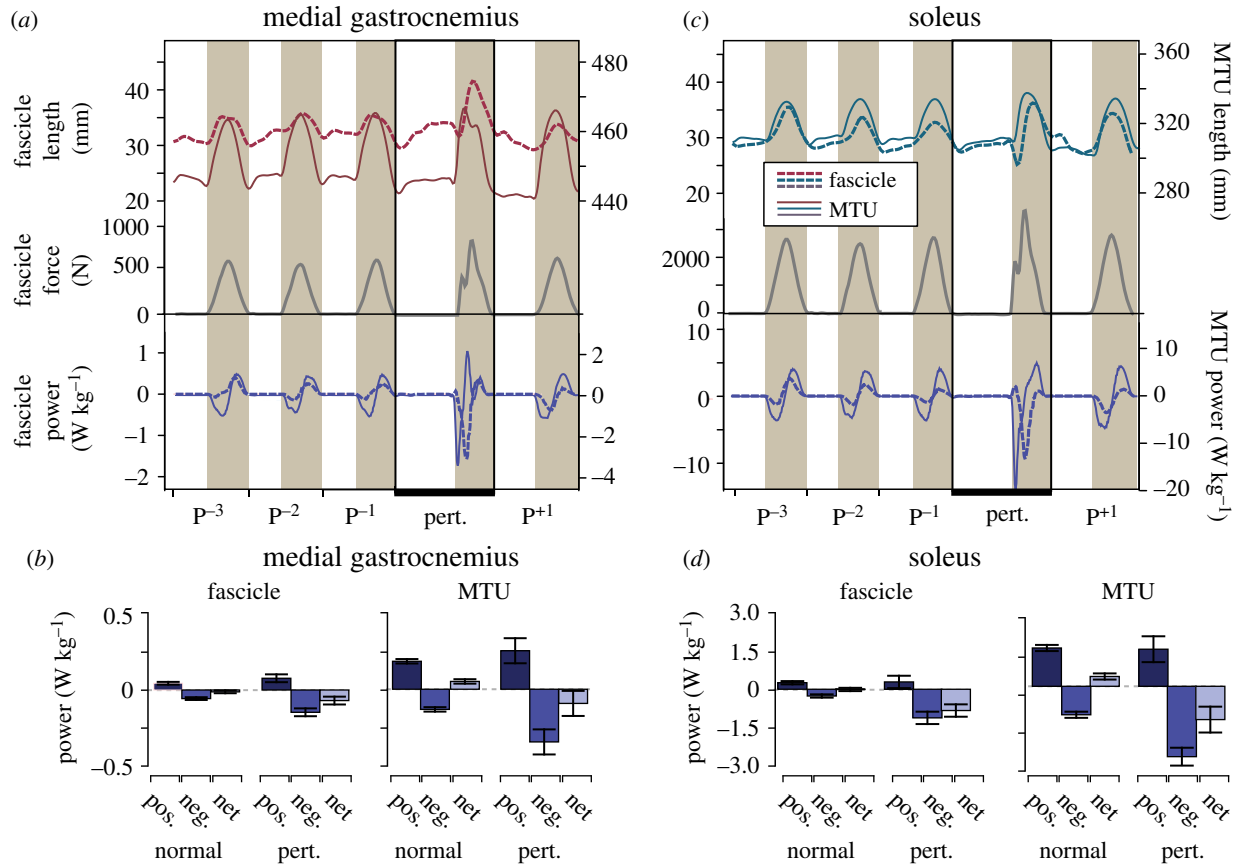

Figure 3. The perturbation initiates the cascade of responses that alter muscle length, force and power for more effective energy dissipation. Time-varying muscle–tendon unit (MTU) and fascicle lengths (top row; red (MG), green (SOL)), forces (middle row; grey) and powers (bottom row; purple) during normal hopping (P^{-3}, P^{-2}, P^{-1}) and the perturbation (Pert.) for the MG (*a*) and SOL (*c*). Shaded regions represent the ground contact phases. Group mean ± s.e. average positive power (darker purple), negative power (purple) and net power (lighter purple) (W kg^{-1}) produced at the fascicle and the MTU for normal hopping and the perturbation (Pert.) in the MG (*b*) and SOL (*d*). Data are normalized to body mass. For the fascicle and MTU, there was a significant ($p < 0.05$) increase in negative power and decrease in net power during the perturbation compared to normal hopping. (Online version in colour.)

the perturbation was on average 53% longer in duration compared to normal hopping. These changes in force, together with alterations in the timing and magnitude of fascicle and MTU length changes, led to changes in the mechanical power of the MTU and fascicle (figure 3*a*,*b*). For both the fascicles and the MTU, we found a significant increase in negative power during the perturbation (fascicle: $p = 0.008$; MTU: $p < 0.001$) when compared with normal hopping that was dependent on muscle (fascicle: $p = 0.018$; MTU: $p = 0.003$), highlighting an increased role of the SOL for absorbing energy of the fall (figure 3*b*,*d*). There was no significant difference in positive power between normal hopping and the perturbation (fascicle: $p = 0.448$; MTU: $p = 0.873$), nor any interaction (fascicle: $p = 0.86$; MTU: $p = 0.685$). Combined, this resulted in a significant reduction in net power (fascicle: $p = 0.008$; MTU: $p = 0.001$) for the perturbation and a significant interaction (fascicle: $p = 0.011$; MTU: $p = 0.002$), reflecting the changes in negative but not positive fascicle power.

4. Discussion

Here, we examined how neuromuscular control and muscle–tendon dynamics are altered in the ankle plantar flexors during recovery from an unexpected drop in substrate height (i.e. a fall into a hole). As predicted, we show that a drop in ground height leads to an automatic phase shift that modulates the timing of muscle activation relative to MTU dynamics. This altered timing initiates a cascade of responses that influences muscle lengths, forces and powers, enabling the plantar flexors to more effectively dissipate energy and recover from the fall. Additionally, we highlight another mechanism whereby an increase in plantar flexor–dorsiflexor co-activation enables internal shortening of the plantar flexor muscle fascicles prior to perturbation ground contact. This antagonist muscle activation in the pre-contact phase of hopping likely increases the plantar flexor's capacity to absorb energy upon ground contact, and may also be a mechanism to avoid potentially damaging fascicle lengthening strains during rapid energy dissipation tasks (figure 4).

Previous studies have shown that successful recovery from a perturbation is accompanied by alterations in the timing and magnitude of muscle activity relative to ground contact in muscles that cross the ankle joint [8,9,31]. Similar to walking perturbations, here, we find increases in the magnitude of muscle activity in the plantar flexors (MG and SOL) and dorsiflexors (TA) both before and after the initial ground contact of the perturbation (figure 2*e*,*f*). We also demonstrate a phase shift in the onset timing of MG and SOL muscle activity relative to ground contact (figure 2*a*,*b*), in part elicited by the extended aerial phase of the perturbation, which is consistent with the neuromuscular response during an unexpected loss of ground support in human walking [9]. In this study, we have combined muscle activation profiles with *in vivo* measurements of muscle–tendon

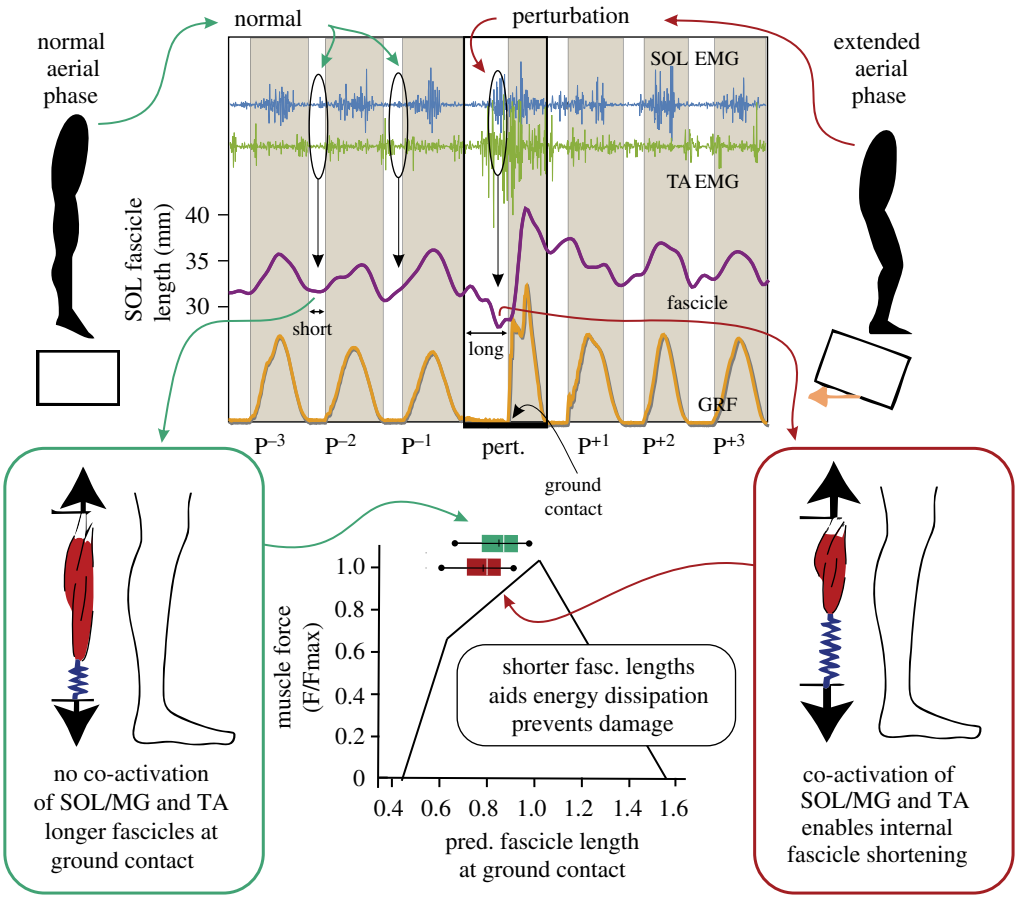

Figure 4. An unexpected drop in ground height extends the aerial phase and leads to an increase in plantar flexor–dorsiflexor co-activation which enables internal fascicle shortening prior to perturbation ground contact. The time-varying EMG signals for the SOL (blue) and TA (green) with the associated SOL fascicle length changes (purple) with vertical ground reaction forces (GRF, orange) are shown in the top panel. Shaded regions represent the ground contact phases. During normal hopping, there is limited co-activation of the plantar flexors and dorsiflexors. The co-activation present during the longer aerial phase of the perturbation enables muscle fascicles to shorten and shift leftward on the force–length relationship prior to ground contact, effectively altering the muscle states for optimal energy dissipation. (Online version in colour.)

dynamics to show that this altered timing leads to effective energy dissipation through a more optimal alignment of active muscle force and fascicle lengthening for net negative work (figure 3). Specifically, changes in the timing and magnitude of muscle activity, combined with the elastic properties of tendons and other in series tissues, enable plantar flexor muscle fascicles to do more negative work without risking potentially damaging long muscle fibre lengths. This emergent property is consistent with studies in isolated compliant MTUs [13] whereby adjusting muscle stimulation phase (akin to muscle activation) shifts a muscle from producing energy (net positive work) to absorbing energy (net negative work). Using an *in vitro* work loop approach in a relatively compliant MTU, they highlight that maximum energy absorption in the MTU and fascicles occurs at 0% phase which aligns active muscle force with MTU and fascicle lengthening. Here, we show, using an *in vivo* 'under the skin' approach in humans, that vertical perturbations induce an automatic phase shift that effectively facilitates an ankle plantar flexor muscle–tendon interaction for effective perturbation recovery.

During unperturbed hopping, muscle activity in the ankle plantar flexors and dorsiflexors is out of phase; however, during the perturbation, there is an increase in the co-activation of the plantar flexors and the antagonist TA (figure 4). Specifically, we find that during the perturbation, the MG, SOL and TA are active at a similar time, which is consistent with a series of studies that have elicited small (less than 5 cm), unexpected losses of ground support during human locomotion [8,9,31]. These studies suggest that this co-activation strategy enables stabilization through stiffening the ankle joint. Here, we also demonstrate that co-activation at the ankle joint, in the absence of ground contact, enables the plantar flexor muscle fascicles to internally shorten prior to ground contact of the perturbation (figure 4). Thus, the presence of plantar flexor–dorsiflexor co-activation may be a strategy that not only functions to stiffen and stabilize the ankle joint as previously thought, but one that also modulates the intrinsic force–length dynamics of the plantar flexor muscles. Theoretically, this would enable a leftward shift on the force–length relationship prior to ground contact, effectively altering the muscle states for optimal energy dissipation (figure 4). Additionally, this may be a mechanism to protect the muscle fascicles against potential damage from rapid and forceful lengthening during energy dissipation tasks. These altered force–length dynamics may function to enable the plantar flexor fascicles to remain within a favourable operating range on the force–length relationship to produce force and power for successful recovery towards stable rhythmic hopping following the fall.

How does the nervous system control this 'falling in a hole' perturbation response? These responses could be mediated through stretch reflex pathways of the ankle muscles. Nakazawa *et al*. [31] proposed that during human

walking, stretch reflexes facilitate ankle stabilization when a supportive surface is unexpectedly destabilized immediately after heel strike. Yet, others suggest that balance recovery during human walking cannot be attributed to stretch reflexes because the TA and MG do not lengthen [8]—however this idea was not supported with direct *in vivo* recordings of muscle lengths. It is possible that a reflex response plays a role in modulating TA muscle activity during the aerial phase prior to perturbation landing. The effects of MG and SOL activation in the absence of ground contact were apparent in the ankle kinematics. It is possible that this brief period of ankle plantar flexion causes the TA fascicles to lengthen and facilitate the stretch reflex (44–51 ms in humans; [32]). However, ultrasound recordings of TA fascicle length changes are necessary to confirm this. Future studies that incorporate *in vivo* recordings of TA muscle length, force and muscle activity patterns are required for a more comprehensive understanding of the possible role of sensory feedback and reflex mechanisms during rapid, unexpected perturbations. Additionally, we found no difference in the timing or magnitude of muscle activity, or the subsequent changes in muscle–tendon dynamics, when comparing these values between normal hopping and the perturbed condition relative to the non-existent, but expected ground contact with the platform. This may suggest that the nervous system does not intervene prior to the expected timing of ground contact, but rather the response is elicited within the extended aerial phase period prior to the new position of the ground.

Our muscle-level results indicate that successful recovery from a vertical perturbation leads to rapid, intrinsic changes in the ankle plantar flexor muscle–tendon force–length dynamics. The importance of the ankle for energy dissipation is supported by external joint-level mechanics where we show that during a 20 cm perturbation, negative ankle power increases by approximately 2.7 W kg^{-1} in comparison to 0.34 W kg^{-1} for the knee and 0.54 W kg^{-1} for the hip (fig. 5 in [18]). The ankle plantar flexors are characterized by a pennate fibre arrangement and a long compliant tendon, and studies have shown that this design favours both power amplification [6] but also energy dissipation [15,16,33]. A series of studies have highlighted the ability for tendons to uncouple fascicle and MTU length changes and absorb energy during eccentric contractions [15,16,33–35]. Konow and co-workers [15,16] used controlled drop landings in turkey's to demonstrate that the series elastic element attenuates the rate of energy dissipation by active muscle fascicles. They suggest that the elastic properties of tendons serve a protective mechanism to reduce the risk of muscle fascicle damage during rapid lengthening. Here, we find that during the perturbation, the magnitude of length change was greater in the MTU compared to the individual muscle fascicles (figure 2*a–d*). This suggests that the series elastic element plays an increased role in energy absorption, through uncoupling MTU and fascicle length changes, during the perturbation compared to unperturbed hopping. However, these fascicle length change patterns are inconsistent with perturbation studies in bipedal birds [14,15] and expected drop landings in humans [36,37] whereby muscle fascicles initially shorten upon ground contact to generate positive work. Our results display that the fascicles initially lengthen, which is consistent with previous ultrasound recordings of the SOL during human hopping [25]. This may be due to the differences in lower-limb posture and morphology between humans and birds. Future studies, combining direct and indirect experimental measures of *in vivo* muscle–tendon behaviour in both animal and human models for a broader range of non-steady and perturbed behaviours, will provide rich insights into the mechanisms for locomotor stability and the control of movement.

We acknowledge potential limitations in our analysis and the interpretation of muscle fascicle dynamics. While we were able to measure the dynamics of the muscle using ultrasound, certain measurements were simplified or calculated based on common assumptions. We did not measure optimal fascicle length directly but rather projected the length of muscle fascicles onto a general force–length relationship (figure 4). In addition, moment arms were geometrically derived and thus may not capture the full complexity of the ankle joint. Finally, due to our inability to directly measure muscle force, we used joint moments and assumed a distribution of force among synergistic plantar flexor muscles based on the relative muscle PCSAs of the ankle plantar flexors from imaging studies.

The biological behaviour with which lower-limb MTUs store and return energy has inspired the design of assistive devices capable of augmenting walking [38], running [39] and hopping [25] gaits. However, designing exoskeletons and prostheses capable of imitating the neuromuscular response to rapidly dissipate energy, for example, during an unexpected hole in the ground, remains an open challenge [40]. Our results suggest that when designing an ankle exoskeleton capable of performing in unexpected, variable environments, scientists need to consider the timing, magnitude and direction (e.g. extension or flexion) of actuation at a joint, as well as the physiological effects (i.e. strains and strain rates) of the device on the user's underlying MTUs. Existing passive devices may not be suitable, given that exoskeletons supporting force in parallel to biological ankle plantar flexors during walking [41] and hopping [25] tend to increase muscle strains which would only act to exacerbate the lengthening strains placed on the muscle fascicles during a perturbation, as shown here. To our knowledge, this is the first study to investigate *in vivo* muscle–tendon dynamics during unexpected human perturbation responses. The results provide updated mechanistic understanding of the neural control of muscle–tendon interactions in unsteady movement as well as an applied platform for the bio-inspired design of legged systems such as humanoid robots and lower-limb assistive devices, including prostheses and exoskeletons, that can perform within varied and unpredictable environments.

Ethics. Subjects provided written informed consent and all protocols were approved by the Institutional Review Board at the University of North Carolina at Chapel Hill, USA.

Data accessibility. Data are available as part of the electronic supplementary material.

Authors' contributions. T.J.M.D., L.K.P. and G.S.S. conceived of the study and designed the experimental protocol; T.J.M.D. and L.K.P. carried out the experiments; T.J.M.D. and C.J.C. analysed the data and drafted the manuscript; T.J.M.D., C.J.C., L.K.P. and G.S.S. edited the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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