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Afferent Contribution to Locomotor Muscle Activity During Unconstrained Overground Human Walking: An Analysis of Triceps Surae Muscle Fascicles

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[1]*Center for Sensory–Motor Interaction, Department of Health Science and Technology, Aalborg University, Aalborg;* [2]*Danish National Research Foundation;* [3]*Department of Exercise and Sport Sciences and Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark;* [4]*Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland; and* [5]*Osaka University of Health and Sport Sciences, Osaka, Japan*

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af Klint R, Cronin NJ, Ishikawa M, Sinkjaer T, Grey MJ. Afferent contribution to locomotor muscle activity during unconstrained overground human walking: an analysis of triceps surae muscle fascicles. *J Neurophysiol* 103: 1262–1274, 2010. First published December 23 2009; doi:10.1152/jn.00852.2009. Plantar flexor series elasticity can be used to dissociate muscle–fascicle and muscle–tendon behavior and thus afferent feedback during human walking. We used electromyography (EMG) and high-speed ultrasonography concomitantly to monitor muscle activity and muscle fascicle behavior in 19 healthy volunteers as they walked across a platform. On random trials, the platform was dropped (8 cm, 0.9 *g* acceleration) or held at a small inclination (up to ±3° in the parasagittal plane) with respect to level ground. Dropping the platform in the mid and late phases of stance produced a depression in the soleus muscle activity with an onset latency of about 50 ms. The reduction in ground reaction force also unloaded the plantar flexor muscles. The soleus muscle fascicles shortened with a minimum delay of 14 ms. Small variations in platform inclination produced significant changes in triceps surae muscle activity; EMG increased when stepping on an inclined surface and decreased when stepping on a declined surface. This sensory modulation of the locomotor output was concomitant with changes in triceps surae muscle fascicle and gastrocnemius tendon length. Assuming that afferent activity correlates to these mechanical changes, our results indicate that within-step sensory feedback from the plantar flexor muscles automatically adjusts muscle activity to compensate for small ground irregularities. The delayed onset of muscle fascicle movement after dropping the platform indicates that at least the initial part of the soleus depression is more likely mediated by a decrease in force feedback than length-sensitive feedback, indicating that force feedback contributes to the locomotor activity in human walking.

INTRODUCTION

Stable gait demands that muscle output is tuned to the environment. Feedback from proprioceptive sensors is integrated with descending drive to enhance and modulate this locomotor activity (Pearson 1993, 2004). In human walking, locomotor activity is widely accepted to be influenced by proprioceptive feedback (for review see Duysens et al. 2000; Rossignol et al. 2006). Ideally, this proprioceptive input to the CNS would best be measured directly as the subject is walking, but this is not possible with current microneurography recording techniques. Instead, indirect techniques have been used to investigate afferent feedback in human walking. For example, it is commonly assumed that spindle activity is correlated with the muscle–tendon behavior (e.g., Dietz et al. 1984; Sinkjaer et al. 1996; Yang et al. 1991). However, the series elasticity of the tendon can dissociate the muscle–tendon movement from the muscle fibers (Loram et al. 2004; Maas and Lichtwark 2009), which may make the assumed correlation between muscle–tendon and spindle activity erroneous. High-speed ultrasonography of muscle fascicles may allow a better estimate of the spindle output during a dynamic movement as it records the mechanical changes to which the spindles are sensitive and this technique has been used in static, walking, running, and jumping situations (e.g., Cronin et al. 2008; Ishikawa et al. 2005b, 2007; Lichtwark and Wilson 2006).

When investigating the role of afferent feedback in unperturbed walking, it is important to make the investigations in as natural conditions as possible. The afferent activity should reflect the normal activity faced during unperturbed walking to avoid the risk of the activity being integrated differently in the experimental condition (Morita et al. 1998; Nielsen and Sinkjaer 2002). Previously, we have attempted to mimic the natural variations in the ground surface by imposing small-amplitude, slow-velocity perturbations on the ankle joint (Mazzaro et al. 2005a) and by asking the subject to walk over small unpredicted inclinations in the ground surface (af Klint et al. 2008). The latter study showed that the minimal changes in ground surface modulated the locomotor activity in correlation to changes in estimated Achilles tendon force and muscle–tendon lengths. This indicates that length-sensitive group II and force-sensitive group Ib afferent activities have a graded contribution to the locomotor activity—more specifically, that the afferent activity not only contributes to the locomotor drive but the contribution is graded with the mechanical input. However, as Maas and Lichtwark (2009) correctly pointed out, the muscle spindle activity is sensitive to the muscle fascicle movement, which was not measured. Previously, gastrocnemius muscle fascicle behavior has been described for sustained inclined walking (Lichtwark and Wilson 2006). However, neither soleus nor gastrocnemius muscle fascicle behavior has been described for single steps on a surface with an unpredicted variation in inclination; thus no estimate of the mostly length sensitive group II or the mostly velocity sensitive group Ia afferent activity has been reported in these conditions.

The contribution of afferent feedback to the locomotor drive is best investigated by transiently decreasing proprioceptive feedback by rapidly unloading the triceps surae muscle–tendon complex (af Klint et al. 2009; Grey et al. 2004, 2007; Sinkjaer et al. 2000). The transient removal of proprioceptive feedback

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results in a depression of the soleus activity, termed the unload response (Sinkjaer et al. 2000). Sinkjaer et al. (2000) demonstrated that the unload response was not the result of reciprocal inhibition due to a stretch of tibialis anterior and that the response was preserved after blocking the largest of the group I afferents, suggesting that the largest group Ia afferents do not contribute substantially to the background locomotor muscle activity. In a subsequent study, it was demonstrated that the response was also independent of muscle and cutaneous afferents of the foot and ankle (Grey et al. 2004). However, contributions of the group Ib and group II pathways have thus far not been dissociated. Characterization of muscle fascicle movement during the drastic unloading of the triceps surae is therefore essential because it may allow the separation of the decrease in load from muscle fiber length changes. Such a separation could potentially indicate how spindle and tendon organ afferents contribute to the locomotor activity in human overground walking.

In the present study, we investigated the within-step contribution of afferent feedback to the locomotor activity in unrestrained overground walking. The study was divided into two parts. First, we hypothesized that the within-step modulation of locomotor activity would coincide with changes in muscle fascicle and tendon length as assessed by high-speed ultrasonography. To test this hypothesis, we designed a walking protocol in which the sensory input was graded using slight changes in the inclination of a stable supporting surface ($\leq \pm 3°$). Second, we hypothesized that a rapid decrease in ground reaction force (GRF) would be a sufficient mechanical stimulus to shorten the soleus fascicles. The timing of the fascicle shortening could then be used to estimate the first possible change in spindle afferent activity. As discussed earlier, the length (group II) and force (group Ib) information constitute the two most likely feedback signals that are removed from the locomotor electromyograph (EMG) to produce the unload response. The contribution of these afferents can then be discriminated by examining the onset latency of the fascicle shortening with respect to the change in GRF and comparing these onsets with the timing of the unload response. The muscle fascicle shortening would need to precede the unload response sufficiently for this disfacilitation to generate the unload response. Thus we hypothesized that if group II afferents contribute to the locomotor activity then the delay between the muscle fascicle shortening and the unload response should be larger than the shortest latency group II–mediated response, i.e., the medium-latency stretch reflex response (Grey et al. 2001; Nardone and Schieppati 1998). These hypotheses were tested by transiently decreasing the GRF by accelerating the support surface downward during the single support phase of overground walking.

METHODS

Nineteen healthy volunteers (6 female and 13 male, mean age: 27 ± 3.4 yr, range: 20–33 yr) with no known history of neuromuscular disorder participated in this experiment. The study conformed to the Declaration of Helsinki and was approved by the local ethics committee (VN-20080019). All subjects provided informed written consent.

The experimental setup is illustrated in Fig. 1. All subjects walked barefoot at a self-selected speed (4.5–5.5 km/h) on a 10-m path over a robotic platform mounted flush in the floor of the laboratory. The robotic platform, with 4 degrees of freedom, is composed of a force plate (OR6-5; Advanced Mechanical Technologies, Westminster, CO) mounted on hydraulically actuated pistons (van Doornik and Sinkjaer 2007).

Ultrasound was used to visualize the gastrocnemius medialis and soleus muscles of both legs during standing, to determine which leg to instrument based on the quality of the muscle fascicle images acquired (10 right and 9 left legs were used). The position of the ultrasound probe was marked and the subjects' leg was instrumented with surface EMG electrodes on the heads of the soleus (SOL), tibialis anterior (TA), and gastrocnemius medialis (GM) muscles (interelectrode distance 2 cm; NeuroLine 720, Ambu A/S, Ballerup, Denmark). The EMG signals were transmitted and band-pass filtered (10–1,000 Hz) using a wireless recording system (Telemyo 2400, Noraxon, Scottsdale, AZ). Ankle and knee excursion of the instrumented leg were recorded using goniometry (models SG150 and SG110/A, Biometrics, Gwent, UK). All signals were sampled at 2 kHz and stored for further processing.

Two-dimensional fascicle lengths of the GM and SOL muscles of the instrumented leg were determined using ultrasonography (B-mode, 7.5-MHz probe; model α-10, Aloka, Tokyo). The probe was positioned over the midbelly of the GM muscle and the optimal depth was adjusted to enable the visualization of fascicles in both GM and SOL. The longitudinal axis of the probe was approximately in the direction between the insertion of the Achilles tendon on the calcaneus and the GM midbelly. In most subjects (13) this position allowed a clear view of both the GM and the SOL muscle fascicles; in the remaining 6 subjects the probe was repositioned for a better view of the SOL muscle fascicles after half of the trials from protocol 1 had been recorded. The probe was then positioned medially and about one third of the shank length proximally to the medial malleolus over the soleus muscle. The probe was secured with a custom-made Styrofoam cast and wrapped tightly around the shank to minimize any probe movement, similar to prior investigations during locomotion (Ishikawa et al. 2005a, 2007). Images of the fascicles during quiet standing were used to normalize fascicle behavior between subjects.

Protocols

All subjects were instrumented and trained for about 5 min to touch down centered on the platform with the instrumented leg. The subjects started with an uneven surface protocol and followed this by a drop protocol. One subject did not participate in the second protocol. During the training and subsequent uneven surface protocols, the subjects wore taped glasses to block the lower part of their field of view. They were instructed to look at a mark on the opposing wall when approaching the platform so that the platform movement was shielded from their field of view at a distance of about 3 m. For safety, the taped glasses were not worn for the drop protocol. However, to make the head orientation consistent throughout the experiment, the subjects were still instructed to look at the mark on the opposing wall while taking the step onto the platform.

PROTOCOL 1: UNEVEN SURFACE. Similarly to the study of af Klint et al. (2008), subjects walked over the platform at a self selected speed (5.2 ± 0.1 km/h) with the addition of the ultrasound probe mounted on the shank. On random trials the platform rotation was changed to one of three different inclinations comparable to irregularities that are normally experienced during overground walking (0 and $\pm 3°$ rotation in the parasagittal plane producing increased dorsiflexion or plantar flexion of the ankle). When the subject was about 1.5 m from the platform, inclination was randomly adjusted for each trial and maintained throughout the step. Knowledge of the platform inclination was prevented by the taped glasses and by masking the noise from the rotation. Subjects walked until ≥ 30 trials were recorded for each inclination, which amounted to nearly 150 trials in total. For those six subjects where the quality of the soleus fascicle imagery was deemed too poor when the probe was positioned over the gastrocnemius,

ultrasonography of GM was acquired for approximately the first 75 trials and of SOL for the last 75 trials. Ultrasonography was acquired during all trials at a frame rate of 150 Hz.

In six subjects, the ultrasound probe was also positioned over the gastrocnemius muscle tendon junction to measure the tendon junction movement during the step. Reflective markers (Ø 14 mm) were attached on the Achilles tendon insertion on the calcaneus, on the lateral and medial malleoli, laterally and medially centered on the knee joint, and three markers on the probe (Fig. 1*C*). The plane defining the view of the ultrasound probe was calculated and the distance from the calcaneus marker to the tracked muscle tendon junction estimated as a straight line. The probe's rotation along the longitudinal direction of the shank, and the angle between the plane defined by the four markers on the shank and the viewing plane of the probe, were also calculated. The marker positions were recorded using a motion-capture system (eight-camera setup, frame rate 240 Hz; Qualisys Pro Reflex, Qualisys AB, Gothenburg, Sweden) centered on the platform. The platform rotations were presented as outlined earlier and data were acquired until ≥10 records of each condition had been presented.

PROTOCOL 2: PLATFORM DROPS. On random trials the platform was dropped vertically by 8 cm with a constant acceleration and deceleration of 0.9 *g* similarly to the procedure used by af Klint et al. (2009). This produces the overground equivalent of the unload response in the soleus muscle, i.e., a short-latency depression in the muscle activity following an unloading of the muscle–tendon unit. The subjects were allowed to view the platform as they approached, but were instructed to look at a mark on the opposing wall when stepping onto the platform. The movement of the platform was initiated at a preset latency after heel touchdown corresponding to mid and late stance (~40 and ~60% of stance, respectively) as determined by the force plate. The perturbations were presented randomly with three of five trials being nonperturbed (control) trials to prevent subject anticipation. Data were acquired until ≥30 trials of each perturbation were recorded.

For this protocol, the ultrasonography investigation was focused on the soleus muscle because previous reports have shown reliable unload responses in SOL but not in GM (af Klint et al. 2009; Grey et al. 2007). Furthermore, a higher frequency of acquisition (200 Hz) was used to capture short-lasting changes in fascicle length. Ultrasonography of the soleus muscle fascicles was recorded for at least the first 50 trials, i.e., about 10 trials for each perturbation and about 30 control trials. The position of the probe was left as in protocol 1, i.e., in 12 subjects the probe was fixed over the GM and in 6 subjects the probe was over SOL. After a sufficient number of ultrasound trials were collected, the ultrasound acquisition was stopped, but the probe was left in place. The ultrasound probe connector was disconnected from the ultrasound machine and held by the investigators behind the subjects while the last 100 trials (approximately) were acquired.

Data analysis

Data analysis was conducted off-line. For the kinematic and muscle activity records the processing steps used in af Klint et al. (2008) were used for protocol 1 and the processing steps in af Klint et al. (2009) were used for protocol 2's data records. Briefly, the EMG records were rectified and low-pass filtered (20 and 40 Hz, first-order Butter-

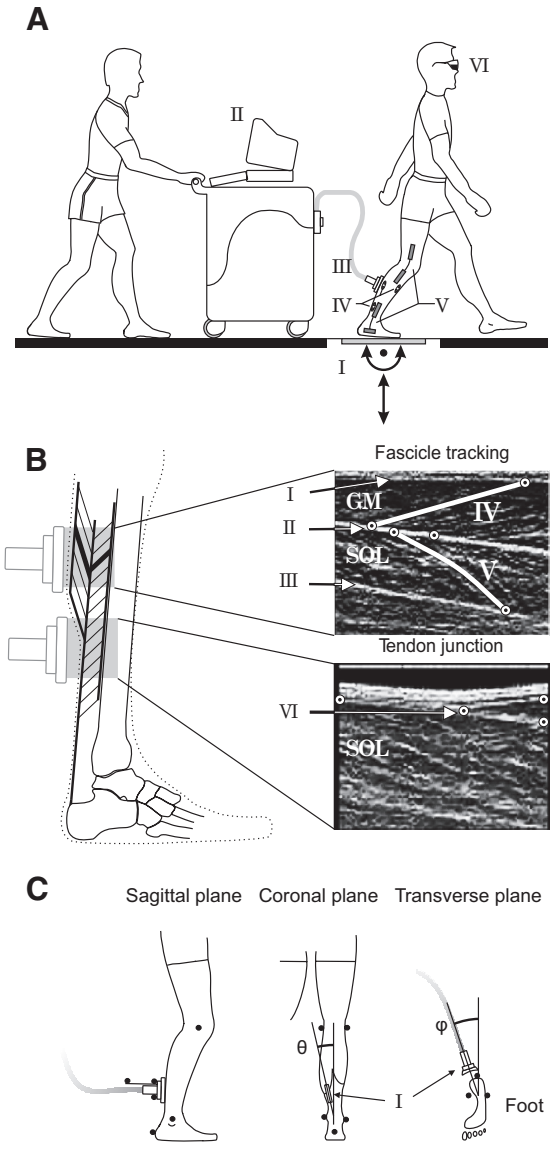

FIG. 1. *A*: subjects walked over a hydraulically actuated platform (I) that could randomly rotate in the parasagittal plane or induce a controlled drop of 8 cm at 0.9 *g* acceleration. The drops were presented during the stance phase, whereas the rotations were initiated and concluded prior to heel contact, so that the subjects stepped on a stable but inclined surface. The subjects were instrumented with an ultrasound probe (III) positioned so that the soleus and gastrocnemius medialis muscle fascicles were visible on the ultrasonograph (II). The ultrasound acquisition frame rate was 150 or 200 Hz, depending on the protocol (see METHODS). Surface electromyograms (EMG) of soleus (SOL), gastrocnemius medialis (GM), and tibialis anterior (TA; IV) as well as goniometry of the ankle and knee (V) were recorded (2 kHz) and synchronized with the ultrasound data. Additionally, in protocol I, knowledge of the platform inclination was prevented by the taped glasses (VI) and by masking the noise from the rotation. *B*: drawing of the lower leg with the ultrasound recording sites highlighted in gray. Thick lines represent tendon and aponeuroses and thin lines represent muscle fibers. An example of fascicle tracking and gastrocnemius tendon junction tracking (150 Hz) is shown on the *right*. The superior and inferior aponeuroses were identified (I–III) for the SOL (*bottom compartment*) and GM (*top compartment*). The lengths of the gastrocnemius (IV) and the soleus (V) fascicles were identified as the distance between points positioned on the aponeuroses, also highlighted in the drawing. Two or 3 tracked points were used for each fascicle, depending on the curvature of the fascicle. The gastrocnemius tendon junction (VI) was identified as the point at the intersection between the distal Achilles tendon and the aponeuroses of the GM (see points). *C*: approximate position of the ultrasound probe (I) and the reflective markers in the sagittal, coronal, and transverse planes as the GM tendon junction was investigated while stepping on inclined surfaces. To determine the length of the gastrocnemius tendon, the movement of the probe in relation to the shank was monitored in 3 dimensions using motion capture (240 Hz). Three markers on the probe and 4 markers on the shank defined the position and rotation of the probe and shank. The tendon length was defined as the straight line from the marker on the Achilles tendon insertion point of the calcaneus to the tracked tendon junction position in the plane of the probe's field of view. The rotation of the probe around the shank (φ) and the tilt of the probe (θ) with respect to the shank's midline were also monitored.

worth for protocols 1 and 2, respectively) to extract an amplitude envelope. The low-pass frequencies used differed between the protocols because protocol 2 (platform drops) investigated a transient effect, whereas protocol 1 (uneven surface) investigated a continuous modulation. The muscle–tendon lengths of the soleus and GM muscles, normalized to the length of the tibia, were estimated from the ankle and knee angular position records using the Hawkins and Hull model (Hawkins and Hull 1990). All records were ensemble-averaged to create a single set of records for each subject and condition.

Trials were manually inspected for each subject to exclude any step from the analysis that was not completely on the platform, as evidenced from the vertical force deviating from the ensemble-averaged records. Similarly, the raw and processed EMG records for each condition were visually inspected to exclude trials that exhibited large spikes deviating from the average activity prior to the initiation of the perturbations. In all cases, ensemble averages were calculated from no fewer than 28 data records for protocol 1 and 20 data records for protocol 2 for each condition.

ULTRASOUND ANALYSIS. The ultrasound videos were manually analyzed frame by frame to extract the fascicle or tendon junction movement (Fig. 1*B*). The superior and inferior aponeuroses were identified and fascicle length was defined as the distance between the two aponeuroses in the direction of the fascicle. Fascicle velocities were calculated by differentiating fascicle amplitudes with respect to time. The GM tendon junction was tracked by placing three markers on the proximal aponeuroses and on the distal tendon (Fig. 1*B*). Lines were drawn between the three markers and a central marker was placed at the tendon junction. The tendon junction marker was placed so that the lines were colinear with the aponeuroses and tendon.

PROTOCOL 1: UNEVEN SURFACE. To investigate the effect of the surface inclination on locomotor muscle activity, the area under the curve of the EMG records was calculated in a window placed 15–60% into stance (Fig. 2*A*). For each muscle, the area measurement was normalized to the control-step (0° inclination) stance phase peak EMG activity to compare the overall influence of the inclination on the triceps surae.

A frame-by-frame analysis of the ultrasonograms was carried out on three trials for each of the three inclinations (−3°, 0°, +3°) and muscles (SOL and GM). To avoid over- or underemphasizing the effect of the platform inclination, trials were selected that best represented the mean activity in the analysis window for the specific inclination and muscle. In other words, for each inclination (−3°, 0°, +3°) and muscle the three trials with the mean EMG activity closest to the median of the activity level of that class were selected to represent the fascicle behavior for that inclination and muscle. The same fascicle was tracked in all trials and its behavior was measured during the full extent of the stance phase in each of the chosen trials. To get a common time base, all trials were time normalized to the stance as determined by the vertical GRF. The trials were also normalized to the standing length of the muscle fascicles to reduce the intersubject variability, and ensemble averaged to create one set of

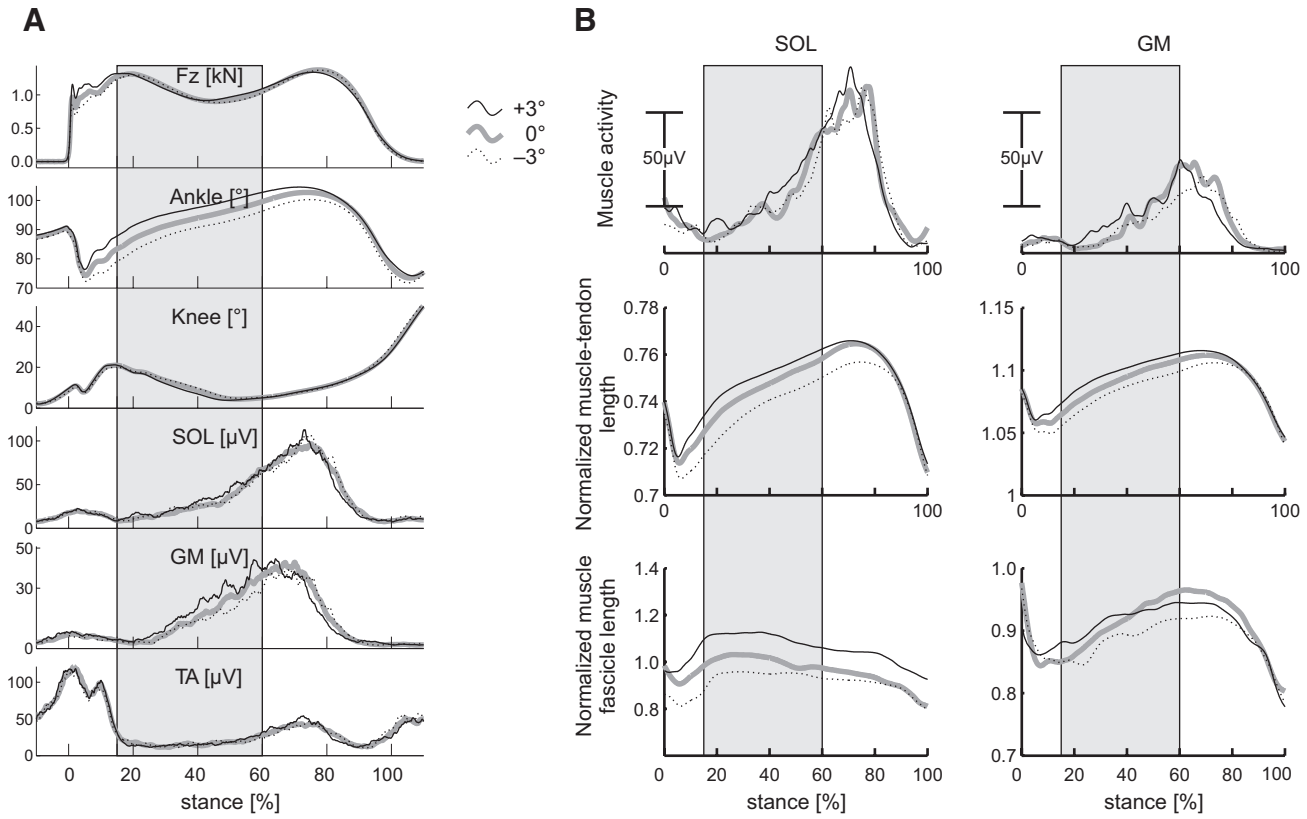

FIG. 2. Analysis of the effect of stepping on an inclined surface in a single representative subject. Subjects stepped on an inclined surface in the middle of a 10-m walkway. Knowledge of the platform's inclination was prevented by occluding the subject's peripheral view with taped glasses. *A*: ensemble-averaged ($n > 30$) vertical ground reaction force (Fz), ankle and knee excursion, and EMG activity of soleus (SOL), gastrocnemius medialis (GM), and tibialis anterior (TA) of a representative subject is presented for 0° (gray), +3°, and −3° (thin and dotted lines, respectively) inclinations. Although most clearly seen in GM, both SOL and GM muscle activity were modulated by the inclination of the platform within the window of analysis (gray area, 15–60% of stance). *B*: muscle activity in SOL and GM (*left* and *right columns*, respectively), normalized muscle–tendon length, and normalized muscle fascicle length ensemble averages of 3 representative trials (see METHODS) for the same representative subject as in *A* are presented for 0, +3, and −3° inclination of the platform. The muscle activity increased with the positive inclination of the platform, as did muscle–tendon length. The mean muscle fascicle length was also modulated with the platform inclination. However, the mean velocity of the muscle fascicles was not significantly altered by the inclination of the platform (not shown).

fascicle trajectories per condition and subject. In Fig. 2*B*, analysis of the representative trials is presented for the same subject as in Fig. 2*A*.

Similarly for the tendon length estimates, the three trials with the smallest difference from the median of the gastrocnemius muscle activity were chosen for each condition. The position of the gastrocnemius muscle tendon junction was tracked during the full duration of stance as determined by the vertical GRF. The length of the distal tendon was estimated by calculating the length of the straight line between the marker placed on the insertion point on the calcaneus and the tracked point placed in the viewing plane of the probe (Fig. 1*C*). The contractile portion of the GM was estimated by subtracting the estimated tendon length from the Hawkins and Hull model (Hawkins and Hull 1990) estimate of muscle–tendon length.

PROTOCOL 2: PLATFORM DROPS. The onset of the platform movement was determined from the vertical component of the GRF (Fig. 3). The perturbations produced muscle activity responses that were evident in the ensemble EMG records and quantified by calculating the difference between the perturbed and control step records. The latency of the response was assessed through visual inspection, by determining when the perturbed trial's EMG significantly deviated from the control within a window of 35 to 70 ms immediately after the perturbation onset. This time window was chosen to correspond to known values of the response (af Klint et al. 2009; Grey et al. 2004), which also encompasses the short-latency responses mediated by large myelinated proprioceptive afferents. To quantify the response, the relative difference in the area under the curve between the perturbed and the control trials was used.

As in protocol 1, muscle fascicle behavior was analyzed in three representative trials for each condition. The ankle excursion during the full stance was used to judge which control trials would best represent the kinematics during the control steps. For the perturbed steps, however, only the relevant kinematics just prior to or after the perturbation should influence the choice of representative trials because, for example, late aftereffects in the kinematics would not be able to influence the short-latency response in the soleus. Thus for the trials where ultrasonography of the soleus was acquired, the mean square error (MSE) of each trial's ankle trajectory to the ensemble average of its condition was calculated for the whole stance phase and for a 120-ms window centered on the perturbation for the control and perturbed trials, respectively. For each condition, the three trials with the smallest MSE values were chosen for fascicle tracking to represent the behavior of that condition. For the perturbed trials, fascicle behavior was measured in a window of 160 ms starting 60 ms prior to the onset of the drop of the platform. This window was chosen to determine the initial difference between the control and perturbed set of trials and to be sufficiently long to detect the effects of the decreased GRF. The control fascicle behavior was tracked during the same instances in time as for the mid- and late-stance perturbed trials.

The onset latency of the muscle fascicle shortening with respect to the platform drop was estimated by two different procedures: a manual visual inspection and a statistical method on the ensemble averages (see details in the following text). Because a priori there was no indication of which method would best describe the fascicle behavior both estimates were implemented. However, only the short-

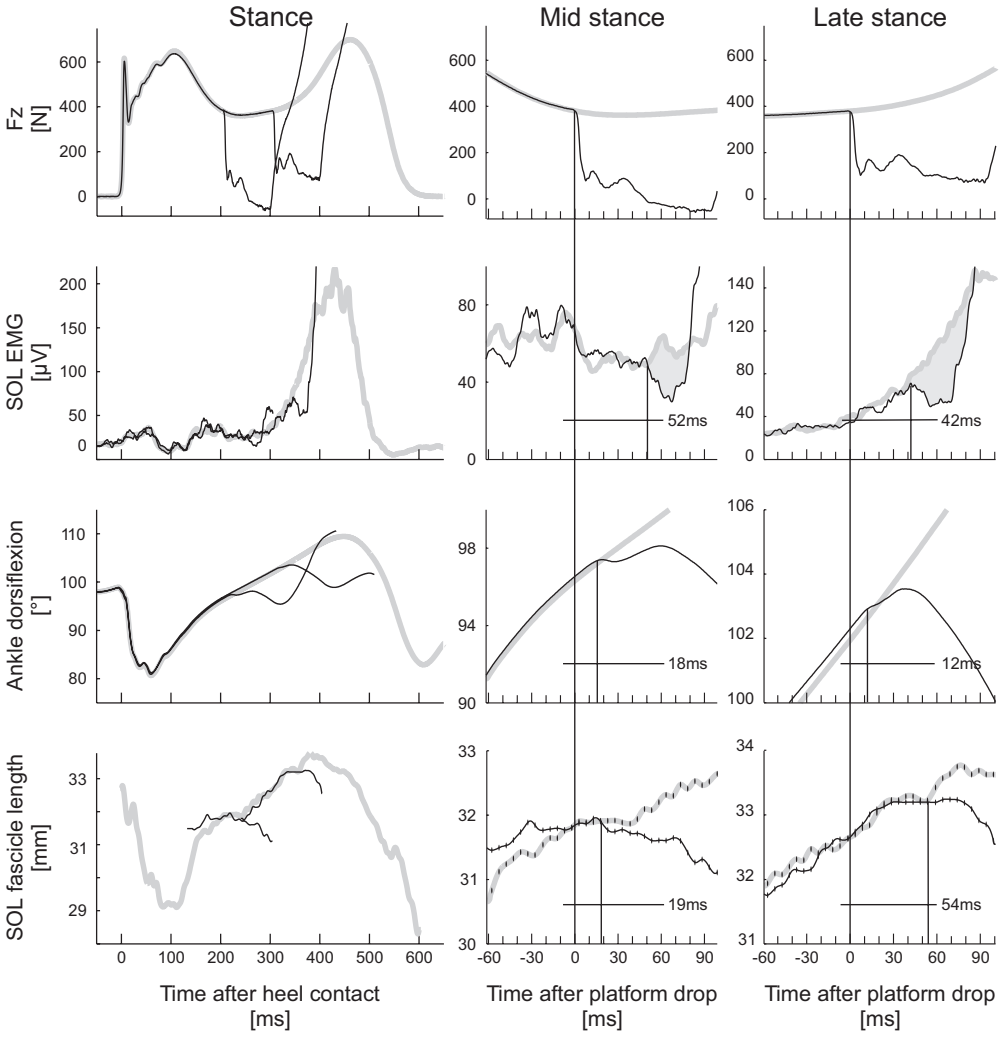

FIG. 3. Ensemble-averaged ($n > 30$) vertical ground reaction force (Fz), soleus activity (SOL EMG, low-pass 40 Hz), ankle excursion, and soleus muscle fascicle length ($n = 3$) for a representative subject shown for the stance phase (*left column*) and for a window (−60 to 100 ms) surrounding the mid- and late-stance perturbations (*middle* and *right columns*, respectively). The vertical lines indicate the first major deflection from the ensemble average of the control steps for vertical ground reaction force (GRF), soleus EMG, ankle movement, and fascicle length (visual inspection). The latency from the drop of the platform to the onset of the unload response (gray area), ankle movement, and fascicle shortening are indicated to the *right*.

est latency estimate was used for the later discussion, to avoid overestimating the delay from perturbation to the resulting length changes in the muscle fascicles.

For the first method, onset latency for each subject was visually determined by choosing the first point after the perturbation in which two or more of the perturbed trials exhibited faster muscle fascicle shortening compared with that of the control trials. Three of the coauthors independently made this estimate for each subject and each perturbation and the mean value for each subject was used to estimate the onset latency.

The second estimate of the onset latency of muscle fascicle shortening was made on the ensemble averages of muscle fascicle length normalized to the standing length of each subject. For each subject, the difference between the ensemble average of control and perturbed trials was calculated and low-pass filtered (80 Hz, dual-pass, first-order Butterworth). By differentiation, the velocity with which the control and perturbed trials deviated was calculated and low-pass filtered (40 Hz, dual-pass, first-order Butterworth). Differences in velocity before the perturbation were removed for all subjects to eliminate any initial divergence of velocity not attributed to the perturbation. The grand-average velocity was calculated across all subjects and the SD of the grand-average velocity was calculated for the data points prior to the perturbation. To limit overestimation of the onset latency, the first point at which the grand-average velocity deviated >1SD from its initial value was chosen as the latency with which the fascicle length change commenced as a result of the platform movement.

Statistics

Prior to all analyses, the distribution of the variables was tested for normality. When a normal distribution was found, or a transform that made the variable normally distributed, repeated-measures ANOVA (rmANOVA) was used to test for significance. For all ANOVAs, Geisser–Greenhouse adjustments were made if the covariance matrix sphericity assumption was violated (denoted by GG following the F test). When the result of an ANOVA test was statistically significant, Tukey–Kramer post hoc tests were used to determine whether there were any group differences. When no transform was found that made the variable normally distributed, nonparametric Wilcoxon signed-rank tests were used to test for differences between groups and the significance levels were Bonferroni corrected. All tests were conducted at a significance level of $P = 0.05$.

RESULTS

Protocol 1: uneven surface

MUSCLE ACTIVITY MODULATION. Evidence for an afferent-mediated graded contribution to the soleus and gastrocnemius muscle activity was seen in the activity modulation with the inclination of the platform [two-way rmANOVA, interaction muscle × inclination: $F_{(2,36)} = 49.75$, GG; $P < 0.001$]. The normalized muscle activity within the analysis window was increased for the positive inclinations of the platform and decreased with the negative inclinations (Tukey–Kramer, $P < 0.001$; see Fig. 4*A*). In GM, the modulation was highly significant, in that all levels of the inclination (−3°, 0°, +3°) were significantly different at $P < 0.001$ (Tukey–Kramer, Fig. 4*A*). However, the modulation in soleus was not as strong. No significant decrease was found between the levels of −3° inclination and 0° inclination of the platform (Tukey–Kramer, $P > 0.05$), but the two other comparisons were significant at $P < 0.002$ [Tukey–Kramer (−3°, +3°) and (0°, +3°)].

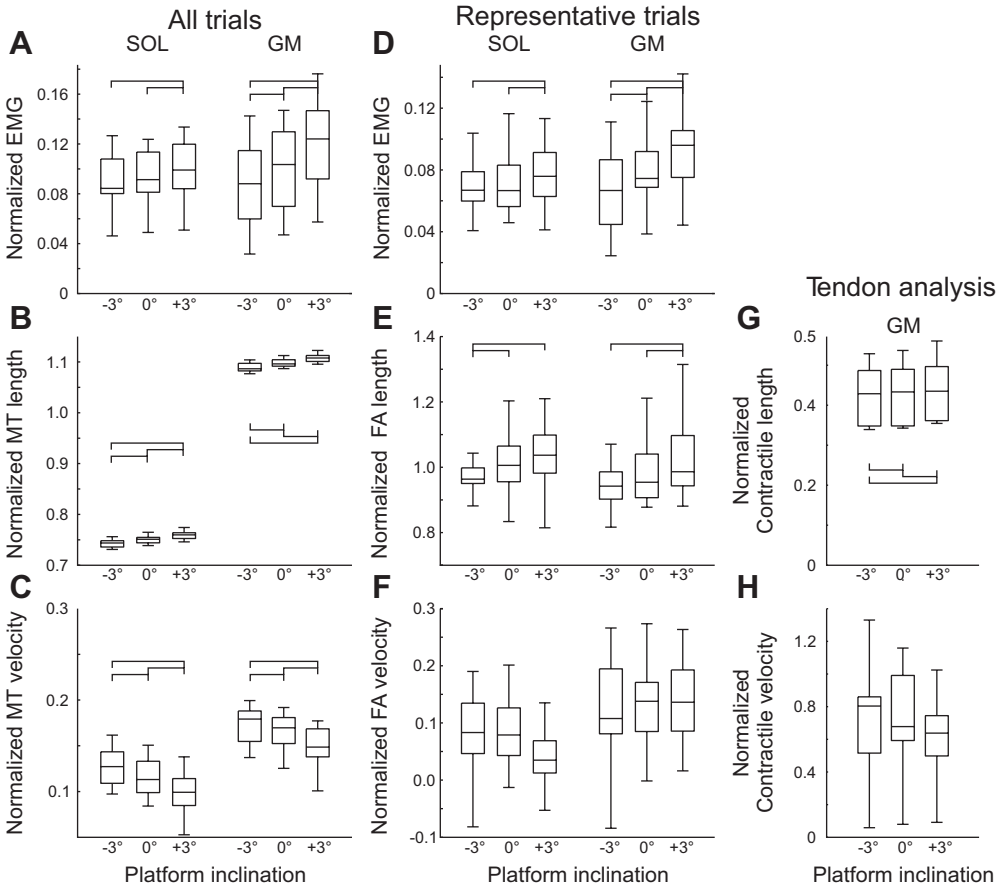

FIG. 4. Summary of analyzed muscle parameters based on all acquired trials (*left column*) and representative trials (*middle column*) in 19 subjects. The normalized EMG activity increased with the inclination of the platform in the ensemble averages of all trials (*A*) and of the representative trials (*D*); horizontal lines indicate significant differences between groups. Similarly, both muscle–tendon (MT) length (*B*) and fascicle (FA) length (*E*) increased when stepping on the platform when it was more inclined. The MT lengthening velocity decreased with increasing inclination of the platform (*C*) and no differences were found for the FA lengthening velocity between different inclinations (*F*). Independent estimates of GM contractile length and GM contractile lengthening velocity were obtained by subtracting the length of the distal tendon from the muscle–tendon length (*right column*). *G*: the platform inclination lengthened the contractile component of the GM. Furthermore, no change in lengthening velocity was observed (*H*), as also seen in the fascicles of both muscles. The concomitant changes in muscle activity, muscle fascicle length, and contractile muscle length indicate that an afferent that covaries its firing rate with the length of the muscle fascicle could make a graded contribution to the locomotor output through an excitatory feedback loop.

The representative trials chosen for muscle fascicle analysis showed similar modulation of the muscle activity as the ensemble averages of all trials, as they were selected on the basis of their EMG activity (see METHODS). The normalized muscle activity in the representative trials showed an interaction between muscle and platform inclination [two-way rmANOVA, $F_{(2,36)} = 32.10$, GG; $P < 0.001$]. The modulation of the GM muscle activity was significant, with all inclinations significantly different at $P = 0.0001$ (Tukey–Kramer, Fig. 4*D*). As for the analysis of the ensemble averages of all trials, the modulation for SOL was not as clear. The difference in activity was increased for the positive inclinations and decreased for the negative inclinations of the platform [Tukey–Kramer ($-3°$, $+3°$), $P = 0.0003$; ($0°$, $+3°$), $P = 0.026$; Fig. 4*D*]. However, there was no significant decrease in soleus activity between the $0°$ inclination and the $-3°$ inclination of the platform ($P > 0.97$).

MUSCLE–TENDON BEHAVIOR. The normalized mean muscle–tendon length within the analysis window was affected by the inclination of the platform [two-way rmANOVA, interaction muscle × inclination: $F_{(2,36)} = 227.97$, GG; $P < 0.001$]. This mean muscle–tendon length increased with the positive inclinations and decreased with the negative inclinations of the platform (all groups different at Tukey–Kramer, $P < 0.001$; Fig. 4*B*). The mean normalized muscle–tendon lengthening velocity was not normally distributed but a paired *t*-test was used to determine the difference between the muscles as that difference was normal. The paired *t*-test showed that the lengthening velocity for GM was on average faster than that for SOL for all inclinations of the platform (*t*-test: $P = 0.0001$). Thus the analysis was performed independently for the two muscles. In both muscles there was a decrease in muscle–tendon lengthening velocity with increased platform inclination [Fig. 4*C*, Wilcoxon signed-rank test: ($-3° > 0°$), ($0° > +3°$), and ($-3° > +3°$), $P < 0.001$ for both muscles, Bonferroni adjusted].

MUSCLE FASCICLE BEHAVIOR. Changes in muscle–tendon length and velocity do not necessarily represent muscle fiber length changes (Loram et al. 2004; Maas and Lichtwark 2009). To better estimate the spindle activity ultrasonography was used to measure muscle fascicle length changes in representative trials for each subject (i.e., the three trials with the smallest deviation from the median muscle activity for each condition and muscle). The following analysis is based solely on the three representative trials that were used for the ultrasonography. The mean normalized muscle fascicle length measured in the analysis window was not normally distributed, thus preventing parametric analysis. Instead, the mean muscle lengths for both muscles were investigated separately for the three different inclinations ($-3°$, $0°$, $+3°$). For both SOL and GM the muscle fascicle length significantly lengthened with the increasing inclinations of the platform [Fig. 4*E*, Wilcoxon signed-rank test ($-3° < +3°$): $P = 0.016$ and $P = 0.002$ for SOL and GM, respectively, Bonferroni adjusted]. However, the muscle fascicle length compared with the $0°$ inclination was not as clear [Wilcoxon signed-rank test, SOL ($-3° < 0°$): $P = 0.034$; ($0° < +3°$): $P = 0.081$; GM ($-3° < 0°$): $P = 0.16$ ($0° < +3°$): $P = 0.008$, Bonferroni adjusted]. See horizontal lines in Fig. 4*E* for a graphical representation of the significant group differences.

The normalized muscle fascicle mean lengthening velocity was significantly greater for GM compared with that for SOL (paired *t*-test: $P < 0.001$). No significant change in lengthening velocity was found when examining the difference between all levels of inclination of the platform for both muscles independently (Fig. 4*F*, paired *t*-test: $P > 0.05$).

ACHILLES TENDON. The independent measure of the gastrocnemius tendon junction showed a tendency similar to that of muscle fascicle tracking. For the six subjects in whom the gastrocnemius muscle–tendon junction was tracked, the estimated contractile component of GM was lengthened as the inclination of the platform was increased [one-way rmANOVA, $F_{(2,5)} = 8.67$, $P = 0.0066$; Fig. 4*G*]. The $-3°$ inclination of the platform significantly shortened the contractile component of the GM muscle with respect to the $0°$ and $3°$ inclinations [Tukey–Kramer ($0°$, $-3°$): $P = 0.040$, ($3°$, $-3°$): $P = 0.0061$]. No significant difference was found between the levels $0°$ and $+3°$ ($P > 0.05$). The lengthening velocity of the contractile component of the GM muscle was not significantly altered by stepping on the inclined surfaces [one-way rmANOVA, $F_{(2,5)} = 0.98$, $P = 0.41$; Fig. 4*H*]. Neither the rotation around the shank (φ) nor the probe's tilt against the shank's midline (θ) were significantly affected by the inclination of the platform. The mean normalized tendon length increased between the $-3°$ and $0°$ inclinations in all subjects. Also, four of the six measured subjects showed increasing tendon length between the $0°$ and $+3°$ inclinations, indicating changes in Achilles tendon force.

In summary, triceps surae muscle activity increased concomitantly with muscle fiber lengths and Achilles tendon load, as measured by the muscle fascicle mean length and Achilles tendon length. However, muscle–tendon lengthening velocity was inversely related to the muscle activity and no significant differences were found between muscle fascicle lengthening velocities. This indicates that the firing rates for force- and length-sensitive afferents are likely correlated with the muscle activity, whereas this may not be the case for the firing rate of the lengthening velocity sensitive afferents.

Protocol 2: platform drops

In 15 of 18 subjects, the drop of the platform produced soleus activity that deviated significantly from the control EMG within the 35- to 70-ms window following the mid- and late-stance perturbations. The three subjects that lacked a response in mid-stance were not used for further analysis of the magnitude and latency estimates of the unload response, although they were included in the ultrasound analysis as the mechanical consequence of the drop of the platform on the muscle fascicle behavior was not significantly different from that of the subjects showing an unload response.

The deviation of the soleus activation after the perturbation is clearly shown for a typical set of ensemble-averaged data from a single subject in Fig. 3. In this case, the subject was walking at about 5.3 km/h and the traces of the perturbed trials (thin lines) are superimposed on the control trace (thick line). The platform was rapidly dropped in mid and late stance (203 and 304 ms after heel strike, 0 ms), which caused a depression of the SOL EMG activity (see Fig. 3, shaded areas). The onset of the platform movement was determined by the vertical GRF (vertical line) and latencies were calculated with respect to this

time. For this subject, visual inspection of the soleus muscle fascicles showed that shortening of the muscle fascicles started at latencies of 19 and 54 ms after the onset of the platform drop for the mid- and late-stance perturbations, respectively (Fig. 3, *bottom row*).

A depression in the soleus muscle activation was seen in all subjects at a latency of 48 ± 7 ms, ranging from 37 to 62 ms, measured from the onset of the platform drop. The latencies for the mid-stance perturbations (50 ± 7 ms; range, 39–62 ms) and the late-stance perturbations (47 ± 6 ms; range, 37–56 ms) were not significantly different [one-way rmANOVA, $F_{(1,14)} = 3.41$, $P = 0.09$]. The perturbed steps' soleus EMG amplitude stayed below the level of the control step for 28 ± 6 ms (range, 20–47 ms). The response in the soleus EMG was significantly shorter in mid stance (26 ± 5 ms) than that in late stance [31 ± 7 ms, one-way rmANOVA, $F_{(1,14)} = 7.52$, $P = 0.016$, Tukey–Kramer, $P = 0.016$]. The area under the curve was used to quantify the depression in EMG, calculated for a window of analysis starting at the onset of each subject's depression in EMG. The width of the analysis window was chosen to correspond to the shortest duration found in the subjects' depression of the EMG (i.e., 20 ms). Across all subjects and times of perturbation, the relative change in area under the curve of the soleus EMG for the perturbed trials was −14.5% (confidence interval [CI]: −6.2 to −33.8%). There was no significant difference between the responses to perturbations in mid- and late-stance [one-way rmANOVA, $F_{(1,14)} = 0.77$, $P = 0.40$, −13.7 and −15.4% for mid and late, respectively].

When the platform is dropped in mid or late stance the normally occurring ankle dorsiflexion is stopped and, in some instances, the ankle undergoes a short plantar flexion (see Fig. 3). This change in the ankle rotation is not imposed by the platform movement, but rather is the consequence of a decrease in GRF (see Fz in Fig. 3), whereas the moment generated by the triceps surae muscle remains stable, as evidenced by the SOL activity following the control trials up until the time of the depression. Across all subjects and times of perturbation, this change in ankle trajectory started 18 ± 7 ms (range, 3–38 ms) after the platform began to move. No significant difference was found between mid- and late-stance perturbations (19 ± 9 and 17 ± 6 ms, respectively). The onset of ankle trajectory change preceded the depression in soleus activity by 30 ± 9 ms (30 ± 11 and 30 ± 7 ms for mid and late stance, respectively).

For each subject a soleus muscle fascicle was tracked during the control and perturbed trials using high-speed ultrasonography. Representative examples of single-trial fascicle lengths are shown in Fig. 5*A* for control (thin black) and perturbed (dotted) steps of a single subject. These traces are shown superimposed over the ensemble averages of the subject's control and perturbed trials (thick black and gray lines, respectively). The general trend in all subjects is that there is a shortening of the fascicle after the perturbation, as seen in the ensemble averages for this representative subject. This is also clearly visible in analysis of the normalized ensemble-averaged trials (Fig. 5, *B* and *C*), where the differences between the control and perturbed trials are shown for all subjects. As the muscle fascicles shorten with the drop of the platform it is evident that the change in muscle–tendon length is not solely the result of a shortening of the tendon. Thus it would be expected that spindle discharge rates decrease as the length of

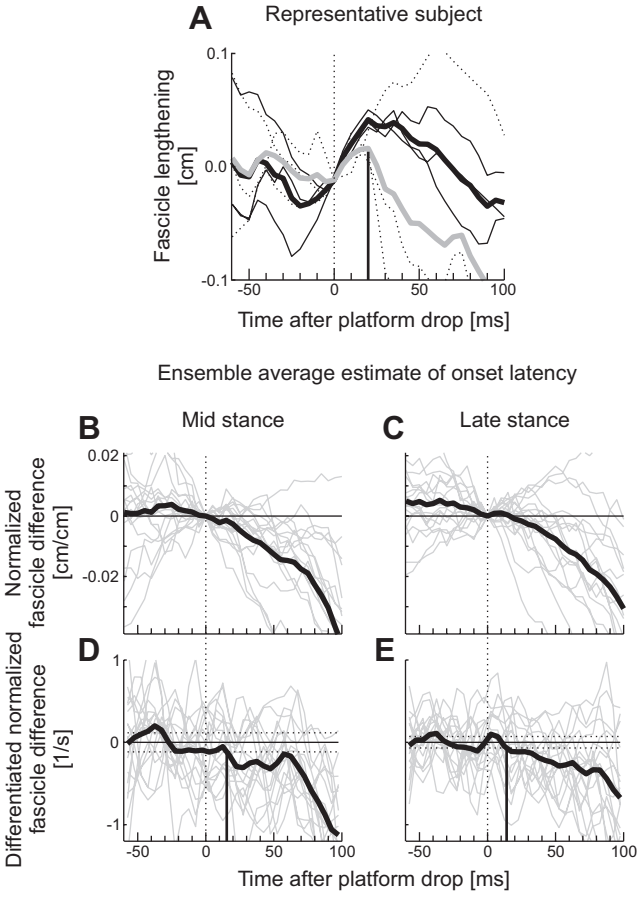

FIG. 5. Analysis of fascicle length changes after platform drops. *A*: an example of fascicle length change from a representative subject. The lengthening trend of the ensemble-averaged control trials was removed and the bias at the time of the perturbation (0 ms) was also removed. Control trials (thin black lines) and perturbed trials (dotted lines) were ensemble averaged (thick black and thick gray for control and perturbed, respectively). In the perturbed trials, the fascicles shortened with respect to the control trials and the estimate of the onset of this shortening is marked by a solid vertical line. For the mid- and late-stance perturbations, the differences between the normalized ensemble averages of control and perturbed trials are shown in *B* and *C* (*gray traces*, $n = 18$). For the sake of clarity, the offset was removed at the time of the perturbation (dotted vertical lines). The shortening of the perturbed muscle fascicles as a result of the lowered GRF is clearly shown by the downward trend of the group-averaged traces (*B*, *C*, *thick trace*). The velocity with which the ensemble-averaged control and perturbed traces diverged was calculated by differentiating the difference traces in *B* and *C* (40 Hz, dual-pass low-pass filtered). The mean velocity before the perturbation was removed for each individual subject (*D, E, gray traces*) and the group-average calculated (*thick traces*). The SD of the group-averaged velocity before the perturbation is indicated by the horizontal dotted lines. As can be seen in the length traces (*B*, *C*), the velocity at which the normalized group averages of the perturbed trials diverged from the controls increased after the perturbation. The onset was estimated as the instance in time when it diverged from zero by >1SD (vertical lines, 15.4 and 14.1 ms for mid and late stance, respectively).

the fascicles diverge from the control trials. The latency of the start of this divergence was estimated by two independent methods.

The manually assessed estimate of the onset latency of fascicle shortening ranged from 5 to 75 ms across all perturbations. The mean onset latency of the fascicle shortening for the mid-stance perturbations (25.1 ± 12.6 ms) was not significantly longer than the latency for the late-stance perturbations [22.2 ± 8.5 ms, one-way rmANOVA, $F_{(1,17)} = 0.73$, $P =$

0.40]. The timing between the depression in soleus activity and the onset of fascicle shortening was also not significantly different for the mid and late perturbations [24.9 ± 18.2 and 22.3 ± 8.4 ms for mid and late stance, respectively; one-way rmANOVA, $F_{(1,14)} = 0.30$, $P = 0.59$].

The second method to estimate the onset latency at which the perturbed fascicles diverged from the control fascicles is illustrated in Fig. 5, *D* and *E*. The method differentiates in time the difference between normalized muscle fascicle lengths in control and perturbed trials (Fig. 5, *B* and *C*), to estimate the velocity with which the two ensemble averages diverge (i.e., velocity of divergence). The onset latency is the time when the average velocity of divergence exceeds the level of 1SD of the average velocity of divergence computed over 60 ms prior to the perturbation (Fig. 5, *D* and *E*, dotted line). The onset latency for the mid-stance perturbation was 15.4 and 14.1 ms for late-stance perturbations. Furthermore, at 20.1 ms for mid stance and at 33.1 ms for late stance, the mean velocity of divergence had passed the lower 95% CI from the baseline. Thus the difference between control and perturbed fascicle lengths does significantly change as the platform is dropped, which is also evident from the unprocessed muscle fascicle length traces (Figs. 3 and 5, *B* and *C*). The onset of fascicle shortening preceded the depression in soleus activity by 34 ± 7 ms (36 ± 7 and 33 ± 6 ms for mid and late stance, respectively).

DISCUSSION

In contrast to previous studies on afferent feedback in walking (af Klint et al. 2008, 2009; Berger et al. 1984; Grey et al. 2002, 2007; Sinkjaer et al. 2000; Yang et al. 1991), we have attempted to better estimate spindle afferent output by measuring the muscle fascicle behavior. With this estimate we addressed two important characteristics of afferent feedback during unconstrained walking—that afferent feedback does indeed contribute to the locomotor activity and that its contribution is graded with respect to the mechanical constraints of the environment in human walking. The aim of this study was to better estimate the spindle afferent output by measuring muscle fascicle lengths, in contrast to previous investigations that have assumed muscle fiber and muscle–tendon complex lengthening to match (af Klint et al. 2008, 2009). To mimic a natural walking situation, the study was restricted to self-selected walking speeds at a moderate pace and the effects of walking speed were not specifically addressed. In the first protocol, modulation of the soleus and gastrocnemius muscle activity was linked to stepping on a surface with different inclinations in the parasagittal plane. Muscle activation was significantly increased with positive inclinations and decreased with negative inclinations, which is comparable to our previous study (af Klint et al. 2008). The changes in muscle activity covaried with significant changes in the length of the muscle fascicles and the length of the Achilles tendon. In contrast, no significant differences in fascicle lengthening velocity were found for the three tested conditions. Given that group Ia afferents are mostly sensitive to the lengthening velocity of the muscle, this indicates that velocity-sensitive afferents are most likely not important in the ongoing adaptation of the muscle activity to small deviations of the ground surface. However, the changes in length and force estimates indicate that both excitatory feedback from group II length-sensitive afferents and group Ib force-sensitive afferents may contribute to the within-step modulation of the locomotor activity.

In the second protocol, the contribution of afferent feedback to the locomotor activity of the soleus muscle was addressed by rapidly decreasing the ground reaction force (GRF). In the perturbed trials, soleus muscle fascicles did shorten with respect to the control trials. However, the latency with which the perturbed fascicles started diverging was significantly delayed with respect to the perturbation; the shortest estimated delay was 14.1 ms. This delay was substantial enough to indicate that the feedback from length-sensitive afferents would be unlikely to contribute to the depression in the soleus activity. We have previously demonstrated that the reduction in soleus EMG following a rapid plantar flexion perturbation during walking covaries with Achilles tendon load (Grey et al. 2007) and that it is unaffected by depression of cutaneous and the largest of the group I afferents (Grey et al. 2004; Sinkjaer et al. 2000). Considering this evidence together with that of the present investigation, the most likely cause of the depressions in the soleus locomotor activity would be decreased firing of group Ib force-sensitive afferents as the GRF is transiently decreased.

Methodological limitations

Ultrasound imaging of muscle fascicle movement during dynamic situations is an emerging field. It has been used to characterize fascicle movement during various types of highly dynamic tasks such as drop jumping (Ishikawa and Komi 2004), running, and walking (e.g., Ishikawa et al. 2007; Lichtwark and Wilson 2006). During ultrasound acquisition the image signal-to-noise ratio decreases as acquisition frequency is increased. As the noise level is increased, the video analysis becomes more difficult and more prone to error. In contrast, when the acquisition frequency is decreased, short-lasting events can be missed and the estimation of event onsets becomes more prone to error. We used relatively high acquisition frequencies (150 and 200 Hz) to minimize the synchronization error between the ultrasound acquisition and the other recorded signals and to have sufficient time resolution to view the short-lasting effect of dropping the platform. Although a lower frequency of acquisition could have been chosen for the uneven ground protocol, we deemed the synchronization error (average 3.3 ms at 150 Hz) to be more important.

Accurate muscle fascicle tracking in ultrasonographic images is an extremely time consuming process involving the manual placement of several markers in all the analyzed frames. Automatic methods for fascicle tracking have yet to be developed using the current high-frame rates (150 and 200 Hz). Although some automatic tracking methods have been used to track length changes of tendinous tissues (Magnusson et al. 2003) and the contractile component of muscles (Loram et al. 2006), as well as fascicle orientation (Rana et al. 2009), they are not suitable for the current investigation of fascicle length. Thus manual tracking was the most reliable method, given the image quality at the high sampling rates used in the present study. To further increase the accuracy of our measures, the fascicles were analyzed in three trials per condition and muscle. The reliability of the current ultrasound method was determined by calculating the coefficient of variation between the three trials for each muscle and condition. For protocol 1

the mean coefficients of variation were 4.6, 3.7, and 4.8% (SOL, GM, and the tendon junction, respectively) and for the platform drops (protocol 2) it was 4.2%, all of which were within the range of previously reported values of ≤6% in dynamic conditions (Cronin et al. 2009b; Ishikawa et al. 2003; Kurokawa et al. 2001).

To determine which afferents may contribute to the depression in soleus activity following decreased GRF, a correct estimate of the onset latency of the response is crucial. In the current study this latency was determined by visual inspection of the ensemble averages as described in METHODS. The response onset was estimated as the time at which the perturbed ensemble average started to diverge from the control ensemble average within 35–70 ms following the perturbation. This criterion was chosen to make the results comparable with those of previous studies (af Klint et al. 2009; Grey et al. 2007; Sinkjaer et al. 2000). A statistical method such as using the SD makes for a more objective estimate, but has the drawback of inducing additional latency to the estimate. For this study the onset latency of the unload response would have changed from 50 ± 7 to 53 ± 7 ms in mid stance and from 47 ± 6 to 48.4 ± 6.4 ms in late stance, without significantly changing the conclusion of the study. However, because the exact onset of the start of the downward deflection in the EMG is important, we chose to estimate it visually.

The onset latency estimate for the muscle fascicle shortening was performed using two methods in this investigation: manual visual inspection and a statistical method on the ensemble average differences. Initially, it was unclear whether the statistical method would be able to detect the subtle changes in the muscle fascicle length trajectories. The onset latencies varied substantially between the two methods and it is debatable which of the two estimates better represents the true onset latency. However, only the shortest latency estimate was used in the final discussion to underestimate the delay between the perturbation and the resulting length changes in the muscle fascicles and thus to avoid biasing the results against a possible length-sensitive contribution.

Protocol 1: uneven surface

This experimental setup was designed to study the within-step modulation of locomotor output by mimicking the natural variations in the ground surface that are faced on an everyday basis. Prior investigations with a subject lacking afferents below the neck indicate that the modulation is not likely to be vestibular in nature or generated from neck proprioceptors because no effect was seen in this particular subject (af Klint et al. 2008). It should also be emphasized that the variations in the ground surface were very minimal, bordering on the perception threshold of healthy subjects. The same study also concluded that the modulation in locomotor activity was likely to originate from the graded contribution of group Ib, group II, or possibly cutaneous afferents. Both electrical reflex stimulation of the cutaneous afferents (Duysens et al. 1996; for review, see Zehr and Stein 1999) and cutaneous depression by cooling parts of the sole (Nurse and Nigg 2001) affect the locomotor activity in humans. However, virtually no difference in locomotor activity was noted after an anesthetic block of cutaneous afferents of the foot and ankle (Grey et al. 2004). Although cutaneous afferents do play an important role in the motor control of locomotion (Duysens et al. 1996; Zehr and Stein 1999), the evidence suggests that they are unlikely to provide the within-step enhancement of locomotor activity that is the focus of the present study.

The within-step modulation of locomotor activity with platform inclination was similar to that observed in our previous study (af Klint et al. 2008). Similarly, the measured changes in muscle–tendon length with platform inclination in the present study support the model-based estimates used in the previous study. Our previous study was correctly criticized (Maas and Lichtwark 2009) for extending the muscle–tendon length estimates to the effect on the muscle fascicles and thereby the muscle spindle output, in that we concluded that length-sensitive afferents but not velocity-sensitive afferents may contribute to the enhancement or reduction of the locomotor output. The characterization of muscle fascicle length and lengthening velocity in the present study does confirm our previous assumption that muscle fascicle lengths are influenced by the inclination of the surface onto which the subject steps. However, the negative relationship between muscle–tendon lengthening velocity and the inclination of the platform was not seen in the lengthening velocity of the muscle fascicles. Therefore it is reasonable to assume that the mostly length sensitive group II afferent activity would correlate with the increase in muscle activity, whereas the mostly velocity sensitive group Ia afferents would be significantly less correlated—or even uncorrelated—with the muscle activity. The mean tendon length was shortened for the negative inclinations and increased for the positive inclinations in four of the six subjects who participated in this study, thus demonstrating that Achilles tendon force is well correlated with platform inclinations and muscle activity. Similarly, when estimating Achilles tendon force with an external buckle transducer, the force covaried with the inclination of the platform using the same inclinations of the platform as those in the present experiment (af Klint et al. 2008). Therefore it is reasonable to assume that group Ib afferent firing rates would vary with the applied force, as seen in freely walking cats (Prochazka and Wand 1980) or when electrically activating muscle fibers in series with Golgi tendon organs (GTOs) (Houk and Henneman 1967). The present study confirms that the group Ib and group II afferents are possible candidates for the within-step enhancement or reduction of the triceps surae locomotor activity and our data suggest that the group Ib pathway may play the more prevalent role.

Protocol 2: platform drops

Positive force feedback has recently been shown to exert a major influence on the locomotor activity in walking cats (Donelan and Pearson 2004a,b; Donelan et al. 2009; Duysens and Pearson 1980; Pearson and Collins 1993). Dietz et al. (1992) demonstrated that load-feedback might be important during standing and they postulated that load-feedback would also play a role in the stance phase of walking. Force feedback in human walking has been investigated with various techniques, including body weight support changes (Bachmann et al. 2008; Harkema et al. 1997; Stephens and Yang 1999; af Klint et al., unpublished data), rapid plantar-flexion perturbations combined with inclined walking (Grey et al. 2007), and transient GRF reductions in overground walking (af Klint et al. 2009). All of these techniques are indirect and the propriocep-

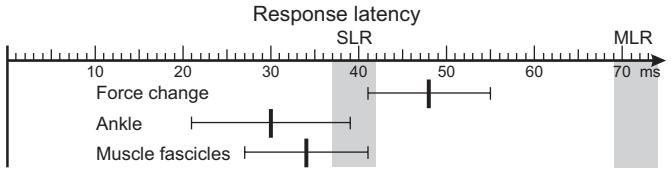

FIG. 6. Analysis of the soleus depression onset latency. The latencies of the soleus depression onset measured from the onset of changes in GRF, in ankle rotation, and in muscle fascicle length (ensemble-averaged estimate) are presented as the combined results of mid- and late-stance perturbations (error bars represent 1SD). Ranges of reported mean onset latencies for the short- and medium-latency stretch reflex (SLR and MLR, respectively) responses (shaded areas) are given as reference for the earliest group Ia and group II response (see DISCUSSION for references). Previous experiments using ischemic depression of group Ia afferents have ruled out the involvement of these fibers in the response (Sinkjaer et al. 2000). Thus given the large discrepancy in timing between the length-related changes and the MLR response it is unlikely that group II afferents contribute to the depression in the soleus following drop of the platform.

tive afferent firing rates are assumed to follow muscle–tendon and muscle force changes. The aim of this study was to better estimate the spindle afferent output as the muscle–tendon complex was unloaded during gait by studying muscle fascicle length changes.

By rapidly decreasing the GRF during human overground walking, we induced an unloading of the triceps surae muscle–tendon complex. Similarly, rapid plantar–flexion perturbations during treadmill walking elicited a depression in soleus locomotor activity that was not affected by ischemia or ankle and foot cutaneous afferent depression (Grey et al. 2004; Sinkjaer et al. 2000). It is therefore unlikely that cutaneous afferents or feedback from the largest group I afferents (i.e., group Ia) contributes to the depression seen in the present study. The reduction in GRF produces an acceleration of the head. However, this acceleration was too delayed, 10 ms, for the vestibular apparatus to significantly contribute to the initial component of the response (af Klint et al. 2009). Similarly, the delayed acceleration of the head would also delay any influence from neck proprioceptors. This delay and the fact that the unload response can be elicited by more focused plantar-flexion rotations make the influence of neck proprioceptors unlikely, although it was not directly tested in this setup. Thus by process of elimination decreased feedback from group II and group Ib afferents may contribute to the depression. In the current experiment the muscle fascicles shortened when the platform was dropped. The onset of this shortening was quantified by two independent methods, with the aim of underestimating the onset latency to avoid underestimating the time that length-sensitive afferents would have to integrate to the response. Muscle fascicle shortening was delayed with respect to the initiation of the platform drop by between 14.1 and 25.5 ms.

The medium-latency stretch reflex response (MLR) is mostly responsive to the group II afferent contribution (Grey et al. 2001). The large and rapid stretches used to elicit the stretch reflex are likely to have a very short delay at the muscle fascicle level. Furthermore, the muscle fascicle delay with respect to rapid plantar-flexion perturbations in sitting and walking are within the temporal resolution of the ultrasound acquisition (frame rates of 100 and 150 Hz, respectively; Cronin et al., unpublished data). Thus the onset latency of the MLR is a reliable characterization of the fastest feedback loop for the group II afferents (see Fig. 6). During treadmill walking, the reported MLR onset was 72–74 ms (Grey et al. 2004; Mazzaro et al. 2005b) and during standing was 69–70 ms (Mazzaro et al. 2005b; Nardone and Schieppati 1998). Given the delay between fascicle shortening and the depression in soleus activity reported in the current study (34 $\pm$ 7 ms), the group II afferents would not have time to contribute to the response. Although the onset delay is consistent with the onset delay of the short-latency stretch reflex response (SLR) during walking (Cronin et al. 2009b; Grey et al. 2004; Sinkjaer et al. 1996), it has previously been shown that group Ia afferents do not contribute to the unload response (Sinkjaer et al. 2000). On the other hand, the GRF does decrease at the time of the perturbation and this would change the moment at the ankle and thereby the output of the Golgi tendon organs (GTOs) at the time of the perturbation. Therefore we believe that a reduction in group Ib force-sensitive afferent firing rates is more likely to contribute significantly to the depression in the soleus activity. However, it should be recognized that at the spinal interneuron level there exists a widespread interconnectivity among both sensory modalities (Jankowska 1992) and muscle groups (Lamy et al. 2008; Nichols 1989; Pierrot-Deseilligny et al. 1981). Reciprocal inhibition from the TA to the SOL is an unlikely cause of the depression in the current investigation because a complete block of the common peroneal nerve did not reduce the unload response to rapid plantar-flexion perturbations (Sinkjaer et al. 2000). We believe that afferent feedback from thigh muscles is also unlikely to be the cause of the response. Knee extension was seen in the current investigation and small knee movements were also generated by the rapid ankle plantar-flexion perturbations used to elicit the unload response during treadmill walking (Grey et al. 2007). However, these knee movements were significantly delayed with respect to the imposed ankle rotations (Grey et al. 2007) and thus changes in afferent activity from the thigh muscles would be unlikely to contribute to the responses in the current investigation. Nevertheless, it is possible that the unload response is caused by a decrease in heterogenic feedback from synergistic muscles. For example, heterogenic excitatory force feedback from the GM muscle during walking has previously been reported by Faist et al. (2006). Additionally, the magnitude of the unload response was found to be correlated to the Achilles tendon force, making homogenic and/or heterogenic feedback from the GM likely (Grey et al. 2007).

The current investigation provides strong additional support to previous conclusions that group Ib and possibly group II afferents make a graded contribution to the within-step soleus locomotor activity to fit the muscle activity to ever-changing biomechanical constraints of the walking environment. This study also demonstrates that the force-sensitive GTOs likely provide the feedback signal to which the unload response is most sensitive. This indicates that group Ib activity may contribute to the overall locomotor activity during human walking. On the other hand, spindle activity as measured by muscle fascicle dynamics is important for compensatory responses to lengthening perturbations both in sitting (Cronin et al. 2008) and in walking (Cronin et al. 2009a,b) and may well contribute to the locomotor activity during running where plantar-flexor muscle fascicles go through more abrupt stretches than those during walking (Dietz et al. 1979).

GRANTS

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