Longitudinal Changes in Muscle Activity during Infants’ Treadmill Stepping

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ABSTRACT

Previous research has described kinetic characteristics of treadmill steps in very stable steppers, in cross-sectional designs. Here we examine longitudinally muscle activation patterns during treadmill stepping, without practice, in 12 healthy infants at 1, 6, and 12 months of age. We assessed lateral gastrocnemius (LG), tibialis anterior (TA), rectus femoris (RF), and biceps femoris (BF) as infants stepped on a treadmill during 12, 20-second trials. Infants showed clear changes in kinematics, such as, increased step frequency, increased heel contact at touch down, and more flat-footed contact at mid stance. Electromyographic data showed high variability in muscle states (combinations), with high prevalence of all muscles active initially, reducing with age. Agonist-antagonist muscle co-activation also decreased as age increased. Probability analyses showed that across step cycles, the likelihood a muscle was on at any point tended to be <50%; LG was the exception, showing an adult-like pattern of probability across ages. In summary, over time, healthy infants produce a wide variety of muscle activation combinations and timings when generating stepping patterns on a treadmill, even if some levels of muscle control arose with time. However, the kinematic stability improved much more clearly than the underlying kinetic strategies. We conclude that while innate control of limb movement improved as infants grow, explore and acquire functional movement, stepping on a treadmill is a novel and unpracticed one. Hence, developing stable underlying neural activations will only arise as functional practice ensues, similarly to that observed for other functional movements in infancy.

Keywords: longitudinal, EMG, infant, interlimb coordination, stepping
INTRODUCTION

Across the first year post birth healthy infants tend to perform steps when supported upright on a motorized treadmill (Thelen 1985; Thelen & Ulrich 1991; Yang et al. 1998). Kinematic analyses of these steps show quite clearly that significant changes occur over these 12 months in infants' leg movement patterns and interlimb coordination (Groenen et al. 2010; Thelen & Ulrich 1991; Vereijken & Thelen 1997), as is true of all other motor behaviors when assessed over such a wide developmental time frame in infancy (e.g., Adolph et al. 1998; Freedland & Bertenthal 1994; Haehl et al. 2000; Konczak & Dichgans 1997; Lynch et al. 2008).

There are periods of time over the first year when most healthy babies step infrequently when supported in the treadmill context, months one through two or three, followed by another couple of months during which infants show a gradual improvement in proportion of time during a test trial during which they produce steps (Teulier et al. 2009; Thelen & Ulrich 1991). Of those steps produced, about 56 percent over the first three months show the limbs engaged in alternation. The remainder includes parallel steps, in which legs initiate the swing phase at the same time, and single steps, ones that are not overlapped by a step performed by the other leg (Groenen et al. 2010; Teulier et al. 2009; Thelen & Ulrich, 1991). Percent of alternation increases readily after the first few months to about 87% by 7 months (Teulier et al. 2009; Thelen & Ulrich, 1991). Interlimb phase lags for alternation, as well, while demonstrating a mean of 180° out of phase have a very high standard deviation and range from 20% to 80% in their
coupling (Musselman & Yang 2008; Teulier et al. 2009). The treadmill context with partial bodyweight support has proven useful to test theoretical questions about postnatal development of motor control. But it also presents an important opportunity for monitoring the neuromotor control of very young infants at risk for motor disabilities. Assessment can begin with the neonate and continue through the onset of independent walking. Perturbations can be introduced to test the stability of the system. And, many aspects of typical development have been characterized. Thus, the potential to identify delays or aberrant trajectories of neuromotor control, as well as the impact of therapeutic intervention to facilitate gait early in life can be monitored via attempts to elicit stepping in this context. Missing, however, from previous studies has been longitudinal studies of the activity of thigh and shank muscles during infants’ treadmill steps. Only through longitudinal work can the changes in behavior at different points in time and their variability be confidently attributed to development, rather than differences among samples selected for the specific study at each age. Infants with motor disabilities generally produce much greater variability in performance around their group means and in their developmental trajectories than do healthy infants (Deffeyes et al. 2009; Ulrich & Ulrich 1995; van Haastert et al. 2006). Thus, closer examination of the underlying control of treadmill stepping, reflected in EMG activity, across the range of behavioral responses (good steppers and poor steppers) for a randomly selected sample of healthy infants, will enhance our understanding of normal variations across infancy.
Our goal here was to examine, longitudinally, the underlying activation patterns of four primary gait muscles of both legs in 12 infants with typical development, from one month post birth through 12 months.

**METHOD**

**Participants**

Participants were 12 infants (7 female) who were tested longitudinally for their responses to being supported upright on a motorized treadmill without any practice stepping on a treadmill between testing sessions (Teulier et al. 2009). Inclusion criteria were: no known physical or cognitive disabilities and gestational age ≥ 36 weeks (group \( M = 39.5 \) wks, SD =0.74) when entering the study at 1 month of age. Families were recruited by flyers posted throughout the local community.

Infants who were part of this research study had an overall decrease in ponderal index from 1 month 26.77(2.23) to 12 months 24.44(2.49) (6 months=27.21(2.17)), similar to the results reported by Lande et al. (2005). In addition, these infants began to walk independently (defined as 3-5 steps without support) at 12.48(1.52) months.

**Procedures**

All testing occurred in the Developmental Neuromotor Control Laboratory, School of Kinesiology, at the University of Michigan. When families arrived at the lab, we explained procedures and asked parents to sign a consent form approved by the University of Michigan Institutional Review Board. Parents also completed a history survey. Infants were tested at 1, 3, 6, 9, and 12 months of age.
To prepare them for testing, we removed infants’ clothing, shoes and diaper and placed reflective markers (8-mm diameter at ages 1 and 3 months; 18-mm diameter at ages 6, 9, and 12 months) bilaterally on the iliac crest, greater trochanter, knee joint, lateral malleolus, and ventral surface of the third metatarsophalangeal joint. After cleaning the skin with an alcohol pad, we placed pre-amplified bipolar surface EMG electrodes (rectangular patch of 5x2.5 cm with electrodes placed 0.63cm apart, electrode conductive surface diameter of 5mm) over the muscle bellies of the Lateral gastrocnemius (LG), Tibialis anterior (TA), Rectus femoris (RF), and Biceps femoris (BF) muscles of the right leg for set one trials and moved them to the left leg for set two trials. To minimize the movement of wires and interference, a research assistant held the EMG cables well above the treadmill during each trial. Finally, an online screening of EMG values were visualized before each trial to check for clarity of the EMG burst(s) when the baby was moving and to reduce the chance that cross talk was recorded.

We used a custom-made infant-sized motorized treadmill, 18 cm X 42 cm X 82 cm (H,W,L) with a smooth belt surface (30 cm wide). The treadmill was placed on a large table 73 cm X 118 cm X 190 cm (H,W,L) with 3 Peak Motus™ cameras placed on each side of the table to monitor joint marker positions at 60 Hz. A 60 Hz digital video camera was positioned on one side and perpendicular to the table to videotape leg movements for data capture verification purposes (for more details, please refer to Teulier, et al., 2009). The electromyograms (EMG) data were collected at a sampling rate of 1200 Hz using Therapeutics Unlimited Model 67 (containing a built in 40 Hz high-pass filter) and using the Peak Motus™ real-time system to digitize the data. All video camera, motion capture system, and EMG data were synchronized.
We held infants under the axillary regions so they were upright with feet resting on the treadmill belt for 12, 20-second trials. Trials were presented in 2 sets of 6 speeds. Trials 1 and 12 were baseline trials where the treadmill belt was stationary. During trials 2 through 6 and 7 through 11, speed increased from .068 to .22 m/s, in increments of .038 m/s. Between sets, and as needed during testing, infants were given rest breaks. The data presented in this study were part of a larger study looking at the developmental trajectory of stepping in infants with spina bifida. The cohort of infants presented here was used as a control group (see Teulier et al, 2009). Speeds were manipulated in that study to see if the speeds known to stimulate infants with typical development (Thelen & Ulrich, 1991) were stimulating differently infants with spina bifida.

After treadmill testing, we measured infants’ total body weight and length, leg length (greater trochanter to lateral malleolus), thigh length (greater trochanter to the lateral knee joint), thigh and shank circumferences. We also administered the motor subscale of the Bayley Scales of Infant Development II (Bayley, 1993) to assess concurrent levels of functional motor skill.

Data Reduction

For the purposes of the questions addressed here, we focus only on treadmill step parameters and patterns of muscle activity during alternating strides and not on speed adaptation. As EMG signals were quite variable all along year one, we decide to only look at those variables at 1, 6 and 12 months to increase the power of the analysis and to clarify the reading of the results by focusing on three ages separated by acquisition of motor milestones. For additional details regarding overall step frequency,
Identification of stride events and characteristics

Three trained behavior coders identified the occurrence of alternating steps by viewing, frame by frame, the recorded digital videos. They recorded the time (frame) when the following step events occurred: toe-off, touchdown, and end of stance.

For alternating steps, coders identified the part of the infant’s foot that contacted the treadmill belt at touch down and mid stance. Foot contact was coded as toe, flat, heel, lateral, or medial. From this information, we then calculated the percentage of each foot posture that was used for touch down and mid stance. In addition, we coded each infant’s leg posture at mid-swing and mid stance during alternating stepping. Their legs were coded as high flexion (if the knee or hip flexion exceeded 90°) or low flexion. From this information, we calculated the percentage of leg flexion demonstrated by each infant when performing alternating steps. Training required each coder to practice with training tapes and, when tested for accuracy, to obtain a coefficient of agreement of 0.85 (inter-observer reliability coefficient, kappa) to 5 sets of data coming from 5 different infants validated previously by experts in our lab.

Description of the step cycle

In order to account for the variability in absolute duration of stride cycles, we normalized raw gait cycle data by transforming the original data points to a 1000 point distribution using a cubic spline interpolation. Stance (ST) and swing (SW) phase durations were calculated as a proportion of the gait cycle.

EMG data reduction
Because the number of alternating strides produced by infants is often low across the first 6 months post-birth, we used several steps in the process of extracting strides to analyze. To ensure reliability of the data, only steps located in a series of alternating steps were extracted, which reduced the analysis to only 2 to 3 consecutives strides per leg for each infant at each age. For a stride to be included, the entire cycle had to be completed within the 20-sec trial and data for all four muscles had to be free from artifact and high levels of noise. We prioritized strides that occurred at the middle belt speed (.144m/s) because in our previous work we found this to be the optimal speed across the first year (Teulier et al. 2009). We followed by selecting strides occurring at .106m/s and .182m/s if strides were not taken at .144m/s. Last, if an infant produced no strides at these middle speeds, but stepped at the slowest (.068m/s) or fastest (.22m/s) speeds, we included them for analyses in order to be as representative as possible of all infants.

To process EMG data, we first applied a band-pass filter with cutoff frequencies set at 75 Hz and 300 Hz (Spencer et al. 2000; Spencer & Thelen 2000). The low-end frequency reduced electrical noise associated with wire sway and biological artifacts while the high cutoff eliminated extraneous tissue noise at the electrode site. Next, we rectified the data, eliminated any high-frequency components added in the rectification procedure by using boxcar averaging with a window size of seven samples, and converted the EMG signal to an on–off designation.

The goals of using an objective series of steps to determine when increases in muscle activation occurred were to operationalize the procedures used, eliminating subjectivity in conclusions. To determine on–off activity, we began by moving a 50-ms
window, frame by frame, across each EMG signal. If the average EMG activity within a
window exceeded a minimum threshold (noise), the center value of that window was
considered ‘on’. To determine the noise threshold, we computed frequency histograms
of amplitudes for each EMG signal, for each trial. In addition, we normalized the
frequency histograms to the modal amplitude for each trial. We used a cutoff value of
0.15 of the normalized modal frequency to differentiate EMG on–off activity (Spencer et
al. 2000; Spencer & Thelen 2000). Finally, the duration of ‘on’ activity was summed
across small segments of activity if the period of inactivity between segments was less
than 50 ms.

Muscle activation patterns extracted were thus analyzed using three methods.
First, we used a muscle state analysis to determine for each frame within a stride which
muscles were active, that is, the “state” of EMG activity as the stride unfolded (Spencer
et al. 2000; Spencer & Thelen 2000). Because we recorded EMG for four muscles,
there were 16 possible combinations ranging from all ‘off’ to all ‘on’. In addition, this
method allowed us to determine the duration and frequency of occurrence for each
state, reflecting which muscles were active and for how much of the normalized stride
cycle.

Next, we calculated the percentage of co-activation between agonist-antagonist
muscle pairs during the stride cycle. This variable does not address amplitude of muscle
activation, only the presence of it (‘on’ or ‘off’).

Third, to determine if there was an increased likelihood of individual muscles
being active or not at specific times across the stride cycle for all infants, we calculated
muscle activation probability across infants. A probability value of 1 meant that the muscle was always ‘on’ at that point in the cycle for all infants. A probability of 0.5 meant that the muscle was ‘on’ at that point for 50% of the stride cycles produced by infants. Probabilities presented reflect the pooling of all ‘on’ values for each time point during the cycle for all infants and dividing by the total number of cycles included. In order to provide a reference to what a stable, rhythmical stepping pattern with low variability would look like, by contrast to the infants, we also looked at the probability of muscular activation for 4 typical adults who walked on a treadmill at 1.40 m/s for 2 trials of 1 minute.

Data Analyses

For our statistical analyses, we used SPSS version 14 (IBM Corporation, Somers, NY). We used mixed-model regression analysis to determine the influence of Age and Phase (ST, SW) on parametric variables. Use of this method allows for random patterns of missing cells and, thus, is well suited for analysis of longitudinal data where missing data points typically occur. In this statistical model, random effects were specified as Infant and Infant by Age interactions to control for within-participant effects.

Because the EMG was recorded unilaterally, we wanted to identify whether there was any effect of leg on the muscle activation characteristics. We tested the Leg effect and Leg by Age interaction for each of our analysis and no effect was found on any muscles. Therefore, Leg was removed as a factor in order to obtain the best fitted statistical model.

In addition, to determine if the data reduction principles we followed affected the mean speed from which cycles were selected, we conducted a mixed model one-way
ANOVA with repeated measures across Age. Results showed that speed did not vary significantly across ages \( F(2,49) = 113, \ p = .329 \). Differences across months were a maximum of .8mm per second. This small difference could arguably have a minimal effect on our mean values for muscle activation duration and cycle duration. All other gait cycle variables were normalized to a proportion of the gait cycle.

**RESULTS**

**Stride Characteristics**

To provide an overview of what the overt behavior looked like, as infants stepped, we begin by describing stride characteristics and their change over time. We used three, one-way mixed-model ANOVAs to assess the relation between Age and cycle duration, proportion of time spent in stance during the stride cycle, and percentage of the cycle during which the leg was highly flexed. Absolute cycle duration \( (F(2,21)=8.707, \ p = .003) \) and proportion of time spent in stance during the stride cycle increased with Age \( (F(2,21)=9.27, \ p < .001) \) while the percent of time spent with the leg highly flexed decreased \( (F(2,21)=9.27, \ p < .001) \) (see Table 1 for post-hoc results). On average, the stance phase comprised 67.7% of the gait cycle; with an increase from 58.2% at month 1 to 73.3% at month 12.

To examine the part of the foot making contact with the treadmill at touchdown and at mid-stance, we used two one-way MANOVAs with repeated measures on Age. Dependent variables for the first MANOVA (for touchdown) were percentage of heel contact, flat-footed contact, toe contact, and medial or lateral contact. For mid-stance, only flat-footed and toe contact were entered in the analysis as they represented more
than 95% of the occurrences. We obtained a significant Age effect (Wilks lambda=0.242, $F(8,34)=4.392$, $p=.001$)) for foot contact at touchdown. Post-hoc ANOVA results revealed a significant decrease for medial/lateral contact at touchdown with increasing age ($F(2,20)=18.5$, $p<.001$) as well as a significant increase in heel contact ($F(2,20)=4.2$, $p=.029$). For contact at mid-stance, an Age effect was obtained (Wilks lambda=0.287, $F(8,21)=8.21$, $p<.001$)). Post-hoc ANOVAs revealed a significant decrease in toe contact with increasing age ($F(2,20)=20.18$, $p<.001$) as well as a significant increase in flat-footed contact ($F(2,20)=17.5$, $p<.001$) at mid-stance.

**Muscle Activation Characteristics**

In our first analysis of muscle activity we examined simple characteristics, including burst duration and frequency within a stride cycle. We used separate one-way mixed model ANOVAs for each muscle to determine the relation between of Age and the mean muscle burst duration and mean number of muscle bursts of activity per stride cycle. The absolute duration of muscle activation showed a significant decrease from .75s at 1 month to .33s at 12 months for the RF ($F(2,24)=8.50$, $p=.002$). Follow-up univariate analyses showed a significant difference between ages 1 and 6 months ($p=.003$) and between 1 and 12 months ($p=.005$). For frequency of muscle activations, none of the ANOVAs were significant, but we noted a strong trend for the TA ($F(2,18)=3.05$, $p=.072$), LG ($F(2,19)=3.460$, $p=.053$), and RF ($F(2,17)=3.37$, $p=.058$), to increase the number of muscle activations within a cycle as age increased.

**Muscle Activity Patterns**

**Sixteen muscle states**
In order to look at how each muscle was activated in relation to other thigh and shank muscles we examined their state, or combinations, across the cycle. For each possible muscle state, we ran a mixed model 3 (Age) x 2 (Phase- ST & SW) ANOVA on the percent of time the muscle state was present. Figure 1 illustrates the distribution of all states, at each time point. This figure shows that infants spent more time with none of their four muscle activated during the SW phase than ST ($F(1,52)=22.2, p<.001$) at all Ages ($p=.094$). Also at all Ages, the TA was activated significantly more often during SW than ST ($F(1,72)=26.1, p<.001$). The reverse occurred for LG which was significantly more active during ST than SW ($F(1,43)=25.8, p<.001$). For RF, we found an Age by Phase interaction ($F(2,46)=3.6, p=.034$). Inspection of the means revealed a decrease in RF activation during ST with increasing Age, but not in SW. No statistical differences occurred for BF or the muscle combinations of RF+BF, and TA+BF. For the muscle state combinations of TA+LG, LG+RF, and LG+BF, infants showed greater frequency during ST than SW ($F(1,46)=21.7, p<.001$; $F(1,41)=13, p=.001$; $F(1,48)=16.9, p<.001$, respectively). LG+BF also showed a trend for increased presence with increasing Age ($F(2,19)=3.4, p=.053$). For TA+RF, an Age effect ($F(2,72)=3.43, p=.038$) and an interaction effect were found ($F(2,72)=3.13, p=.049$) reflecting an increase during SW, but not in ST as infants got older.

The occurrence of states in which three muscles were active concurrently increased, typically, during ST; for TA+LG+RF ($F(1,45)=28.1, p<.049$), LG+RF+BF ($F(1,72)=13.03, p=.001$) and TA+LG+BF ($F(1,52)=4.7, p=.033$). For TA+LG+BF, we also found an interaction effect, reflecting an increase in appearance with age during ST but not SW ($F(2,52)=4.6, p=.014$). No significant effects were detected for
Interestingly, the muscle state in which all four muscles were activated occurred more frequently in ST than SW ($F(1, 47) = 11.9, p = .001$) and more often at 1 month than at older ages ($F(2, 12) = 6.9, p = .01$).

**Co-activation muscle patterns**

Figure 2 presents co-activation percentages and shows a decrease, with Age, for all agonist-antagonist pairs: $TA+LG = F(2, 22) = 3.77, p = .035$; $RF+BF = F(2, 19) = 9.05, p = .002$; and $LG+RF = F(2, 25) = 8.3, p = .002$). Additionally, all co-contractions were more prevalent during ST than SW: $TA+LG = F(1, 65) = 44.6, p < .001$, $RF+BF = F(1, 16) = 18.05, p = .001$, and $LG+RF = F(1, 12) = 70.5, p < .001$.

**Probability of muscle activity**

Figure 3 presents the probability that a particular muscle was active, at each point across the stride cycle, collapsed over all babies. Graphs show data for infants at 1, 6, and 12 months as well as for 4 young adults. Readily apparent is the fact that only the LG shows a probability values that reached or exceeded 50%. The distribution of probabilities (e.g. their trajectories of rising and falling across the stride cycle) differs significantly for the RF, TA, and BF, with low probability at all ages.

To test the significance of changes in probability data over age we identified 3 phases during which, based on walking in adult skilled performers, one might anticipate contrasting levels of muscle activity across the stride cycle. Within the stance phase, we looked at Initial Stance (IniST, around 0 to 20% of the gait cycle) and Mid Stance (MidST, around 20 to 40% of the cycle). IniST usually represents a peak of muscular activity while MidST represents a decrease in muscular activity (only LG is expected to be highly active). Finally, Mid Swing (MidSW, around 80 to 90% of the cycle) was
chosen to contrast ST and SW when TA is expected to reach peak activity. We then used a 3 (Age) by 3 (Phase) mixed model ANOVA for each muscle to determine if significant differences emerged as a function of events within the cycle or with age. Results show that LG (Fig. 3a) decreased probability of activation with Age ($F(2,142)=3.9, \ p=.02$) and demonstrated significant differences in level of activation across sub-phases of the gait cycle ($F(1,134)=67.1, \ p<.001$). Mean values suggest a higher probability of activation during MidST than either MidSW or IniST ($p<.001$ for both), but the probability of activation during IniST was higher than MidSW ($p<.001$). When compared to the probability of muscle activation for adults, the probability of activation for the LG in infants follows a similar pattern despite the tendency for infants to activate their LG earlier in the stride cycle.

The probability for activation for RF (Fig. 3b) showed an Age effect ($F(2,10)=6.7, \ p=.013$), Phase effect ($F(2,98)=23.4, \ p<.001$), and interaction effect ($F(4,98)=3.8, \ p=.006$). Comparison of the means shows a reduction in probability of activation during ST with increasing age, but the probability of muscle activation remained similar across ages during the SW phase. The probability of activation for the BF in infants across the first year of life does not exceed 50%, showing a lack of clear, rhythmical activity across step as it can be seen usually in adult. Similarly, the probability of activation of BF (Fig. 3d) showed a Phase effect ($F(2,104)=14.5, \ p<.001$), but no Age ($p=.648$) or interaction ($p=.331$) effects. Mean values showed higher probabilities during MidSt and IniSt than during MidSW ($p<.001$). But overall, the probability of activating BF, like the one of the RF was weak in infants.
For the TA (Fig. 3c) Age, Phase, and interaction effects were not significant. Overall, TA decreased its probability of being activated over the first year post birth, and no differences among sub-phases were detected in terms of the probability of activation. Figure 3c also illustrates that adults have a very strong probability of activating TA during MidSW and IniST, as they prepare to and initiate foot contact with the floor. This pattern of probability for activation of the TA for adults is clearly not observed in infants at any age across the first year of life, even though by 12 months, all were cruising and some were walking.

**Examples of EMG traces for Individual Infants at 1, 6, 12 Months**

Because EMG data are often presented as ensemble averages, which are not sensible when variability is very high, and because we chose to focus on ways to quantify objectively aspects of the EMG activity produced by infants, we add here examples of the smoothed and rectified data for two infants across all three ages to illustrate the quality of their muscle activity (see Fig. 4). We specifically chose one infant who might be classified as a poor stepper (Fig. 4a), who produced only 3-5 consecutive alternating steps maximum at months 1 and 6, though stepped consistently by month 12 and a good stepper (Fig. 4b), an infant who produced a minimum of 8 consecutive alternating steps at each age. Neither infant was unusual; each simply reflects two common overt behavior types. Each muscle trace shows 2-3 strides and illustrates the enormous variability individuals demonstrated, whether consistent or not in behavioral outcome, in the ways in which they marshaled active and passive forces to produce the step pattern. As the figures show, rhythmic activations were not evident, nor were
consistent firing patterns among muscles to produce one stride, relative to the next, at any age.

**DISCUSSION**

Our goal in this study was to examine longitudinally the muscle activity underlying step patterns of infants, when they were supported upright on a treadmill, regardless of how stable or unstable their step frequencies were, in order to understand the variety of muscle patterns healthy infants demonstrate across this first year post birth. We chose to examine the four major muscles of the thigh and shank, often referred to as core gait muscles in highly skilled walkers, because of the clear kinematic similarity between treadmill steps and steps produced during walking. Overall, our results show that while kinematic behavior improved in terms of the consistency with which infants produced steps, their reduction in overall leg flexion, and improved foot placement patterns, the underlying muscle activation patterns that contributed to these overt trajectories were generally quite inconsistent. We believe our data also suggest a refinement in control of the major muscles of the leg, but because this behavior (stepping) is not one infants practice or use throughout most of this time period, each time they are tested they respond with exploratory behavior that, we argue, reflects a goal of overcome the instability of having their feet moved backward, beyond their center of mass, rather than a desire to “step” or walk.

*Lack of clear patterns of muscle organization across first year*

Lack of muscle organization across the first year post birth was illustrated by several objective and quantitative measures of activity. The muscle state analysis
illustrated clearly that many combinations of muscle activation were produced across
the cycle. Infants spent a considerable amount of cycle time with no muscles active, an
efficient response during swing, but which occurred as well, on average, 20% of the
time during stance. At one month, all four muscles often activated concurrently; during
stance it was as likely that all muscles were active as that none of them was active.
Across combinations of muscle activity, infants seem to be somewhat randomly
activating the options available to them, with small fluctuations in combinations (some
increasing and others decreasing) over time. Probability data further illustrated that for
three of the four muscles, the likelihood at any point in the cycle that the muscle was on,
across cycles and infants, was consistently well below 50%. Only for the rectus femoris
at 1 month and lateral gastrocnemius did this differ and, in fact, the lateral
gastrocnemius demonstrated a pattern of probability approaching that of skilled adult
walkers.

The lack of overall organization at the muscular level is in opposition with the
improvement seen at kinematic level. This cohort of infants was stepping almost
continuously on the treadmill by 12 months of age (0.8 steps per second), and the
percentage of alternative stepping increased from 55% at 1 month to 95% at 12 months
of age ((Teulier et al. 2009), with more foot control and leg control (reduced high flexion
during swing/ more heel to flat transition). These findings are consistent with Chang
(Chang et al. 2006; 2009) and Ivanenko’s (Ivanenko et al. 2005) results in toddlers
learning to walk overground with or without balance support. Stability first arose at the
kinematic level and then at the nervous and muscular level; the plasticity of the nervous
and muscular systems being so enormous that a significant amount of experience/practice to achieve consistency was required.

Other functional motor skills that have been studied longitudinally show similar EMG results. For the development of skill in reaching or seated posture muscle activity is highly variable initially, even when the outcome is successful. Only with weeks to months of goal-directed practice, do rhythmic and stable muscle activity patterns emerge (de Graaf-Peters et al. 2007). Perhaps the clearest picture of infants’ muscle activity across the first year is represented by the muscle traces in Figure 4 for two individual infants, a good stepper and a poor stepper. By looking simply at the traces themselves, it would be difficult to guess which infant was more likely to step a lot and which one not. Each demonstrated normal variability in active force production and no obvious pattern of improvement, even by 12 months of age.

Given the variability of timing and duration of individual “gait” muscles, it seems reasonable to point out that there are many other muscles that we did not monitor, that likely contributed to the net flexor and extensor torques responsible for the kinematic flexions and extensions created. Even when none of the muscles we monitored showed activity, combinations of deeper muscles may have been at work. Without a stepping “goal” and significant practice intentionally producing this goal, settling into a stable and efficient force production pattern may not emerge. Yang and Gorassini (2006), in their review of studies comparing availability of a spinal pattern generator for stepping in primates and other mammals, of the type defined by Grillner (Grillner et al. 1981), concluded that, “There is a general sense that the pattern generator is either not as important for human walking or not as easy to activate or both.” (p. 379)
On the surface, at least, the variability we observed in early muscle activity during stepping seems to contrast with that reported recently by Dominici and collaborators (2011). These researchers studied the EMG activity during stepping in 3-day-old neonates and walking in toddlers, using mathematical techniques to extract commonalities. They concluded, and we agree in the abstract, that neonates’ leg behaviors show more extension than flexion in stance, and more flexion than extension during swing, and that by 12 months muscle activation patterns show greater differentiation among muscles. But they also concluded that both age groups showed sinusoidal muscle activity across strides at both ages, which we did not observe. Differences in our data may reflect age differences or the support surfaces (ours was moving and theirs was stationary) or the fact that their conclusions were based on a compression of data which masks the underlying variability.

Refinement of control in gait muscles, more generally

What is clear is that all of these infants were practicing some functional motor skills across this first year and, thus, might be assumed to have improved their level of lower limb control. By 6 months all were sitting alone, some were crawling, and by 12 months all were cruising, with some beginning to walk. Further, the quality of their limb postures (less flexion, more extension) and foot placements (less lateral and toe contact at touchdown, more flat-footed stance) suggests better foot control, and perhaps increased muscle strength. An overall improvement in their ability to generate or inhibit activity in their lower limb muscles is observed in everyday life, as they
intentionally get into seated postures, move toward objects of interest and avoid collisions and change directions. This refinement in their use of muscle force and to adapt to novel contexts is apparent here in their reduction in co-activation of agonist and antagonist muscles, across ages, during supported treadmill stepping and was also reported on stepping on a firm surface (Okamoto et al. 2003). Reduction in co-activation occurred most dramatically during swing, from about 40% to an average of less than 10%. Though reduced, co-activation during stance remained at approximately 30%, even at 12 months. Although cross talk cannot be ruled out with absolute certainty when working with infants, the low average correlation we obtained for filtered and smoothed data of $r^2 = .13$ (SD .13) for the LG-TA pairs and $r^2 = .17$ (SD .13) for the RF-BF pair, suggests that the high level of co-activation we report in this study across the different ages is not significantly affected by cross talk. Moreover the electrode conducting surface of 5 mm used in this study is typical from what has been reported to previously in the literature to properly pick motor activity in infants in the same age range (Okamoto et al. 2003; Lamb & Yang 2000; Hadders-Algra et al. 1992). Co-activation is one way a system can increase stiffness, which has been argued to be a response elicited by humans during gait when they perceive their stability is threatened (Holt et al. 2006; Ulrich et al. 2004). Standing on the moving belt of a treadmill, even with researchers holding their trunks, is likely to feel like a very unstable posture, thus demanding more co-activity than they will ultimately use as skilled walkers overground.

Probability graphs, as well, showed a reduction in mean values, with age, especially from month 1 compared to months 6 and 12. This is particularly easily seen in the RF muscle in stance, which also showed a significant reduction in burst duration
with age. This reduced activation probability likely also reflects the reduction in co-
activation, and the capacity to activate muscle groups in isolation and more “at will”. Our
muscle state data (see fig 1) indicates that infants increased, generally, the frequency
with which single muscles were active or active in pairs, rather than activating all
muscles concurrently. Studies on spontaneous kicking also show that infants refine their
intralimb coordination with age (Thelen et al. 1981, Jeng et al. 2002) by decoupling the
hip, knee and ankle joints. This decoupling can also be seen in our data (reduce burst
duration and co-activation, increase frequency) and shows that the development of
stepping is parallel to the development of basic leg control.

Similarly, researchers working with other species have shown that spinal and
supraspinal reorganization occurring during the neonatal period induced a reduction of
sensitivity of the motor units in other species with age (Bradley & Smith 1988; Cazalets
et al. 1990). Hadders-Algra and colleagues hypothesized that the same process
engendered a reduction in duration of muscle activation in infants’ spontaneous kicking
(Hadders-Algra et al. 1992).

While level of EMG activity decreased with age (burst duration, co-activation,
probabilities across the stride cycle), infants nevertheless showed a scale up in activity
at an appropriate time in the stride cycle. For most muscles, the peak of
activity/probability of being active occurred well into stance. This suggests that their
behavior reflects weight acceptance and efforts to support their bodies in upright
position. Significant increases in muscle activation at touchdown have been reported
many times for infant stepping (Forssberg 1985; Ivanenko et al. 2005; Okamoto et al.
2003; Yang et al. 2005), and were primarily associated with hypersensitivity of the
segmental reflex (Forssberg, 1985; Okamoto et al. 2003). Our data, in line with Ivanenko (2005) suggest several arguments against reflexive muscle activity, certainly at the initiation of stance. First, the inconsistency of the activity at touchdown suggests that if contact transmits input to reflex receptors, they are not consistently activated. Second, muscle burst activity we observed seems to persist longer than a reflex response would be expected to do so. For example, our results showed that the range of mean burst duration varied between 0.29 to 0.60 s (95% confidence interval) for the biceps femoris to .60 to .92 s for the rectus femoris at 1 month of age. These durations are clearly greater than the 10 to 15 ms reflex response that has been recorded in adults (Cheng et al. 1995; Gottlieb et al. 1979) or infants (Myklebust 1990; Myklebust & Gottlieb 1993; Myklebust et al. 1986; O'Sullivan et al. 1991; Teulier et al. 2011). Third, the responses at initial stance, seen in Figure 3, tended to be lower in probability than ones later in stance.

Also, of all four gait muscles, one seemed to reflect a better control, with higher level of probability, and a pattern quite similar in overall pattern to that of skilled adult stepper, the lateral gastrocnemius. We hypothesize that the capacity to increase activation of this muscle in stance as early as month one may be because infants discover and exploit plantarflexion (pushing) against the uterine wall to aid in repositioning pre-birth. This hypothesis is supported by two recent findings. The first one showed that in rats, experience in utero contributes to the development of coordinated motor behavior before birth that is maintained post birth (Robinson 2005). Second, Musselman and Yang (2008) found that post birth, in humans, rhythmical and coordinated interlimb movements were transferable to different leg movements. In fact,
they showed that infants who were able to practice alternating or synchronous weight bearing movements were able to transfer those interlimb coordinations into spontaneous kicking, leading these researchers to hypothesize that interlimb coordinations might share similar neural substrates. In utero, when babies push against the uterine wall and effect valued outcomes (e.g., repositioning) synaptic linkages among relevant neurons and motor units contributing to the kick/leg extension and the sensory receptors activated upon contact with the wall as movement occurs are strengthened. These same neural substrates may be utilized when the baby’s foot strikes the surface at touchdown, eliciting lateral gastrocnemius’s biarticular capacity to both stabilize the knee and produce antigravity forces at the ankle. Later, creeping and crawling for locomotion and exploration may enhance not only strength but also adaptability to varied contexts. This may account for the fact that peak activation of the lateral gastrocnemius shifts toward that of skilled walkers, that is later in stance, with age (see Figure 3). That this is the most stable occurrence among the four gait muscles does not mean it is stable across infants and consecutive strides. As the profiles of two of our infants show, many cycles still fail to show lateral gastrocnemius bursts in stance, but the likelihood seems to increases by 12 months.

**Development of flexor-extensor synergies**

When considering the overall profile of EMG activity, our results also show a lack of clear alteration between flexor and extensor muscle activity that others have reported (Berger et al. 1984; Leonard et al. 1991). In fact, at 1 mo, flexors and extensors spent 50% of the stance phase being co-activated and 20% being not activated at all. More interestingly, our longitudinal data seems to show that even for infants who master the
alternation pattern at an interlimb coordination level (95% of alternating steps)

underlying alternation between flexor and extensors at the muscular level is still
questionable. In fact, even though we observed a decrease in co-contraction, the tibialis
anterior and lateral gastrocnemius are still co-activating 30% of the time at 12months of
age and the very weak probability of muscle activation for the tibialis anterior across
points in the cycle reinforces the plasticity of the neuro-muscular system in infants,
allowing them to find an enormous number of solutions to create their kinematic
movements, but the lack of experience in this specific context does not allow them to
stabilize to any one solution.

Over the first year of life it seems that lower limb control emerged from the
repeated perception and action cycles (spontaneous kicking/crawling in various
environments) based on the availability of a complex neural network undergoing
dynamic structural reorganizations, sensitive to practice and experience. As infants in
this study did not practice stepping on a treadmill between visits to the lab, we argue
that our results reflect an improvement in adapting to an unusual environment
threatening infant’s stability by better controlling lower limb motion rather than a clear
development of a goal-orientated stepping pattern. Nevertheless we believe that with
practice the development of a more rhythmical neuro-muscular pattern on treadmill will
develop, developing in a quite similar manner as the ones seen in toddlers learning to
stated that, “Morphological plasticity in the brain occurring in response to an increase in
the complexity of the environment appears to reflect brain substrates of adaptation to
the demands and opportunities provided by experience, including both relatively typical
forms of learning and memory and adjustments associated with fundamental processes such as sensory, motor and cognitive processing." (p.2) We hypothesize that the same ubiquitous role of experience should not be seen differently when looking at the acquisition of a functional motor skill like walking.

**Conclusion**

To conclude, our goal here was to examine, longitudinally, the patterns of muscle activation produced by healthy infants, over the first year of life, when stepping while supported upright on a treadmill. Further, we intentionally included infants, regardless of whether they responded with many steps or few because this range is typical in healthy infants. In order to compare the neuromuscular development of atypically developing infants to healthy ones, this diversity in sample is critical. Interestingly, this continuum of ability in our sample did not affect the underlying and main conclusion: across these ages, step cycles were created by infants by a wide variety of muscle combinations and timing of their activations. Nevertheless, some levels of muscle control were evident, such as stable probability of gastrocnemius activity and reduction in coactivation with age. However, kinematic stability improved much more clearly over the first year than did the kinetic strategies that underlie this overtly patterned and stable (by 12 months) behavior. Thus we conclude that while innate control of their limb movements improve as infants grow, explore, and acquire functional movements, the treadmill context is a novel and unpracticed one. As observed for other functional skills of infancy, underlying neural activation of muscles used to walk (on or off the treadmill) will resolve into more stable patterns, as functional practice ensues.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES


FIGURE LEGENDS

Figure 1a, b. Muscle activity patterns for sixteen muscle states, by phase, *significant Age or Interaction effects.

Figure 2. Percent of stride cycle during which agonist-antagonist muscles were simultaneously co-activated.

Figure 3a, b, c, d. Probability of muscle activity across stride cycle, by age.

Figure 4a, b. Smoothed, rectified EMG from 2 infants a) EMG traces from an infant who produced alternating steps consistently at all ages; b) EMG traces from an infant who took fewer consecutive, alternating steps at 1 and 6 months. Y axis values represent the percent of maximal amplitude for that muscle. Maximum amplitude was identified for each baby, at each test session, for each muscle, as the highest peak value across all steps produced by that infant; ST=Stance phase; SW=Swing phase.
Table 1. Description and statistics for stride parameters, by age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 month</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cycle duration (s)</strong></td>
<td>1.72 (0.62)**</td>
<td>1.95 (0.32)**</td>
<td>2.12 (0.43)</td>
</tr>
<tr>
<td><strong>Stance phase (% cycle)</strong></td>
<td>58.2 (14.0)</td>
<td>66.8 (7.0)*</td>
<td>73.3 (6.1)*</td>
</tr>
<tr>
<td><strong>Percent Leg flexed in Swing (%)</strong></td>
<td>73.3 (18.1)</td>
<td>60.4 (21.8)</td>
<td>45.5 (36.1)*</td>
</tr>
<tr>
<td><strong>Interlimb Phase Lag (%)</strong></td>
<td>50.8 (15.0)</td>
<td>50.4 (14.7)</td>
<td>51.6 (10.8)</td>
</tr>
<tr>
<td><strong>Foot Contact at Touch down (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heel</td>
<td>5.7 (1.0)</td>
<td>10.2 (1.8)*</td>
<td>13.2 (2.6)*</td>
</tr>
<tr>
<td>Flat</td>
<td>23.0 (4.0)</td>
<td>31.4 (5)</td>
<td>40.5 (6.2)</td>
</tr>
<tr>
<td>Toe</td>
<td>47.4 (4.6)</td>
<td>50.4 (7.0)</td>
<td>40.5 (7.3)</td>
</tr>
<tr>
<td>Medial/Lateral</td>
<td>23.8 (4.0)</td>
<td>7.8 (2.7)*</td>
<td>5.7 (1.3)*</td>
</tr>
<tr>
<td><strong>Foot Contact at Mid-stance (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flat</td>
<td>21.6 (5.5)**</td>
<td>33.7 (7.5)**</td>
<td>70.4 (5.5)</td>
</tr>
<tr>
<td>Toe</td>
<td>73.5 (5.2)**</td>
<td>63.9 (8.5)**</td>
<td>27.1 (5.8)</td>
</tr>
</tbody>
</table>

* Significantly different from 1 month.

** Significantly different from 12 months.