**Role of activity in the optimization of neural and non-neural systems in infants**

by

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## DEDICATION

For my parents, Gwang Yang and Myeong Sook Lee, and fiancé Jin Tack Lim.

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## Abstract

From basic neuroscience evidence and theoretical explanation of the development of neuromotor skill, we know that the emergence of behavior is a self-organized process that results from the cooperative tendencies of multiple, heterogeneous subsystems within contextual demands. Via sufficient repeated cycles of perceiving and acting, many intrinsic and extrinsic resources are intertwined to produce new behavior patterns eventually becoming stable, functional skills. This means that through exploration and experience, underlying factors can be strengthened to foster the functional foundation for neuromotor and non-neural areas. This foundation leads to a proactive view on neuromotor rehabilitation for the development of functional motor skills in infants with neurologic problems: suggesting ways to optimize residual recourses (i.e., neural, muscular, and skeletal systems), to increase functionality, to maximize neuromotor control, and to minimize the cascading effects on other systems. Therefore, multilayered reciprocal interactions among underlying factors should be studied during the emergence and control of behavior longitudinally for populations with neuromotor difficulties.

The overall goal of this dissertation was to design empirical studies to identify changes in underlying subsystems in response to motor activity and to address how rigorous practice affects the development and recovery of neuromotor function in infants. With this series of studies, we examined changes in subsystems at both the neural level and the non-neural level: At the neural level, we tested the integrity of spinal-level reflexes and concurrent functional skills. At the non-neural level, we determined simple behavioral adaptations to upright activity practice and the impact of practice on bone mineral content.

Our results showed improvements in neuromotor and non-neural areas via massive repetitions of specific activities in infants with Myelomeningocele (MMC) as well as with typical development (TD). But, each subsystem showed its own unique rate of change in response to the massive activity. Gradual changes emerged first at the behavioral level such as walking skill changes with toddler or stepping pattern changes with MMC infants, followed by non-neural levels, such as bone mineral content, and then slowly at the neural level such as integrity of sensorimotor loops. In addition, type of activity and intensity were important factors to consider in order to optimize each subsystem's development. The study results will help researchers to design better, assertive early intervention protocols for infants who have neuromotor disabilities.

# CHAPTER I

## INTRODUCTION

Only a few decades ago, the predominant view was that early motor skills simply emerge as infants get older, much like a prescribed machine with controls or programs for the acquisition of motor skills. Now, basic neuroscience evidence and theoretical explanations for the emergence of behavior support that the acquisition of motor skills is not innately designed. Across the discipline, a number of different theories are used in science to explain the emergence of behavior; the most compelling arguments come from the dynamic systems theoretical framework (Thelen & Smith, 1994), developmental systems approach (Adolph & Robinson, 2008; Oyama, Griffiths, & Gray, 2001; Spencer et al., 2009), probabilistic epigenesis (Gottlieb, 2007), neuroconstructivism (Denis et al., 2007; Karmiloff-Smith, 2006, 2009) and the theory of neuronal group selection (Edelman, 1987). The terms used are different across disciplines, but the core principles are fairly consistent. All these approaches posit that the emergence of new behavior is a self-organized process that results from the cooperative tendencies of multiple, heterogeneous subsystems within contextual demand. Via sufficient repeated cycles of perceiving and acting, many intrinsic and extrinsic resources are intertwined to produce a new pattern of action, eventually becoming a stable, functional behavior. Thus, new skills are not directed by a neural maturation plan for their emergence.

Depending on the types of intrinsic and extrinsic factors, we sometimes observed unique behaviors that may not be considered typical patterns in motor skill acquisition. For example,

infants with Down syndrome (DS) showed different ways of shifting their posture from prone to seated. Healthy infants put their legs together to one side and then swing them as one unit. Infants with DS used exaggerated hip abduction to split the legs and then swing them forward individually to sit (Lydic & Steele, 1979). Given different intrinsic factors such as extreme laxity in the hip joints and poor control of trunk rotation, this prone-to-seated pattern emerged uniquely, but they accomplished the same functional goals as the pattern discovered and used by healthy children. That is, there is more than one way to accomplish the same functional goals. Commonly, we observed unique developmental sequences and progressions in motor skill acquisition in populations with neurologic disabilities because they tend to work with different underlying subsystems. Researchers should closely examine the contribution of underlying subsystems to the emergence of behavior because their contributions may sometimes become more obvious with longitudinal examination.

Not only underlying systems but also history matters for developmental sequences in motor skill acquisition. That is, when a poor foundation occurs in infants for neurological development, the divergent organization may become compounded over time to have increasingly deleterious effects on future development in motor, cognitive, or growth areas. This effect is referred to as "cascading effects" (Karmiloff-Smith, 2009). For example, infants with neuromotor disabilities show impaired (less frequent) early spontaneous leg movements, which affects their subsequent ability to build strength and control for early motor milestones, which, in turn, reduces their ability to locomote upright (Chapman, 2002; Rademacher, Black, & Ulrich, 2008; Smith, Teulier, Sansom, Stergiou, & Ulrich, 2011). Although walking may sometimes become possible much later in development than typical, the diminished early spontaneous

movement fosters atypical trajectories in motor control acquisition (neural level) and growth, such as body composition (non-neural level), throughout the lifetime.

These notions lead to exciting new options for how we can design optimal neuromotor rehabilitation for the development of functional motor skills, especially for infants with neurologic problems. When the neuromotor control is interrupted or delayed, we can proactively work to optimize residual recourses (i.e., neural, muscular, and skeletal systems), to encourage more function, to maximize neuromotor control, and to minimize the cascading effects on other subsystems. Through exploration and experience--sufficient repetition of perceiving and acting within contextual demands--underlying factors can be strengthened to foster the functional foundation for neuromotor and non-neural areas. Therefore, multilayered reciprocal interactions among underlying factors should be assessed and tested in the emergence and control of behavior.

The overall goal of this dissertation was to design a series of empirical studies to test behavioral and underlying subsystems' changes in response to repeated cycles of activity within contextual demands. I elected to study a population with myelomeningocele (MMC) because MMC is a neural tube defect that leads to developmental neural impairments, causing different trajectories of motor skill acquisition and disrupting neurophysiological function. Most frequently, lesions occur in either the lumbar or the sacral regions, innervating the pelvis and lower limbs. Due to lesions in the spinal cord, the MMC population is prone to difficulties in neuromotor strength and control in the lower extremities, contributing to a cascading sequence of secondary complications throughout life. Starting in infancy, reduced frequency of spontaneous movements leads to decreased neuromotor strength and control ability, which results in diminished capacity to develop self-locomotion (Rademacher et al., 2008). This diminished capacity in the neuromotor systems fosters atypical trajectories and delays in motor skill

acquisition and eventually causes cascading effects on not only neuromotor strength and control (neuromuscular changes) in the lower extremities but also non-neuronal systems, such as bone strength and growth.

In four studies, I monitored changes in underlying subsystems in response to motor activity to address how rigorous practice (repeated cycles of perceiving and acting) affects the recovery of neuromotor function in infants. Changes in subsystems were measured at both the neural level and the non-neural level. At the neural level, I assessed the integrity of spinal-level reflexes and concurrent behavior adaptation over time. At the non-neural level, I studied quality of stepping behavior and changes in bone mineral content as a function of age and upright activity.

To design intervention programs to enhance neuromotor control and strength, researchers should first understand the integrity of the peripheral neural sensorimotor loops or residual conductivity - reduction in neural transmission from the spine to muscle and from peripheral sensory receptors to the spine. The integrity of peripheral neural sensorimotor loops is typically grossly approached in clinics, by neurologists. However, this traditional method makes it impossible to understand which muscles of the leg can utilize varied types of sensory information to generate motor output. Assessing the activity of integrated motor neuron and spinal circuits with muscle fibers requires a more controlled method, such as concurrent assessments of multiple muscles and pathways, particularly for populations with interrupted neuronal pathways. Therefore, in my first study I examined the accessibility of 1a proprioceptive pathways to the primary gait muscles in infants with MMC over the first 10 months of life. The results revealed that Ia-proprioceptive pathways to homonymous and heteronymous muscles were functioning in some of the MMC babies tested, but the gain setting of these pathways was

generally depressed for many and require more functional stimuli and experience to enhance the gain on the sensitivity of these neural pathways.

Because locomotor reflexes were unstable and did not change over 10 months in the MMC population, I proposed that locomotor practice was needed to stabilize theses pathways. I tested the effect of massive practice (e.g., cruising and walking) on neuronal level – the integrity of peripheral 1a pathways to the gait muscles. From previous researches, we know that peripheral 1a pathways will eventually (by 2 - 6 years) be fine-tuned via massive practice with relevant sensory input (Leonard, Hirschfeld, Moritani, & Forssberg, 1991; Leonard, Matsumoto, & Diedrich, 1995), but when these improvements begin and what types of sensory input contribute to the organization of proprioceptive feedback loops are undefined. I hypothesized that reflex behavior processes undergo rapid and continuous development during the first three months of independent walking experience. By studying gait development during this period immediately before and after the acquisition of independent walking, it was possible to determine possible influences of locomotion (massive practice) on the functioning of 1a-mediated reflex pathways (neuronal level). The results indicated that throughout the walking experience, the ratio of reflex responses was increased for the agonist and decreased for the antagonist reflex loops. Moreover, each tested muscle displayed a reduction in the variability of reflex response. However, the impact of walking experience seems to be slowly emerging and, depending on muscles tested and pathway types, developmental progress can be quite different.

As I explained earlier, infants with MMC need therapy to help them learn how to control their bodies by strengthening the foundation of their underlying subsystems. As detailed in my final two chapters, I tested the effects of repeated cycles of perceiving and acting on behavioral and non-neural levels – stepping behavior on a treadmill (chapter 4) and bone mineral content

(chapter 5), respectively. Contemporary theory and basic science argue for early, aggressive, and functionally relevant activity protocols to maximize recovery and function (B. D. Ulrich, 2010). However, the current approach for infants with neuromotor disabilities typically delays intervention until motor milestones are missed or to wait until they have emerged and then try to correct motor skills. In addition, therapy tends to be passive and less focused on functional skills (e.g., passive range of motion movements or the correction of sleeping positions) than when therapy is prescribed for older children or adults. The disadvantage of the typical approach is that it starts relatively late, from the point of neuroplasticity, and misses opportunities to accumulate a history of skills, building the foundation for the next skills to follow. Based on treadmill studies with DS (D. Ulrich, Lloyd, Tiernan, Looper, & Angulo-Barroso, 2008; D. Ulrich, Ulrich, Angulo-Kinzler, & Yun, 2001) and MMC (Pantall, Teulier, Smith, Moerchen, & Ulrich, 2011; Teulier et al., 2009), we hypothesized that the use of a treadmill to promote the stepping response (repeated cycles of perceiving and acting) might present an effective early therapeutic intervention. In the study described in chapter 4, I determined the impact of early individualized practice being supported by parents on a treadmill, on stepping behavior (behavioral level) over the first six months of this in-home intervention. The results indicated that treadmill practice did not clearly affect step rates over the first half of intervention, but steps infants produced were of a much higher quality, suggesting improved neuromotor control and strength.

Finally, in chapter 5, I studied the impact on a non-neuronal subsystem (bone mineral density). Due to reduced spontaneous leg movements and weight-bearing behavior, children with MMC have lower bone mineral density (BMD) than children with TD in the upper body (Quan, Adams, Ekmark, & Baum, 1998), but it is much lower in the lower body (Ausili et al., 2008; Rosenstein, Greene, Herrington, & Blum, 1987; Szalay & Cheema, 2011; Valtonen et al., 2006).

However, there are no published data for BMD or bone mineral content (BMC) in infants with MMC. Consequently, how early and how rapidly this reduced BMD emerges in children with MMC is not known. Therefore, I monitored changes in BMC over the first 18 months of life in healthy and MMC infants. The effects of activity on BMC were also tested in MMC populations. Considering all normalization procedures, all groups showed increase in whole body BMC with age. For arms and legs, analyzed separately from whole body, lower values for infants were observed in infants with MMC compared to those with TD. But, in MMC infants, weight-bearing exercise seemed to improve bone strength at all sites measured.

Overall, the results I obtained help us understand some of the effects of upright activity on underlying subsystems at multiple levels. My results show evidence for neuroplasticity, which drives changes in neural tissues (i.e., the integrity of spinal level reflex), behavior adaptation (i.e., stepping behavior on a treadmill), and non-neural tissues (i.e., BMC), in response to functionally relevant practice. But, each subsystem shows its own unique rat of changes in response to the massive activity.

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## Chapter II

## FUNCTIONING OF PERIPHERAL IA PATHWAYS IN INFANTS WITH MYELOMENINGOCELE


### Abstract

The goal was to examine the accessibility of la-proprioceptive pathways to motoneurons of leg muscles associated with gait in infants with Myelomeningocele (MMC). Participants were 15 MMC infants, ages 2-10 months. We assessed over repeated trials, the tendon reflex (T-reflex), vibration-induced inhibition of T-reflex (VIM-T-reflex), and tonic vibration-induced reflex (VIR) when computer controlled stimuli were applied to the three gait muscles of each leg. Only one third of MMC infants exhibited motor responses following the mechanical stimuli with sufficient frequency to be judged functioning as in typically developing (TD) infants. Age and lesion level were not apparently associated with response frequency, but scores on the gross motor portion of the Bayley Scale was a reasonable predictor. For those in which responses were frequent, the pattern of reciprocal excitation was similar to that of age-matched TD infants. 4 of the 10 non-responders who were also tested for their responses to being supported on a pediatric treadmill in a companion study showed voluntary muscle activity in all three gait muscles and a vibration-induced contraction was observed for some of the non-responders. Ia-proprioceptive

pathways to homonymous and heteronymous muscles are functioning in some MMC babies, but the gain setting of these pathways were generally depressed and for many there was no evidence that the pathways were intact, although for some group more functional stimuli may be needed to elicit responses and experience may be needed to enhance the gain on the sensitivity of these neural pathways. More research is needed to understand how to optimize outcomes via rehabilitation.

Keywords: MMC, T-reflex, Tonic vibration reflex, Development, Proprioception,

## Introduction

Myelomeningocele (MMC) affects approximately 1500 to 2000 babies per year (Spinal Bifida Fact Sheet, 2007). MMC is the most common of all neural tube defects and the most severe congenital defect that affects mainly sensorimotor mechanisms. Over the last 13 years, primary preventive strategies using folic acid supplementation and advanced treatments of the frequently developing hydrocephalus with ventriculoperitoneal cerebrospinal fluid shunting have contributed, respectively to a birth rate decline, and long-term survival increase of individuals with MMC (Edmonds, Flores, Kirby, Rasmussen, & Williams, 2005). But, the physically inactive lifestyle remains an issue for individuals with MMC, particularly those who are non-ambulators.

The most critical characteristic of MMC is the defective fusion of vertebral arches. During the first four weeks of pregnancy, one or more of the posterior vertebral arches fail to close, commonly in the lumbar or sacral region of the spinal cord. Real time ultrasound examinations of fetuses with thoracic and lumbar lesions revealed that spontaneous lower limb movements were observed from 18 to 36 weeks of age and quality of movement was similar with those of fetuses without neurological disorders (Korenromp, van Gool, Bruinese, & Kriek, 1986;

D. Sival et al., 1997). However, Sival et al. (1997; 2004) reported that those spontaneous movements were present until postnatal day 1, and then movements tended to diminish after the first postnatal week. Others have reported as well that over the first 6 months, infants with MMC show less-spontaneous leg activity, compared with infants with typical development (Chapman, 2002; Rademacher, Black, & Ulrich, 2008). Although reduced amplitude and frequency of spontaneous leg activity has been identified as a characteristic of infants with MMC over developmental time, it is unknown how these fewer leg movements relate to the functional integrity of peripheral sensorimotor mechanisms, such as proprioceptive reflexes, which contribute to posture and locomotion control in adults.

Over the past 20 years, the developmental trajectory of the stretch reflex in infants during the first year of life has been outlined. Stretch receptors detect muscle proprioceptive information mediated by monosynaptic and polysynaptic pathways to homonymous and heteronymous motoneurons. In young infants, myotatic responses in lower limb muscles elicited by tendon taps are quite variable from the first week of life (Mayer & Mosser, 1969; Myklebust & Gottlieb, 1993; Prechtl, Vlach, Lenard, & Grant, 1967) up to10 years of age (Myklebust, 1990). Reciprocal excitation of antagonist muscles and irradiation to distant muscles are commonly observed due to the excitatory projections of primary afferents onto heteronymous α motoneurons (Leonard, Matsumoto, & Diedrich, 1995; Myklebust & Gottlieb, 1993; Myklebust, Gottlieb, & Agarwal, 1986; O'Sullivan, Eyre, & Miller, 1991). Most studies reported that a gradual reduction in simultaneous excitation (reciprocal excitation and reflex irradiation) is observed in infants with typical development (TD) over developmental time (Myklebust, 1990; O'Sullivan et al., 1991; O'Sullivan et al., 1998), but the age at which stable spinal reflexes emerge is still being debated (Myklebust, 1990). Only few study, examined the stretch reflex in

infants with MMC (Geerdink, Pasman, Rotteveel, Roeleveld, & Mullaart, 2008; D. Sival et al., 2006). The corresponding results indicate that the patellar tendon reflex gradually disappears in infants with MMC during the first week of life post-birth.

Teulier and colleagues (2010) were the first to examine the functioning of both mono- and poly-synaptic reflexes pathways in infants across the first 10 months of life. They tested the stretch reflex, the vibration-induced modulation of the stretch reflex and vibration induced reflex contractions in the stimulated muscles (TVR) and their antagonists (AVR). They found that TVR and AVR could be evoked by 2 months of age. Tendon vibration superimposed to tendon taps could induce either inhibition or facilitation of the T-reflex and the pattern of responses varied significantly as a function of time. Overall, instability of reflex responses was commonly observed even by 10 months of age. Given this data in infants with TD, the emerging question is what early reflex behaviors may be present in infants with MMC who are known to have partial spinal lesions and relatively difficulty to control their legs, compared to infants with TD.

As indicated above, infants with MMC exhibit less-spontaneous movements, which influence negatively their ability to learn to walk (Chapman, 2002; Rademacher et al., 2008). While their spinal peripheral nervous system is compromised, the extent of loss is difficult to specify at those early ages. Less-spontaneous movement alone is not sufficient to guide therapeutic interventions or to predict subsequent acquisition of motor control. Understanding the integrity and change over time of proprioceptive neural pathways is critical to determining possible neural sources of support for the development of sensory and motor functions in infants with MMC. Hence, the goals of this study were to examine accessibility of la-proprioceptive pathways to primary gait muscles (gastrocnemius, tibialis anterior, and quadriceps) in infants with MMC over the first 10 months of life. Infants with MMC generally suffer secondary

medical complications (such as hydrocephalus, Chiari II malformation, and club foot) and neuronal structural deterioration, reducing the gain settings of sensorimotor Ia pathways; thus, we expected to see depressed and high variability of reflex responses, compared to a previous report for same aged infants with TD (Teulier et. al 2011). Here, our data are presented mostly in descriptive format. Based on the results for TD infants, we also hypothesized that integrity of proprioceptive neural pathways in the gait muscles will not be influenced during the first year post birth by age. Standardized stimuli were delivered to elicit tendon reflexes, vibration-induced modulation of the tendon reflex, and tonic vibration-induced reflexes. Reflex responses were quantified by ratios, amplitude, and distribution, across primary walking muscles of both sides. We will expect fewer or no responses when compared to TD infants.

## Method

### Participants

Participants were 12 infants with MMC. Six infants were tested at a younger age (3 males, 3 females, 2–6 months of age). Six additional infants were tested at an older age (7–10 months of age); three infants from the younger age group were tested at least 3months later and included the older group as well, created a total of 9 infants (5 males and 4 females). We recruited all participants from fliers and MMC clinics in hospitals throughout the S.E. Michigan and N.W. Ohio areas. Inclusion criteria were: only lumbar and sacral level lesions, gestational age at birth >28 weeks, free of medical issues not characteristic of this population. The institutional review board of the University of Michigan Medical Sciences approved the experimental protocols for this study. Prior to participation, written consent was obtained from the parents or legal guardians of the babies. Each family received a monetary gift for participating in this study.

**Procedure**

The detailed test procedures were identical to those reported previously in Teulier et al. 2011; thus, only a concise summary of our protocol is presented here. To increase diversity and numbers of participants, we conducted the testing either in the developmental neuromotor control laboratory (33.3%) or family homes (66.7%) if they were not able to come to the laboratory. Upon entering a quiet room in the laboratory/ family home, infants were allowed a period of time to become comfortable with the testing room and experimenters. Each infant was prepared for testing by removing all clothing from the waist down, except diaper. To record electromyographic activity (EMG), preamplified pediatric bipolar electrodes (Noraxon$^{TM}$272, 1.7cm center to center) were placed on four muscles of both legs: gastrocnemius (GA), tibialis anterior (TA), quadriceps (QA), and hamstring (HA). The stimulated muscle and its antagonist (GA/TA, TA/GA, and QA/HA) were monitored in pairs simultaneously to identify the distribution of responses for all stimuli types. All infants were seated in our custom-made padded chair accommodating differences in leg length and posture of infants. Depending upon the muscle tested, the inclination of the seat-back support and leg position was changed for appropriate stimulation (Fig. 1). The monosynaptic tendon reflex (T-reflex), the vibration-induced modulation of the tendon reflex (VIM-T-reflex), and the tonic vibration-induced reflex (VIR) were elicited in three muscles (GA, TA, and QA) of each leg by electromagnetic stimulators (Ling Dynamic V203) equipped with smooth hammer heads. A custom-designed Labview$^{TM}$ virtual instrument run on a lap-top computer generated the stimulation signals, including tendon taps (5 ms pulse) and vibration (80 Hz sine wave). Each signal was transmitted to each stimulator by a power amplifier, whose gain was adjusted for each infant before the experimental session. The magnitude of stimulation was set to approximately 1.2 times the

response threshold level and, for each muscle, was maintained constant throughout the experiment. Furthermore, as justified by the pattern of responses obtained in age matched TD infants obtained in our previous investigation (see Teulier et al., 2011), the eventual mechanical spread to reciprocal muscles cannot be considered as a major source of cross-talk in the present results. Finally, it is understood that in infant, the use of a recruitment curve to determine the intensity of T-reflex stimulation is not practical. Nevertheless, since the stimulation was maintained constant and each infant was considered as its own reference for response comparison between the two conditions (T-reflex and VIM-T-reflex) a specific normalization was not necessary.

The order in which muscles were tested was randomized across infants, but for each infant and each muscle tested, T-reflex, VIM-T-reflex, and VIR were measured within a series of consecutive stimuli in that order. Hence, long lasting post-vibration effects which might cause interference with the monosynaptic responses were prevented (Roll, Martin, Gauthier, & Mussa Ivaldi, 1980). For the T-reflex, 20 taps were delivered to the distal tendon of the stimulated muscles with a minimum inter-stimuli interval of 8s. For the VIM-T-reflex, 20 taps were delivered during a concurrent vibratory stimulation applied to the same tendon. To elicit VIR, vibration was applied during 2 periods of 20s, preceded and followed by 10s of no vibration, to assist in distinguishing more clearly voluntary muscle activity from reflex responses.

Stimuli were applied when infants were calm and legs relaxed to reduce inherent response variations associated with changes in infant behavior. Via video recordings of the test session we coded infants' arousal levels post data collections. Infants arousal levels varied over the test period; during test trials the average arousal level for all infants was in the 3.04 ~ 3.42 range, based on a scale from 1-6 where 1 = asleep, 2 = drowsy, 3 = awake, 4 = awake and

moving, 5 = fussy, mild crying, and 6 = crying. In a few cases we did not complete testing of all muscles in all conditions, due to arousal levels being out of range (asleep or crying). In the case of absence of monosynaptic response (T-reflex) to the 20 stimulations, no attempt was made to test the influence of vibration (VIM-T-reflex) on the T-reflex.

We recorded the infants' medical history, including lesion level, brain morphologic abnormalities and musculoskeletal conditions. Descriptive medical characteristics for individual infants are summarized in Table1. To assess concurrent motor skill-development level, we administered the Motor Scale of the Bayley Scales of Infant and Toddler Development III (Bayley, 2006).

**Data Processing**

Using a custom-designed Labview$^{TM}$ virtual instrument (detailed in Teulier et al., 2011), we quantified reflex responses occurring in the stimulated muscle and its antagonist. All responses were quantified by the ratio of responses relative to the number of stimuli and the average amplitude of responses. In addition, response patterns for each muscle pair (the stimulated muscle and its antagonist) were examined.

## Results

Typical reflex responses obtained in each condition are illustrated in Figure 2. 2 (T-reflex, VIR-T-reflex) and 3 (VIR), respectively. Reflex responses previously obtained from infants with TD are also illustrated as a reference. The figures show that T-reflex time profiles are similar for TD (Figure 2.2a) and MMC (Figure 2. 2b/c) infants.

Table 2. 2 presents the distribution of responses associated with the different stimuli for the 15 infants tested. Compared to infants with TD at these same ages under identical test conditions (Teulier et al., 2011) fewer responses were obtained in infants with MMC.

Stimulations failed to evoke any muscle activation in four infants. Among the other participants, variability within and across infants dominated, with evidence of responses radiation to the antagonists of the stimulated muscles, as seen in infants with TD at these ages. Most interesting was that the dependent variables did not appear to be influenced by age or lesion level. Given the high variability of responses and the infants' medical characteristics, as well as the sample size, the results presented here are largely descriptive. We proposed that statistical treatment would not add understanding but, rather, mask the profiles we observed for infants' sensorimotor responses.

**Distribution of Reflex Responses**

First, we detail responses of infants with MMC for each reflex tested. Only a few infants exhibited reflex responses and a large variability of ratio and amplitude in the reflex responses was observed within and across infants. Therefore, we focused primarily on the ratios of reflex responses rather than the average of peak to peak amplitude of all the responses to a same stimulation type.

**Tendon reflex.** Regardless of age group, five babies exhibited a high ratio of responses in the stimulated muscles and their respective antagonists (see Table 2. 2). Two babies exhibited a few T-reflex responses in the stimulated muscles and their respective antagonists which lead to response ratios considerably lower for these two babies than the other five. No T-reflex was observed in the remaining eight babies.

**Vibration-induced modulation of T-reflex.** When tendon taps were concomitant with the vibratory stimulus, this vibration induced either an inhibition or a facilitation of the T-reflex. Among the five participants exhibiting a high ratio of T-reflex responses, vibration did generally produce an inhibition in the QA of either leg. Facilitation of the reflex responses was rarely

observed for the other 10 participants; when T-reflex stimulation fails to elicit a response, VIM-T-reflex was generally not tested. In addition due to some uncertainty about the presence of the T-reflex in some infants VIM-T-reflex was not tested for the GA and TA in these cases.

**Tonic vibration-induced reflex.** The TVR or AVR or both were observed in 11 participants. Among them, five of the babies who exhibited monosynaptic (T-reflex) and VIM-T-reflex responses also exhibited polysynaptic responses in each tested muscle pairs. These responses were less consistent in the other six participants. Neither the TVR nor the AVR were observed in any of the muscles for the other four babies (see Table 2. 2).

The results may be summarized as follows: (1) for all muscles tested, the response ratios for each stimulation situation (T-reflex, VIM-T-reflex, and VIR) showed variation in response consistency within and between infants. Moreover, as illustrated in Table 2. 3, even for those who were high responders, reflex responses as well as amplitude varied greatly from tap to tap. (2) When a muscle was stimulated by either a tap or vibration, simultaneous excitation of stimulated muscle and its antagonist was commonly observed, but occasionally, reflex responses appeared for either a stimulated muscle or its antagonist. (3) When T-reflex was elicited under concurrent tendon vibration, facilitation in GA and inhibition in QA were commonly observed in infants who exhibited T-reflex responses, but not in other infants who did not show T-reflex.

**What Distinguishes Levels of Response?**

As we indicated in the overview and summarized in Tables 2. 2 and 3, no relation was observed between the ratios of response and age or lesion level. To pursue further the factors underlying poor responses in MMC infants, we organized the data in three categories associated with the ratios of responses to distinguish four groups of infants. The High Responders Group (n=5) consists of babies for whom T-reflex, VIM-T-reflex, and VIR responses occurred in most

tested muscles. The Moderate Responders Group (n=4) corresponded to babies for whom vibration induced a modulation of the T-reflex or a few VIR responses in each muscle tested. Low Responders Group (n = 2) corresponded to babies for which a few VIR responses for each muscle tested were observed. The No Response Group (n = 4) corresponded to infants that did not show any responses in any of the muscles tested.

**Profile of infants in the high responder group.** High responder infants in our MMC sample showed a pattern of responses (distribution of responses in antagonist muscle groups) largely similar to the patterns observed in infants with TD described by Teulier et al (2011). From Teulier et al study, 2- to10-month old infants with TD showed 1) accessibility of the Ia-presynaptic inhibition and the Ia-mediated polysynaptic pathway and 2) the random distribution with inconsistency across T-reflex, VIM-T-reflex, and AVR. Table 2. 3 presents the mean ratios and amplitudes of reflex responses for the high responders group. Because the amplitude could not be normalized, the results below focus on response ratios.

***T-reflex.*** With few exceptions, infants in this group showed muscle activations in the stimulated muscles or their respective antagonist or both. Although the response ratios were lower for MMC than TD infants, the patterns were similar. That is, like infants with TD, these infants with MMC exhibited reciprocal excitation. For instance, for most babies, tendon taps to the QA often elicited reflex responses in the QA as well as the HA. However, other tested muscles (GA or TA) did not generally demonstrate distributed reflex excitation: T-reflex of GA and TA were evoked either in the stimulated muscle or its antagonist, but seldom in both.

***VIM-T-reflex.*** Modulation of reflex responses by the vibratory stimulus seems to vary with the muscle simulated. A facilitation of the T-reflex was observed in the GA while the response was inhibited in the QA and was not affected in the TA (see Table 2. 3).

***VIR.*** Results for this stimulation condition suggested that vibration induced contractions, TVR or AVR, occurred primarily simultaneously in the high responder group of our infants with MMC. The other babies often showed polysynaptic responses in either the stimulated muscle or its antagonist but seldom in both simultaneously.

**Relation between reflex response ratios and individual profiles.** Table 2. 4 presents the classification of infants' responsiveness (from high to none) to stimuli, along with their individual characteristics, within their age group. Four subgroups, as defined earlier, were identified. Similar rank orderings are presented for age at testing, lesion level, total score for motor items on the Bayley scale and subscore for lower body skills only of the motor items on the Bayley scale.

As can be seen, age, lesion level, and total score on Bayley scale did not seem to be highly correlated within age groups. However, despite a lack of correlation between responsiveness and lesion level it is worth noting that high responders had no lesion above the sacral level, while responsiveness was reduced for one infant with a sacral lesion. Generally, older age group (7-10 months) at testing or lower lesion level may not predict responsiveness of Ia pathways in all muscles combined. For example, infant $3^{a,b}$, who has a lesion level at S1, did not exhibit any type of response, whereas $2^{a,b}$, who has an L2 lesion level, showed some tonic responses to vibratory stimuli. In addition, infant $3^{a,b}$ was tested twice, at 3 months and 10 months of ages; while ratios of mono- and poly-synaptic responses did not change, he seemed to demonstrate development of functional neuromuscular control as reflected in improvements in performance on the Bayley scale. The lower body score on the Bayley scale tends to be the most predictive overall in that it shows the closest association with leg muscle reflex responses. That is, infants who had reached more advanced motor milestones in the lower body tended to be in

the group with the most reflex responses. The total Bayley scale may not be correlated with the development of lower limb reflex responses because they measure not only lower body motor skill but also upper body motor skill, for which these scales contain more items, proportionately. Including the Bayley scale's scores for the upper body, which is less affected for infants with MMC, made it difficult to describe activity patterns related to neuromotor function for the lower limbs.

## Discussion

The purpose of this study was to assess the functioning of peripheral Ia pathways via mechanical stimuli in infants with MMC over the first 10 months of life. Our results revealed three major behaviors/aspects of the tested reflex responses. First, responses associated with all tested pathways were observed only in few infants (high responder group), however the respective ratios of responses were lower for these infants than TD infants. Only one third of infants exhibited frequent motor responses following the mechanical stimuli, whereas, the other two third did not exhibit sufficient responses for each tested pathway. Second, for those in the high responder group, the pattern of reciprocal excitation of stretch reflex in the QA was similar with that of age-matched infants with TD. Third, lower body activity, as determined using Bayley scale, appeared to be more associated with Ia pathways functioning than age, lesion, and total Bayley scale.

### High Responders

Within the high responder group, the reflex patterns are in line with those of age-matched infants with TD. Stretch reflex responses were observed in antagonist muscle pairs and these responses were more frequent in the QA than TA and GA. That is, Ia pathways to the stimulated

muscle and its antagonist do exist in these MMC babies, but the gain of the responses was generally lower in TA and GA than in QA muscle.

However, the ratio of stretch reflex responses was lower in MMC than TD infants. The stretch reflex ratio in each stimulated muscle was less than half of the same ratio for infants with TD (e.g., for TA, 32% for infants with TD and 10% for high responder infants with MMC). This indicates the gain setting of the Ia monosynaptic pathway was generally lower for MMC than TD infants. It may be assumed that the descending drives/influences acting on α-motoneurons and the associated γ system are lower in MMC than TD, which would reduce the excitability of motoneurons and/or sensitivity of muscle spindle endings, respectively.

As in infants with TD (Teulier et al, 2011), the responses obtained in infants with MMC were generally inhibited in the tested muscles during the vibration-induced modulation of T-reflex test. However, some facilitation was also observed in the GA for the higher responders in MMC population. This inhibitory effect suggests that the Ia pre-synaptic mechanism inducing primary afferent depolarization (Jankowska, 1992) seems to be functioning in high responder infants (see Teulier et al., 2011).

In contrast with the almost exclusive TVR observed in adults (Martin & Park, 1997; Roll, Gilhodes, & Tardy-Gervet, 1980), the TVR and AVR were expressed in infants with TD (Teulier et al., 2011). These responses were also observed in high responder infants with MMC. Their expression indicates that Ia polysynaptic pathways to homonymous and heteronymous muscles are functioning in these MMC babies.

**Other Groups**

Few or no responses to stimulations were observed in two-third of the infants tested. This result is not likely due to methodological issues since responses could be obtained consistently in

a group of infants and less frequently in others, but rather to several factors that may account for low responsiveness. It may be that weak responses did not emerge sufficiently from noise to be identified according to our selection criteria. In addition, the quantification/qualification of the effects of a vibration superimposed to stretch reflex stimulation (VIM T-reflex) are difficult since few responses were obtained without vibration, which consisted of the baseline in that context. Hence the strength of the Ia afferent outflow was probably not strong enough to elicit motor responses or modulate the activation of α-motoneurons.

Weak or absent T-reflex responses are commonly observed in infants with MMC (Geerdink et al., 2008; D. Sival et al., 2006). Geerdink et al. (2008) measured T-reflex responses in the GA and QA muscles in 2 days old (median age) infants with MMC. In 31 newborn infants with MMC, these reflex responses were observed in 16 of them. Siva et al. (2006) reported that during the first postnatal week, QA reflex responses gradually decreased in six of seven neonates with MMC. After the first week, QA reflex responses could be elicited only in one of five neonates. These studies indicate that the monosynaptic pathway may be functioning within the seven days following birth and is strongly inhibited after.

Despite the paucity of responses associated with the functioning of Ia pathways, voluntary muscle contractions were present when infants were supported upright on the treadmill. Of the seven infants who participated in the treadmill stepping study (Pantall et al., 2011), all seven (3 – high responder infants and 4 - low or none responder infants) exhibited voluntary muscle contractions to produce steps when the belt was moving. This suggests that the absence of reflex responses is neither associated with disrupted descending motor pathways or motoneurons themselves, nor interrupted integrity of a sensorimotor function. In addition, decreases in the number of reflex responses were observed in babies who exhibited spontaneous

leg movements during seven-day observation immediately following birth (D. Sival et al., 2006). Given the documented voluntary muscle contractions in these studies, we may assume that since motor commands are executed at the motoneuron level, the accessibility of Ia pathways to motoneurons is strongly inhibited, or the pathways may be partially disrupted or underdeveloped due to MMC, which would decrease the number of Ia fibers responding to the simulation and thus the strength or their influence on motoneuron excitation.

**Variability**

Inter-and intra-subject variability was considerable in infants with MMC. The variability in reflex responses was defined by large changes in amplitude of reflex responses between consecutive responses and randomness of reflex responses. Such variability is also observed in infants with TD (Teulier et al. 2011) and children who were independent walker (Leonard et al., 1995); however, variability is more pronounced in MMC. Variability of reflex behaviors may result from abrupt changes in the gain of sensorimotor pathways, which also reflects changes at the central level, and thus contribute to instability in the control of sensorimotor feedback to leg muscles. Variability persists as an identifiable T-reflex behavior until 4 to 6 years of age (Myklebust et al., 1986), but variability start to decrease significantly during the second year when children start to walk independently (Leonard et al., 1995). Moreover, presence of facilitation as well as inhibition of T-reflex responses induced by vibration indicates that the gain of mono- and polysynaptic pathways is not yet stereotypic and is plastic in infants with MMC and TD during the first year of life. This unstructured gain setting could contribute as well to the ineffectiveness of the Ia-presynaptic inhibition. Variability persists even with infants with TD, but the degree of variability was wider in infants with MMC. It may be resulting from a reduction of sensory information to the CNS, which affects the ability of MMC babies to

produce motor responses. This in turn may contribute to a great instability in the reflex gain setting.

Since, the delivery of mechanical stimuli was standardized, and limb and head position as well as arousal level were controlled, these factors may have a small influence on variability. However, their possible contribution to the lack of responses in some infants may not be excluded. The infants tested are likely to have a wide range of cerebral or spinal deteriorations, reducing the gain settings of sensorimotor pathways and/or neural connections of Ia pathways. Accordingly, the degree of neuronal structural deterioration needs to be considered as a causal factor of variability. Furthermore, motor coordination in infants with MMC has been associated with the integrity of upper and lower motoneurons (D. Sival et al., 2006). In combination with the above mentioned factors, reflex responses were observed in few infants, but not all.

**Predicting Factors**

Our research highlight that the lesion level was partly associated with a sensorimotor impairment. To be more specific, the functioning of Ia mediated pathways, represented by reflex ratio of mono- and poly-synaptic responses, closely relates to the degree of the leg movement, but not distinctly with spinal cord lesion level, age, or total Bayley scale. The higher scores for lower body functions correspond to the high and moderate responder groups. These findings are in line with results from a 5-year observational study of children with MMC followed since the time of birth (McDonald, Jaffe, Shurtleff, & Menelaus, 1991). The study showed that, muscle strength could be a better predictor of ambulatory status than the neurological level of lesion. Similarly, muscles strength is a better predictor of sensorimotor responses than lesion level (Bartonek & Saraste, 2001). Furthermore, assessments of musculoskeletal deformity and neurological deficiencies have been proposed to determine ambulation in the clinical setting

(Samuelsson & Skoog, 1988). Therefore, lower body activity, as determined by Bayley scale, appeared to be a better indicator of Ia pathways functioning.

**Clinical Implication**

Taken together these results suggest that an early therapeutic sensorimotor experience should be provided to infants with deficient Ia-mediated responses. Therapeutic training involving repetitive muscle stretch, which in turns activates the muscle spindles, may eventually induce changes in reflex behavior. For children with cerebral palsy (CP), in particular, 12-week treadmill training resulted in changes in the reflex modulation during walking (Hodapp, Vry, Mall, & Faist, 2009). Treadmill training induced change in modulation of the monosynaptic reflex in the soleus muscle during the swing phase of walking. Training led to the complete suppression of that reflex, which eventually contributed to a functionally useful walking pattern (e.g., walking velocity). Despite the fact that successful treadmill training have been documented in population with CP, the underlying neuronal mechanisms responsible for the beneficial effects of early therapeutic intervention (e.g, treadmill training) are still poorly understood in infants with MMC. The plasticity of the CNS can be potentiated by activity, so that early interventions could enhance the capacity to compensate for sensorimotor impairments or imbalance effects (Aimonetti, 1999; Cooke & Bliss, 2006; Wang & Sun, 2011).

**Potential Limitation**

The period of observation of peripheral reflex pathways, which was limited to the first 10 months of life, needs to be extended in further studies. Along with the results of Teulier and colleagues (2010), no difference in reflex responses with age was observed over the first 10 months of life. Generally, reciprocal inhibition and homonymous TVR are not systematic in newborns. Structural changes such as increase in number of connection and corticospinal tract

changes contribute to evolution in the processing of sensorimotor information over the first year and beyond. These structural changes (maturation) alone do not seem to be sufficient to explain development of the plastic organization of the CNS. It was assumed by Teulier et al (2011), that with neuronal maturation and experience, the CNS develops the ability to control the gain of peripheral pathways and thus the functional organization of reflex mechanisms. Leonard et al. (1995) suggested that functioning of monosynaptic pathways should be examined until the second year of life when children begin to walk. When compared with infants with TD, infants with CP exhibited similar reflex behaviors (reciprocal excitation and irradiation) during the first year of life. However, these early stretch reflex behaviors in lower extremities remained the same in children with CP, but disappeared during the second year of life in children with TD.

Hence, peripheral Ia pathways functioning may change after the first year of life. In infants with MMC, sensorimotor pathways are primarily depressed, which could be indicative of disrupted sensorimotor pathways or insufficient or delayed neuronal maturation due to less-spontaneous movements, or both. Our primary focus was to examine accessibility of la-proprioceptive afferents to lower limb muscles in infants with MMC. Since our investigation was limited to the first 10 months of life, to gain further understanding of reflex organization and depression in MMC, we suggest that in further study, the developmental trajectory of reflex behaviors over the first two years of life should be examined in relation to other sensorimotor impairments.

## Conclusion

Ia mono- and polysynaptic pathways to homonymous and heteronymous muscles are functioning in some of the MMC babies tested, but the gain setting of the Ia-proprioceptive pathways was generally depressed for many and require more functional stimuli and experience

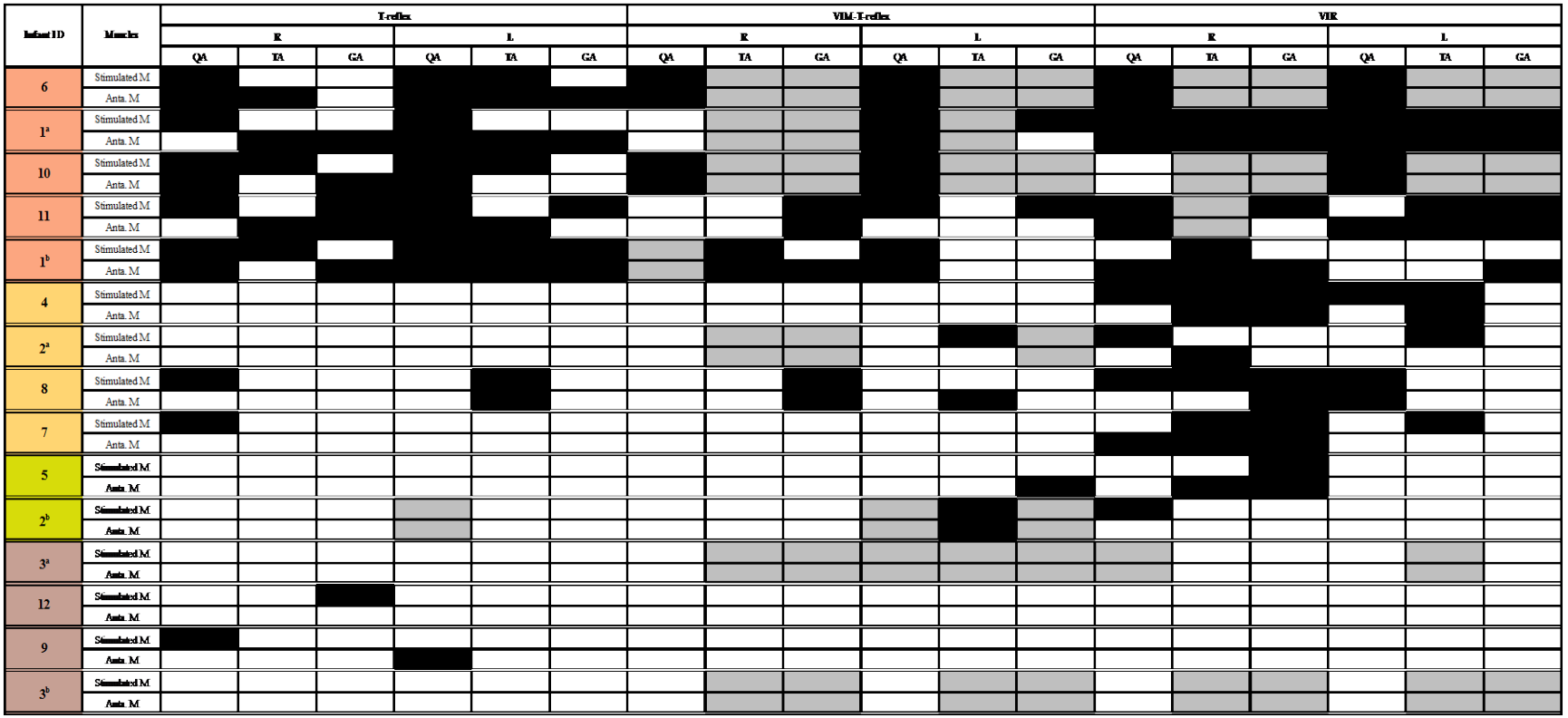
to enhance the gain on the sensitivity of these neural pathways. Understanding the level of leg muscle reflex responses is critical to identifying factors that can affect improvement through stimulation and rehabilitation.

Table 2. 1. Medical Characteristics of Participants with MMC

| | | | | | Brain Morphologic Abnormalities | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | ID | Gender | Gestational Age (weeks) | Lesion Level | Hydrocephalus | Shunt | Arnold Chiari II | Foot Deformity | Hip Status |
| Younger group | 1[a] | M | 35 | S1-S4 | Y | Y | - | - | - |
| | 2[a] | F | 37 | L2 | Y | Y | Y | Bilateral clubfeet | - |
| | 3[a] | M | 36 | S1 | Y | Y | Y | Bilateral clubfeet | Bilateral dislocated |
| | 4 | F | 38 | S1 | - | - | Y | Bilateral clubfeet | - |
| | 5 | F | 39 | L2 -L3 | Y | Y | Y | Bilateral clubfeet | R dysplasia L dislocated |
| | 6 | M | 39 | L5, S1 - S2 | Y | Y | Y | - | - |
| Older group | 7 | M | 35 | S1 -S2 | Y | Y | Y | - | - |
| | 1[b] | M | 35 | S1-S4 | Y | Y | - | - | - |
| | 2[b] | F | 37 | L2 | Y | Y | Y | Bilateral clubfeet | - |
| | 3[b] | M | 36 | S1 | Y | Y | Y | Bilateral clubfeet | Bilateral dislocated |
| | 8 | F | 37 | L4 -L5 | Y | Y | - | L clubfoot | - |
| | 9 | F | 38 | L3- L4 | Y | Y | Y | Bilateral clubfeet | L dislocated |
| | 10 | M | 39 | L5 -S1 | Y | Y | - | - | - |
| | 11 | M | - | S1 - S2 | Y | - | Y | Bilateral clubfeet | - |
| | 12 | F | - | L4 | Y | Y | Y | Bilateral clubfeet | - |

[a] Infants 1, 2, and 3 are babies who were tested twice, once at young age (a) and once at older age (b). L = left, R = right, Y = yes.

Table 2. 2. Overall Reflex Response Patterns across Stimulation Situations




Black boxes indicate that reflex responses occurred; white boxes indicate that no reflex response occurred; gray boxes indicate that reflexes were not tested due to fussiness or that no reflex responses was elicited for the first tested muscle. Based on the ratios of reflex response, all infants were subdivided into four groups: high responders (pink color), moderate responders (yellow color) low responders (green color), and no response (purple color).

Table 2. 3. Detailed Responses of Infants in the High Responders Group

| Variables | Ratio(%) | | | Amplitude(mV) | | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Range** | **Mean** | **SD** | **Range** |
| **T-reflex** | | | | | | |
| QA | 55% | 35% | 4 ~ 100% | 417.05 | 250.50 | 110.34 ~ 884.95 |
| GA | 13% | 27% | 0 ~ 78% | 309.07 | 72.61 | 241.2 ~ 385.64 |
| TA | 10% | 12% | 0 ~ 35% | 126.45 | 29.09 | 94.75 ~ 168.24 |
| **VIM-T-reflex** | | | | | | |
| QA | 30% | 26% | 0 ~ 64% | 229.41 | 132.08 | 95.95 ~ 453.78 |
| GA | 33% | 34% | 0 ~ 68% | 473.79 | 14.07 | 463.841 ~ 483.733 |
| TA | 8% | 7% | 0 ~ 12% | 113.50 | 37.62 | 86.9 ~ 140.10 |
| **VIR** | | | | | | |
| QA | 45% | 44% | 0 ~ 100% | - | - | - |
| GA | 50% | 45% | 0 ~ 100% | - | - | - |
| TA | 70% | 45% | 0 ~ 100% | - | - | - |

Table 2. 4. Relation between Subgroups Affiliation and Potential Predictors (rank ordered)

a. Younger Group

| Subgroups | ID | Age (days) | Lesion | Bayley (SUM) | Bayley (lower body) |
|---|---|---|---|---|---|
| High Responders Group | 6 | 199 | S1-S4 | 43 | 7 |
| | 1[a] | 142 | S1 | 25 | 5 |
| Moderate Responders Group | 4 | 132 | S1 | 23 | 5 |
| | 2[a] | 108 | L5, S1-2 | 22 | 4 |
| Low Responders Group | 5 | 116 | L2-L3 | 21 | 4 |
| No Response Group | 3[a] | 108 | L2 | 17 | 0 |

b. Older Group

| Subgroups | ID | Age (days) | Lesion | Bayley (SUM) | Bayley (lower body) |
|---|---|---|---|---|---|
| High Responders Group | 10 | 343 | S1-S4 | 59 | 19 |
| | 11 | 328 | S1-S2 | 59 | 17 |
| | 1[b] | 313 | S1-S2 | 58 | 16 |
| Moderate Responders Group | 8 | 304 | S1 | 52 | 12 |
| | 7 | 303 | L5-S1 | 50 | 11 |
| Low Responders Group | 2[b] | 297 | L4-L5 | 48 | 11 |
| No Response Group | 3[b] | 292 | L4 | 44 | 10 |
| | 12 | 270 | L3-L4 | 39 | 9 |
| | 9 | 217 | L2 | 38 | 8 |

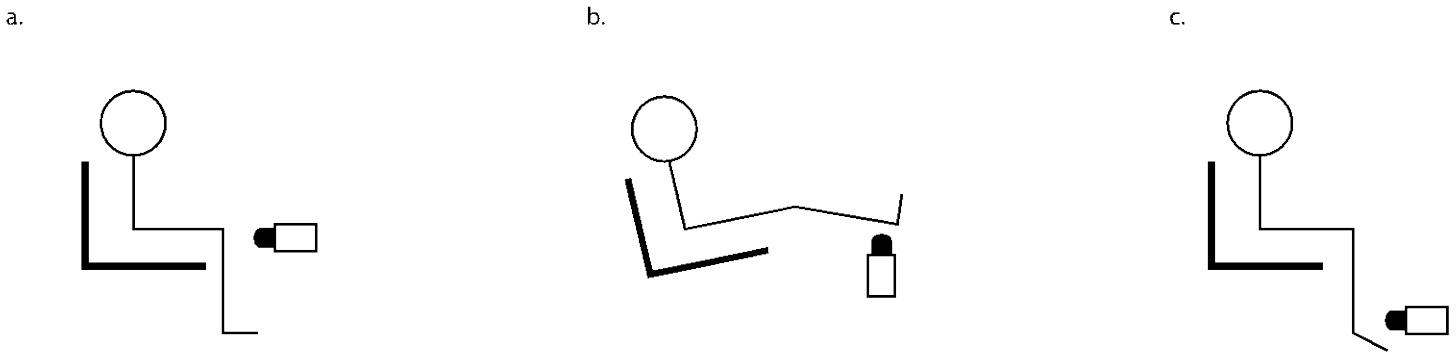



Figure 2. 1. Illustration of infant's position during stimulations.

Inclination of the seat and leg position correspond to the stimulation of quadriceps (a), gastronemies (b), and tibialis anterior (c) muscles.

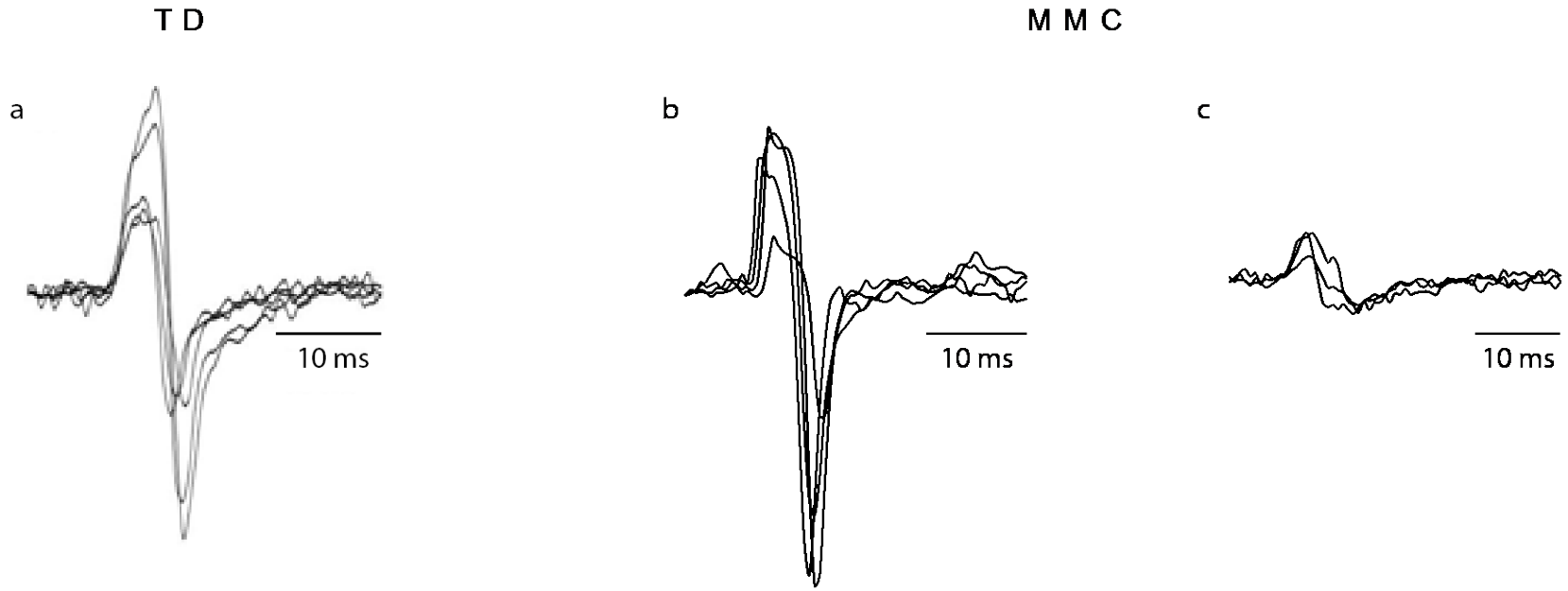



Figure 2. 2. Examples of tendon reflex responses elicited in a stimulated muscle.

The GA muscle of a TD infant in the T-reflex (a) and the QA muscle of an MMC infant in the T-reflex (b) and VIM-T-reflex (c) condition. T-reflex time profiles are similar for TD (a) and MMC (b) infants. Reflex response amplitude is relatively smaller during concomitant vibration stimulation (b vs. c). Figure 2. 2. a is adapted from Teulier et al. 2011.

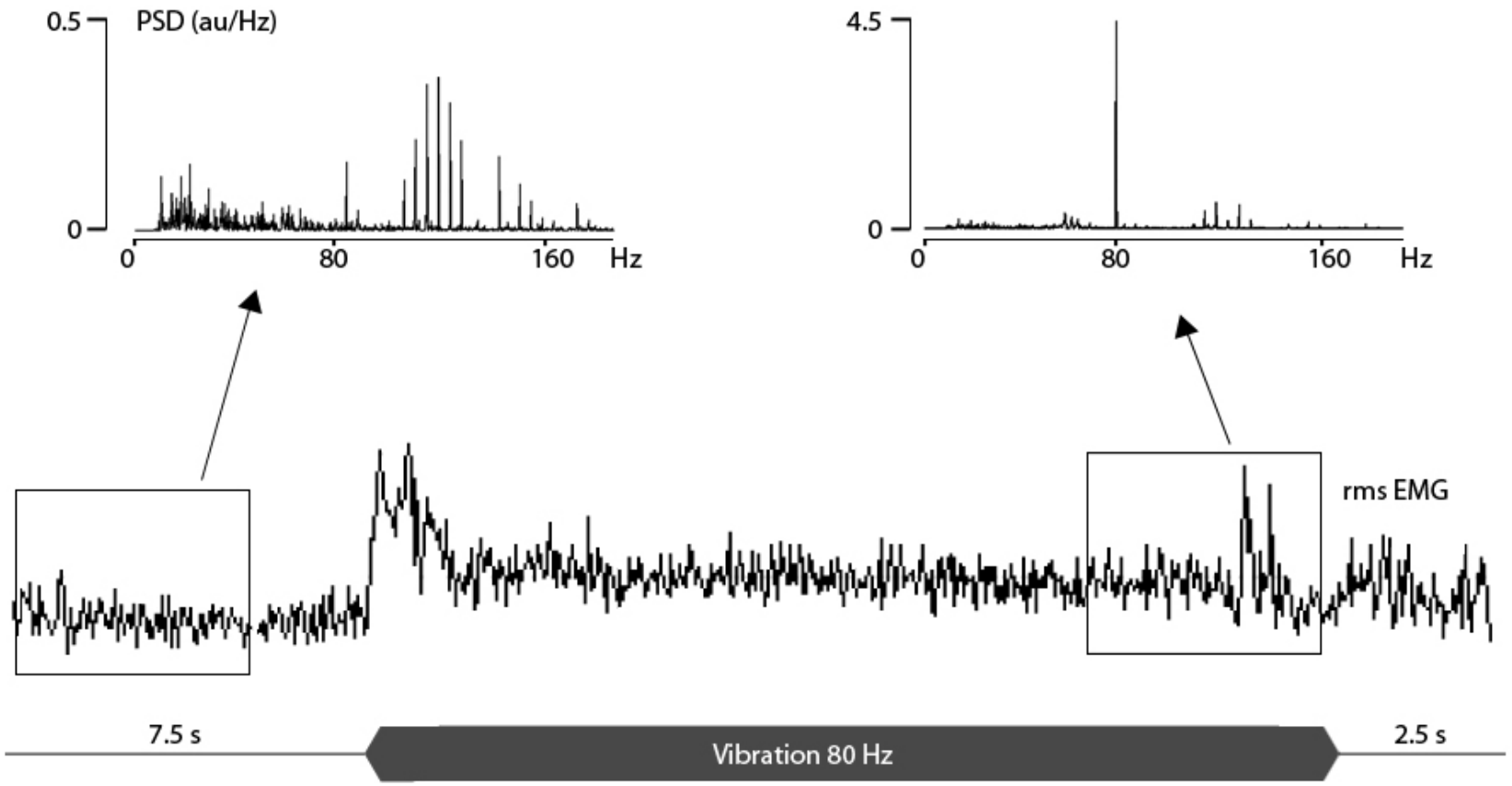



Figure 2. 3. Example of a tonic vibration reflex response obtained from the TA muscle of one MMC infant.

Increase of the EMG activity is observed in the stimulated muscle during vibration. The power spectra (PSDs) obtained from the raw EMG signal illustrate the synchronized muscle activity with vibration frequency (80Hz).

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## Chapter III

# SPINAL-LEVEL RESPONSES IN LEG MUSCLES OF NEWLY WALKING TODDLERS


## Abstract

Spinal-level responses in leg muscles during the first year after birth differ in significant ways from those produced by healthy young adults. Infants demonstrated quite variable reflex responses with reciprocal excitation and irradiation to antagonist and distal muscles. Here we asked, when and why do infants' reflex responses resolve to the consistency seen in adults? We assessed the potential role of walking experience in improving the stability of spinal-level reflexes in three primary gait muscles. Reflex responses in multiple Ia pathways - Tendon reflex (T-reflex), vibration-induced inhibition of T-reflex (VIM-T-reflex), and tonic vibration-induced reflex (VIR)- were elicited by mechanical stimuli applied to the distal tendons of gait muscles in 22 infants, aged 9-16 months. Walking skills were collected. Generally, the impact of walking experience seems to be slowly emerging and, depending on muscles tested and pathway types, developmental progress can be quite different. Amplitude and latency of reflex responses were more clearly impacted by age or leg length, while the ratio or distribution pattern of the reflex response was impacted by walking experiences. As walking experience increased, the ratio of reflex responses was increased for the agonist and decreased for the antagonist reflex loops and the distribution of the pattern shifted from multiple types of responses toward a single type of

reflex response in all tested muscles. A description of reflex reposes associated with the multiple Ia pathways provides a better understanding of reflex mechanisms organization in core gait muscles.

## Introduction and Purpose

Significant effort has been devoted to mapping, in adult humans, the spinal-level reflex responses to specific types of stimuli, in order to describe what is typical and ultimately test the integrity of the peripheral neuromotor mechanisms involved in controlling movement and adapting to perturbations (Hultborn, Meunier, Pierrot-Deseilligny, & Shindo, 1987; Katz, Meunier, & Pierrot-Deseilligny, 1988; Lacquaniti, Borghese, & Carrozzo, 1991; Morita et al., 1995). Most commonly reflex testing involves recording the muscle activity (via electromyographic [EMG] recordings) that occurs in homonymous and heteronymous muscles in response to mechanical stimulation (e.g., via a percussive tap or vibration) applied to the muscle tendon, thus activating sensory receptors with mono- or poly-synaptic pathways. The monosynaptic reflex response is evoked by tapping the tendon, creating dynamic stretch that activates muscle spindle primary endings and elicits a prolonged discharge of Ia-afferents. When vibration stimulation is applied to the muscle tendon and is sustained for more than 10-20s, several interneurons are integrated in the signaling pathways and this polysynaptic pathway induces reflexive contractions in the stimulated muscle (TVR) and/or the antagonist muscle (AVR)(Person & Kozhina, 1992). The monosynaptic pathway is also regulated peripherally by an autogenic presynaptic inhibitory mechanism involving depolarization of Ia afferents, when tonic vibration is applied to the tendon of the examined muscle (Lance, 1968; Romaiguere, Vedel, Azulay, & Pagni, 1991; Schieppati, 1987).

Testing the integrity of the peripheral neuromotor mechanisms requires use of more than one stimulus type because each evokes different pathways; that is, Ia-pathways are modulated by context (Desmedt, 1978; Roll, Gilhodes, & Tardy-Gervet, 1980; Roll, Martin, Gauthier, & Mussa Ivaldi, 1980). Researchers have demonstrated in adults, for example, that the tendon tap (T-reflex), a monosynaptic reflex, invokes the peripheral mechanisms involved in compensating for perturbations, muscle stiffness regulation during motion, and balance control. The presynaptic inhibition test evokes the peripheral mechanisms that control the gain of the monosynaptic reflex and decreased presynaptic inhibition causing exaggeration of stretch reflex is present in most spastic patients (Ashby, Verrier, & Lightfoot, 1974; Faist, Mazevet, Dietz, & Pierrot-Deseilligny, 1994; Nielsen, Petersen, & Crone, 1995). The la-polysynaptic pathways provide assistance to motor responses (Feldman & Latash, 1982; Roll, Martin, et al., 1980) and locomotor-like leg movements (Gurfinkel, Levik, Kazennikov, & Selionov, 1998), and maintain congruence between the desired motor outcomes and the perceptions during postural perturbation (Calvin-Figuiere, Romaiguere, Gilhodes, & Roll, 1999; Kavounoudias, Gilhodes, Roll, & Roll, 1999) via polysynaptic reflex modulation in which role of facilitory or inhibitory can be reversed as a function of context (Feldman & Latash, 1982).

To date, much less research has focused on the development of these spinal level reflexes; most of the focus has been directed toward the T-reflex. Perhaps this is because it is the one used most often in standard clinical neurological assessments and it is the easiest to administer. It is clear, however, that T-reflex responses during the first year after birth differ in significant ways from those produced by healthy young adults. When tendon tap stimuli are applied to the muscle tendons in the lower limbs of infants, the response is disperse activation including the agonist (stimulated), antagonist (reciprocal excitation), and distal muscles (reflex

irradiation). Further, the pattern of excitation over multiple stimulation trials is quite variable, compared to that of healthy adults, sometimes all muscles respond, sometimes none, and sometimes agonist or antagonist (B. M. Myklebust, 1990; B. M. Myklebust & Gottlieb, 1993; Teulier, Ulrich, & Martin, 2010). Reciprocal excitation and reflex irradiation have been attributed to unstructured supraspinal inhibition and to excitatory projections that outweigh inhibition (Leonard, Matsumoto, & Diedrich, 1995; B. M. Myklebust, Gottlieb, & Agarwal, 1986; O'Sullivan, Eyre, & Miller, 1991). Variability in T-reflex responses overall has been attributed to incomplete myelination of the spinal cord and peripheral nerves (Leonard & Hirschfeld, 1995; Leonard, Hirschfeld, Moritani, & Forssberg, 1991).

The gap in our knowledge between infancy and the preschool years is large. Several studies have focused on three to four-year olds (B. M. Myklebust, 1990; O'Sullivan et al., 1991; O'Sullivan et al., 1998). Gradual reduction in reciprocal excitation and reflex irradiation is observed in infants with typical development over developmental time, particularly when the children acquire functional motor skills. Based on these results, Leonard et al, (1995) concluded that experience with upright locomotion was critical to resolving the dispersion of spinal reflex responses for the lower limb. Myklebust (1986) suggested that Ia excitatory projections become restricted during the early years of life. However, the time course of improvements in response, even for the monosynaptic reflex, seems complex. For example, Myklebust (1990) reported the case that reciprocal excitation/irradiation was not found in children age 1 year, but Leonard et al (1995) reported evidence for reciprocal excitation/irradiation in children up to 6 years of age. The fact that walk onset occurs at a range of ages notwithstanding, if Leonard's hypothesis is correct, upright locomotion onset alone may not be sufficient to drive mature reflex behavior.

Based on review of monosynaptic reflex literature, Myklebust (1990) recommended that more efforts should be devoted to studying the time course of development in reflex responses.

Given the gaps in our knowledge of early development of the more simple monosynaptic spinal reflexes, it is not surprising that even less has been documented concerning the emergence of polysynaptic reflexes. Our research team began recently to map the development of polysynaptic reflex responses in the lower limb- the vibration-induced modulation of the T-reflex (VIM-T-reflex) and the tonic vibration-induced reflex (VIR), along with the monosynaptic tendon reflex (Teulier et al., 2011). We studied the gastrocnemius-soleus (GS), tibialis anterior (TA) and quadriceps (QAD) muscles in healthy infants ages 2 through 10 months. Tendon taps alone elicited responses in either, both, or neither of the agonist/antagonist pairs. The homonymous response of TA in T-reflex was elicited a lower percentage of time than it was in the GS or QAD. And when vibration was superimposed on tendon taps the result was primarily inhibition of the monosynaptic response although facilitation also occurred in either the antagonist or agonist. Vibration alone produced a tonic reflex response in the vibrated muscle and/or the antagonist muscle. Of all the muscles and conditions tested, only the TVR for the TA muscle showed a significant improvement with age. These results suggest that although this period of life is one in which neural development is generally progressing very rapidly increases in processing speed and myelination, while perhaps contributing to the improvement that will ultimately emerge, there may be other factors that drive the functional tuning of these neuromotor responses.

To invoke the neural plasticity, the rapidly changing nervous system requires periods of massive amounts of practice (Johnson, 2001; Karmiloff-Smith, 2009; Stiles, 2008). New walkers, for example, practice stepping many times daily (up to 14,000 steps; Adolph et al.,

2012; Adolph, Vereijken, & Shrout, 2003). With practice, many gait parameters show rapid improvements, such as stride length, step width, and consistency of interlimb phasing (Black, Chang, Kubo, Holt, & Ulrich, 2009; Grimshaw, Marques-Bruna, Salo, & Messenger, 1998; Hallemans, Clercq, & Aerts, 2006). Yet for months after walking onset toddlers continue to misstep and fall frequently (17 falls per hour; Adolph et al., 2012; Joh & Adolph, 2006). Underlying this new neuromotor skill is gradual improvement in organization and activation of the primary gait muscles. Chang & colleagues (2006, 2009) showed that after one month of walking, infants' muscle activations were arrhythmic and highly inconsistent; toddlers used many combinations of muscle activations to produce the net effect of flexion and extension. Three to four months of practice were needed for muscles to settle into recognizable patterns of excitation, with the gastrocnemius-soleus group showing rhythmicity first, followed by the quadriceps, hamstrings, and tibialis anterior (Chang et al., 2006).

We propose that the sequential improvements observed for gait in new walkers indicates that the global behavioral characteristics stabilize first (i.e., functional convergence of limbs, joints, muscles into step production), with resolution of the deeper levels- stride lengths, step widths, joint coordination patterns, and muscle sequencing- resolving in gradual order. Further, we propose that the neuromotor capacity to fine tune these muscle activations in response to perturbations, to maintain appropriate levels of stiffness as function occurs in the dynamic environment, is the last to resolve and require more time and much practice to emerge.

Thus, our purpose in this study was to determine possible influences of locomotion on functioning of Ia-mediated reflex pathways. Standardized stimuli were delivered to elicit the T-reflex, VIM-T-reflex, and VIR across primary walking muscles of both sides. Responses recorded simultaneously in antagonistic muscle pairs were quantified in ratios, amplitude,

latency, and distribution of response patterns. We hypothesized that organization of proprioceptive feedback loops would be stabilized over the first 3 months independent walking experience. Experienced walkers have higher ratios of reflex responses in multiple Ia pathways compared to novice walkers. Over walking experiences, the distribution of the pattern would shift from multiple types of responses toward a single type of reflex response in all tested muscles. A description of reflex reposes associated with the multiple Ia pathways provides a better understanding of reflex mechanisms organization in core gait muscles and possibly determines the neural plasticity in associated with neuromotor skills (e.g., walking).

## Method

### Participants

Twenty-two cruisers and toddlers with typical development ranging in age from 9 to 16 months participated in the study. We recruited all participants through flyers and word-of-mouth communication in the S.E. Michigan area. Participants were White (n = 21) and other/Unidentified (n = 1); all were born full term without known cognitive, sensory, or motor impairments. Approval for the experimental protocol of this study was granted through the Institutional Review Board of the University of Michigan. Prior to participation, written consent was obtained from the parents. Each family received a small monetary gift for participating in this study. Participants' age, gender, and anthropometric characteristics are summarized in Table 1.

**Classification of participants.** We chose cruisers and toddlers who had from 0 to 3 months of walking experience as our target participants. Toddlers continuously and rapidly develop their walking pattern over the first 3 months after their initial independent steps (Clark, Whitall, & Phillips, 1988). The first 3 months of walking practice are an important period of

rapid transition into adult-like patterns and can lead to dramatic changes in gait patterns. We hypothesized that reflex behavior processes undergo rapid and continuous development during the first 3 months of walking experience. By studying gait development during this period immediately before and after the acquisition of independent locomotion, it was possible to determine influences of locomotion on functioning of Ia-mediated reflex pathways.

The cruisers and new walkers were recruited by walking experience, with 4 groups: cruisers, 1 month walkers, 2 month walkers, and 3 month walkers. To be included, in the cruiser group, infant had to be able to cruise continuously along the furniture at least 2 meter with their bodies oriented frontward. If babies showed excessive reliance on any part of the furniture or required two hands to maintain balance throughout cruising, they were excluded. For walker groups, we recruited parents prior to their baby's walk onset and remained in weekly contact or ask them to call/email when their child took first independent steps. Researchers randomly assigned target month of walking experience (1mo, 2mo, and 3mo) and scheduled infants to be tested at the lab when child achieved that amount of walking experience.

**Procedures**

For each infant we collected both neurological reflex assessment and gait parameters during the same visit to our lab (Developmental Neuromotor Control Lab). The detailed procedures we followed for spinal reflex assessment were identical to those published previously (Lee, Teulier, Ulrich, & Martin, 2013; Teulier et al., 2011) thus, we present a condensed description of our protocol here.

**Spinal-reflex assessment.** All participants were seated in our custom-made chair, which we designed to allow adjustments that accommodate each participant's unique leg length, pelvis and trunk size, and the optimal leg posture for eliciting spinal-level lower limb reflexes for each

tendon. The inclination angle of the seat-back support and leg position were changed for each tendon (see Figure 1). We prepared participants for testing by removing their clothing from the waist down, except the diaper, which was loosened at the waist to avoid undue pressure on pelvis and abdomen. In order to elicit optimal reflex responses, participants' legs needed to be relaxed. Therefore, we only initiated trials when their arousal states were quiet and alert; arm could be moving but not the test leg. Cruisers and toddlers were not tested if they fell asleep or cried because their emotional state would mask the stimulation response. In addition, all reflex testing was conducted in a warm and quiet sound attenuated room to reduce environmentally induced response variations.

We used an electromagnetic stimulator (Ling Dynamic V203) equipped with a smooth probe (hammer head), to elicit the monosynaptic tendon reflex (T-reflex), the vibration-induced modulation of the tendon reflex (VIM-T-reflex), and the tonic vibration-induced reflex (VIR). We stimulated the distal tendons of three muscles on both sides: gastrocnemius-soleus (GS), tibialis anterior (TA), and quadriceps (QAD). All mechanical stimuli, including tendon tap (5 ms pulse), vibration (80 Hz sine wave), and the tendon tap superimposed on vibration were generated by signals from our custom-designed Labview$^{TM}$ virtual instrument (National Instruments Corp., Austin, Texas). Via a power amplifier, each signal was transmitted to the probe. The magnitude of stimulation required by each child was adjusted to their sensitivity levels before the experimental session; once a participant adjusted to the threshold level of stimulation intensity, we increased it approximately 1.2 times from the response threshold level and maintained that level throughout all sections. While each muscle might have a different threshold level of magnitude, most cruisers and toddlers tested had a similar magnitude among the muscles tested and all tested babies.

To record muscle activations in response to tendon stimulations, preamplified pediatric bipolar electrodes (Noraxon$^{TM}$ 272, 1.7cm center to center distance – Noraxon USA Inc., Scottsdale, Arizona) were placed on the belly of four muscles of both legs: GS, TA, QAD, and Hamstring (HA). To monitor the potential cross-talk issues across the leg, all four major muscles on the side of the leg being tested were assessed simultaneously.

Muscles were tested in random order. To avoid the potential for a long lasting post-vibration effect, within the muscle stimulus order was always T-reflex, VIM-T-reflex, and VIR (Roll, Martin, et al., 1980). For the T-reflex and VIM-T-reflex, 20 taps were delivered to the distal tendon of the stimulated muscles with a minimum inter-tap interval of 8s. VIM-T-reflex taps were delivered during a concurrent vibratory stimulation to the same tendon; muscle activation due to reflex stimulus (T-reflex and VIM-T-reflex) was monitored during the 0.5 s recording, the onset of which was triggered by the stimulation. To elicit VIR, vibration was applied during 2 trials; each trial began with 5s of rest, followed by 20s stimulation, then another 5s rest. The initial rest period was used as baseline for subsequent muscle activity. EMG was monitored throughout the 30s VIR trial.

**Gait assessment.** We recorded spatial and temporal gait parameters via a GAITRite® system (CIR systems Inc., Sparta, New Jersey). Consistent with reflex testing, infants were tested wearing only a diaper. Participants walked barefoot across our GAITRite mat (3.66m × 0.61m) laid over the floor. The GAITRite mat is pressure-sensitive and registers the location and time of each footfall transmitted to our laptop at 80Hz. For cruisers, we provided low walls (boxes) that spanned the length of the entire walkway, so they were able to assess a minimum amount of postural support by touching the top of the wall their hands while cruising (see Figure 2. a). For walker groups, support walls were not provided (see Figure 2. b). Before test session

commenced, skill levels were confirmed via one or two practice trails. A parent and investigator stood at opposite ends of the GAITRite mat and encouraged the child to cruise or walk the entire length of the mat. All participants completed at least 5 consecutive trials. Participants were asked to repeat any trial that had less than 4 steps on the walking or if they paused more than 1s. All trials were also recorded via a video camera placed at walk onset of the walkway, to allow us to confirm GAITRite data mapped onto behavior observed.

**Data Reduction**

**Reflex responses.** Using custom-written Labview$^{TM}$ virtual instrument programs (detailed in Teulier et al., 2011), all raw EMG data were examined to assess the peak-to-peak amplitude, and latency to the first peak of muscle activation, for each individual stimulus presentation. Responses were summarized for each condition and muscle obtained by the ratio of responses relative to the number of stimuli presented, the average amplitude, and the average latency of responses. Differences in response magnitude between T-reflex and VIM-T-reflex were computed to determine the inhibitory effect resulting from vibration exposure. In addition, the distribution of response patterns for each muscle stimulated and its antagonist muscle pair were examined to identify reciprocal radiation: a response in the stimulated muscle alone (Ag), a response in the antagonist muscle alone (An), a simultaneous response in both muscles (S), and no response at all (N).

**Gait parameters.** To quantify spatiotemporal gait parameters, GAITRite software (version 3.8) was used to quantify toddlers' footfalls over each trial. All cruising or walking trials were required to have at least five consecutive footfalls without gait disruptions; if participants stepped with one or both feet outside the active sensor section of the gait mat or stopped for longer than 1s, the trial was excluded from analysis. In addition, the first and last two

steps (when babies were speeding up to walk or slowing down to stop) were removed prior to analysis. Mean values for cadence (steps/min), step length (in centimeters), stance phase (in percentage of gait cycle), and double-support phase (in percentage of gait cycle) were calculated for each trial, for each child, via GAITRite software. To control for differences in the infants' leg length, the step length was normalized by each infant's leg length, and cadence was normalized by using the following formula (Hof, 1996):

$$Normalized\ cadence = \frac{cadence(steps\ per\ minute)}{\sqrt{gravity / leg\ length}}$$

**Data Analysis**

To check for normal distribution of dependent variables, the Kolmogorov-Smirnov test was applied (SPSS version 17.0, SPSS Inc., Chicago, Illinois). While the dataset of response amplitudes and latencies were normally distributed, the majority of response ratios and distribution pattern variables were not a normally distributed (all *p*s < .05). Therefore, when normality of distribution was violated we applied non-parametric statistic tests; otherwise we used parametrics. For quality of reflex response data (amplitude/latency), analyses of variance (ANOVA) were conducted to test (a) the main effects of walking experience on amplitude and latency of T-reflex and VIM-T-reflex and (b) the main effects associated with muscles (QAD, TA, and GS) and vibration (no vibration, vibration) on amplitude and latency of T-reflex and VIM-T-reflex responses. Post hoc tests (Tukey Honestly Significant Differences-HSD- for multiple comparisons) were used to compare response amplitudes/latencies among muscles and months of walking experiences. Repeated-measures ANOVAs were applied to test for significant differences in amplitude and latency of T-reflex and VIM-T-reflex responses between antagonistic muscle pairs (QAD/HA, TA/GS, GS/TA). Pearson correlations were applied to

examine relations between age, leg length, and amplitude and latency of T-reflex and VIM-T-reflex responses.

Because response ratio datasets and the distribution of response patterns were non-normally distributed (determined via a Kolmogorov-Smirnov test (all *p*s < .05)), the Kruskal-Wallis test was used to test for the main effect of walking experience on ratios and patterns of responses (e.g., Ag, S) for each muscle tested. Post hoc tests (Mann-Whitney tests –non parametric) were applied to compare changes among responses ratios and distribution patterns across levels of walking experience. Wilcoxon signed-rank test was used to analyze vibration effects on simultaneous response (S pattern) between T-reflex and VIM-T-reflex. The Kendall's tau was conducted to find (a) association between response ratios and walking skill (e.g., normalized step length) and (b) correlation between distribution of response pattern and walking skill. For gait parameters, ANOVAs were conducted to test for differences by walking skill (means and variability) across the developmental range. The significance level was 0.05 (2-tailed).

## Results

### Changes in Quality of Responses - Amplitude and Latency

As illustrated in Figure 3, across all levels of walking experience, toddlers generally showed higher amplitudes of response to the T-reflex than the VIM-T-reflex in the stimulated muscle. In addition, as months of walking increased toddlers showed less variation in the pattern of amplitude and latency and increased consistency in the quality of responses following T-reflex and VIM-T-reflex stimuli, compared with those of novice walkers. On the contrary, cruisers tended to show an amplitude of response to the T-reflex that was similar to that of VIM-T-reflex and the quality of responses was not consistent following tendon stimuli.

A series of one-way ANOVAs indicated that the main effects of walking experiences had no statistically significant effect on amplitude and latency of T-reflex and VIM-T-reflex responses (all *p*s > .05). For variability change (standard deviation) over walking experience, more experienced toddlers tended to produce less variable amplitude and latency than less experienced infants. One-way ANOVAs showed that the main effects of walking experience significantly reduced the variability of amplitude in stimulated muscle following tendon stimuli to GS ($F(3, 29) = 3.582$, $p = .03$). However, main effects for walking experiences on variability of amplitude and latency in T-reflex and VIM-T-reflex responses of QAD and TA did not reach statistical significance (all *p*s > .05).

Changes in amplitude and latency data showed relationship with changes in age and leg length. Pearson correlations revealed that the main effects of age and leg length were significantly related to amplitude and latency of the response to the mechanical stimuli. Age was significantly related to latencies following the tendon tap stimuli to GS and TA. As age increased, toddlers reduced response latencies for GS (agonist: $r(36) = -.42$, $p = .009$) and antagonistic muscle pairs in TA (agonist: $r(33) = -.35$, $p = .038$, antagonist: $r(31) = .-35$, $p = .045$). Leg length was significantly related to amplitude of QAD activation following the T-reflex elicitation and to latencies of QAD and TA activation following VIM-T-reflex stimuli. When the participant's leg length was longer, response amplitude increased following the tendon stimuli to QAD antagonist ($r(34) = .39$, $p = .019$) and reduced response latencies for stimulated muscle of QAD and TA following VIM-T-reflex (QAD: $r(36) = -.44$, $p = .006$, TA: $r(27) = -.368$, $p = .049$).

**Differences in amplitude and latency among three muscles tested (QAD vs. TA vs. GS).** One-way ANOVAs indicated that among the muscles tested, there are significant

differences in amplitude ($F(2, 111) = 5.64$, $p = .005$ for T-reflex, $F(2, 101) = 3.38$, $p = .038$ for VIM-T-reflex) and latency ($F(2, 111) = 14.76$, $p = .000$ for T-reflex, $F(2, 101) = 20.57$, $p = .000$ for VIM-T-reflex).

**T-reflex.**

***amplitude.*** For the T-reflex, mean response amplitude for each stimulated (agonist) muscle was 618.51mv(QAD), 541.86mv(GS), and 350.75mv(TA). The amplitude was significantly larger for QAD than TA (Tukey HSD, $p = .004$), but GS was not significant different from the other two amplitudes, all $p$s >.05. Repeated-measures ANOVAs were applied to test for significant differences of amplitude in each agonist/antagonist muscle pair (QAD/HA, TA/GS, and GS/TA). Stimulated muscles showed larger amplitude than their antagonist muscle for the QAD/HA (antagonist = 512.00mv, $t(35) = 4.255$, $p = .000$), the GS/TA (antagonist = 392.96mv, $t(32) = 3.957$, $p = .000$), and for the TA/GS (antagonist = 334.95mv, $t(30) = -3.07$, $p = .005$).

***latency.*** In T-reflex, the mean reflex response latency for each stimulated muscle was 16.86ms (QAD), 21.8ms(GS), and 23.32ms(TA). As might be expected due to the distance from the spinal cord, the latency was significantly shorter for the QAD than GS and TA (Tukey HSD, all $p$s < .000); no significant difference in latency occurred between the GS and TA, $p$ >.05. Repeated-measures ANOVAs were applied to test for significant differences in latency for each agonist/antagonist muscle pair (QAD/HA, TA/GS, and GS/TA). Results showed that stimulated muscles produced shorter latencies than their antagonist muscle for the QAD and GS, but no significant difference was observed for the TA. For the muscle pair of QAD/HA ($t(35) = -3.50$, $p = .001$), latency was significantly shorter in QAD than in the HA ($M = 19.02$ms). For the GS/TA ($t(32) = -4.33$ $p = .000$), the GS showed significantly shorter latencies than TA ($M = 25.51$ms).

However, for the TA/GS ($t(30) = .24$, $p = .813$), the latency tended to be longer in TA than the GS ($M = 22.90$ms), but the difference was not statistically significant.

**VIM-T-reflex.**

***amplitude.*** In VIM-T-reflex, the mean reflex response amplitude for each stimulated muscle was 512.01mv(QAD), 392.96mv(GS), and 337.73mv(TA). The amplitude was significantly larger in QAD than TA (Tukey HSD, $p = .04$).

***difference in amplitude between T-reflex and VIM-T-reflex.*** Over the walking experience, the effects of vibration tended to show reduced amplitude in VIM-T-reflex (see Figure 3), but due to the variability of responses amplitude, the difference was not statistically significant in muscle response amplitudes among the stimulated muscles (all $p$s > .05).

***latency.*** In VIM-T-reflex condition, the mean latency of muscle responses was 16.19ms(QAD), 20.97ms(GS), and 25.10ms(TA), resulting in a significant difference. Post hoc tests (Tukey HSD) indicated shorter latency in QAD than TA and GS (all $p$s < .001).

**Change in Response - Ratios for Agonist and Antagonist Responses**

Given the high variability of responses ratios among participants and muscles, the ratio results presented from these data will be largely descriptive to show as much of the raw data as possible. We propose that statistical treatment would mask the profiles we observed for toddlers' sensorimotor responses. We mainly observed low and very gradually changing relationships between 1.) walking experience and response ratios following mechanical stimuli and 2.) walking skill and response ratios/ the distribution pattern of reflex response across muscles.

A series of one-way ANOVAs were used initially to confirm significant walking experience differences for each gait parameter (all $p$s < .05). That is, infants showed improvements in their mean values for walking skill over months of walking experience (see

Table 2). Variability of normalized step length (standard deviation) was significantly decreased over walking experience ($F(3, 40) = 3.99$, $p = .01$) while others did not. We chose normalized step length as a predictor of response gain, because among the walking skill, infants demonstrated the most significant change in normalized step length over the walking experiences.

**Response Ratios as a Function of Walking Experience.** Figure 4 illustrates response ratios for individual participants as well as group means as a function of walking experience. High variability of responses was observed across stimulus conditions and muscles tested. Kruskal-Wallis test was used to find main effect of walking experience on ratios for each tested muscle and stimuli.

***T-reflex.*** As walking experience increased, toddlers generally exhibited an increased ratio of T-reflex responses in two of the three stimulated muscles, and a decrease in two of the three their antagonists. However, for the TA, the antagonistic muscle (GS) showed an increase ratio of responses rather than a decrease ($H(3) = 9.56$, $p = .02$).

***VIM-T-reflex.*** When tendon taps were administered during an ongoing vibratory stimulus, the result could be either inhibition or facilitation of the T-reflex response. Figure 4. b suggests a decrease in responses for the stimulated muscles for two of the three muscles, with experience walking and a decrease for all three compared to the T-reflex alone (Figure 4 a). Antagonist muscles activations also tended to decease with experience and to be lower than in T-reflex condition.

***VIR.*** The vibration induced contractions, TVR (agonist) or AVR (antagonist) or both, in antagonistic muscle pairs in each tested muscle. Ratio of TVR tended to be decreased in the GS and be increased in the TA ($H(3) = 7.40$, $p = .06$). Following with Mann-Whitney tests for the

TVR of TA, cruisers showed increased responses ratios of TVR compared with those of toddlers with 3mo walking experience ($U = 35.5$, $r = .03$). Ratio of AVR were decreased with the GS and increased with the TA over walking experience. No clear changes were observed in antagonistic muscle pair of QAD.

**Response Ratios as a Function of Walking Skill.** Figure 5 illustrates response ratios for individual participants as well as the slopes, as a function of walking skill was defined by normalized step length. Kendall's tau was conducted to find association between response ratios and normalized step length.

***T-reflex.*** As walking skill increased, two of the three agonist muscles (the QAD and GS) tended to show an increased ratio, while all three antagonist muscles showed small decreases.

***VIM-T-reflex.*** No clear changes of response ratios were observed across stimulated muscles, though they tended to be lower than and not increase with skill level, as did the T-reflex alone. Antagonists exhibited a decreased ratio in the QAD and TA while the GS antagonist showed an increased ratio.

***VIR.*** Agonist and antagonist muscle pairs exhibited an increased ratio as skill increased for the QAD and TA (agonist: $\tau = .245$, $p = .04$); a reverse pattern (decrease) was found in the GS muscle pair (antagonist: $\tau = -.243$, $p = .04$).

**Changes in Distribution among Possible Responses to Agonist and Antagonist**

When toddlers' muscle tendons were stimulated mechanically via T-reflex, VIM-T-reflex, or VIR, the response was seldom the same over the sequence of applications of the stimulus (i.e., only agonist or only antagonist). Rather they exhibited varied combinations of responses, simultaneous responding with both agonist and antagonist, only agonist, only

antagonist, or no response. We calculated the distribution of our participants' response patterns as a percentage of all stimulations, for each of the four different combinations possible.

When plotted in relation to months of walking experience there is no clear relationship with pattern of distribution responses for the T-reflex, VIM-T-reflex, and VIR. Walking skill however, seems more sensitive to changes in the activation distribution patterns. Hence, we illustrate in Figure 6 the relation between walking skill and the distribution of response patterns across all conditions and muscles tested. Again, we used normalized step length to reflect improvement in walking skill. Differences in the distribution of the response pattern were observed with walking skill for all of the muscles tested.

**T-reflex.** Again, variability within/between babies was high for all stimulated muscles. But, as participants showed improved step length, they also demonstrated an increase in the frequency with which the response activation was Ag only (particularly in the QAD ($\tau = .234$, $p = .03$) and GS) and a clear decrease in the frequency of simultaneous response (S pattern) in all tested muscles. That is, while step length was increased, the distribution of the pattern shifted from multiple types of responses to a single type of T-reflex response in all tested muscles.

**VIM-T-reflex.** When vibrations were superimposed on the tendon tap at the QAD, GS, and TA, the distribution patterns were also changed as step length increased. Cruiser/novice walkers used all possible patterns of response in the QAD, TA, and GS, while experienced walkers generally exhibited a less simultaneous response in both antagonistic muscle pairs (S pattern) in all tested muscles. That is, the frequency of concurrent activation of the antagonistic muscle pairs was decreased as a function of walking skill; decrease in reciprocal excitation was mainly observed in the QAD, TA, and GS. Through Wilcoxon signed-rank test, the QAD and GS showed less frequency of simultaneous response during tendon vibration condition compared to

T-reflex condition, suggesting an inhibitory of effects on vibration (QAD: $z$ = -3.93, $p$ = .000, GS: $z$ = -3.51, $p$ = .000).

**VIR.** With improved walking skill, the QAD and TA showed an increase of frequency in Ag pattern while the GS exhibited reverse pattern (a decrease of frequency in Ag pattern). But, the frequency of reciprocal excitation was decreased during mechanical vibration to the GS, but was increased in the QAD and TA. We found a difference in distribution patterns of responses between the T-reflex and the VIR. VIR induced significantly more simultaneous response (S pattern) than those of T-reflex in GS ($z$ = -2.39, $p$ = .01), but did not affect the distribution pattern in the QAD and TA (all $p$s > .05). That is, reciprocal excitation is more frequent for polysynaptic than monosynaptic responses in the GS.

## Discussion

Our data show developmental changes in the integrity of the spinal-level reflex with respect to the walking experience. When taken together, the profile of the set of indicators shows slow, gradual improvement during the first 3 months of the walking experience. Generally, throughout the walking experience, the ratio of reflex responses was increased for the agonist and decreased for the antagonist reflex loops. Moreover, each tested muscle displayed a reduction in the variability in reflex response; the distribution of the pattern shifted from multiple types of responses toward a single type of reflex response in all tested muscles. However, the impact of walking experience seems to be slowly emerging and, depending on muscles tested and pathway types, developmental progress can be quite different. The integrity of the T-reflex tended to be strengthened in QAD and GS as walking experience increased, but TA seems to require more walking practice to be improved and stabilized. The function of presynaptic inhibition and polysynaptic pathways tended to remain unstable even with three months of

walking experiences or improved overt walking skills, and participants showed slower developmental progress than observed for the integrity of the T-reflex, regardless which muscles were tested. Interestingly, the differences presented in some reflex variables showed a clearer relation to general growth than to walking experience. Amplitude and latency, for example, were more clearly impacted by age or leg length, while the ratio or distribution pattern of the reflex response was impacted by gait characteristics.

**Changes in Amplitude and Latency in Relation to Neurophysiologic Characteristics**

Generally, the amplitudes and latencies of reflex responses showed basic neurophysiologically expected characteristics. That is, changes in amplitude and latency were more clearly impacted by general growth, such as chronological age or leg length, than by walking experience or walking skills. In GS and TA, the latency of the T-reflex significantly shortened as age increased. Our finding is in line with the response latency of the T-reflex previously reported by Myklebust (1986). In the GS T-reflex, onset latencies ranged from 25.3 ± 4.5 ms for an 8-month-old child to 16.51 ± 1.0 msec for an 11-month-old child. This interpretation is most likely due to changes in nerve conduction velocity and muscle size over developmental periods. During the first few years of life, the synapses' formation and myelination become a vigorously active process (de Graaf-Peters & Hadders-Algra, 2006). Also, the most striking development of synaptic connections occurs during this period. The myelination of neuronal pathways tends to increase conduction velocity and, thereby, shorten the latency of the T-reflex responses.

Depending on muscle size and the distance of the stretched muscle from the spinal cord, amplitude and the latency of the T-reflex was affected. With regard to muscle size, amplitudes of EMG responses were largest in the largest muscle group (QAD) and smallest in the smallest

muscle group (TA); even when the amplitude was reduced by superimposing the T-reflex elicitation on existing vibration stimulation, the same pattern held. Latency of the T-reflex response reflected the distance from the spinal cord to the site of the tendon; QAD, the most proximal muscle, had shorter latencies than TA and GS, the more distal muscles. Anatomically, the distance between the site of the tendon stimulated and the spinal level for each muscle are different. The QAD innervation is at L2-L4 spinal levels, while TA and GS are derived from the L4-L5 and S1-S2 spinal levels, respectively (Eccles & Lundberg, 1958). That is, QAD is closest to its spinal origin among the three gait muscles that we tested. The tendons of GS and TA are the same distance from the spine, but GS's connection (spinal circuit) is closer to the muscle than TA's connection: the TA travels to a higher level L4-L5 than GS, which travels to S1–S2.

In antagonistic muscle pairs, the latency of the reflex response showed shorter latency for the stimulated muscles than its corresponding antagonist muscles (QAD/HA and GS/TA, but not TA/GS). A similar finding was reported by Teulier e al. (2010). They found that for the T-reflex responses of the antagonist muscle pairs (QAD/HA and GS/TA), infants between the ages of 2-10 months showed shorter latency for the stimulated muscles (QAD and GS) than their antagonist muscles (HA and TA), but TA (stimulated)/GS (antagonist) showed a reversed pattern. It seems that toddlers still showed similar reflex response patterns with those of infants, aged 2-10 months old, although our participants achieved independent walking and showed steady improvement in gait parameters. It is not clear why the TA (stimulated)/GS (antagonist) condition showed different latency patterns unlike with QAD/HA or GS/TA; it is also unclear as to why this trend held even by toddlers who exhibit the acquisition of upright locomotion. Along with the explanation from the Teulier et al. study, we believe that the role of the oligosynaptic pathways (Lacquaniti et al., 1991) and the antigravity role of the GS, requiring different

heteronymous projections (Meunier, Pierrot-Deseilligny, & Simonetta, 1993) may contribute to this trend.

**Ratio of Reflex Response in Relation to Walking Experience/Walking Skills**

Regardless of months of walking experience, the ratios of T-reflex responses in QAD and GS were always higher than those of TA. That is, the circuit of the spinal-reflex in QAD and GS development as toddlers show proficient walking skills, but the function of TA shows slower development. Our data are in line with the results from infants aged 2-10 months old, reported by Teulier et al. (2011): the ratio of the T-reflex in QAD and GS were demonstrated to be higher than that of TA. In core gait muscles, the muscle activation timing of the GS while babies step on a treadmill even at one month of age is similar to that of adult walking (Teulier, Sansom, Muraszko, & Ulrich, 2012). They concluded that the fetus discovers and utilizes the function of GS (plantar flexion) by pushing against the uterine wall to aid in repositioning. Further, early childhood locomotion, such as creeping and crawling, may enhance the strength of the GS neuromuscular circuit. Perhaps for the same reason, this base of GS development helped create similar changes, in which the integrity of the T-reflex in QAD and GS emerged earlier than TA over the first 3 months of walking practice: the gain of the spinal circuit for TA is not yet stereotypic, even though acquisition of independent walking has occurred. We hypothesize that when the toddler acquires increased balance that enables more fine-turning of ankle movement (such as heel-strike movement present in adults) during walking, the integrity of the TA T-reflex may also be strengthened. However, we cannot overlook the fact that in adults, the TA has an extremely high threshold, resulting in lower ratios of myotatic reflex (Gottlieb & Agarwal, 1979; B. M. Myklebust, Gottlieb, Penn, & Agarwal, 1982).

**Faster Improvement at Behavior Level and Slower Changes at Muscular Level**

Our results showed that the impact of walking experience seems to be very slowly emerging in the development of the spinal-level reflex. We expected that the first 3 months of walking (when infants acquire independent walking and practice it) is an incredibly critical period to improve the integrity of the spinal-level reflex as they improve their walking skills, such as increased step length. There are the predicted trends of the improved integrity of the spinal-level reflex, but the amount of change does not bring the neural responses very close to the level of 4–6-year-olds or adults (Leonard & Hirschfeld, 1995; B. M. Myklebust, 1990; B. M. Myklebust et al., 1982). Adolph and colleagues (2012) report that after the onset of walking, 12- to 19-month-old toddlers, on average, step 2368 times per hour, so that by six hours of play (half of infants' walking day), they step approximately 14,000 times and travel the length of 46 football fields. However, they also continue to fall 17 times per hour and incur more than 100 falls daily. That is, toddlers' overt gait skills improve with walking practice, but their underlying control system (such as muscle activation) still struggles to settle into a consistent unerring walking pattern for generating movement through space. Therefore, during the first 3 months of independent walking, the function of their spinal-reflex also showed great variability.

Kinematic analyses of independent walking show quite clearly that significant changes occur over the first 3 months of walking experience. However, the organization at the muscular level lags with the improvement seen at the kinematic level. This means that stability arises first at the kinematic level and gait characteristics at the behavioral level, and then subsequently at the nervous and muscular level, via a significant amount of experience to achieve a stable pattern. That is, muscle activation patterns may be one of the slowest systems to stabilize. Generally, three months of walking practice induced significant changes in the spatiotemporal gait

parameters in terms of step length, step width, and stance phase (Adolph et al., 2003; Bril & Breniere, 1992; Chang et al., 2006). The interlimb phasing (Clark et al., 1988) and thigh, shank, and foot segmental angle trajectories achieved adult-like patterns by the first few months of practice (Ivanenko, Dominici, Cappellini, & Lacquaniti, 2005). Unlike with the improvement of walking skills, muscle activation in major gait muscles (e.g., quadriceps) is only slowly settling into some level of stability over the walking practice. Chang and colleagues (2006; 2009) reported that after one month of walking experience, only gastrocnemius showed rhythmic muscle bursts, and over the first 3 months of walking practice, reduced co-contractions and changes in combinations of concurrent muscle activations were observed. At a minimum of six months of walking practice, toddlers showed efficient synergy among muscles, allowing for a rhythmic and stable muscle activation pattern at a specific timing during walking. The interpretation is most likely due to the enormous degrees of freedom at the level of the muscles, to resolve synergies in muscle activation.

Like with the results from the Chang et al. study, we determined that with the first 3 months of walking experience, walking parameters tend to stabilize more quickly than neural organization (spinal-level reflex integrity). We could not observe dramatic changes in the ratios of T-reflex, VIM-T-reflex, and VIR responses. However, we did observe reduced reciprocal excitation (co-contractions) through the distribution of response pattern analysis. Although the statistical power of this analysis was lower and reduction of reciprocal excitation did not reach significance, the slow change of the reflex behavior showed a specific trend: while walking skills improved, the distribution of the pattern shifted from multiple types of responses to a single type of T-reflex/VIM-T-reflex responses in all tested muscles. Followed by the slow developmental progress known to emerge at the muscular level, we propose that longitudinal studies of the

spinal-level reflex development over at least six to 12 months of walking practice, a more clear and dramatic reduced reciprocal excitation could be seen.

**Possible Underlying Mechanisms**

The current study provides the first data on development of the integrity of spinal-level reflexes in relation with age, walking experience, and walking skills—step length, cadence, stance phase, and double-support phase. Historically, many researchers have measured the function of myotatic reflex—the ratio of reflex response over chronological age (though mostly older ages) rather than over walking experience or walking skills. We found that, elicited neuromuscular responses have many complex components—ratio, amplitude, and latency of reflex responses, thus development of patterns of reflex behavior need to be observed in multiple ways. For example, amplitude and latency of reflex responses may be more affected by age rather than walking skill level. However, age alone is not sufficient. Ratios or distribution patterns of reflex responses seem to be more affected by amount of walking experience or walking skill level.

We believe that unique differences among individuals actual amount of daily practice might affect their rate of neural development. The amount of walking experience is calculated as the number of days (or months) between the first day of walking and the day (month) of testing. However, toddlers who have the same months of walking experience are not guaranteed to have the same walking skills. Novice walkers do not walk every day after acquiring independent walking, so the amount of their walking experience is a mixture of both walking days and days without walking. Furthermore, each toddler may have accumulated a different number of steps per day, causing some toddlers to show faster rates of improvement in walking. Therefore, we

suggest that based on our data, observation over walking skill is a finely discriminate continuum of individual differences in developmental reflex behavior.

**Limitation**

In our categorical variables, experience was represented as months of walk onset. Months of walk onset seem to be a less precise organizational framework than that of chorological age/walking experience in days, which is finely discriminated in a continuum of individual differences. To take account of difference in individuals' actual amount of daily practice, better would be to report number of steps per day per children. We applied relatively strict rules to recruit our participants into four distinct groups as levels of walking experiences, but parental reports for walk onset may induced imprecision in classification. Less accurate days on walk onset may cause an overlap in participants' walking skills among groups. Therefore, in some of the gait characteristics, walking groups were not clearly representative of children's walking skills. As a result, some children showed proficient walking skills with less practice time, while more practiced children showed less proficient walking skills. For example, children in the 2 month walking group demonstrated longer step length compared with those of children in 3 month walking group. With only five to seven participants per group and less clear divisions on the amount of walking experience (in months), it was not easy to find the relationship between the walking experience and the integrity of the spinal-level reflex. Nevertheless, power analyses for some of the group analyses suggest that with 15 participants per group, we would have found significance. We strongly believe that by increasing the number of participants per group, the trends of improved integrity of the spinal-level reflex should be demonstrated noticeably. In addition, testing children with 6, 9, or 12 months experience may be able to explore further change in neural responses in multiple Ia pathways.

Table 3. 1. Characteristics of Participants

| Groups | Sex | Age (days) | Weight (kg) | Height (cm) | PI[a] | Leg length (cm) |
|---|---|---|---|---|---|---|
| **Cruiser (n = 7)** | 3 male | 280 - 429 (*M* = 330.14) | 7.93 - 9.52 (*M* = 8.72) | 67.30 - 73.90 (*M* = 69.99) | 22.54 - 28.11 (*M* = 25.55) | 24.30 - 30.80 (*M* = 27.46) |
| **1month walker (n = 5)** | 3 male | 312 - 485 (*M* = 413.20) | 8.68 - 10.75 (*M* = 10.10) | 68.80 - 79.60 (*M* = 74.30) | 20.68 - 27.63 (*M* = 24.77) | 25.30 - 33.05 (*M* = 29.52) |
| **2months walker (n = 5)** | 3 male | 364 - 488 (*M* = 410.80) | 8.30 - 10.40 (*M* = 9.36) | 72.50 - 76.40 (*M* = 74.24) | 20.90 - 25.22 (*M* = 22.86) | 27.70 - 30.35 (*M* = 28.75) |
| **3months walker (n = 5)** | 3 male | 385 - 462 (*M* = 431.00) | 8.39 - 12.90 (*M* = 10.71) | 65.00 - 79.60 (*M* = 75.10) | 19.79 - 38.78 (*M* = 27.63) | 27.25 - 33.45 (*M* = 30.20) |

[a] Ponderal index (PI) is an indicator of body proportion, by using the following formula: $\frac{weight\,(g)}{length(cm)^3} \times 100$

Table 3. 2. Mean Values and Standard Deviations for Gait Parameters

| | Cruiser | | 1month walker | | 2month walker | | 3month walker | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Normalized Step Length | 0.50 | 0.18 | 0.62 | 0.18 | 0.91 | 0.10 | 0.93 | 0.25 |
| Normalized Step Width | 0.50 | 0.14 | 0.80 | 0.35 | 1.02 | 0.20 | 1.02 | 0.33 |
| Normalized Cadence | 0.25 | 0.06 | 0.42 | 0.11 | 0.56 | 0.09 | 0.56 | 0.06 |
| Stance (%) | 66.24 | 5.63 | 62.32 | 3.57 | 54.81 | 2.15 | 52.41 | 4.22 |
| Double support (%) | 33.61 | 10.70 | 25.73 | 7.70 | 10.10 | 2.67 | 11.14 | 5.85 |

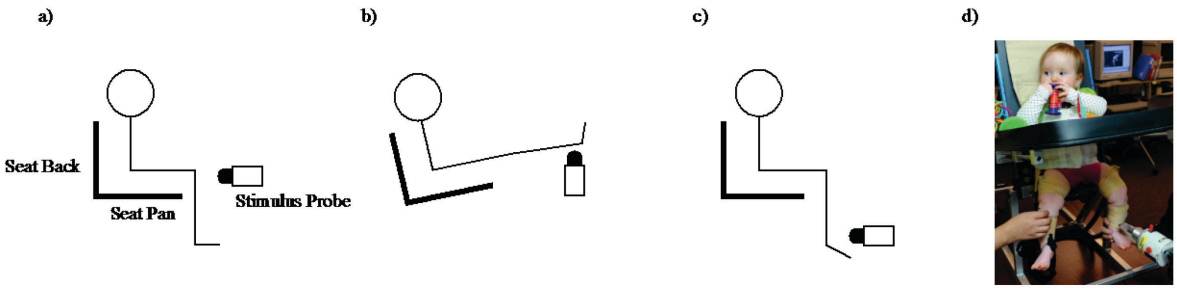


Figure 3. 1. Illustration of infant's position during stimulations

Inclination of the seatback support and leg position correspond to the postures we used to asses a.) quadriceps (QAD), b) gastronomius-soleus (GS), and c) tibialis anterior (TA) muscles. Fig 3. 1. is adapted from Lee et al. 2012. d) shows on infant being tested.

a. b.

Figure 3. 2. Illustration of an infant cruising (a) and a toddler with 3 month walking experience walking (b).

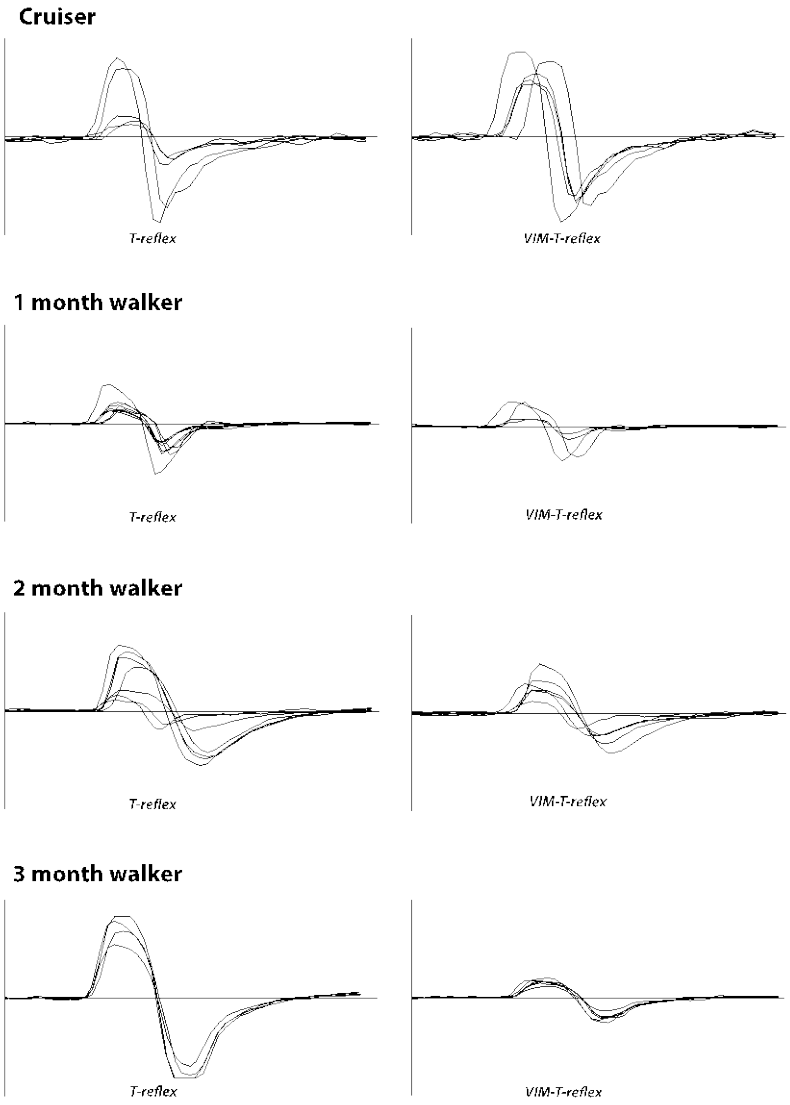


Figure 3. 3. Exemplar T-reflex and VIM-T-reflex responses elicited in the QAD muscle

Illustrate differences in the quality of EMG activation to the T-reflex and the VIM-T-reflex in the agonist muscle across walk experience. T-reflex time profiles are similar to those of the VIM-T-reflex. Reflex response amplitude and latencies were relatively varied over the walking experience, but more experienced walkers tended to show less variation in amplitude and latency and consistent quality of responses following stimuli, compared with those of novice walkers or cruisers.

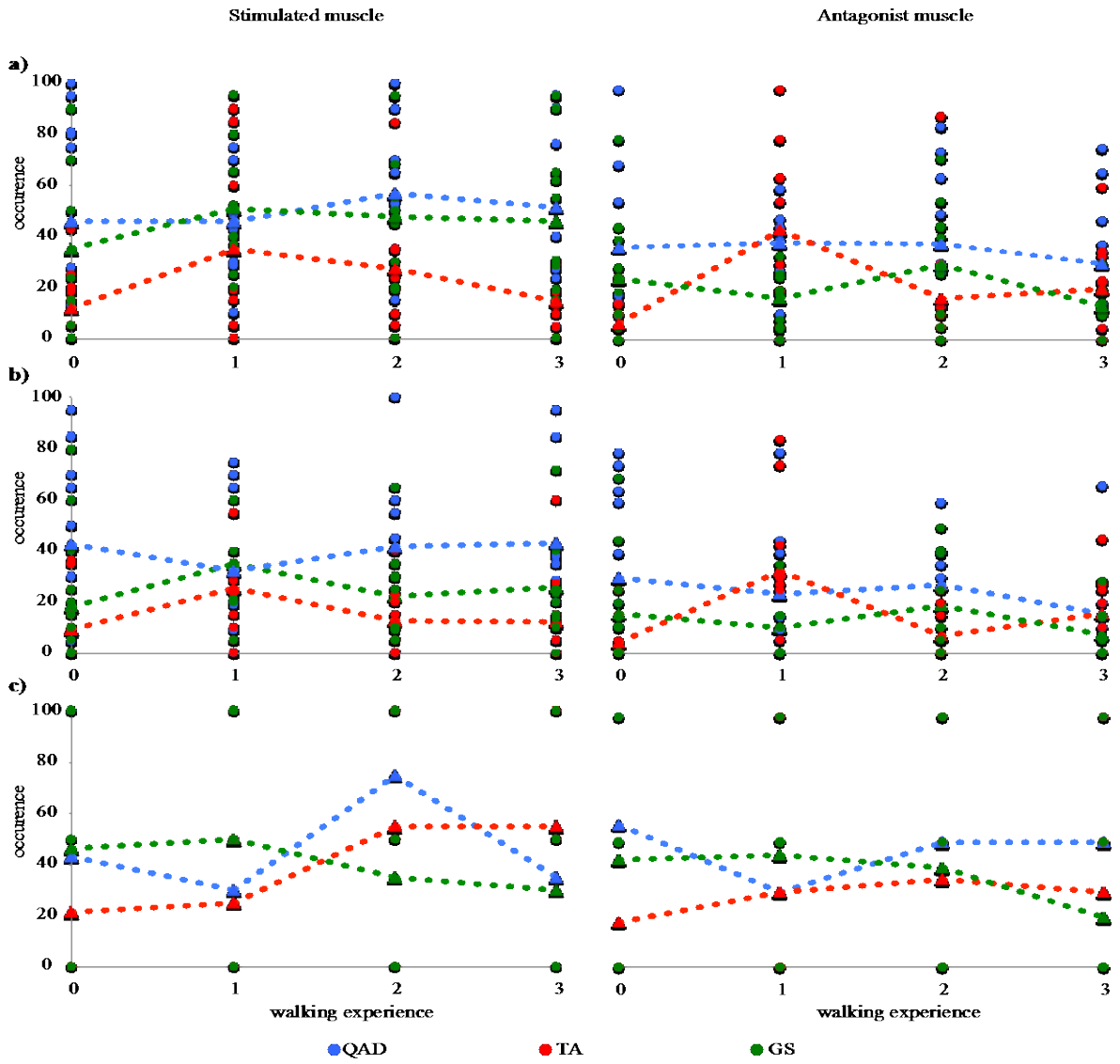


Figure 3. 4. Ratio of EMG responses to stimuli presented to antagonist muscles pairs for a) T-reflex, b) VIM-T-reflex, and c) VIR

QAD (blue circles), TA (red circles), and GS (green circles). Larger circles reflect individual participants. Smaller triangles-dotted lines response the means across experience levels. Variability of response patterns was mainly observed between conditions and muscles tested.

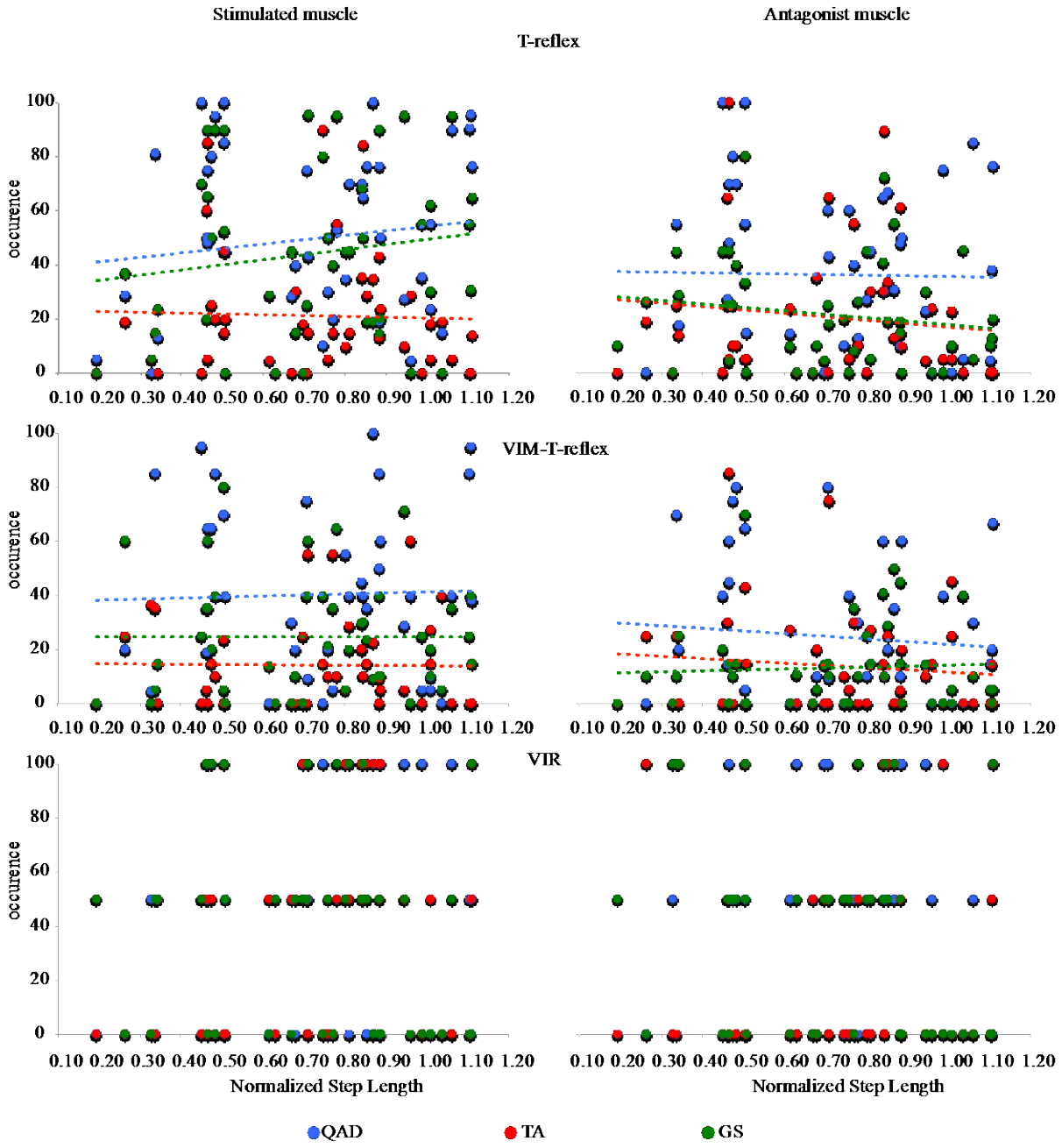


Figure 3. 5. Relationship between walking skill and response ratios for each stimulated muscle and stimulation type

QAD (blue circles), TA (red circles), and GS (green circles). Larger circles reflect individual participants. Smaller circles-dotted lines response the slopes across normalized step length.

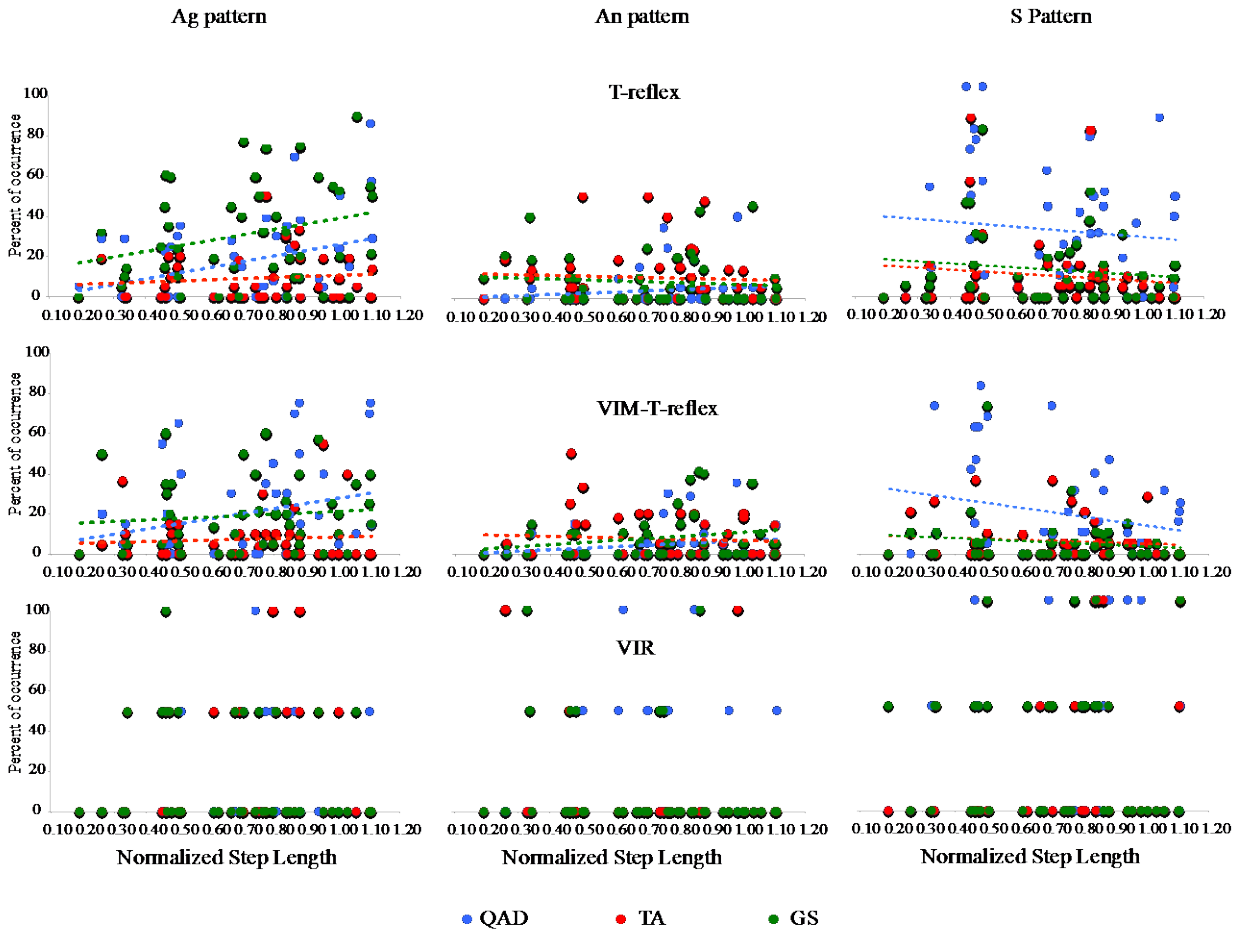


Figure 3. 6. Relationship between walking skill and response pattern occurrence for each stimulated muscle and stimulation type

QAD (blue circles), TA (red circles), and GS (green circles). Larger circles reflect individual participants. Smaller circles-dotted lines response the slopes across normalized step length. A response in the stimulated muscle alone (Ag pattern), a response in the antagonist muscle alone (An pattern), and a simultaneous response in both muscles (S pattern).

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## Chapter IV

# IMPACT OF TREADMILL STEPPING PRACTICE ON THE BEHAVIOR AND UNDERLYING MECHANISMS IN BABIES BORN WITH MYELOMENINGOCELE


## Abstract

Purpose: On average, infants with myelomeningocele (MMC) learn to walk about two years later than infants with typical development (TD). Our purpose was to determine the effect of early and aggressive treadmill practice on stepping in infants with (MMC) when supported on a motorized treadmill.

Methods: 10 infants comprised the intervention group whose results we compared to data published previously for 12 infants (Teulier et al, 2009), but who did not receive the intervention. All infants had lumbar or sacral MMC. Intervention group protocol was treadmill stepping practice 5 days per week, 10 minutes per day starting at 1-2 months of age (mean age = 63.3 days) for 12 months. Bimonthly, we assessed treadmill step behaviors via a motion capture system. For the first 6 months of intervention, step frequency, interlimb stepping patterns, and step parameters were examined and compared to comparison group infants.

Results: Across the first 6 months of intervention, intervention infants did not significantly increase step frequency and their developmental trajectory of step frequency were similar with that of comparison infants. But, in quality of steps, intervention infants produced mainly alternating steps and tended to contact the surface of treadmill with the heel or flat part of foot

supported by weight while comparison infants produced a large proportion of parallel steps with toe contact. Temporal parameters of alternating steps did not change over practice and had no difference between groups.

Discussion and conclusion: This study demonstrates that parents and infants are able to perform early aggressive intervention that was home-based, parent-administered, starting at 1month post birth. Although treadmill practice did not clearly affect step rates over the first half of intervention, steps infants produced were of a much higher quality, suggesting improved neuromotor control and strength. From our pilot work as a starting point, early aggressive treadmill training can be a potential supplement for regularly scheduled physical therapy intervention to improve both neuromotor strength and control in the lower extremities for infants with neuromotor disabilities.

## Introduction

Myelomeningocele (MMC) is the most serious and frequent form of spina bifida, which encompasses a broad range of malformations of the neural tube. In this condition, during the first trimester of pregnancy between the $26^{th}$ and $30^{th}$ day of gestation, one or more of the vertebral arches fails to close properly, causing an extrusion of the protective covering (the meninges) from a dorsal opening in the spine, and incomplete development of the fetus's spinal cord. In the United States, approximately 1,500 to 2,000 of 4 million live births present with MMC each year, and approximately 166,000 individuals live with this form (Spina Bifida Fact Sheet, 2007). From 1998 – 2006, mandatory folic acid food fortification programs have helped to reduce the prevalence of MMC by 30% to 50% (Castilla, Orioli, Lopez-Camelo, Dutra Mda, & Nazer-Herrera, 2003; De Wals, Rusen, Lee, Morin, & Niyonsenga, 2003; Edmonds, Flores, Kirby, Rasmussen, & Williams, 2005; Honein, Paulozzi, Mathews, Erickson, & Wong, 2001) and

helped to improve first-year survival rate of 92.1 %, compared with a 90.3 survival rate for those born before fortification (Bol, Collins, & Kirby, 2006). But, a physically inactive lifestyle remains an issue for individuals with MMC, particularly those who are non-ambulators (Buffart, van den Berg-Emons, van Meeteren, Stam, & Roebroeck, 2009).

In MMC, the degree of loss of sensorimotor units is impacted by the spinal column's lesion level. Lesions can occur at all levels of the spinal cord, but most frequently they occur in the lumbar and sacral regions, which are associated with lower body function (Emery & Lendon, 1973). Therefore, while infants with MMC demonstrate relatively typical development of strength and motor milestones for the head and upper-extremities, they do not develop typically in the lower-body; strength, control, and mobility are more affected in lower extremities, compared to the upper body.

Leg activity in infants born with MMC becomes less frequent and less vigorous compared to that of infants with typical development (TD) during their first year of life post birth. For infants with thoracic and lumbar lesions, at least, spontaneous leg movement is present and is as frequent in utero as for fetuses with TD. But it becomes depressed or disappears post birth (Rademacher, Black, & Ulrich, 2008; Sival et al., 1997; Sival et al., 2006; Smith, Teulier, Sansom, Stergiou, & Ulrich, 2011). After birth, all infants need to produce sufficient muscle activity and force to generate antigravity movements, but their muscles may not be strong enough to move their legs as frequently as they could in utero, when movements were stimulated and supported by movement of surrounding fluid systems, sporadic uterine contractions, and maternal motor activity. This early depressed leg activity in infants with MMC leads to cascading effects on functional motor skills and body composition in the lower body. If infants born with MMC acquire sufficient trunk and lower body strength and control, they learn to sit and crawl at

a median age of 1 year, stand alone at a median age of 2 years, and walk independently at a median age of 3 years (Findley et al., 1987; Swank & Dias, 1992; Williams, Broughton, & Menelaus, 1999). By late childhood or early adolescence, many of them stop walking and switch to wheelchairs as the favored mobility mode (De Souza & Carroll, 1976; Iborra, Pags, & Cuxart, 1999; van den Berg-Emons et al., 2001).

In addition to the delayed functional motor skills and reduced mobility during early childhood, these factors lead in a cascading manner to secondary consequences related to body composition and growth. By the end of the first year, infants born with MMC exhibit shorter leg length and smaller shank circumference than infants with TD (Teulier et al., 2009). Children with MMC are prone to acquire osteoporosis in their hips and legs, causing a high incidence of bone fractures (Lock & Aronson, 1989; Swank & Dias, 1992; Valtonen et al., 2006). Musculoskeletal deformities such as scoliosis and joint contractures are the other major issues in non-ambulatory children with MMC (Ausili et al., 2008; Marreiros, Loff, & Calado, 2012; Mayfield, 1991). Such growth impairment and bone weakness believed to be driven by inherently weaker muscles in the lower limbs and reduced time spent in antigravity activity. These cascading complex problems, starting from infancy, are associated with a sedentary lifestyle, which leads to a high prevalence of obesity in the MMC population (Ausili et al., 2008; Buffart et al., 2009; Hayes-Allen, 1972; Shurtleff, Walker, Duguay, Peterson, & Cardenas, 2010). Together, these outcomes suggest the need to increase the level of active leg movements (e.g., spontaneous activity and weight-bearing movements) early in life, creating a strong foundation of muscle strength and control in the lower body of infants with MMC during the first year of life.

Early active and aggressive neuromotor therapies emphasizing functionally relevant tasks are recommended for pediatric neuromotor rehabilitation (Kleim & Jones, 2008; B. D. Ulrich,

2010). Theoretically, intervention at an early age, when the brain and spinal cord are very plastic, should be more effective than intervention which starts beyond infancy, because with sensorimotor activity, neuronal connections become strengthened and pruned at amazing rates through the first few years of life. The first year of life may be considered the critical period of motor development, offering the best opportunities for effective intervention of motor development for children whose neural development specifically is interrupted via spinal lesion (Sharkey et al., 1990; Shonkoff & Hauser-Cram, 1987). Clinically, physical therapy should begin early and intensively for rehabilitation. In addition, therapeutic protocols should emphasize continual self exploration and selection of the movement patterns, eventually facilitating functionally relevant motor behavior (such as locomotion) and continuing the long term effects on motor behavior throughout the lifespan (Edelman, 1989; Hadders-Algra, 2001, 2011). Ramey & Ramey (1998) strongly suggested that based on successful intervention protocols, interventions that begin earlier in development and consist of a higher intensity may deliver greater and longer positive outcomes to infants with disabilities than do those that begin later and at lower intensities. However, physical therapy typically begins in infants with MMC when the physical therapist can document delayed development of motor milestones with an accepted assessment instrument. The disadvantage to this approach is that it starts relatively late from the point of neuroplasticity, so that it misses opportunities to accumulate history of skills, control, and strength, building the foundation for the next skills to follow. Indeed, pediatric physical therapy tends to be more passive, less aggressive, and less focused on functional skills (e.g., passive range of motion movements or correction of sleeping positions) than when prescribed for older children (K. Hinderer, Hinderer, & Shurtleff, 2006). Given the principle of neuroplasticity, history of skills, and the importance of aggressive intervention, current therapeutic approaches

(such as physical therapy (PT) sessions or biweekly Early on® Michigan service (http://www.1800earlyon.org)) are not sufficient intervention to improve neuromuscular strength and control. However, pediatric physical therapists do recognize the importance of significant levels of intervention very early in life for optimizing residual neuromuscular strength and control (B. D. Ulrich, 2010). Therefore, innovative ways to provide early intensive therapy need to be identified and tested for infants with MMC.

For the pediatric population, stepping practice on a treadmill has been established as beneficial for accelerating the acquisition of walking in infants with Down syndrome (DS) (D. Ulrich, Lloyd, Tiernan, Looper, & Angulo-Barroso, 2008; D. Ulrich, Ulrich, Angulo-Kinzler, & Yun, 2001). Newborn babies perform stepping patterns that disappear over the first few months (about 4-6 weeks post birth) and reappear closer to 1 year, the typical onset of walking (Peiper, 1928 cited in Peiper, 1963). However, deliberate stepping practice can lead to continuous stepping patterns throughout the first year (Super, 1976; Zelazo, Zelazo, & Kolb, 1972). Ulrich et al. (2001) provided stepping practice on a moving treadmill to infants with DS, starting when infants could produce 30 seconds of independent sitting and continuing until the onset of independent walking. Infants with DS who received 8 minutes of treadmill stepping practice 5 times a week, at home, walked 4 months earlier than peers who did not receive stepping practice. In addition, infants with DS who received more intensive and individualized treadmill protocol (.22m/s in addition to progressively increased ankle weights) showed greater increase in the frequency of (alternating) steps and began independent walking sooner than those who received less intensive treadmill practice (D. Ulrich et al., 2008). A task-specific practice such as treadmill stepping intervention is prone to augment neuroplastic changes within motor cortex and central nervous system (Adkins, Boychuk, Remple, & Kleim, 2006). By allowing infants to take as

much as weight as possible on their lower body, infants are able to improve muscle strength and have opportunities to explore and practice the functional patterns used in walking, before the skill itself becomes functional. This, in turn, contributes to improvements that will support actual, functional locomotion. An added benefit of treadmill stepping training is that it provides a structured commitment for parents that is easy to follow and contributes to their perception that they are contributing directly to their infants' progress in motor development. Based on DS studies, it is possible that infants with other disabilities who receive early treadmill stepping practice may come closer to maximizing their potential for improvement in motor control, leading to better physical health-related quality of life in later childhood.

Recent longitudinal research has demonstrated that infants with MMC can, without practice, respond to being supported on a moving treadmill by producing a variety of step types (Pantall, Teulier, Smith, Moerchen, & Ulrich, 2011; Saavedra et al., 2012; Teulier et al., 2009). Infants with MMC showed, however, a significantly lower number of steps and lack of improvements in step rate across the first year of life compared to infants with TD (Teulier et al., 2009). Research subsequently showed that by enhancing sensory input such as visual flow (i.e., a checkerboard belt pattern) and friction (i.e., tacky belt surface) on the treadmill's surface, infants with MMC increased step frequency as compared to the baseline (Pantall et al., 2011). Thus, when the treadmill stepping rate remains low, the combination of visual flow and friction conditions may facilitate step responses on a motorized treadmill. Moreover, Moerchen et al. (2011) reported a case study in which 18 weeks of treadmill stepping practice combined with over-ground stepping with the support of a walker (assistive device) induced a 23-month-old toddler with MMC to increase leg activity and to learn to coordinate leg movements, which gradually facilitated the acquisition of independent walking sooner than other toddlers with L4-

and L5-level lesions. These results are promising that treadmill practice may enable infants with MMC to increase their step frequency on a motorized treadmill by improving neuromuscular strength and control, and eventually accelerate the acquisition of independent walking over developmental time.

Given the results of treadmill studies to date in infants with DS and MMC, treadmill practice can be hypothesized to be a viable early intensive intervention for infants with MMC. However, because infants with MMC generally have more severe problems in terms of sensorimotor function than do infants with DS, the optimal level of intensity and length of treadmill training for infants with MMC and the impact on growth and development need to be studied. The goal in this study was to monitor the impact of early aggressive individualized treadmill training via assessments of treadmill stepping behavior (i.e., step frequency, interlimb stepping patterns, foot position during steps) over the first 6 months of intervention. Specifically, we wanted to compare outcomes of this intervention with a comparison group tested recently in (Teulier et al., 2009): for all of the variables assessed here, we have published longitudinal data for 12 age-matched infants with MMC who did not receive treadmill practice. Our hypotheses were that over the first 6 months of intervention, infants who received early aggressive intervention would show a steep increase in total step rate, which is accompanied by mostly alternating steps, than would those in the comparison group.

## Method

### Participants

Two cohorts of participants from two studies were included: the first is our current study, the treadmill intervention group, and the other is a comparison group from another study (Teulier et al., 2009), who did not receive treadmill intervention, but were tested longitudinally using the

same testing method as described for the intervention group. In the intervention group, 10 infants with MMC participated in a 12-month intervention study. Of 10 participants, 6 infants with MMC received this intervention at 1 month post-birth, and 4 infants began participating later than 1 month post-birth due to medical complications or late identification (e.g., 1 infant at 2 months, 2 infants at 3 months, and 1 infant at 6 months post-birth). We recruited infants up to the age of 6 months. Two additional infants were enrolled but were excluded from this study because one of them moved from the geographical area and the other did not follow our intervention protocol (lack of compliance). Therefore, a total of 10 infants with MMC who were enrolled in 12 months of intervention study, and whose data we analyzed here, were from the first 6 months of intervention. In the comparison group, a total of 12 infants were tested at 1, 3, 6, 9, and 12 months of age. Exclusion criteria for infants in both groups included any central nervous system or chromosomal abnormalities beyond those associated with MMC (e.g., hydrocephalus with or without shunt and Arnold Chiari II syndrome), lesion levels higher than the lumbar region, or a gestational age at birth of 32 weeks. If gestational age was less than 37 weeks, we applied a corrected age for all assessments. Lesion levels were based on the fusion sites recorded by neurosurgeons. Table 4.1 provides participant characteristics for both groups. Families were recruited by neurosurgeon or physiatrist referrals from Southeast Michigan (Ann Arbor, Lansing, and Detroit). The study was approved by the Institutional Review Board at University of Michigan, and a written informed consent was obtained from parents before their infant's participation in the intervention study. Families were given a T-shirt for their child on the first visit; at each assessment session they were given a small monetary gift.

## Procedures

**Intervention.** We provided a custom-engineered pediatric treadmill (an infant-sized treadmill) (Carlin's Creations, Sturgis, MI) to each family in the intervention group, for the duration of the training (i.e., 12 months). The pediatric treadmill has 4 wheels, so the family can easily move and store it. A bench also was provided to the family so they could sit in the front or rear of the treadmill while holding their baby upright. After the two days of pretesting, we trained parents to understand and use the correct procedure for practice (such as how to hold the infant on the treadmill). In addition, each family received a manual (with pictures and text) and a DVD in which the instructions for the practice are described. The family was given a "baby practice log" in which they recorded notes about the timing, duration, and quality of their daily practice.

During the intervention, infants received practice 10 minutes per day, 5 days per week at a belt speed of .144 m/s. Our intervention protocol was progressive with variations in training accommodated to each baby's individual rate of stepping response/neuromotor strength and control. We based our exercise training on the results from our previous longitudinal study of infants with MMC (see Pantall et al., 2011; Teulier et al., 2009). In the beginning of intervention, a combination of a.) newborn step elicitation, b.) treadmill step elicitation, and c.) bouncing on lap was used. This combination of newborn step and bouncing maximizes babies' opportunities to activate muscles, eventually responding well to the treadmill. For newborn step elicitation, when holding the baby upright while tilting the baby's trunk forward slightly on a firm stationary surface, the parent(s) move the child slowly forward over the surface to help the child lift their legs and step alternately. For bouncing, while parents hold the baby upright so the feet rest on the parent's thighs, the parents move the baby's trunk rhythmically downward and upward to encourage them to extend their legs and accept weight on the legs. At the beginning of practice, a

checkerboard patterned material made from dycem (Dycem Ltd, Warwick, Rhode Island) covered the treadmill belt to facilitate stepping responses. When the babies took continuous alternating steps, extra sensory input was no longer provided because infants were able to elicit treadmill stepping with the baseline of the treadmill belt. Depending on the babies' achievement with treadmill stepping, the conditions of the treadmill belt speed and duration of stepping were individualized and updated to progress the treadmill portion of the intervention. On top of our intervention program, the majority of our participants received pediatric physical therapy twice a week, or biweekly sessions of the "Early on Michigan program" in their home or school setting when they reached 2 months old or more. Total eight infants received pediatric physical therapy or "Early on Michigan program" (3 for pediatric PT, 3 for Early on program, and 1 for both) and two infants did not receive any therapeutic treatment.

To monitor adherence to the intervention, parents were asked to write a log of daily stepping practice: duration practiced per day, the types of exercise practiced per day, infants' general response to the treadmill, or the days on which exercise occurred. A small gauge on the side of treadmill also recorded the amount of time the treadmill was used, in minutes. Researchers visited each family's home every other week to check gauge values, review the parents' log, answer questions from the family, and offer advice for optimizing practice sessions.

**Assessment battery.** We conducted two sets of assessments: home visit assessment and pre- and bimonthly treadmill testing throughout the duration of practice. Each assessment had its own frequency of administration and level of involvement. We conducted all assessments at the time of day the parent reported as optimal relative to their infant's mood; as needed to keep infants' wake-alert state, feeding or rests (such as short nap time) were provided during the testing session.

***Home visit assessment.*** We assessed anthropometrics, motor skill acquisition, and medical history and nutrition survey data/updates biweekly for the first 3 months, and monthly thereafter (see Appendix B.).

We assessed the following anthropometrics: body weight and length; thigh length (greater trochanter to knee (the proximal-lateral border of the tibia)); shank length (knee to lateral malleolus); foot length (heel to big toe); thigh, shank, and head circumferences; malleolus, knee, and hip widths; and shank, thigh, and umbilicus skinfolds. The purpose of these measurements was to determine whether some were impacted by therapy or affected level of response, and also for the normalizing of some stride parameters. We assessed the concurrent motor skill acquisition level by administering the Motor Subscale from the Bayley Scales of Infant Development III (BSID III). Parents completed their infant's medical history survey and nutrition survey of infant and mother (if nursing).

***Treadmill stepping elicitation (Pre- and post, bimonthly).*** We conducted treadmill stepping elicitation in a developmental neuromotor control laboratory (School of Kinesiology, at the University of Michigan) or the testing in families' homes to support families' accommodation (reduce extra trips to come laboratory for family).

For the pre- and 6-month testing, we measured treadmill step responses in our laboratory via a six-camera Peak Motus$^{TM}$ (Peak Motus -Vicon Performance Technologies, Oxford Metrics Group, Oxford, United Kingdom) or an eight-camera motion capture system (MotionAnalysis, Corp., CA) sampling at 60Hz. We calibrated the test space prior to each session testing. Upon arrival at the laboratory, parents/researchers removed all of the infant's clothing and cleaned all skin surfaces where the markers would be attached with alcohol pads. The Spherical reflective markers (8 mm diameter- pretest and 23 mm diameter- thereafter) were attached bilaterally on

the following leg joints: lateral malleolus, lateral knee joint line, greater trochanter, and iliac crest. Pediatric bipolar surface electrodes (rectangular patch 2.2 x 2.2 cm) were positioned bilaterally over the muscle bellies of the tibialis anterior, lateral gastrocneminus, rectus femoris, and biceps femoris. We placed a ground electrode over one of the vertebral arches, above the infants' surgical incision line.

Our custom-made motorized treadmill (Carlin's Creation, Sturgis, MI) was 18 cm high, 42 cm wide, and 82 cm long, with a smooth, polyvinyl chloride black belt. We placed the treadmill on a large table (117 cm wide and 188 cm long). We held the baby upright in a partial body-weight-supported position (Figure 4.1). Treadmill testing consisted of four trials at 0.144 m/s; each trial was 60 seconds long, with breaks between trials as needed. A digital video camera (60Hz, Canon ZR 960) was positioned at the side of the table perpendicular to the treadmill (at the right side of the infant, at chest level) to record stepping behavior for behavior coding purposes. An audio signal was used to synchronize with video recording, motion capture system, and EMG data.

Immediately following 2 and 4 months of intervention, we assessed treadmill step responses in the family's house. Instead of three-dimensional configurations via a six-camera system (Peak Motus$^{TM}$) or an eight-camera system (Motion capture system), we used two digital cameras (60Hz, Canon ZR 960) to record 2-D leg movements. One camera was placed on each side of the treadmill, perpendicular to the right/left sagittal plane of the infant, at chest level. We put our test treadmill on a portable table (61 cm wide and 122 cm long) and calibrated the test space prior to testing with a Peak Motus$^{TM}$ calibration frame. Two digital cameras and EMG data were synchronized with audio signal. With the only difference being in space configuration

between the laboratory and the family's home, we conducted treadmill step elicitation in the same manner as we did in the lab setting.

After completion of treadmill step elicitation, we conducted anthropometrics, motor skill acquisition via Bayley, and medical history and nutrition survey data/updates in same manner as we did home visit assessment.

**Data Reduction**

For this paper, we mainly focused on quality and frequency of treadmill stepping behaviors over the first 6 months of intervention study. The results for kinematics and EMG of treadmill stepping elicitation, and survey questions will be analyzed in a separate article. We analyzed stepping behaviors in 3 ways--step frequency, interlimb stepping behavior, and step parameters--over the first half of intervention. All of the variables assessed here were compared with longitudinal data for 12 age-matched infants with MMC who did not receive treadmill practice published in (Teulier et al., 2009).

**Step rate and interlimb stepping patterns.** To determine the frequency and type of steps taken by infants during treadmill stepping elicitation, we behavior coded the videotapes using frame-by-frame analysis (60Hz) with Peak Motus Version 8 software. Three behavior coders (student assistants) were trained to code the data tapes. They practiced with training tapes for at least 6 weeks before validity testing, for which each needed to achieve a coefficient of agreement of 0.85 (interobserver reliability coefficient, kappa) to match their work with that of previously validated coders for the same set of trials on training tapes. Detailed descriptions of training procedure and interobserver stability were presented in B. D. Ulrich, Ulrich, & Collier (1992). Validated behavior coders identified all occurrences of steps produced in each test trial and categorized steps as one of 4 interlimb stepping patterns (see Thelen & Ulrich, 1991):

alternating (a step of one leg is followed by a step of the opposite leg with temporary overlap), single (a step of one leg does not overlap with a step of the opposite leg), parallel (both legs swing forward together to step), and double ("stutter" step within a sequence of alternating steps). The step rate was defined as the total number of steps taken per trial for each baby, divided by the trial duration in seconds. For interlimb stepping patterns, we calculated the percent of occurrence of each interlimb patterns per trial for each session.

**Step parameters.** In addition, coders identified the time (in frame) when events occurred: toe off, touch-down, and end of stance for alternating steps. If infants did not produce alternating steps within a trial, events within single steps were identified for subsequent analyses. These step events were used to calculate step cycle, swing, and stance phase durations. Because of differences in body size across infants (such as leg length) and with age, we normalized the total cycle, stance, and swing duration times to infant's leg length with the following formula:

$$\text{Normalized cycle duration} = (\text{cycle duration})/\sqrt{((\text{leg length})/\text{gravity})}$$

Next, the behavior coders identified the part of foot that contacted the treadmill initially at touch-down and in mid-stance for alternating steps: toe, flat, heel, lateral side, or medial side. We calculated the percent of occurrence of each foot posture across all alternating steps.

**Other parameters.** In anthropometric measurement, we used the 2009 Centers for Disease Control (CDC) "Birth to 24 months" growth charts to assess growth patterns of weight for length in our participants across the first half of practice (Appendix C, Figure C. 1)

## Results

### Step Rate

First we tested whether intervention affects total steps taken during the first half of treadmill exercise. The individual infants' step frequencies are presented in Figure 4. 2 with the

average of total steps in the comparison group. In the intervention group, each baby showed a fair amount of variability in step rate over the intervention. Only three (infant # 2, 7, and 8) showed a rapid increase in total steps following 6 months of exercise, but the others did not. For those who produced few steps, their step frequency taken at pretest decreased in the middle of intervention (i.e., 2– and 4-month intervention) and then slightly increased at 6 months test. Therefore, the average total steps of the intervention group remained relatively unchanged over practice, and the trajectory of step rate was similar to that of infants in the comparison group. Compared to age-matched peers without exercise, the intervention infants begin with a comparatively moderate step rate at 1 month old, followed by a period of decline in steps and then increase steps at 8 or 9 months old. This means that treadmill practice did not affect developmental process of total steps taken following the first 6 months of exercise. However, for most, the step frequency begins to increase steeply after 6 months of testing (Appendix C, Figure C. 2); of 4 graduated infants, 3 infants (infant # 6, 9, and 10) started to show a dramatic increase in step rate by 8 months of testing, and continuously improved their steps until post test. That is, treadmill practice had a much larger effect on step frequency in the second half of the intervention.

**Interlimb Stepping Patterns**

We next reviewed whether treadmill exercise affects their quality of steps as reflected in the proportion of each interlimb stepping pattern in the first half of intervention: 4 possible patterns were reviewed including alternating, single, double, and parallel. In the intervention group, infants began with a mixture of single, parallel, and alternating steps at pretest, but over the course of intervention, alternating and single steps increased whereas parallel and double steps gradually decreased (Figure 4. 3). Thus, most infants showed predominantly strong

preference for alternating and single steps over the course of 6 months of exercise. In follow-up observation, the preference of alternating steps continuously increased with 12 months of practice (Appendix C, Figure C. 3). In the comparison group, infants began with a high proportion of single steps at 1 month old, and this pattern was largely preserved until 12 months old. In comparison, the preference of alternating steps was notably higher in the intervention infants than that of infants without practice at a given time. Additionally, trajectories of the preference of interlimb patterns were quite different between groups over the course of our observation: intervention infants increased alternating steps and decreased parallel steps over the course of practice, while infants without exercise slightly decreased single steps and maintained the same level of parallel steps. Nevertheless, in both groups, double steps incidentally emerged and never became the preferred steps at any point in time.

We reviewed the change of step frequency in relation to the preference of the interlimb patterns. Although no change was observed in number of steps taken, most infants with practice increased their preference for alternating steps over 6 months of intervention (see Figure 4. 2 and Figure 4. 3). Only three infants (infant # 2, 7, and 8) who had consistently increased step rate, demonstrated an increase in the number of alternating steps in the first 6 months of exercise. This indicated that treadmill practice had effects on reducing the repertoire of interlimb patterns and settling into a high proportion of alternating steps. This effect seems to be stronger than number of steps taken over the first half of intervention.

**Step Parameters – Temporal and Foot Position**

Here, we reported the parameters of alternating steps. Figure 4. 4 demonstrated the average mean intervention infant's results of temporal parameters in the first half of exercise with the mean of the comparison group for the first year of life: dimensionless cycle, stance

phase, and swing phase durations and percentage of stance phase in cycle duration. In the intervention group, practice did not affect changes in temporal parameters: slight increases were observed in cycle, stance phase, and swing phase durations at 9 months old, but the rate of changes was relatively low compared to that of the pre-test. Between groups, we also found no differences of temporal parameters of alternating steps. Figure 4. 5 a. and b. showed foot posture at touch-down and in the stance phase, respectively. With practice, infants contacted the surface of the treadmill mostly with the heel of the foot followed by a fair amount of flat part contact. Between groups, intervention infants showed a high proportion of heel and flat part contact than the comparison group across our observation period. However, for all infants, regardless of practice, variability of foot posture was predominantly observed and could not be resolved in our observation period. This means that even with practice, infants still showed a mixture of all possible foot contacts at touch-down. But, in both groups, all infants performed middle-foot contact only incidentally throughout our observation.

In infants with exercise, from initial contact to the middle of the stance phase, the proportion of heel contact was reduced whereas the proportion of flat part contact was increased. This indicated that right after initial contact on the surface of the treadmill, the intervention infants settled into the flat part of the foot, likely inducing weight-bearing on their foot. In mid-stance, the proportion of flat foot contact was much higher in intervention infants than that of comparison infants over our observation period. In the comparison group, the foot contact was made frequently with the toes, which infants with practice rarely did. Given the difference in predominated foot contact during the stance phase between groups, for all infants, regardless of practice, a mixture of foot preference was still preserved over our observation period.

## Discussion

This study demonstrates that parents and infants with MMC are able to perform very early intervention that was home-based, parent-administered, starting at 1month post birth and continuing for 12 months. However, our data showed that during the first 6 months of intervention, treadmill practice did not increase overall step frequency. However, over this time period infants produced steps that were of a much higher quality in terms of interlimb patterns and foot position, which suggests improved neuromotor control and strength.

### Overall Step Frequency

As individuals and as a group, the intervention infants had similar step rates to that of the comparison group that did not receive treadmill practice. Neither group increased in step frequency over the course of our observation. At the pretest, infants in the intervention produced a slightly higher number of total steps, but the mean of total steps decreased in the middle of intervention and then slightly increased at 6 months test. Therefore, the mean of total steps became similar to that of age-matched peers who did not receive treadmill practice. This is a somewhat unexpected result. In general, step rate increased with age in healthy infants without practice (Thelen & Ulrich, 1991). We suspect that the variability of individual medical characteristics and parent compliance (e.g., amount of practice time, difficulty "enabling" babies to accept weight, and motivating such young babies) across our infants may contribute to a low progress of step frequency in the intervention group.

There are several individual characteristics of our population that may affect the rate of change in total steps across infants with MMC: lesion level, foot deformities, weight gain over intervention, and age at which they enrolled in the intervention. For example, lesion level was related to step rate across the first year of life when MMC infants were tested longitudinally on

the treadmill without practice (Teulier et al., 2009). Given the variability of medical characteristics and small sample size in our participants, it was not possible to find a statistically significant predictor. However, we did observe the tendency that an infant's weight gain uniquely affects developmental trajectories of stepping behaviors over the first half of intervention. For example, infant #4 and infant #10 had fairly similar birth characteristics: both were premature and have similar spinal level lesions. They started the treadmill training at 1 month corrected age and continued for 12 months, and their parents showed relatively high compliance of treadmill practice. The major difference between #4 and #10 was weight gain. #10 gained weight properly, but #4 did not and became quite overweight. We believe that it is primarily due to weight gain, but also to parents' tendency to limit baby's weight acceptance when upright; therefore, their developmental trajectory of treadmill step frequency was different. #4 and #10 showed quiet similar step rates at 1 month post birth. Although both demonstrated variability of their responses over the first half of intervention, #4 (over 80 % weight for length in CDC chart at 6 months of intervention - see Appendix C, Figure C. 1) showed decreases in step rate while #10 (non-overweight baby) presented an increase in step rate at 6 months testing. We suggested that asynchronous development of muscle mass and concomitant strength in lower limb could explain lack of increases in total steps. This account is in line with previous findings. Thelen and colleagues illustrated that by 2 to 3 months of age, newborn stepping behaviors were depressed due to weight gain. During infancy, gains in fat mass outstrip gains in muscle mass, so that infants' legs were not strong enough to perform stepping behavior (Thelen, 1983; Thelen & Fisher, 1982; Thelen, Fisher, & Ridley-Johnson, 1984; Thelen, Fisher, Ridley-Johnson, & Griffin, 1982). In addition, Luo et al. (2009) and Pantall et al. (2011) observed a negative association of step frequency with total body fat as determined by skinfold thickness or PI score

respectively: having a higher level of adipose tissue without concomitant muscle strength contributed to low step frequency on the treadmill. Nevertheless, although weight gain seems to tax infants moving their legs and producing steps, it is unlikely to be a sole predictor; more plausibly, several factors interactively contribute to the unchanged or decreased step rate over the first 6 months of practice. For instance, you may argue that spinal lesion levels may contribute the rate of progress as documented by (Teulier et al., 2009), but because highest-level lesion we had was L3, an association could not be examined in our study.

**Variability**

In the comparison study, Teulier et al. (2009) reported that without treadmill practice, infants with TD resolved the variability in their step rate in the second half of the first year, while infants with MMC could not resolve the variability across the entire first year of life. Similar findings were observed in our treadmill practice group of MMC infants. During the first 6 months of treadmill practice, the intervention infants demonstrated a high variability in their step rate, as individuals and as a group. But, when we followed the step rate beyond 6 months, the group mean for step rate was no longer flat and each individual showed an increase in step rate. For example, when we continued to monitor step rate for the 4 infants who completed the 12-month program, they showed an increase of step rate and resolved the variability of step rate in the second half of the intervention program. Why did intervention infants resolve the variability and show an increase of step rate in the second half of intervention?

One might reasonably expect that the intensity (10 minutes/day) and frequency (5 times/week) of our protocol might not be sufficient to improve muscle strength and limb control, which are needed to change step rate. Compared to the previous research of treadmill practice in DS (D. Ulrich et al., 2008; D. Ulrich et al., 2001) and MMC (Moerchen et al., 2011), our

participants were much younger and the intensity they received is relatively aggressive and individualized treatment: the speed of treadmill belt and duration of treadmill stepping practice are tailored to each child's rate of progress. Nevertheless, compared to new walkers who practice an average 9000 step per day to improve their walking skills (Adolph, Vereijken, & Shrout, 2003), repetition of our protocol is far less: our infants generally produced a daily mean average of 214 steps[1] on the treadmill. Currently, there are no data to confirm necessary and sufficient intensity and frequency of therapy (i.e., number of repetition and duration) for infants as young as 1 month and with MMC to increase muscle strength and neuromotor control sufficiently to increase stepping. Therefore, we are not sure that our protocol--10 minutes a day and 5 days per week--was sufficient to affect the central nervous system and peripheral nervous system in these young MMC infants. Nevertheless, based on no definitive changes in the first half of exercise, we could argue that with our protocol, intervention should be provided more than 6 months to observe the changes.

Alternatively, we may need to modify exercise protocol in terms of exercise schedule or goal directed movements with motivation. We mixed three different exercises (bouncing, newborn stepping, & treadmill stepping) in the beginning of intervention and the intensity of intervention was tailored to each child's rate of progress, but we were not able to create a sufficiently motivating task for these babies. To improve motor skills in young infants, it requires some level of "goal" directed movements with motivation. We suggest that these very young infants would have been better to vary the practice schedules with periods of intensity alternating with break periods, to maintain enthusiasm and allow sufficient recovery. For example, infants may receive

[1] Based on total step intervention infants produced at each treadmill testing session, we calculated group mean per minute and then multiped by average practice time (7 minutes).

exercise every other day with a day off in between for recovery, just as adults employ for exercise training. In addition, because such young infants had difficulty socializing with their parents during treadmill stepping practice, perhaps in beginning of intervention, we should start to provide exercise with visual and auditory rewards for large leg movements such as kick start gym while in supine position.

The other possibility we should consider that our infants ages 1-6 months may be too young and fragile to get maximum benefits during the first half of intervention. Over the intervention, 4 infants were ill and underwent subsequent series of medical treatment (such as shunt revision) or orthopedic treatment (such as wearing harness all day), decreasing opportunities to build neuromotor strength. Although most babies resumed the treadmill practice right after the surgeries or after a few days of recovery, those unpracticed periods seemed to cause a decrease in muscle mass and strength that they gained prior to surgeries. In addition, one infant (infant #10) wore a harness 23 hours a day for 10 weeks. This treatment was to align her hip joint properly without surgery, but limited her general spontaneous leg activity, which eventually slowed her rate of improvement in muscle strength and limb control. You may argue that our population may be exceptionally fragile, having been hospitalized several times. But, in general, infants with MMC undergo 1-2 surgeries post birth. About 40% of all infants who have shunts due to hydrocephalus experience shunt failure (blockage), requiring revision within the first year of life (Dias, 2012). So, we believe that our participants do represent the MMC population. In our argument, the MMC population mostly receives medical treatment, which interrupts and holds back the developmental progress of neuromotor control. As researchers when examining the utility of exercise treatments, we need to acknowledge these issues when designing intervention

protocols. Thus, infants should have enough time to get benefit of exercise, even when they experience interruptions in their capacity to comply with the protocol.

Last, but less direct to exercise, nutrition status should be considered along with intervention protocol. Many of our participants were premature, so pediatricians had prescribed high calorie formula or extra supplements throughout the first year of life. It seems that being less spontaneously active (Rademacher et al., 2008) and taking high calorie formula seriously increase the chances of infants becoming overweight within a few months post birth. Four infants were above the 80th percentile in their weight for length in the first 6 months of exercise (see Appendix C, Figure C.1). Many of those babies who were in the higher weight percentile had high calorie formula throughout these months. We are not arguing that these infants do not need high calories formula because of inherently less spontaneous activity level. High calorie intake may be critical for those premature babies to catch up, but should be monitored to maintain healthy weight. The point is that even though they received early aggressive exercise daily, they still had a high chance of being overweight. This result may explain the high obesity rates within the MMC population in childhood and adulthood (Ausili et al., 2008; Buffart et al., 2009; Hayes-Allen, 1972; Shurtleff et al., 2010). Since there is an increased chance of being overweight in the MMC population, early aggressive exercise protocols with nutrition management should be considered in future research.

**Quality of Steps: Interlimb Stepping Patterns and Step Parameters of Alternating Steps**

Although 6 months of treadmill practice was not sufficient to increase step frequency, the quality of step patterns did improve. Intervention infants generally produced more alternating steps over the treadmill practice while infants in the comparison group produced a large proportion of parallel steps, which tends to be viewed by developmentalists as a simpler pattern

to control. Alternating steps require continuous proprioceptive dialogues between the stretch of one leg and the pattern in the opposite leg, while simple steps such as single or parallel require mainly a mechanical stretch of extensors provided by the moving belt of a treadmill (Thelen & Ulrich, 1991). Thus, producing alternating steps involve more leg control and muscle strength than producing single or parallel steps. Typically, by 6 to 7 months old, alternating leg movements are dominant in healthy infants without treadmill practice (Thelen & Ulrich, 1991). At all data points we tested, the developmental trajectories of alternating steps in intervention infants are very similar to those of age-matched peers who are healthy and did not get practice (Teulier et al., 2009). We argue that daily treadmill practice helped infants develop rhythmic coupling between movements by stabilizing upright posture and increasing limb control. Several studies reported that a high proportion of alternating steps was associated with age of independent walking onset in pre-term and full-term infants at 7 months of corrected age (Luo et al., 2009), and in DS infants at 11 months old (B. D. Ulrich et al., 1992). This suggests that the ability to create rhythmic coupled movements is essential in acquiring upright locomotion. In relation to this mechanism, we predict that our infants with practice would have achieved onset of walking earlier than their peers who generally achieve it at a mean age of 3 years (Williams et al., 1999). From the follow-up phone call that we collected after intervention, we know that at least 2 infants (infant # 6 and 9) took the first independent steps before 24 months old.

Bouncing and newborn stepping in the earlier stages of exercise, and mainly treadmill stepping thereafter, establish a repertoire of perceiving and acting, eventually developing their capacity to organize alternating leg movements in response to being upright and moving. A greater proportion of alternating steps may explain why the total steps remained unchanged over the first 6 months of exercise. For infants in the intervention group, an increase in response

frequency came first in the form of the most advanced pattern, alternating step, which requires utilizing more complex control and coordination of their limbs. So, for them, increasing total steps means performing alternating steps. Interestingly, our results were in contrast to the data on MMC and TD infants without treadmill practice: these infants generally showed an increase of step frequency with simple patterns, single or parallel steps (Pantall et al., 2011; Teulier et al., 2009).

Not only did our infants with treadmill practice produce more advanced interlimb stepping patterns, when stepping, they tended more often to bear their weight on their heel or the sole of their foot. Usually, without treadmill practice, infants with MMC and TD used a mixture of possible foot postures (e.g., heel, toe, lateral, middle, and flat part of foot) to contact the surface of the treadmill at the touch-down and during the stance phase. When dominantly producing alternating steps on the treadmill, healthy infants used the heel of the foot to bear their weight (Thelen, 1983; Thelen, Ulrich, & Niles, 1987). Over the course of 6 months of exercise, infants settled into an advanced foot position--using the heel or flat part of the foot supported by body weight--to contact the surface of the treadmill and during stance. Infants decreased lateral contact and increased heel contact at touch-down, but decreased heel contact during the stance phase. Infants made contact with the heel and then rolled their foot into a flat position to bear their weight in mid-stance. This is even true of infants who had foot deformities such as club foot. With daily weight-bearing exercise, even infants with club foot could utilize the flat part of the foot instead of the lateral part of the foot. This example demonstrates the possibility that exercise can contribute to better joint alignments via continuous repetitions of weight-bearing on their bones, particularly the foot, during the first year of life.

**Parental Compliance**

Our intervention is believed to be the first to attempt an early aggressive and long term approach in a home-based exercise program carried out by parents of infants with MMC. Based on parents' comments, they seem to enjoy the dedication to development of their infant. The counter numbers from the treadmill and parent log allowed the tracking of each baby's compliance (the duration of daily treadmill practice) over intervention. Unfortunately the compliance of treadmill training seems to vary across the babies over the first 6 months of intervention. In the beginning of intervention, parents seem eager to provide 10 minutes of exercise a day without it being burdensome. Compliance with treadmill exercise fell off in the middle part of intervention, however: it began quickly requiring tremendous attention and time commitment to implement the treadmill exercise. Therefore, parents provided exercise on average of 6 - 8 minutes daily, which was lower than the requested 10 minutes a day (see Appendix C. Table C. 1).

Several factors could have contributed to the relatively low compliance from our participants such as home environment, frequent medical appointments, number of siblings, or family income. In addition, it might be case that parents believed they were providing longer time of practice, but truly they never get full required minutes per day. It is hard to find the most important factor for maintaining adherence to intervention, but family income and no siblings seem to be salient to aid compliance. For example, infant #10, who is the only child of an average-middle class family, practiced an average of 9 minutes a day, which is above the group mean. Meanwhile, infant # 9, who had siblings and came from a lower income family, faced obstacles often and showed struggle at times to keep practicing as scheduled. Although we visited each family's house biweekly and provide feedback on their child's progress, it may have

been insufficient for families who were dealing with multiple challenges. As researchers, it is necessary to encourage family participation to maintain adherence to our exercise protocol throughout the intervention. We proposed that weekly phone conversation, sharing our exercise protocol with a pediatric PT if they saw one regularly, or better instrumentation to measure compliance should be added for further work to optimize effects of treadmill training. In addition, we will conduct a survey for any parent whose child graduated from our intervention, and answers will be shared with the public elsewhere. We hope that their responses to our survey questions will help researchers to design home-based intervention programs for children with neuromotor disabilities.

**Limitation**

As we discussed a bit in each discussion section, our study had limitations that should be considered. The sample size in our study was relatively small, and the medical profiles of each participant were varied. In addition, low compliance of treadmill practice time may have affected the outcomes of the intervention group. Thus, definitive conclusions could not be drawn over the first 6 months of intervention.

## Conclusion

Our study provides a foundation to build on for future studies. We have demonstrated a.) young infants can tolerate regular upright activity without apparent adverse effects b.) parents can, with biweekly “coaching”, provide meaningful activity therapy and c.) even less than 10 minutes/day, 5 times/week can increase neuromotor control. Given our results from pilot work as a starting point, optimal training protocol can be designed for infants with motor disabilities. We highlight considerations for future studies, such as compiling nutrition and body weight for intervention with activity. In addition, we encourage future researchers to address the impact of

age of onset of activity therapy, ways to enhance infants' goals/motivation, and the impact of mixing the intensity of training.

Table 4. 1. Medical and Anthropometric Characteristics of Participants

| Participants No. | Surgery | Fusion Level | Hydrocephalus | Shunt | Arnold -Chiari Malformation | Clubfeet | Gestational Age (wk) | Birth Length (cm) | Birth Weight (kg) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | IU | L3 | Y | | Y | | 36 | 49.53 | 3.288 |
| 2 | EU | L3 - L4 | Y | Y | Y | | 39 | 48.26 | 2.663 |
| 3 | EU | L4 | Y | Y | Y | Y | 39 | 53.34 | 3.458 |
| 4 | EU | L4 - L5 | Y | Y | Y | | 31 | 39.37 | 1.2 |
| 5 | EU | L4 - L5 | Y | Y | Y | | 38 | 50.8 | 3.571 |
| 6 | EU | L4 - L5 | Y | Y | Y | | 39 | - | 3.345 |
| 7 | EU | L5 | Y | Y | Y | | 39 | 49.53 | 3.515 |
| 8 | EU | L5 - S1 | Y | | Y | | 37 | 45.72 | 3.27 |
| 9 | EU | L5 - S1 | Y | Y | Y | | 40 | - | - |
| 10 | EU | S1 | Y | Y | Y | | 34 | 38.1 | 1.473 |
| **MEAN** | | | | | | | 37.20 | 46.83 | 2.86 |
| **SD** | | | | | | | 2.82 | 5.45 | 0.91 |

*Note.* [a] EU = extrautero (surgery to close myelomeningocele performed after birth), IU = intrautero (surgery to close myelomeningocele performed in utero).

Table 4.1. Continued

| Participants No. | Surgery | Fusion Level | Hydrocephalus | Shunt | Arnold -Chiari Malformation | Clubfeet | Gestational Age (wk) | Birth Length (cm) | Birth Weight (kg) |
|---|---|---|---|---|---|---|---|---|---|
| 11 | EU | L1 - L2 | Y | | Y | | 37 | 53.34 | 3.52 |
| 12 | IU | L2 - L3 | Y | | | | 34 | 44.45 | 2.10 |
| 13 | EU | L3 | Y | Y | Y | Y | 38 | 47.00 | 3.06 |
| 14 | EU | L3 | Y | Y | | | 40 | 54.61 | 3.81 |
| 15 | EU | L4 | Y | Y | Y | Y | 38 | 48.26 | 3.46 |
| 16 | IU | L4 | Y | | Y | Y | 37 | 50.80 | 3.77 |
| 17 | IU | L4 | Y | | Y | Y | 37 | 50.17 | 3.12 |
| 18 | EU | L4 - L5 | | | Y | | 38 | 45.72 | 3.27 |
| 19 | EU | L5 -S1 | | | | | 37.5 | 55.88 | 3.52 |
| 20 | EU | L5 -S1 | Y | Y | | | 36.5 | 48.90 | 2.95 |
| 21 | EU | L5 -S1 | | | | | 37 | 48.26 | 2.83 |
| 22 | EU | S1 | | | | | 36.5 | 49.53 | 3.71 |
| **MEAN** | | | | | | | 37.21 | 49.74 | 3.26 |
| **SD** | | | | | | | 1.39 | 3.47 | 0.49 |

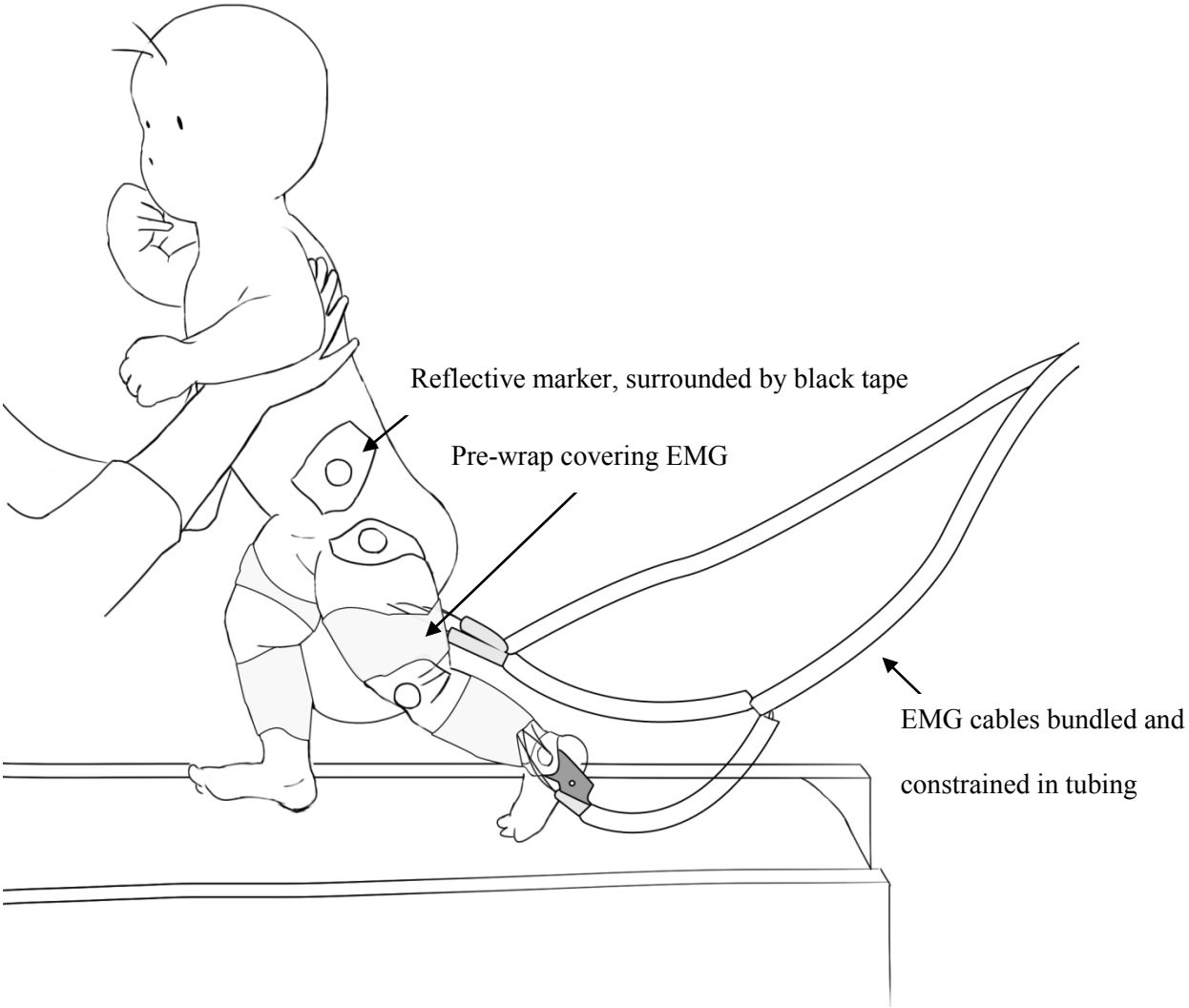


Figure 4. 1. Illustration of infant being held on the small motorized treadmill.

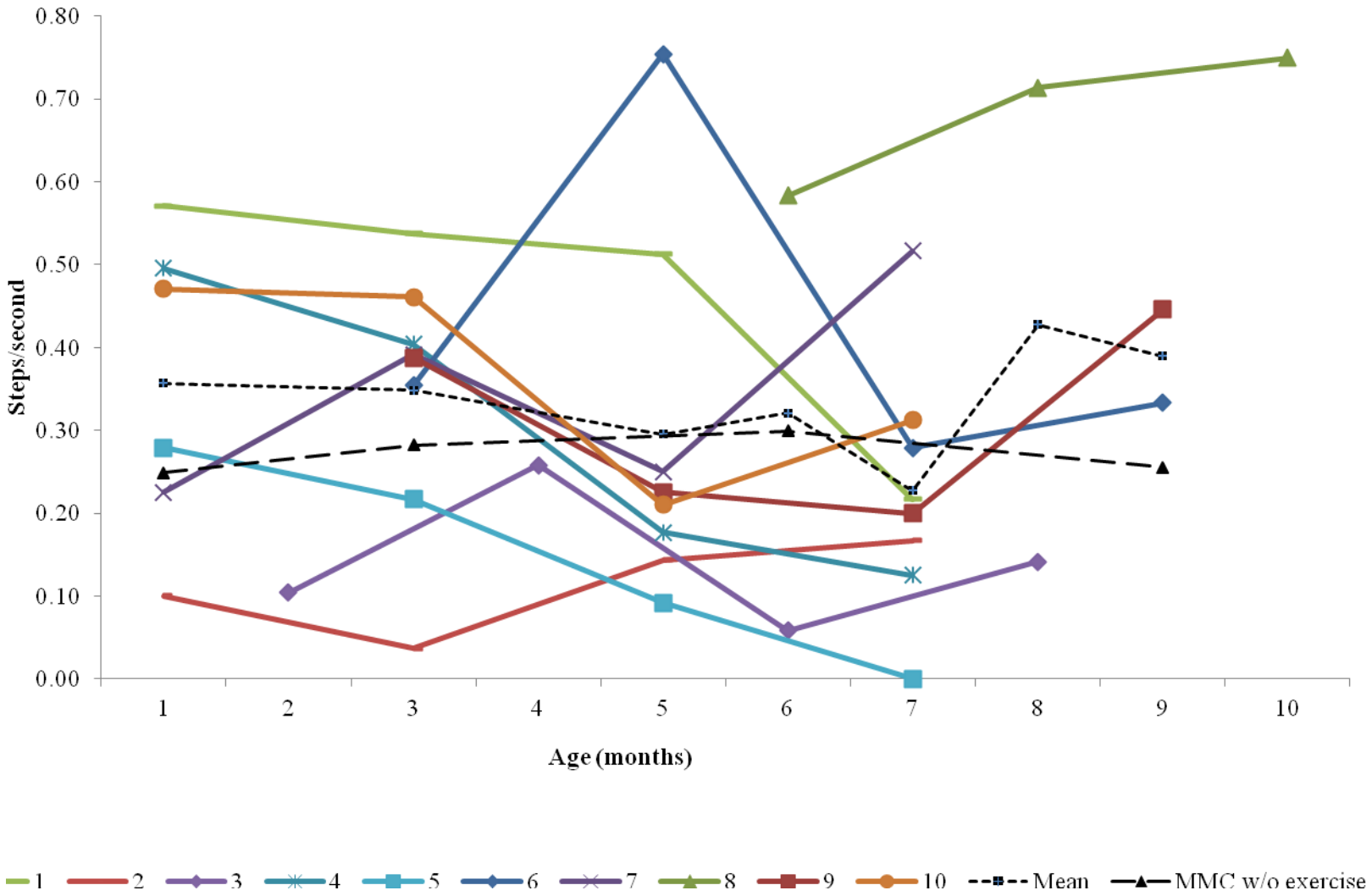


Figure 4. 2. Number of steps taken per second over the first 10 months of life.

Colored lines represent data for each baby in intervention group; black lines show means for intervention and comparison groups.

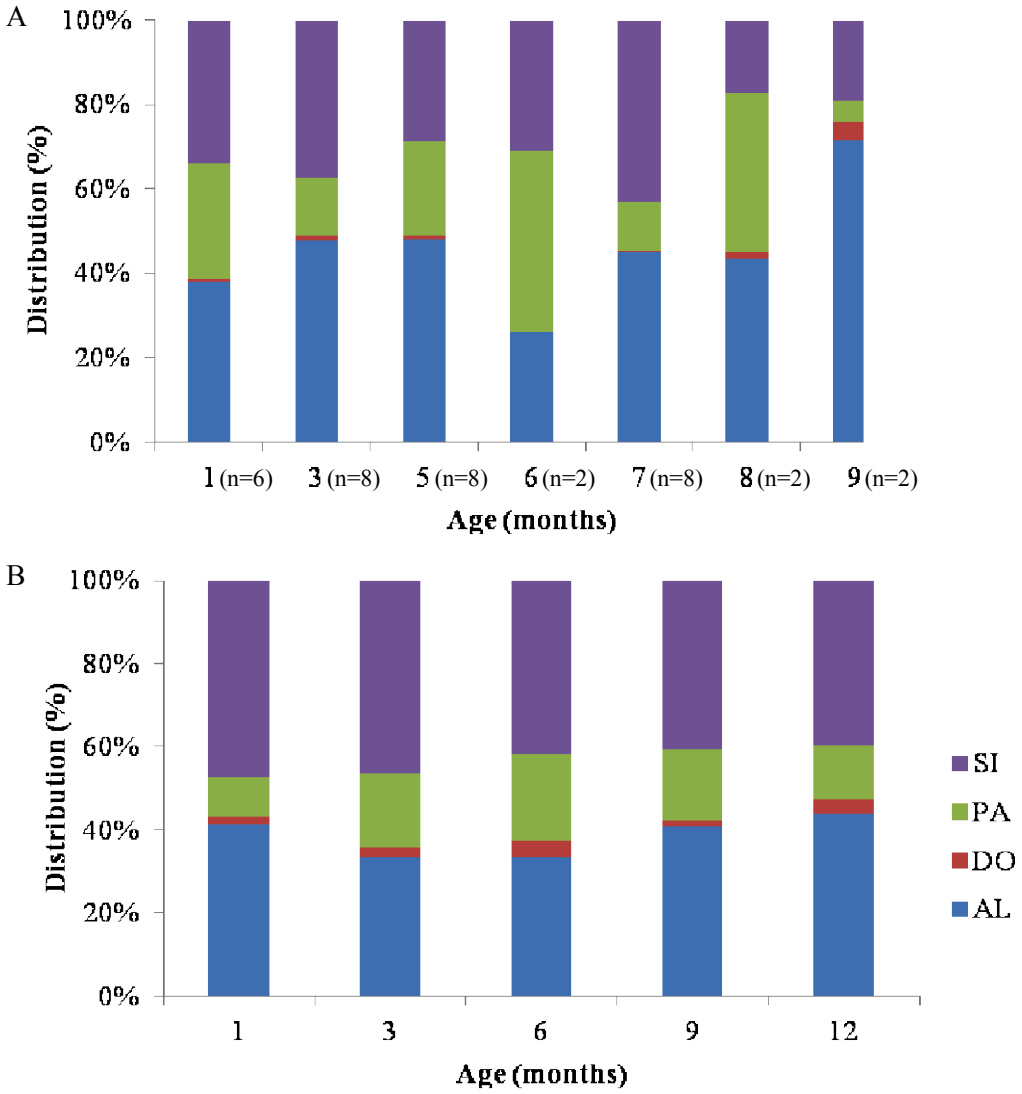


Figure 4. 3. Distribution of stepping patterns produced by infants in intervention group (A) and comparison group (B).

Alternating (AL), double (DO), parallel (PA), and single (SI).

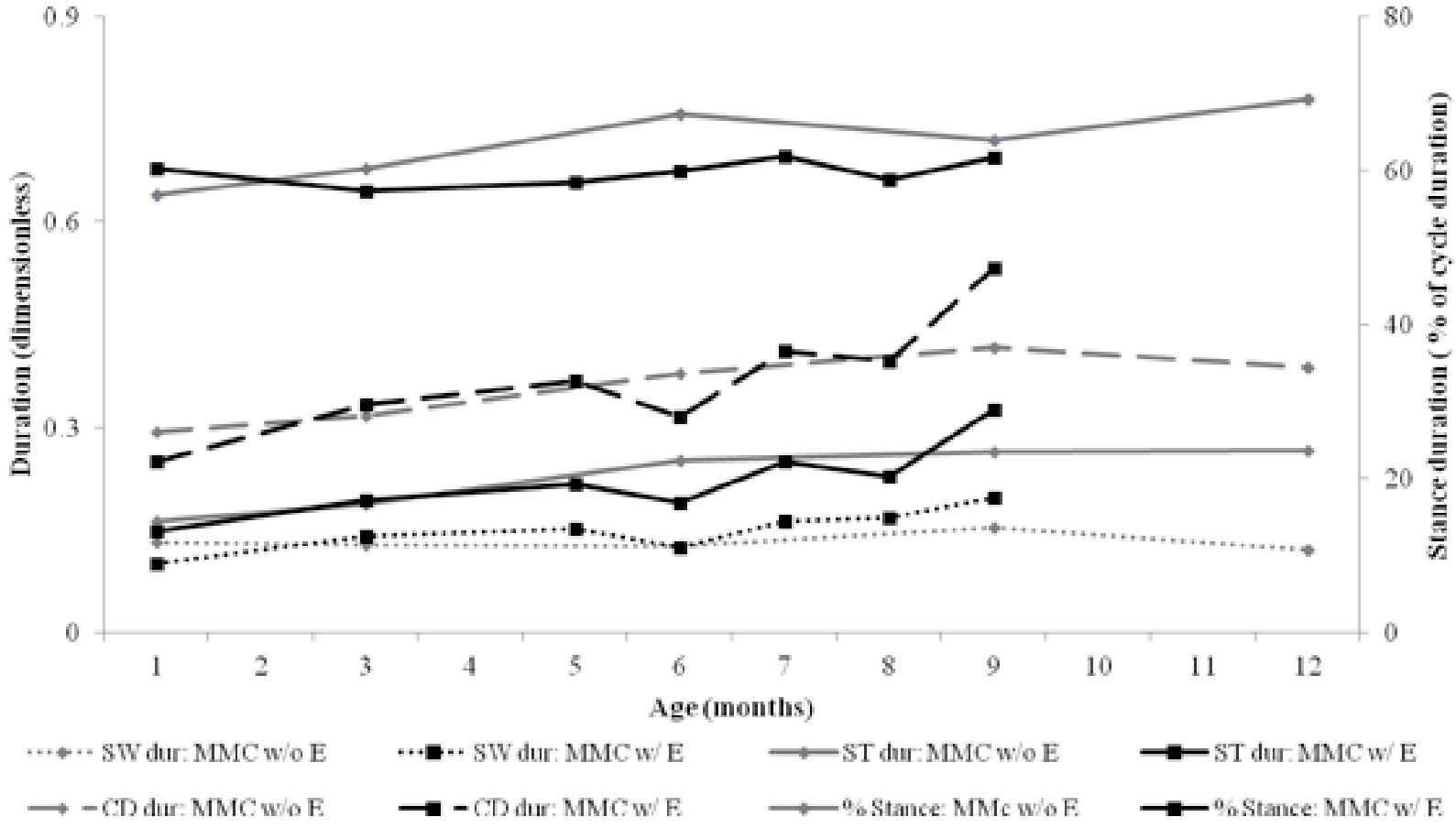


Figure 4. 4. Temporal parameters for alternating steps.

Mean of normalized cycle duration, swing duration, stance duration, and percent stance per cycle.

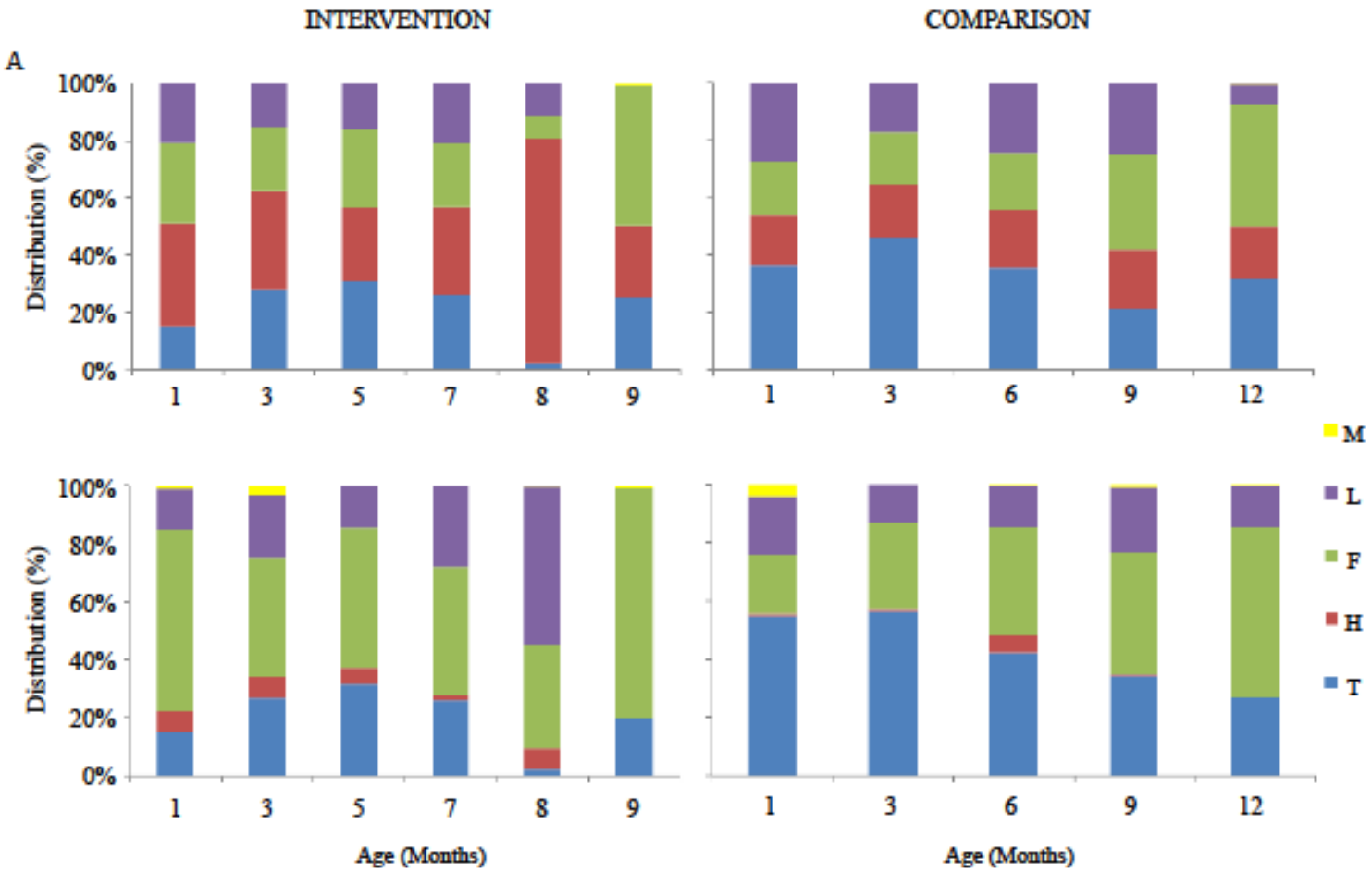


Figure 4. 5. Foot position of alternating steps.

Mean percentages of foot positions at touch-down (A) and mean percentages of foot positions during mid-stance (B): toe (T) heel (H), flat (F), lateral (L), medial (M).

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## Chapter V

# DEVELOPMENTAL TRAJECTORY OF BONE MINERAL CONTENT IN INFANTS WITH TYPICAL DEVELOPMENT AND MYELOMENINGOCELE


## Abstract

Purpose: The aim of the present study was to document changes in bone mineral content (BMC) over the first 18 months of life both in infants born with myelomeningocele (MMC), and in healthy infants. Due to the lack of normative data tables for infants, we compared alternative ways to normalize raw data obtained via a dual energy X-ray absorptiometry (DXA) scan – infancy mode. We compared the changes in BMC among infants who are developing typically, ones with MMC, and ones born with MMC who received early and sustained activity intervention.

Method: We tested 18 infants with MMC (9 in the intervention group, 9 in the control group) and 13 infants with typical development (TD) across ages 1-18 months. BMC measurements of the whole body, using a whole body DXA scanner (GE Lunar Prodigy Advance Plus, equipped with GE Lunar enCORE software for infants), were obtained. From the raw whole body BMC, raw segmental BMC data were extracted as were the software-estimated bone mineral density (BMD) values for whole body and body segments. Using anthropometric measures and published algorithms for segment mass, we normalized the BMC values to each infant's unique size.

Results: Considering all normalization procedures, generally, all groups showed increases in whole body BMC with age; infants with MMC's values being only slightly lower than those for

TD infants. BMC values for legs and arms were more clearly lower for infants with MMC compared to infants with TD. However, between the two MMC groups, only the group with upright activity intervention showed clear increases in BMC with age. Normalization to length and widths seemed to be appropriate to express bone mineralization across age and population. Discussion and conclusion: DXA scan data can be useful in activity and growth in research, when values are normalized to skeletal length to track BMC in infants with TD and MMC. Our data showed that in the MMC population, the process of bone mineralization begins to lag during infancy with greater decrements in the legs and arms than whole body. With a larger database these reference datasets of BMC can help clinical professionals identify significant deviations from typical values across populations, and help to diagnose and prevent osteoporosis and risk for pathologic fractures. This technique also holds the potential to assess the impact of early activity and nutrition interventions for infants with and without bone growth problems during infancy.

## Introduction

MMC is the most serious form of spina bifida, which is the most common neural tube defect today. In the United States approximately 1,500 to 2,000 babies are born with MMC each year (Spina Bifida Fact Sheet, 2007). Defining the etiology of this disability is the fact that during the first few weeks of gestation one or more of the vertebral arches fails to close; subsequently, the spinal cord loops out of this spinal opening and folds, causing sensorimotor deficits below the affected neurological level of lesion. Most lesions occur in the lumbar or sacral regions, affecting the lower extremities and impairing functional ability and ambulation.

During infancy, spontaneous leg movements are depressed and lower limb motor control is delayed or fails to develop (Findley et al., 1987; Rademacher, Black, & Ulrich, 2008; Sival et

al., 2004; Smith, Teulier, Sansom, Stergiou, & Ulrich, 2011). This reduced activity and weight-bearing behavior can lead to poor body composition (i.e., bone mineral content and bone mineral density), and to varying degrees of osteoporosis and pathologic fractures, starting from infancy and sustained throughout the lifespan (Akbar et al., 2010; Anschuetz, 1984; James, 1970; Lock & Aronson, 1989; Teulier et al., 2009; Valtonen et al., 2006). For example, by the end of the first year, infants born with MMC exhibit shorter leg length and smaller shank circumference than those of age-matched healthy infants (Teulier et al., 2009). An incidence of bone fractures between 11.5% and 30% has been reported in children with MMC during childhood, though rates decrease as time spent walking increases (Akbar et al., 2010; James, 1970; Lock & Aronson, 1989).

Despite the fact that the MMC population is prone to osteoporosis and pathologic fractures, only a few studies have reported changes in the bone mineral content (BMC [g]) and bone mineral density (BMD [$g/cm^2$]) of older children and adults with MMC using dual-energy X-ray absorptiometry (DXA) which is a standard of measurement of bone health status as a result of its availability, accuracy, reliability, and low radiation exposure (Salle et al., 1992). Rosenstein, Greene, Herrington, and Blum (1987) reported, based on using I single photon absorptiometry, that BMD of the distal radius, tibia, and first metatarsal in children with MMC may be strongly affected by the neurologic level of spinal cord involvement and ambulatory status. Several other studies also indicated that children and young adults with MMC have lower BMD in both the upper and lower extremities, compared to those of healthy children and young adults (Apkon, Fenton, & Coll, 2009; Ausili et al., 2008; Quan, Adams, Ekmark, & Baum, 1998; Valtonen et al., 2006). Instead of BMD of the whole body, regional BMD of lower extremities only, such as femoral neck, is the most appropriate way to detect a decrease in BMD and acquire

pathological fractures between ambulators and non-ambulators in children with MMC (Apkon et al., 2009; Ausili et al., 2008; Szalay & Cheema, 2011). However, there have been conflicting results in the association of neurologic level of spinal cord involvement, ambulatory status, and BMD in children with MMC: Rosenstein et al. (1987), Apkon et al. (2009), and Szalay and Cheema (2011) found benefits of up-right locomotion on BMD, while Quan et al. (1998) did not support the trend toward improved BMD in relation to either ambulatory status or neurologic level. In addition, because documentation of BMD or BMC is nonsexist in infants with MMC, how early and how rapidly these reduced BMD/BMC occur in the children with MMC is not yet known.

Basic tissue science indicates that BMD increases through reasonable stresses and forces that are produced via muscle activity and weight-bearing activities (Eliakim, Raisz, Brasel, & Cooper, 1997; Karlsson et al., 2002; Slemenda, Miller, Hui, Reister, & Johnston, 1991). Importantly, the growing skeleton has a greater capacity to adapt its stress and forces associated with weight-bearing exercise than the adult skeleton. Bone mineralization begins in utero at about 8 weeks, when reflexive limb movements begin and minerals (especially calcium) begin to replace cartilage (Maltin, Delday, Sinclair, Steven, & Sneddon, 2001). During infancy, when the healthy infants learn to oppose the forces of gravity and control their limbs to sit, stand, and locomote, bone mass and strength are crucially increased. Generally, during infancy those functional motor milestones might augment bone mass and enhance the bone's structural characteristics that contribute to overall bone strength throughout the lifespan. Despite this body of knowledge, we still don't have clinical evidence of the effectiveness of weight-bearing activity, where acquisition of functional motor milestones significantly influenced bone mass and bone strength accrual during infancy. Most DXA studies mainly documented body composition,

including BMC and BMD with children starting from age 2 (Apkon et al., 2009; Ausili et al., 2008; Quan et al., 1998; Rosenstein et al., 1987; Szalay & Cheema, 2011; Valtonen et al., 2006).

Although no empirical data yet exists to prove the enhancement of bone strength via functional activity in young infants (aged 0-12 months), there is substantial literature to support effectiveness of various physical activities on optimizing bone mass and strength in healthy children and children with limited ambulation (Caulton et al., 2004; Chad, Bailey, McKay, Zello, & Snyder, 1999; Fuchs, Bauer, & Snow, 2001; MacKelvie, Petit, Khan, Beck, & McKay, 2004; Schneider & Zernicke, 1992). In healthy children, Fuchs et al. (2001) and MacKelvie et al. (2004) reported an increase (ranging from 1.3 to 3.9 %) for femoral neck BMC following 6 months of exercise compared to controls. Children with limited ambulation also demonstrated that increased duration of standing or physical activity with an emphasis on weight-bearing activity influenced BMD of the spine and femoral neck (Caulton et al., 2004; Chad et al., 1999). Further, there is no data available that identifies the opportune period during which bone adapts most efficiently to loading, or the most appropriate amount of weight-bearing to increase bone mass and strength in the young population-particularly among the population with neurological disorders. Moreover, in studies with healthy children and adults, the minimum effective dose of weight-bearing exercise required to either maintain or enhance bone strength has not been documented.

As general awareness of osteoporosis and fractures within the young population has increased, use of DXA has been extended to the pediatric population, including neurologic disabilities. In the pediatric population, however, interpretation of DXA is complicated by issues related to ongoing skeleton growth and bone mineral accrual. Because DXA measurement is based on the two-dimensional projected area of a three-dimensional structure, both bone growth

and bone mineral accrual may cause erroneous values of BMD along with age progression. For example, aBMD (area BMD) may be lower in infants with short stature even though the vBMD (volumetric BMD) is normal. In addition, no population means are available to compare changes in bone density among the pediatric population. Therefore, unlike with the adults' study, the T-score that compares an individual's BMD with young adult normative data at peak bone mass should not be used for the pediatric population who has not completed either their growth or their bone mineral acquisition. The Z-score (Standard Deviation compared to individuals of the same age) is occasionally applied, but this method is inappropriate for children who are either unusually large or small for their chronological age. Due to those limitations, most DXA studies with infants generally accept BMC as the more appropriate expression of DXA measurement for the infant population, compared to BMD, the common expression in adult DXA measurements (Carter, Bouxsein, & Marcus, 1992). In addition, infant DXA studies mainly focus on identifying other anthropometric factors that predict BMC values (Koo et al., 1996, 1998; Salle et al., 1992). Their results suggest that whole body mass is the strongest predictor of BMC in healthy, full-term neonates and infants, followed by body length. However, these results may simply reflect that bigger babies have larger "skeletons," and thus more BMC but not relatively more BMC, or denser bones. That is, DXA measurement may reflect the changes in body size and composition that occur with body growth (i.e., length or width) more than true changes in BMC. The inabilities of DXA technology to account for the large changes in body size that occur during growth restrict its use in longitudinal studies in the young population, who need to regularly monitor BMC. Reasonable methods for normalization of pediatric BMC to anthropometrics may need to be investigated.

Currently, no normed references of body composition exist for healthy infants or infants with developmental disabilities across the first year post-birth. Specifically for our interests, no researchers have been published documenting changes in bone growth and BMC in infants with MMC across the first year of life. International Society for Clinical Densitometry (ISCD) recommended that BMC of the spine and total body less head should be monitored regularly in the young population if bone health is at risk, but the accuracy of these regional BMC is sometimes decreased due to deformity of bone elements in the MMC population. A reasonable site for regional BMC values needs to be defined and monitored for the MMC population. Indeed, reference datasets of infancy-BMC are urgently needed for clinical professionals to identify significant deviation from typical values across MMC populations, and for diagnosing and preventing osteoporosis and pathologic fractures.

The purpose of our study was to evaluate changes in BMC over the first18 months of life, for both infants born with MMC and healthy infants. First, we compared several options for treating raw BMC data, obtained via DXA scan, of infants using the Infant Protocol (GE Lunar Prodigy Advance Plus, equipped with GE Lunar enCORE software). We compared trends in data over 1-18 months for total body, as well as upper vs. lower body segments, using dependent variables: raw BMC data, "BMD" data calculated by the Infancy software, raw data normalized to anthropometric values such as body (or segment) mass and length, and ankle and knee joint widths. We hypothesized that normalization to segment length (e.g., leg) was a reasonable choice and more useful than normalization to segment mass. Indeed, based on relatively healthy development in functional movements of the upper body in children with MMC, we expected that the arm BMC, normalized to arm length, showed a steady increase with age, and predicted no differences between TD and MMC during the first 18 months of life. Whole body and leg

BMC normalized to length, however, showed no difference at birth, but significant difference with age.

Secondly, we tested the effect of weight-bearing exercise on raw and normalized BMC within the MMC population. Based on evidence of weight-bearing exercise with healthy children, we hypothesized that compared with MMC infants who did not receive intervention, MMC infants with intervention would show a steady increase of BMC with age and their rate of changes in BMC at all measured regions would be more similar to that of healthy infants.

## Method

### Participants

We tested 31 infants (13 TD, 9 MMC, and 9 MMC who received treadmill therapy), ages 1–18 months (Table 5. 1). Infants with TD and MMC without treadmill therapy were tested cross-sectionally by age across the 18 months. Infants with MMC who received treadmill therapy were participants in a study of the effects of early intervention treadmill training. Details of the intervention are provided in chapter 4. Infants in the intervention group were tested first prior to involvement then after 6 months, and 12 months of intervention. Inclusion criteria for all infants with MMC were lumbar- or sacral-level lesions only, gestation age at birth greater than 32 weeks, and no known physical problems beyond those associated with MMC. Infants with TD were full-term with no known cognitive or physical problems. We recruited infants with MMC via medical teams and support groups across Southeast Michigan and Northeast Ohio including the pediatric neurosurgery team at Mott Children's Hospital (University of Michigan), the pediatric physiatrists at the Myelomeningocele Clinic (Detroit Medical Center), Sparrow Hospital MMC Clinic, and the Spina Bifida Association of Toledo. Infants with TD were

recruited from the local community via word-of-mouth and flyers posted in public areas (e.g. daycares, libraries). All procedures were approved by the University of Michigan Institutional Review Board. Consent was obtained from parents prior to their infant's participation in the study. Families were given a monetary gift for participating.

**Procedure**

All DXA scans took place at the Michigan Clinical Research Unit (MCRU) in the Cardiovascular Center at University of Michigan Hospital. We conducted testing sessions at the time of day (between 8am and 5pm) that the parents identified that their infant was most likely to sleep. To ensure quality control of the system (GE Medical Systems Lunar, 2008), the GE DXA technician calibrated the scanner daily using a Lucite calibration block consisting of tissue-equivalent material with 3 bone-simulating chambers of known BMC. Prior to the scan, the baby's weight and length were measured for operation of the scanner software (GE Lunar Prodigy Advance Plus, equipped with GE Lunar enCORE software- Infant Mode). We asked parent(s) to change the baby's diaper and dress their infant in lightweight clothes without any metal parts (e.g., snaps), or to swaddle their infant in a cotton blanket if necessary to keep the infant still. Then, parents encouraged their infant to sleep, placed the baby supine on the scan bed (262.3cm(L) and 90cm(W)) with arms and legs as close to anatomical position as possible (Figure 5. 1). For the infants who would only sleep on their stomachs, we placed them in prone position. Once infants were in a deep sleep, the DXA scan began: a C-shaped arm glided over the bed slowly from head to feet, taking approximately 3 minutes of time. The radiation was approximately 5μSv which is less than the dose of a standard chest X-ray (Blake, Naeem, & Boutros, 2006). During the scan, infants were not repositioned, and remained within the scan-region lines which we marked on the scanner bed to define the borders of the infants' proper

positioning. When excessive movement (movement artifacts) occurred during the scan procedures, creating a blurred image or jagged body segments on the scan monitor, a second scan was attempted.

For normalization and segment mass calculation, we measured infants' anthropometrics: body weight; length of total body (crown to heel), upper arm (acromion process to lateral epicondyle), forearm (lateral epicondyle to styloid process), thigh (greater trochanter to knee joint line), shank (knee to lateral malleolus), and foot (heel to big toe); circumferences in upper arm, forearm, thigh, and shank; and widths of malleolus, knee, and hip. We assessed concurrent motor skill development level by administering the motor subscale items from the Bayley Scales of Infant Development III (BSID III).

**Data Reduction**

Post scan, we reviewed the image created by the software (GE Lunar enCORE) to assure that the body segment template was aligned appropriately over the infant's body and no movement artifact (such as blurriness or broken-up body segments) occurred. As needed, we adjusted the geometric lines on the scan to clearly demarcate each region including leg, pelvis, trunk, arms, and head (Figure 5. 2).

From the raw data we extracted BMC and estimated BMD for individual body segments and for whole body. With anthropometric measures, we calculated estimated body segment mass and normalized BMC values to each baby's unique size with the following formula:

Length

Whole Body BMC [g]/Crown to Heel Length [cm]

Average of Right Leg BMC [g]/Right Thigh + Right Shank Length [cm]

Left Leg BMC [g]/Left Thigh + Left Shank Length [m]

Right Arm BMC [g]/Right Upper Arm + Right Forearm Length [cm]

Mass

Whole Body BMC [g]/Total Body mass [kg]

Average of Right Leg BMC [g]/Calculated Right Leg Mass [kg]

Left Leg BMC [g]/Calculated Left Leg Mass [kg]

Right Arm BMC [g]/Calculated Right Arm Mass [kg]

The formula for Leg mass (Schneider & Zernicke, 1992)

(0.069126 × Age [years] + 2.9582 × Thigh length [m] + 3.1541 × Thigh circumference [m] – 0.67217) + (0.0065138 × Body Mass [kg] + 1.8158 × Shank length [m] + 1.8743 × Shank circumference [m] – 0.35460)

The formula for Arm mass (Schneider & Zernicke, 1992)

(0.012249 × Infant Body Mass [kg] +1.3067 × Upper Arm Length [m] + 0.98645 × Upper Arm Circumference [m] - 0.19376) + (0.0052671× Infant Body Mass [kg] + 0.978584 × Forearm Length [m] + 1.1492 × Forearm Circumference [m] – 0.16886)

Width

Right Leg BMC [g]/Knee Width [cm]

Right Leg BMC [g]/Malleolus Width [cm]

**Data Analysis**

We used PASW version 20.0 statistical software for statistical analyses. All data were reviewed for distribution normality and homogeneity in error variance, ensuring that assumptions of the multiple linear regressions were not violated. There are significant interactions between age and group in each variable of BMC: the regression lines generated by the subgroups within each dependent variable showed significant interaction between MMC groups. Therefore, we applied multiple regressions instead of Analysis of covariance (ANOCA). Our regression model compared BMC at all sites measured across the groups over developmental time. In multiple regression models, we allowed age factor to be varied across the groups, so that we could apply

regression analysis with different distribution of age among the groups. We chose an alpha level of .05 to determine the significance of all dependent variables (two-tailed).

## Results

Our results presented here reflect changes in raw BMC and BMC as normalized by different methods in various regions, across the first 18 months of life in healthy infants and infants with MMC. Within each segment region, we present raw BMC values, and BMC as normalized to lengths, mass, and estimated bone area (GE BMD values). For the leg segments, we also report BMC values normalized to knee and malleolus widths. Results of our data suggest that across the first 18 months of life, raw BMC and normalized BMC changed, but the rate of changes differed among regions (whole body, legs, and arms) and by subgroup (TD, MMC with activity, and MMC without activity).

### Whole Body BMC

Over the first 18 months of life, whole body BMC increased for all subgroups though slight differences were observed across the groups and by normalization procedure (Figure 5. 3). Raw whole body BMC showed healthy infants had higher levels, but was lower in those with MMC, who were in both intervention and non-intervention groups. However, in multiple regression models analyses (factors = group, age, and group × age), the slops of raw whole body BMC were not statistically different among the groups by adjusting age factor to be varied across the groups (Wald Chi- Square = 4.69, $p = 0.096$). Depending on normalization methods, group differences varied. Whole body BMC normalized by skeletal length and mass suggested a trend toward lower values in both groups of infants with MMC compared to infants with TD, but whole body BMC normalized with area (BMD) showed the reverse pattern. In normalized whole body BMCs, the difference was not statistically significant among the three groups (for BMC

normalized by skeletal length: Wald Chi- Square = 3.49, $p = 0.174$, by area: Wald Chi- Square = 1.47, $p = 0.48$, & by mass: Wald Chi- Square = 0.96, $p = 0.618$).

**Leg BMC**

Raw BMC of the legs was greater for healthy infants than MMC infants at all ages, with the difference increasing over developmental time (Figure 5. 4. A). Infants with MMC in the activity group showed lower raw leg BMC than healthy infants, but their slope increased similar to TD infants across age. MMC infants without activity, showed no change in leg BMC over age. A comparison of raw leg BMC regression lines demonstrated a significant difference among groups (Wald Chi-Square = 17.56, $p = 0.000$). Depending on normalized methods, regression line slopes varied. Normalized leg BMC with leg length and knee and ankle widths showed significant differences among the groups, with the lowest BMC in MMC infants without the intervention program over the first 18 months of age (for leg BMC normalized by length: Wald Chi- Square = 6.56, $p = 0.038$, by knee width: Wald Chi- Square = 15.26, $p = 0.000$, & by ankle width: Wald Chi- Square = 16.11, $p = 0.000$) (Figure 5. 4. B – D). However, in normalized leg BMC with area (BMD), the slope of the three regression lines were not significantly different among the groups (Wald Chi- Square = 2.45, $p = 0.293$) (Figure 5. 4. E). Leg BMC normalized by mass showed the reverse pattern: regardless of group, all participants demonstrated a decrease in leg BMC with age (Figure 5. 4. F).

When a comparison of the slope of the three regression lines revealed a statistical difference, we applied post-hoc analysis to compare difference in β coefficients across the group. In raw leg BMC and normalized leg BMC by length and widths, post-hoc analysis indicated that differences were found between MMC infants with and without intervention (for raw leg BMC: Wald Chi-Square = 8.91, $p = 0.003$ and for normalized leg BMC by length: Wald Chi-Square =

6.52, $p = 0.011$). However, there was no significant difference in β coefficients between healthy infants and MMC infants with activity (for raw leg BMC: Wald Chi-Square = 3.64, $p = 0.056$ and for normalized leg BMC by length: Wald Chi-Square = 0.89, $p = 0.343$).

**Arm BMC**

By adjusting the age factor in the regression model, a comparison of raw arm BMC regression lines demonstrated a significant difference among groups (Wald Chi-Square = 7.00, $p = .030$)(Figure 5. 5. A). In MMC infants with exercise, raw BMC was slightly lower than that of healthy infants, but nevertheless, it rose with age, indicating that the rate of accrual of bone mineralization was slower in MMC infants, compared to that of healthy infants. In contrast, there was no rise in raw arm BMC in MMC infants without exercise over developmental time. In all MMC infants, there was a trend showing higher values of raw arm BMC in MMC infants with exercise, but there was no significant difference by participation of intervention program (Wald Chi-Square = 2.15, $p = .143$). None of the normalization procedures revealed statistically significant differences among subgroups for normalized arm BMCs (Figure 5. 5. B - D): a comparison of the three regression lines revealed that β coefficients were not different across the groups (for normalized arm BMC by length: Wald Chi-Square = 4.73, $p = 0.094$, by area: Wald Chi-Square = 0.17, $p = 0.920$, & by mass: Wald Chi-Square = 0.05, $p = 0.974$). Interestingly, there was a trend showing positive relations between age and normalized arm BMC with length or area (BMD), particularly in healthy infants and MMC infants with intervention, but normalized arm BMC with mass showed a strong negative relation with increasing age across groups (Figure 5. 5. D).

## Discussion

To the best of our knowledge, this is the first study to document whole body and body segment bone mineral content in infants with MMC across the first 18 months post birth. It is also the first to assess the impact of physical activity therapy, via treadmill training, on bone mineral content in infants with MMC. As expected for healthy babies, and as we found for infants with MMC in both groups, whole body BMC increased with age. We observed small but not statistically significant differences among these three subgroups, regardless of whether we examined the raw data or data normalized by skeletal length, bone area, or body mass. For upper and lower limbs, analyzed separately from whole body, we observed lower values for infants with MMC compared to those with TD. However, in MMC infants, partial weight-bearing exercise seemed to improve bone strength at all sites measured, but its effect was more statistically pronounced at the lower body.

### Trajectory of Raw BMC over the First 18 Months

Whole body BMC increased steadily during infancy regardless of group, but the rate of change differed slightly among the groups. In all MMC infants, at birth, the degree of whole body bone mineral content was very similar to that of age-matched healthy infants, but with age, differences emerged. While their whole body BMC gradually lagged with age, their BMC for the legs and arms seemed to lag more quickly after birth and remained lower across infancy. Moreover, MMC infants who did not receive weight-bearing exercise showed no change in BMC in the leg and arm BMC. That is, the process of bone mineralization begins to lag during infancy and the most pronounced decrement was happening in the upper and lower limbs with different rates of changes.

Finding lower raw BMC in infants with MMC was not surprising. Previously, several researchers reported that children and adults with MMC suffer statistically low bone mineralization, particularly in the lower body (Apkon et al., 2009; Ausili et al., 2008; Rosenstein et al., 1987; Szalay & Cheema, 2011; Valtonen et al., 2006). Area BMD in any regional site of the lower extremities, including femoral neck, trochanteric region of hip, mid-tibia, and metatarsals were approximately 1 to 2 standard deviations below the mean of age- and gender-matched healthy children. For example, Szalay and Cheema (2011) found that 65.8 % of children with MMC had diminished BMD at the lateral distal femur, specifically, 2 standard deviations below the age- and sex- matched mean. Across numerous different regional sites studied, bone demineralization of the lower body is observed consistently for MMC children. Our results suggest that this starts in infancy; BMC remains low and continues lower than in the healthy population throughout childhood and adolescence, creating the high risk of osteoporosis and fractures.

Low BMC in the arms was not expected in infants with MMC. We hypothesized that infants with MMC who generally develop typical function in the upper body, would, as a result show relatively normal bone mineralization in the upper limbs. Contrary to our hypothesis, our data suggest that MMC infants without intervention did not increase in bone mineralization in the arm with age. As for adults with MMC, they tend to use assistive devices requiring the upper extremities, so that use of the upper extremities in daily life contributes to healthy bone strength, particularly in the upper body. Therefore, BMD of the forearm was nearly within normative values in adults with MMC (Valtonen et al., 2006). However, there are opposite results from Quan et al. (1998) that demonstrate reduced BMD at the distal radius, in children with MMC

compared to healthy children. Given the mixed results, further studies are required to investigate the degree of bone strength at the upper body for the MMC population.

As we reviewed above, an interesting finding from our data was that depending on regional site, BMC values showed different trajectories over developmental growth. Whole body BMC showed only a small difference among groups in slope and amount. However, when closely looking at individual regions, particularly the legs, differences in bone mineralization became more statistically pronounced among the groups. This pattern was also reported by Apkon et al. (2009) for children with MMC. They found that whole body BMD in children with MMC was not different from that of healthy children, but BMD at the femoral neck was significantly lower and varied as a function of ambulatory status and lesion level.

In infants and children, the skull constitutes a substantially greater proportion of the total body (skeletal mass), compared to adults (approximately 25% vs. 12.5%) (Sinclair, 1989). Because of this large head in proportion to the total body, when we conduct whole body BMC scan and compare the values, decrements of BMC in the leg or arms may not be clearly detected in pediatrics. To avoid this issue, there are several studies suggesting direct regional measurement of bone mineralization in the upper or lower body instead of the whole body scan for growing children (Henderson et al., 2002; Kroger, Kotaniemi, Vainio, & Alhava, 1992; McKay et al., 2000). However, unlike with older children, skeletal landmarks in infants may not be well developed to conduct the direct regional measurement (e.g., the distal radius or femoral neck). Thus, a whole body scan may be suggested as an only acceptable alternative for the young infants (Gordon et al., 2008; Taylor, Konrad, Norman, & Harcke, 1997). To identify decrement of sub-regions, we proposed that from the whole body scan, upper and lower bodies should be

demarcated via regional lines and BMC of those sub-regions should be calculated and monitored in young infants.

**Normalized Methods**

A large database to which our MMC participants' data can be compared to establish a normative percentile, does not exist. So, normalized techniques are needed for comparison of BMC to normative values of healthy infants. We applied 3 different methods to normalize the raw data with skeletal length, area, and mass. Depending on the region of interest, some of them seemed to be more sensible than others to assess the trajectory of BMC across the group and age. Due to the small sample size of healthy infants and no validation of our results against a gold standard outcome, it is difficult to conclude the most appropriate way to express bone mineralization. Although it is still limited, the method most consistently aligned with literature and what one might hypothesize for differences among TD, MMC, and MMC infants with activity were normalization to skeletal length and joint widths. The trajectory of normalized BMC to skeletal length or widths seemed quite similar to that of raw BMC at all measured sites. We did not measure radius width for normalization methods of arm BMC, but we suggest that given the results of normalized leg BMC to widths, the normalization to radius width in the upper limbs should be an appropriate way to express the level of bone mineralization in infants. Unlikely with normalization to length or widths, BMD and normalized BMC to mass seemed inappropriate and often showed reverse patterns compared to raw BMC.

Normalization to segmental mass did not prove reasonable for examination of bone mineral content. We observed decrease in arm and leg BMCs normalized with mass across the first 18 months of life. The fact that values for bone mineral reduced with age when normalized to mass does, however, make sense, given the relative changes during infancy in body weight

and body length. From birth to 12 months of age, infants on average increase their skeletal length by 50% while they increase their body mass to values three times what they weighed at birth. Thus, proportionately, while bones grow longer and denser, their relative increases are much smaller than growth in body mass. This might be why normalized leg and arm BMCs with mass decreased with age. There are few studies suggesting that body composition (i.e., lean mass) can explain the changes of total body less head BMC for children and adults (Crabtree et al., 2004). However, measuring body composition is unreliable and less accurate to use for infants who are younger than 2 years old due to their small body size (Hammami, Koo, & Hockman, 2004). Thus, it may not be an appropriate normalization method to use for young infants.

Area BMD also showed a different rate of changes compared to raw whole body BMC or whole body BMC with other normalized methods. Because the small bone status may distort the edge detection of body areas determined by DXA- infant software, BMC at the region of interest may be less accurate, compared to an adult scan. For growing infants under 2 years old, a whole body scan constitutes a different shape of bone, causing more complicated estimation of depth of individual bones. Therefore, even with infant software algorithms, interpretation of bone density results still seemed to be complicated in the young population. This may be why BMC is mostly preferred for assessment of bone status in growing infants, rather than area BMD. Nonetheless, we should not conclude this explanation with a small sample size. It needs to be reviewed with a larger population.

**Effects of Weight-Bearing Exercise on Bone Strength**

Despite the fact that the MMC population is at increased risk for low bone strength as they age, the importance of weight-bearing exercise to increase bone strength has long been neglected, particularly during infancy. Our results suggest raw BMC in MMC infants with

focused weight bearing activity to be higher than those without activity. Interestingly, a slight difference was observed for whole body BMC, but MMC infants with activity therapy showed increased BMC in the arms and legs with age, while MMC infants without activity did not show increased BMC in the limbs with age. These findings are likely to be a result of the benefits of early weight-bearing activity on bone strength during early infancy.

The reduced spontaneous movement characterizing the MMC population may contribute to the lack of bone mineralization in our subgroup of MMC participants who did not receive frequent opportunities to be upright and active. But, a supplementary intervention program, constituting mainly of partial weight-bearing exercise, enabled the second subgroup of infants with MMC to use their legs to bear their weight during steps. Although BMC at all measured sites in MMC infants with exercise were lower than that of healthy babies, this activity, when repeated, eventually contributes to increased BMC, compared to those without exercise. Interestingly, weight-bearing exercise, stimulating mainly muscle activity of the lower body and mechanical compression on joints of the lower body, influenced a systemic effect on the entire body, particularly in the upper extremities, instead of localized effects on lower extremities. That is, weight-bearing exercise could play an important role in reducing the risk of low arm and leg BMC in infants with MMC. Our findings are consistent with previous works showing that daily passive range-of-motion exercise of both the upper and lower bodies increased bone mineralization in premature infants (Litmanovitz et al., 2003; L. Moyer-Mileur, Luetkemeier, Boomer, & Chan, 1995; L. J. Moyer-Mileur, Brunstetter, McNaught, Gill, & Chan, 2000). Ausili et al. (2008) showed an increase in BMD at the lumbar vertebrae and femoral neck regions in adults with MMC who participated in athletic activities in comparison with those who did not.

From our results, we could conclude that effects of weight-bearing exercise would be of help in increasing bone mineralization during infancy. The important question to consider is whether the observed increased level of BMC of the MMC intervention group would translate to increased bone strength, eventually causing reduction in the incidence of pathological fractures later in their life. In other words, the long-term benefits of this exercise are still unknown in young infants. It is important to acknowledge that early weight-bearing exercise may be the optimal approach to prevent secondary conditions (e.g., osteoporosis or fractures) in children with neurological disabilities that lead to a decreased quality of life. Therefore, follow-up studies are needed to continuously review these effects on bone strength even beyond intervention.

**Factors that May Affect BMC**

Medical factors should be taken into consideration for a clinically meaningful interpretation of BMC in a population who are known to be at an increased risk of fractures. For example, the prognostic factors indicate lower BMC in the young infants: whether the low BMC in the MMC population was related to factors such as neurological level of lesion, ambulatory status, or incidence of fracture is unknown. Several studies reported reduced BMC at any site (whole body, arms or legs) was associated with neurological level or ambulatory status in children and adults. For example, neurological level of the spinal cord injury affected bone densitometry at several measured sites, which included the distal radius, femoral neck, and proximal femur in children with MMC (Ausili et al., 2008; Rosenstein et al., 1987). In addition, many studies demonstrated BMD at the femoral neck or hip to be markedly reduced and closely correlated with ambulatory status or incidence of fracture (Ausili et al., 2008; Quan et al., 1998; Rosenstein et al., 1987; Szalay & Cheema, 2011; Valtonen et al., 2006).

Our results suggested that the BMC was more closely related to involvement of weight-bearing exercise. But, risk factor could not be defined to explain the lower BMC in the MMC population. Due to the fact that our MMC infants were lumbar- or sacral-level lesions only and had not yet learned to walk (ambulation status), we could not examine these associations. We believe that if an older population with extended range of spinal level lesions (from thoracic to sacrum) had been studied, the relationship might have existed. However, we speculated that these associations may be only observed for children or adults. During infancy, those factors may not correspond well to reduced BMC and it is most likely that multiple and complicated factors may be associated, but not pronounced until childhood. For instance, the effects of neurological level of spinal cord injury may become more pronounced during childhood when development of bone strength reaches its peak point.

**Limitations**

We recognize the limitations of this study. One of our limitations is the relatively small number of participants in each of the groups. The low number of participants could be due to the difficulties of applying DXA scanning to a pediatric population. The assessment of bone mineral content in the pediatric population with and without congenital disabilities (MMC) could be problematic: keeping participants lying on their backs while sustaining the anatomical position was not easy for infants who were not sedated (Hammami et al., 2004). Moreover, because of clubfeet or muscle deformities characteristic of MMC, assessing and interpreting BMC may be inherently more difficult for the MMC population. However, we believe that even with a small population, our participants were very representative with regard to medical characteristics of MMC. Our first attempt to assess BMC in the pediatric population contributes to building a

normative dataset with healthy infants and to the growing body of evidence that infants with MMC are at an increased risk for low bone mineralization starting from infancy.

## Conclusion

In summary, our study produced the normalized values of BMC for infants and illustrated the relations among several potential normalization techniques to compare BMC values across age in infancy. Although the sample was small, the results suggest that infants with MMC have BMC that is lower than those with TD over the first 18 months post birth. Whole body BMC is an important base measure for evaluating BMC values across age and populations. But, BMC in the arm and leg segments may be critical because not all body parts grow and develop at uniform rates. Most importantly, early weight-bearing exercise can increase bone mineralization in infants with MMC during the first18 months of life. Lastly, among the several normalization techniques, normalization to length and widths seemed to be an appropriate tool to compare BMC over age.

Table 5. 1. Medical and Anthropometric Characteristics of Participants

| Group | Participant No. | Age (days) | Weight (Kg) | Length (cm) | Gastational Age(wk) | Fusion Level | Hydrocephalus | Shunt | Arnold-Chiari Malformation | Clubfeet |
|---|---|---|---|---|---|---|---|---|---|---|
| MMC (N = 9) | 1 | 42 | 4.56 | 54.15 | 39.00 | L4-L5 | Y | Y | Y | N |
| | 2 | 46 | 4.99 | 56.10 | 39.86 | L5 | Y | Y | Y | N |
| | 3 | 56 | 4.61 | 55.00 | 40.00 | L5 | Y | Y | Y | N |
| | 4 | 62 | 5.60 | 55.00 | 38.00 | L4-L5 | Y | N | Y | N |
| | 5 | 173 | 9.88 | 68.00 | 37.00 | L5-S1 | Y | N | Y | N |
| | 6 | 222 | 9.81 | 71.30 | 38.00 | L3-L5 | Y | Y | Y | Y |
| | 7 | 223 | 7.78 | 67.00 | 39.57 | L4-L5 | Y | Y | Y | N |
| | 8 | 294 | 7.59 | 66.00 | 34.00 | L2 | Y | Y | Y | Y |
| | 9 | 361 | 8.10 | 71.00 | 39.00 | S1-S2 | Y | Y | Y | N |
| | **MEAN** | 164.33 | 6.99 | 62.62 | 38.27 | | | | | |
| | **SD** | 119.12 | 2.12 | 7.38 | 1.88 | | | | | |

| Group | Participant No. | Age (days) | Weight (Kg) | Length (cm) | Gastational Age(wk) | Fusion Level | Hydrocephalus | Shunt | Arnold-Chiari Malformation | Clubfeet |
|---|---|---|---|---|---|---|---|---|---|---|
| MMC with activity (N = 9) | 10[a] | 17 | 2.82 | 45.30 | 33.86 | S1 | Y | Y | Y | N |
| | 10[b] | 189 | 5.58 | 59.60 | | | | | | |
| | 10[c] | 390 | 7.16 | 65.30 | | | | | | |
| | 11[a] | 22 | 4.27 | 50.20 | 31.00 | L4-L5 | Y | Y | Y | N |
| | 11[b] | 197 | 8.25 | 63.00 | | | | | | |
| | 11[c] | 395 | 9.64 | 72.00 | | | | | | |
| | 12[a] | 43 | 5.18 | 53.40 | 38.00 | L4-L5 | Y | Y | Y | N |
| | 12[b] | 237 | 8.87 | 69.00 | | | | | | |
| | 13[a] | 48 | 3.34 | 50.60 | 39.20 | L3-L4 | Y | Y | Y | N |
| | 13[b] | 226 | 5.57 | 61.00 | | | | | | |
| | 14[a] | 50 | 5.15 | 56.30 | 36.60 | L3 | Y | N | Y | N |
| | 14[b] | 222 | 11.40 | 70.10 | | | | | | |
| | 15[a] | 63 | 3.38 | 58.00 | 37.00 | L3 | Y | Y | Y | Y |
| | 15[b] | 252 | 11.16 | 72.39 | | | | | | |
| | 16[a] | 64 | 4.38 | 54.40 | 39.00 | L4 | Y | Y | Y | Y |
| | 16[b] | 239 | 7.57 | 65.50 | | | | | | |
| | 17[a] | 79 | 6.00 | 59.10 | 39.00 | L4 -L5 | Y | Y | Y | N |
| | 17[b] | 251 | 9.30 | 69.20 | | | | | | |
| | 17[c] | 446 | 11.50 | 70.10 | | | | | | |
| | 18[a] | 92 | 6.14 | 59.40 | 40.00 | L5-S1 | Y | Y | Y | N |
| | 18[b] | 264 | 9.00 | 70.00 | | | | | | |
| | 18[c] | 459 | 10.08 | 76.00 | | | | | | |
| | **MEAN** | 192.95 | 7.08 | 62.27 | 37.07 | | | | | |
| | **SD** | 140.27 | 2.75 | 8.37 | 2.93 | | | | | |

*Note.* [a] pretest. [b] 6 months of intervention. [c] 12 months of intervention.

(Table 5. 1. Cont.)

| Group | Participant No. | Age (days) | Weight (Kg) | Length (cm) | Gastational Age(wk) | Fusion Level | Hydrocephalus | Shunt | Arnold-Chiari Malformation | Clubfeet |
|---|---|---|---|---|---|---|---|---|---|---|
| TD (N =13) | 19 | 30 | 4.82 | 57.20 | 41.00 | N/A | N/A | N/A | N/A | N/A |
| | 20 | 34 | 3.91 | 53.70 | 39.00 | | | | | |
| | 21 | 48 | 5.82 | 60.00 | 40.40 | | | | | |
| | 22 | 55 | 5.40 | 59.20 | 41.00 | | | | | |
| | 23 | 65 | 5.88 | 60.50 | 39.86 | | | | | |
| | 24 | 116 | 6.62 | 63.00 | 40.43 | | | | | |
| | 25 | 151 | 8.66 | 70.40 | 40.50 | | | | | |
| | 26 | 167 | 7.96 | 69.00 | 38.00 | | | | | |
| | 27 | 187 | 5.88 | 63.10 | 42.29 | | | | | |
| | 28 | 215 | 8.34 | 68.50 | 37.50 | | | | | |
| | 29 | 302 | 11.28 | 72.00 | 39.50 | | | | | |
| | 30 | 319 | 8.46 | 74.00 | 39.00 | | | | | |
| | 31 | 504 | 10.49 | 79.70 | 37.00 | | | | | |
| | **MEAN** | 168.70 | 7.20 | 65.41 | 39.65 | | | | | |
| | **SD** | 139.64 | 2.21 | 7.51 | 1.52 | | | | | |


Figure 5. 1. Photograph of a 2-month old infant with MMC placed on the DXA scan bed.

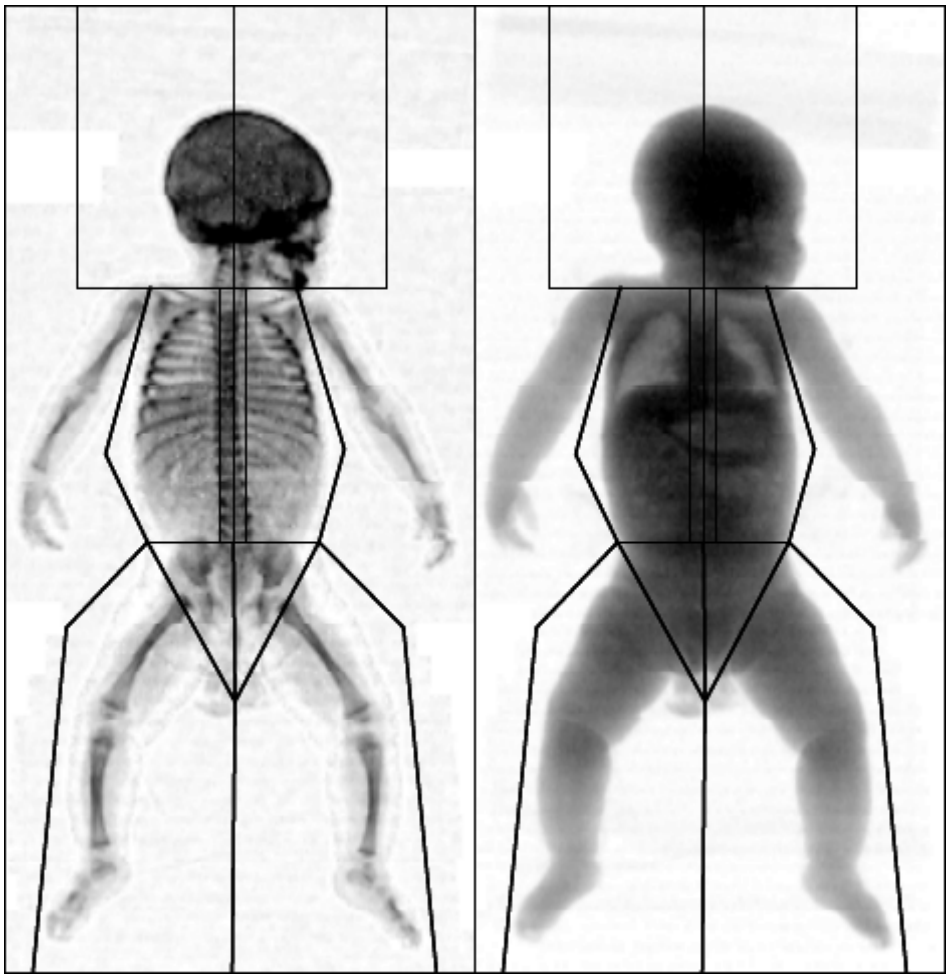

Figure 5. 2. GE Lunar Prodigy ADVANCE Plus DXA scan image of one infant with segment division lines.

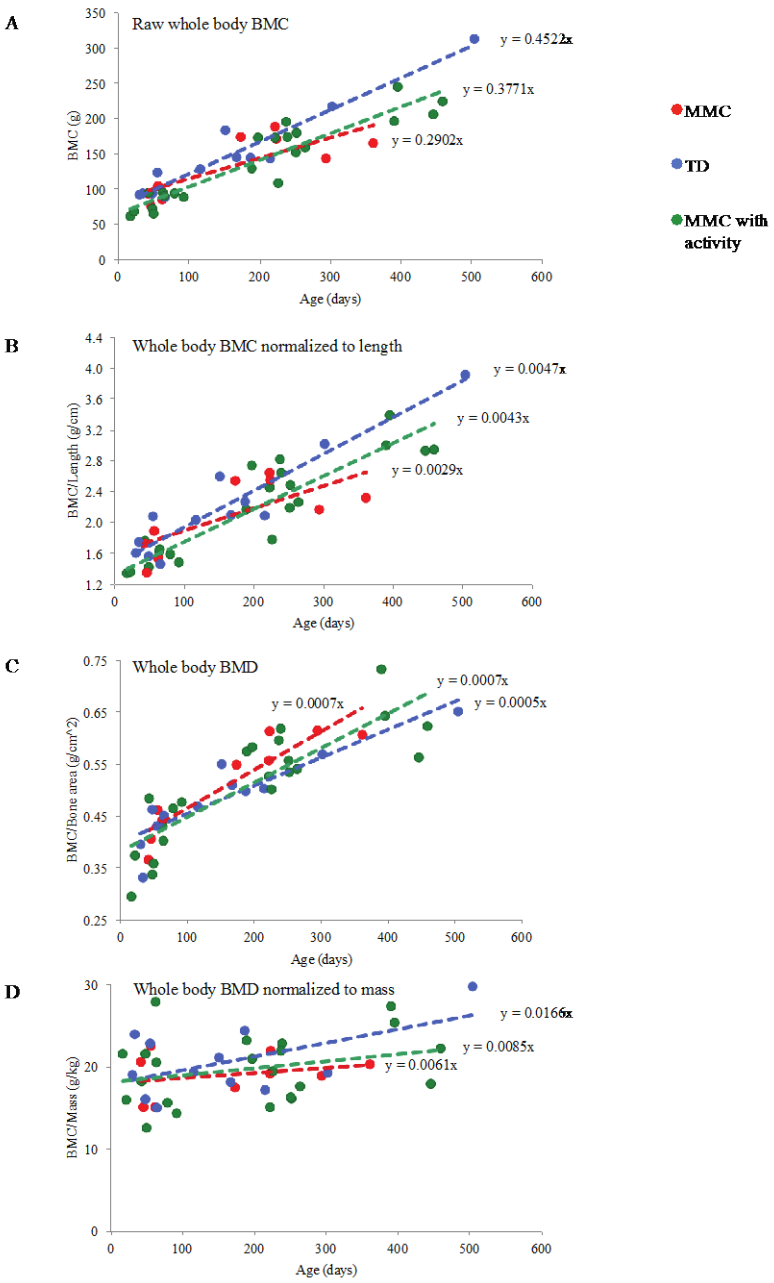


Figure 5. 3. Whole body BMC.

Raw BMC (A), BMC normalized to total body length (B), normalized to total body area as calculated by GE Lunar encore Software-infant Mode (C), and normalized to total body mass (D). Lines represent the line of best fit for each group's data.

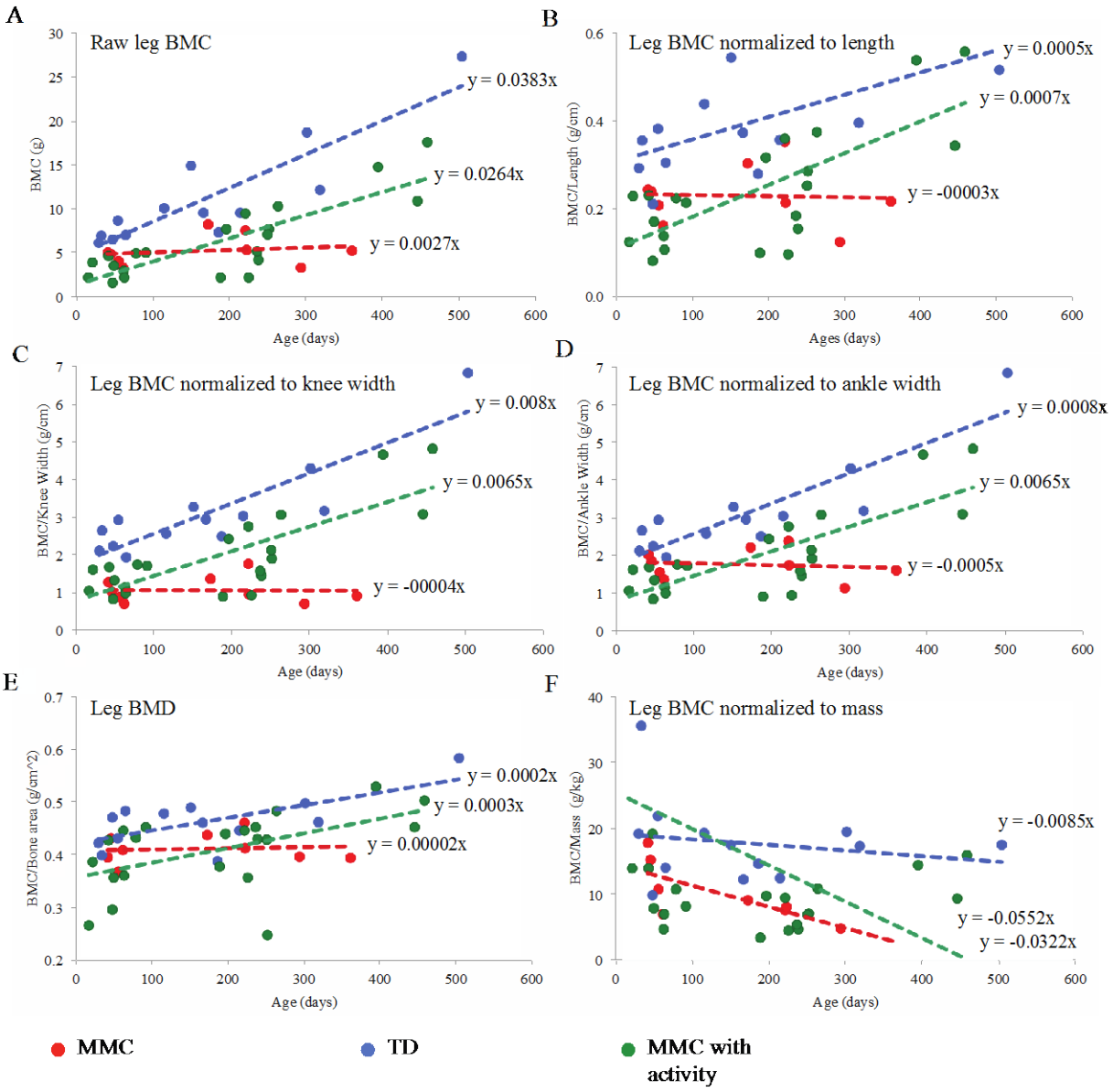


Figure 5. 4. Leg BMC.

Raw BMC (A), normalized to leg length (B), normalized to knee width (C) and malleolus width (D), normalized to lower body area as calculated by GE Lunar encore Software-infant Mode (E), and normalized to lower body mass (F). Lines represent the line of best fit for each group's data.

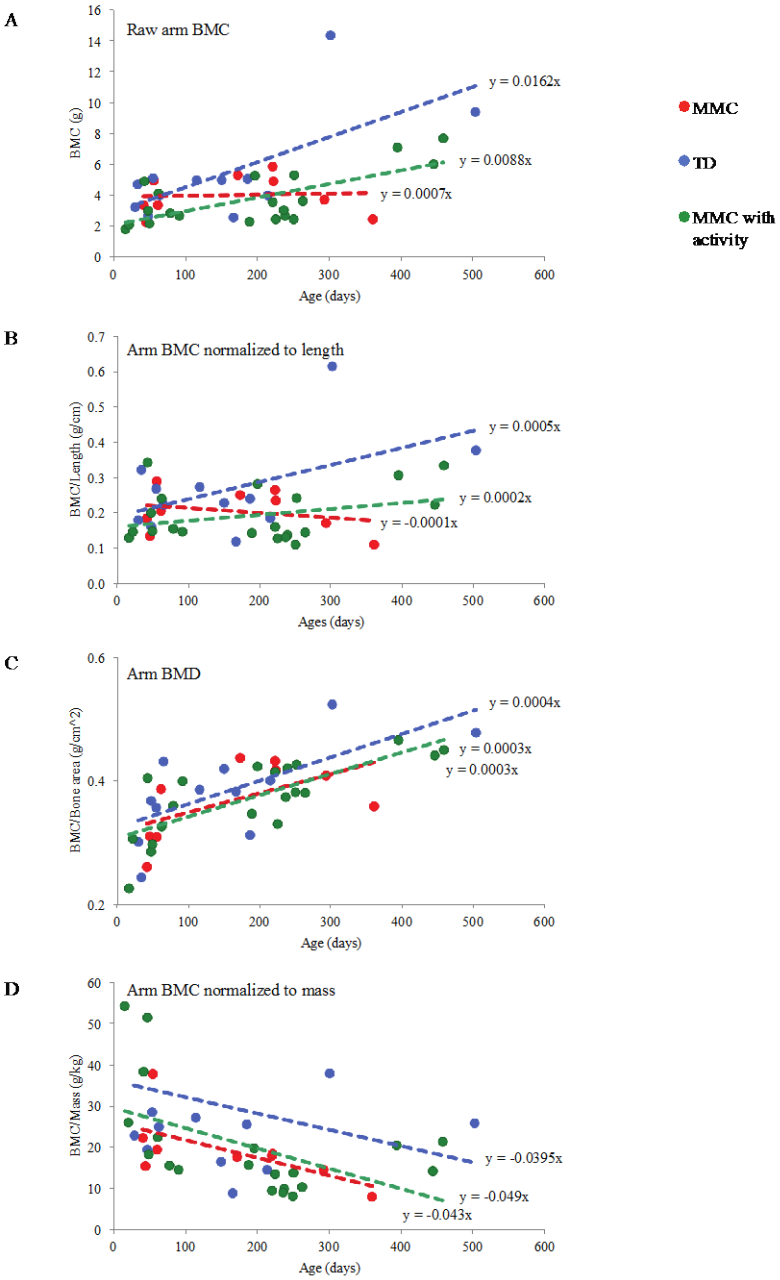


Figure 5. 5. Arm BMC.

Raw BMC (A), normalized to arm length (B), normalized to arm area as calculated by GE Lunar encore Software-infant Mode (C), and normalized to arm mass (D). Lines represent the line of best fit for each group's data.

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# APPENDIX A

Table A. 1. Overall Reflex Ratios across Stimulation Situations for Each Infant.

| Infant ID | Muscles | T-reflex | | | | | |
|---|---|---|---|---|---|---|---|
| | | R | | | L | | |
| | | QA | TA | GA | QA | TA | GA |
| 6 | Stimulated M | 55% | | | 40% | 14% | |
| | Anta. M | 18% | 11% | | 13% | 5% | 95% |
| 1[a] | Stimulated M | 89% | | | 73% | | |
| | Anta. M | | 7% | 3% | 45% | 10% | 41% |
| 10 | Stimulated M | 56% | 27% | | 100% | 10% | |
| | Anta. M | 11% | | 9% | 88% | | |
| 11 | Stimulated M | 16% | | 78% | 100% | | 45% |
| | Anta. M | | 5% | 11% | 63% | 5% | |
| 1[b] | Stimulated M | 4% | 17% | | 19% | 35% | 9% |
| | Anta. M | 17% | | 10% | 4% | 10% | 9% |
| 4 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 2[a] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 8 | Stimulated M | 9% | | | | 6% | |
| | Anta. M | | | | | 33% | |
| 7 | Stimulated M | 3% | | | | | |
| | Anta. M | | | | | | |
| 5 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 2[b] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 3[a] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 12 | Stimulated M | | | 5% | | | |
| | Anta. M | | | | | | |
| 9 | Stimulated M | 5% | | | | | |
| | Anta. M | | | | 5% | | |
| 3[b] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |

(Table A. 1. Cont.)

| Infant ID | Muscles | VIM-T-reflex | | | | | |
|---|---|---|---|---|---|---|---|
| | | R | | | L | | |
| | | QA | TA | GA | QA | TA | GA |
| 6 | Stimulated M | 22% | | | 64% | | |
| | Anta. M | 11% | | | 16% | | |
| 1[a] | Stimulated M | | | | 13% | | |
| | Anta. M | | | | 13% | | |
| 10 | Stimulated M | 60% | | | 63% | | |
| | Anta. M | 10% | | | 42% | | |
| 11 | Stimulated M | | | 30% | 25% | | 68% |
| | Anta. M | | | 10% | | | |
| 1[b] | Stimulated M | | 13% | | 27% | | |
| | Anta. M | | 13% | 11% | 27% | | |
| 4 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 2[a] | Stimulated M | | | | | 14% | |
| | Anta. M | | | | | | |
| 8 | Stimulated M | | | 4% | | | |
| | Anta. M | | | 8% | | 9% | |
| 7 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 5 | Stimulated M | | | | | | |
| | Anta. M | | | | | | 36% |
| 2[b] | Stimulated M | | | | | 30% | |
| | Anta. M | | | | | 30% | |
| 3[a] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 12 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 9 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 3[b] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |

(Table A. 1. Cont.)

| Infant ID | Muscles | VIR | | | | | |
|---|---|---|---|---|---|---|---|
| | | R | | | L | | |
| | | QA | TA | GA | QA | TA | GA |
| 6 | Stimulated M | 100% | | | 50% | | |
| | Anta. M | 50% | | | 50% | | |
| 1[a] | Stimulated M | 50% | 100% | 100% | 100% | 100% | 50% |
| | Anta. M | 100% | 100% | 50% | 100% | 100% | 100% |
| 10 | Stimulated M | | | | 100% | | |
| | Anta. M | | | | 50% | | |
| 11 | Stimulated M | 50% | | 50% | | 50% | 100% |
| | Anta. M | . | | | 100% | 100% | 100% |
| 1[b] | Stimulated M | | 100% | | | | |
| | Anta. M | 100% | 50% | 50% | | | 50% |
| 4 | Stimulated M | 100% | 50% | 100% | 50% | 50% | |
| | Anta. M | | 50% | 50% | | 100% | |
| 2[a] | Stimulated M | 50% | | | | 100% | |
| | Anta. M | | 50% | | | | |
| 8 | Stimulated M | 50% | 50% | 100% | 50% | | |
| | Anta. M | | | 50% | 50% | | |
| 7 | Stimulated M | | 100% | 50% | | 50% | |
| | Anta. M | 100% | 50% | 50% | | | |
| 5 | Stimulated M | | | 50% | | | |
| | Anta. M | | 100% | 50% | | | |
| 2[b] | Stimulated M | 50% | | | | | |
| | Anta. M | | | | | | |
| 3[a] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 12 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 9 | Stimulated M | | | | | | |
| | Anta. M | | | | | | |
| 3[b] | Stimulated M | | | | | | |
| | Anta. M | | | | | | |

Table A. 2. Detailed Responses of Infants in Moderate, Low, and No Response Groups.

**Moderate Response Group**

| Variables | Ratio(%) | | | Amplitude(mV) | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range |
| **T-reflex** | | | | | | |
| QA | 2% | 3% | 0 ~ 9% | 86.40 | 17.25 | 74.20 ~ 98.60 |
| GA | 0% | 0% | 0% | N/A | N/A | N/A |
| TA | 1% | 2% | 0 ~ 5.5% | 180.20 | N/A | 180.20 |
| **VI-T-reflex** | | | | | | |
| QA | 0% | 0% | 0% | N/A | N/A | N/A |
| GA | 1% | 2% | 0 ~ 4% | 183.60 | N/A | 183.60 |
| TA | 2% | 5% | 0 ~ 14% | 83.00 | N/A | N/A |
| **VIR** | | | | | | |
| QA | 19% | 37% | 0 ~ 100% | - | - | - |
| GA | 31% | 37% | 0 ~ 100% | - | - | - |
| TA | 19% | 26% | 0 ~ 50% | - | - | - |

**Low Response group**

| Variables | Ratio(%) | | | Amplitude(mV) | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range |
| **T-reflex** | | | | | | |
| QA | 0% | 0% | 0% | N/A | N/A | N/A |
| GA | 0% | 0% | 0% | N/A | N/A | N/A |
| TA | 0% | 0% | 0% | N/A | N/A | N/A |
| **VI-T-reflex** | | | | | | |
| QA | 0% | 0% | 0% | N/A | N/A | N/A |
| GA | 0% | 0% | 0% | N/A | N/A | N/A |
| TA | 8% | 15% | 0 ~ 30% | 75.37 | N/A | 75.37 |
| **VIR** | | | | | | |
| QA | 0% | 0% | 0% | - | - | - |
| GA | 13% | 25% | 0 ~ 50% | - | - | - |
| TA | 25% | 50% | 0 ~ 100% | - | - | - |

**No Response group**

| Variables | Ratio(%) | | | Amplitude(mV) | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range |
| **T-reflex** | | | | | | |
| QA | 1% | 2% | 0 ~ 5% | 190.40 | N/A | 190.40 |
| GA | 1% | 2% | 0 ~ 5% | 229.50 | N/A | 229.50 |
| TA | 0% | 0% | 0% | N/A | N/A | N/A |
| **VI-T-reflex** | | | | | | |
| QA | 0% | 0% | 0% | N/A | N/A | N/A |
| GA | 0% | 0% | 0% | N/A | N/A | N/A |
| TA | 0% | 0% | 0% | N/A | N/A | N/A |
| **VIR** | | | | | | |
| QA | 0% | 0% | 0% | - | - | - |
| GA | 0% | 0% | 0% | - | - | - |
| TA | 0% | 0% | 0% | - | - | - |

# APPENDIX B

## ***Background Information: Treadmill Therapy Study***

**Child's name**: _____ Today's Date:_____

**General Information:**

1. Date of Birth:_____ (mo) _____ (day) _____ (year) 2. Gender:___ male ___ female
3. Birth weight:_____ (lbs/oz)4. Birth length:_____(in)

**Medical History:**

5. Delivery:___ vaginal ___ C-section
6. Any complications during delivery?_____
_____
7. Date of surgery to close spine? _____
8. Levels of fusion? _____
9. Was your baby born prematurely?_____
10. If yes, how many weeks early? _____
11. Does your baby have a. Hydrocephalus? ____ Shunt? ____ Date of placement?_____
Describe any complications so far? _____
_____
b. Arnold Chiari? ___ Describe any complications so far? (sucking, breathing)?
_____
c. Bladder Status. Do you need to help your baby urinate via a catheter?
_____
d. Bowel complications? __If yes, what are they & how are they being treated?
_____
e. Clubbed feet? _____R _____L Describe_____
f. Hip problems (e.g., subluxation, dislocation)? _____R _____L
12. For any surgeries described above, how mnay days was your baby in the hospital?_____
13. Is your baby currently taking any prescription medications?_____ If yes, please list which ones,
Or what they are for_____
14. Other medical issues?
_____

**Family History:**

15. Parents' highest level of education:
_____ mother _____ father
16. Number of parents/grandparents contributing to daily care of child with MMC _____

Combined annual income of parent(s) supporting child with MMC:

_____ Below $20,000 _____$ 101,000 to $200,000

_____ $20,000 to $50,000 _____$ Above $200,000

_____$ 51,000 to $100,000

17. Please list below any siblings your baby has, their ages, and any medical issues they have.

| Name | Age | Medical Issues |
|---|---|---|
| 1. | | |
| 2. | | |

**Nutrition Survey: Treadmill Therapy Study**

Child's Initials_____ Child's DOB_____Today's Date_____

**Part One: Child's Diet**

1. How many times a day does your baby eat?
2. How much does your baby eat at each feeding?
3. What type of milk or formula do you feed your baby? (check all that apply)
   _____a. Breast milk
   _____b. Iron-fortified infant formula (brand _____)
   _____c. Low-iron infant formula (brand _____)
   _____d. Goat's milk
   _____e. Evaporated milk
   _____f. Whole milk
   _____g. Reduced-fat milk (2%)
   _____h. Low-fat milk (1%)
   _____i. Fat-free milk (skim)
4. Which of the following liquids do you put in your baby's bottle or sippy cup?
   _____a. Formula (brand _____)
   _____b. Milk
   _____c. 100% juice
   _____d. Fruit drinks (such as Kool-Aid, Sunny D, Capri Sun, Tampico, and Hi-C)
   _____e. Soft drinks
   _____f. Coffee or tea
   _____g. Water
   _____h. Other (name_____)
5. How much juice does your child drink every day?
   _____a. 4 ounces
   _____b. 6 oz
   _____c. 8 oz
   _____d. more than 8 oz
6. Do you ever add honey, syrup, or sugar to your baby's bottle?
   _____yes _____no
7. Does your baby ever take a bottle or sippy cup to bed?
   _____yes _____no
8. How much time does your baby spend in a swing, infant seat, or walker?
   _____(hours per day, average)
9. How much time does your baby spend in a play pen or on a mat/blanket on the floor?
   _____(hours per day, average)
10. Does your baby eat solid foods? Check all that apply.

| *Food* | *Age food was introduced* |
|---|---|
| Infant cereal | _____ |
| Vegetables | _____ |

Fruits _____
Eggs _____
Meat _____
Table Food _____
Other _____

**Part Two: Mother's Diet**

***Prenatal Diet***

1. What was a typical breakfast during your pregnancy?
2. What was a typical lunch?
3. What was a typical dinner?
4. Most common snacks you ate while pregnant (e.g., popcorn, potato chips, ice cream, apples, pickles, cookies, etc.)
5. Did you take any supplements (e.g., vitamins, herbals)? _____no _____yes (if so, please list which ones _____)
6. Alcohol consumption:
   _____none
   _____1-2 glasses beer/wine per week
   _____3-4 glasses beer/wine per week
   _____1-2 glasses beer/wine per day
7. Tobacco consumption:
   _____none
   _____1-2 cigarettes per day
   _____3 or more per day

***Current Diet if Nursing***

1. What do you typically have for breakfast?
2. What was a typical lunch?
3. What was a typical dinner?
4. Most common snacks you eat (e.g., popcorn, potato chips, ice cream, apples, pickles, cookies, etc.)
5. Do you take any supplements (e.g., vitamins, herbals)? _____no _____yes (if so, please list which ones _____)
6. Alcohol consumption:
   _____none
   _____1-2 glasses beer/wine per week
   _____3-4 glasses beer/wine per week
   _____1-2 glasses beer/wine per day
7. Tobacco consumption:
   _____none
   _____1-2 cigarettes per day
   _____3 or more per day

# APPENDIX C

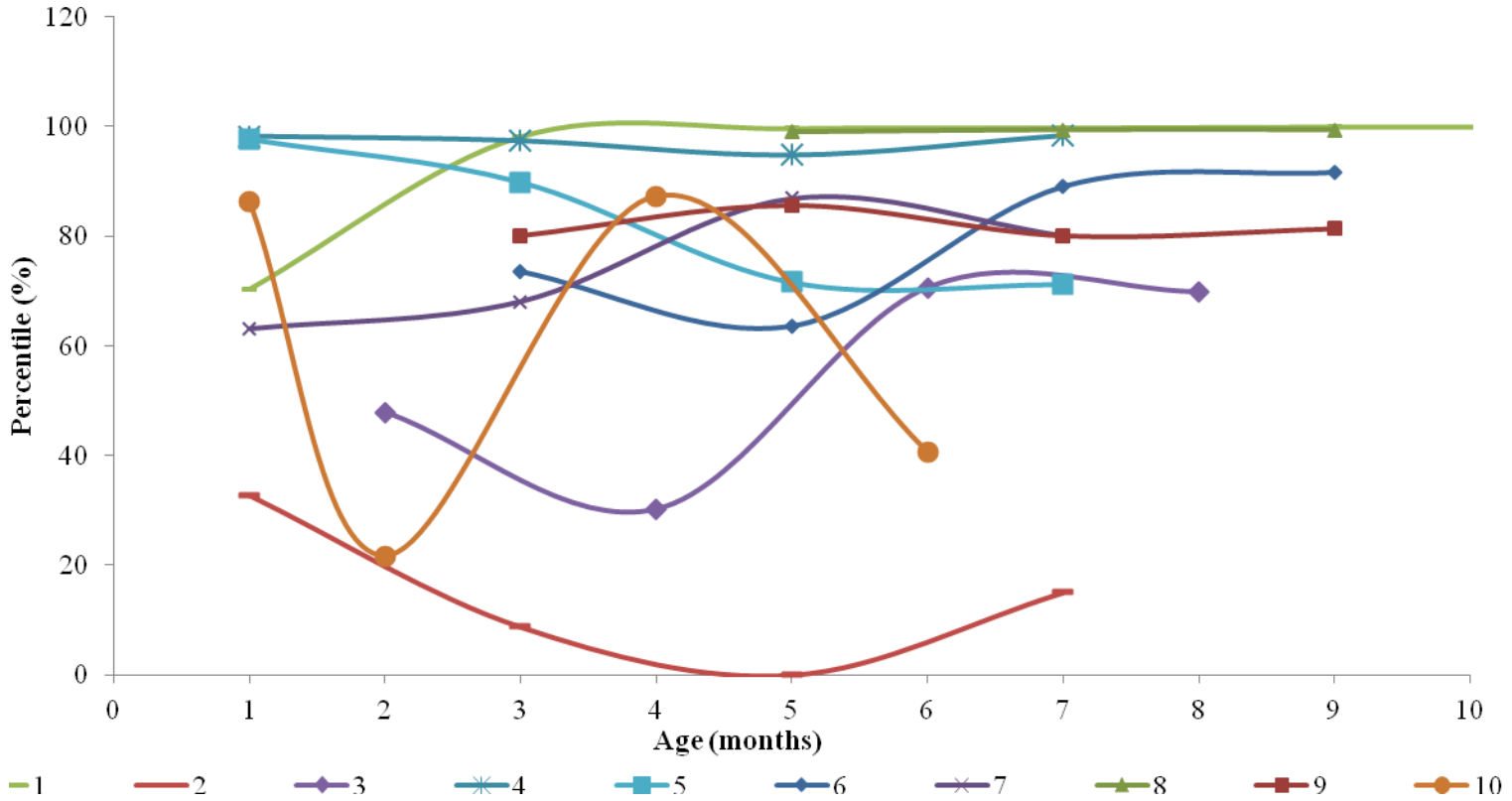



Figure C. 1. Percentage of weight for length in CDC chart.

Colored lines represent data for each baby in intervention group.

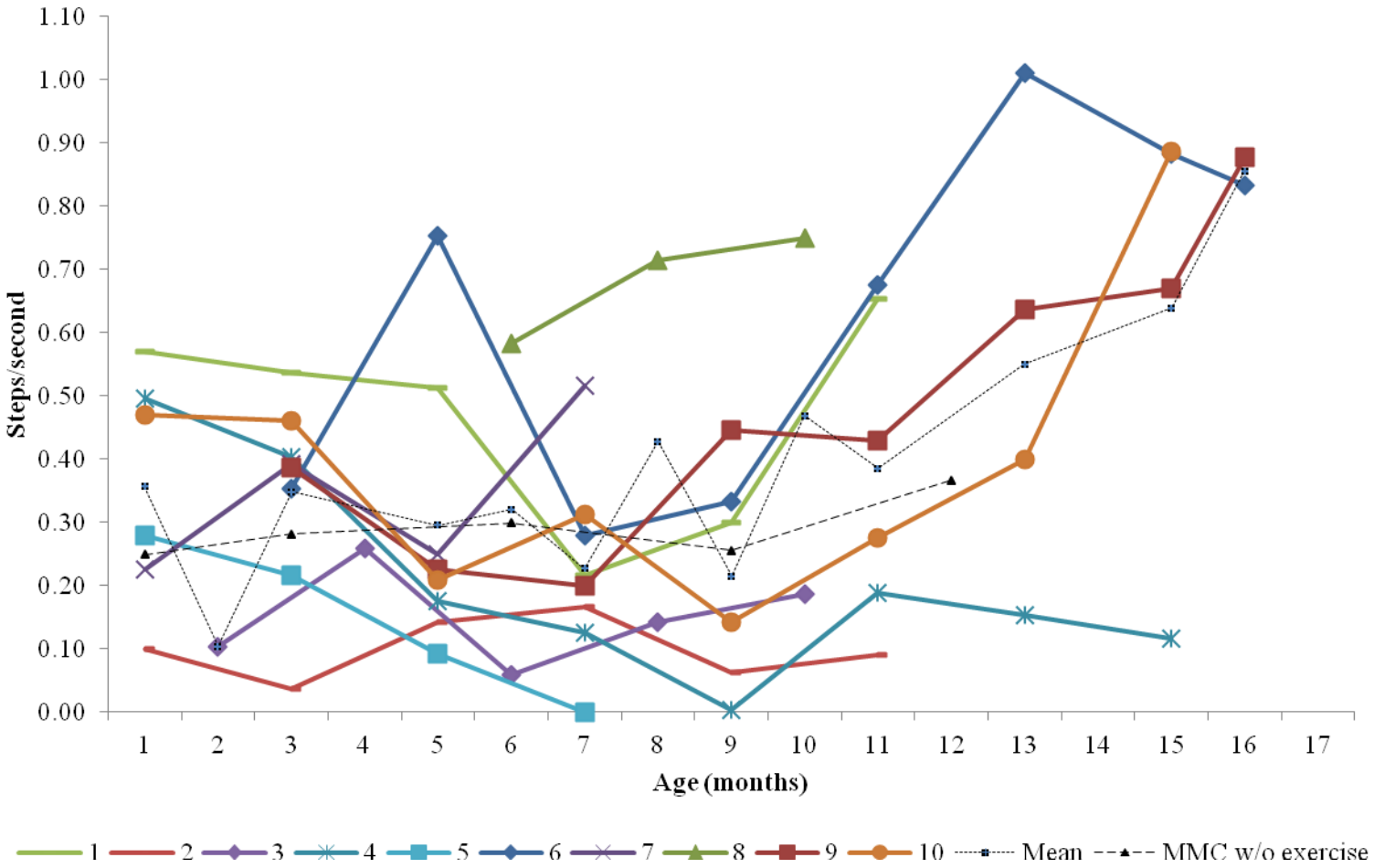



Figure C. 2. Total step rate over 1 year of intervention.

Colored lines represent data for each baby in intervention group; black lines show means for intervention and comparison groups.

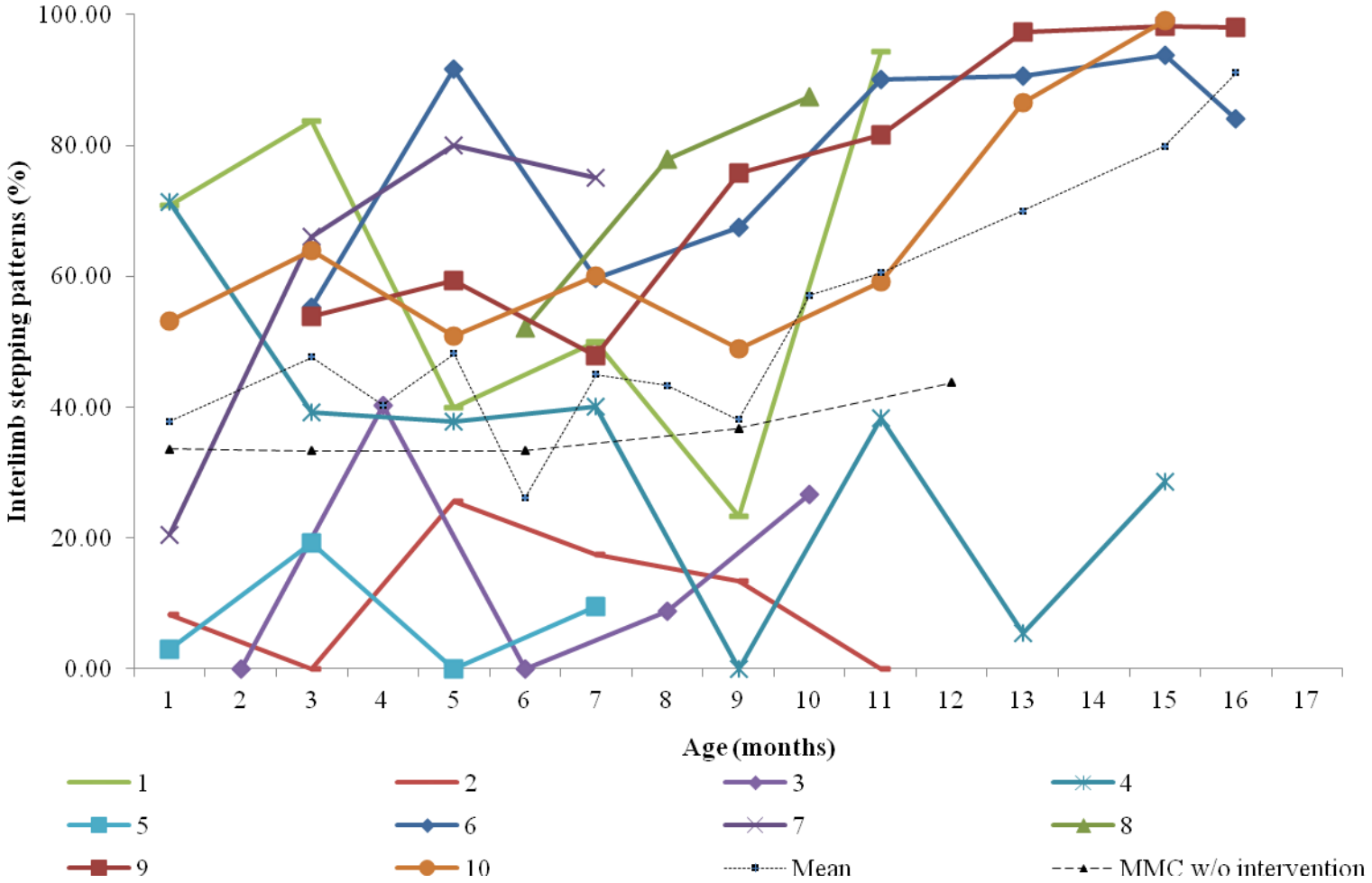
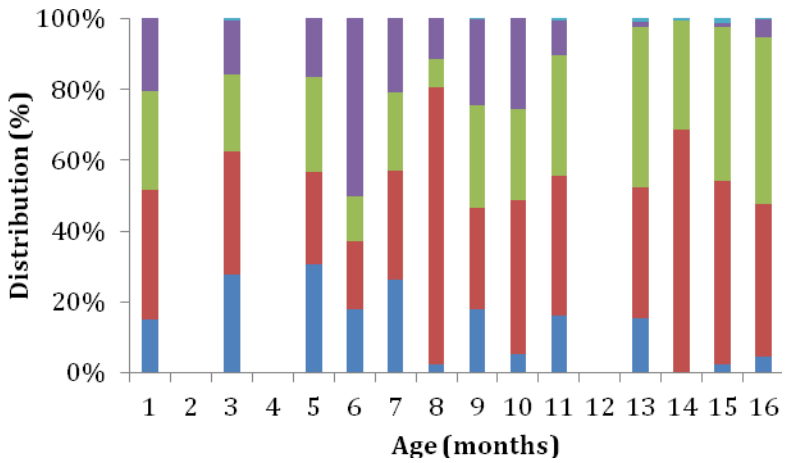



Figure C. 3. Proportion of alternating steps in interlimb stepping patterns (%).

Colored lines represent data for each baby in intervention group; black lines show means for intervention and comparison groups.

A




B

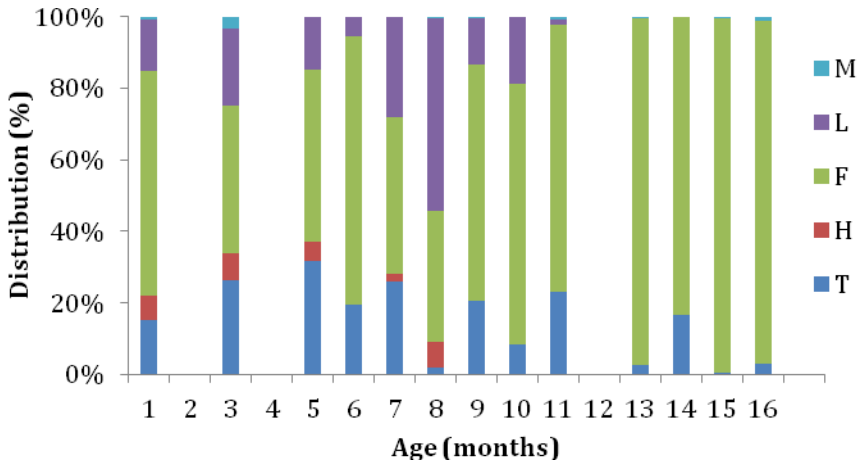



Figure C. 4. Step parameters.

Mean percentages of foot positions at touch-down (A) and mean percentages of foot positions during mid-stance (B): toe (T), heel (H), flat (F), lateral (L), and medial (M)

Table C. 1. Average Practice Time per Day

| Infants' ID | Practice time per day |
|---|---|
| 1 | 10.04 |
| 2 | 8.71 |
| 3 | 3.31 |
| 4 | 8.63 |
| 5 | 5.38 |
| 6 | 7.15 |
| 7 | 3.12 |
| 8 | 2.94 |
| 9 | 6.34 |
| 10 | 11.01 |
| Average | 6.663 |

Table C. 2. Average Step Frequency

| Groups | Month of Age | Mean | Standard Deviation |
|---|---|---|---|
| Intervention | 1 (n = 6) | 0.36 | 0.18 |
| | 3 (n = 8) | 0.35 | 0.16 |
| | 5 (n = 8) | 0.30 | 0.22 |
| | 7 (n = 8) | 0.23 | 0.15 |
| Comparsion | 1 (n = 12) | 0.25 | 0.22 |
| | 3 (n = 12) | 0.28 | 0.24 |
| | 6 (n = 12) | 0.30 | 0.29 |
| | 9 (n = 12) | 0.26 | 0.28 |
| | 12 (n = 12) | 0.37 | 0.38 |

# APPENDIX D

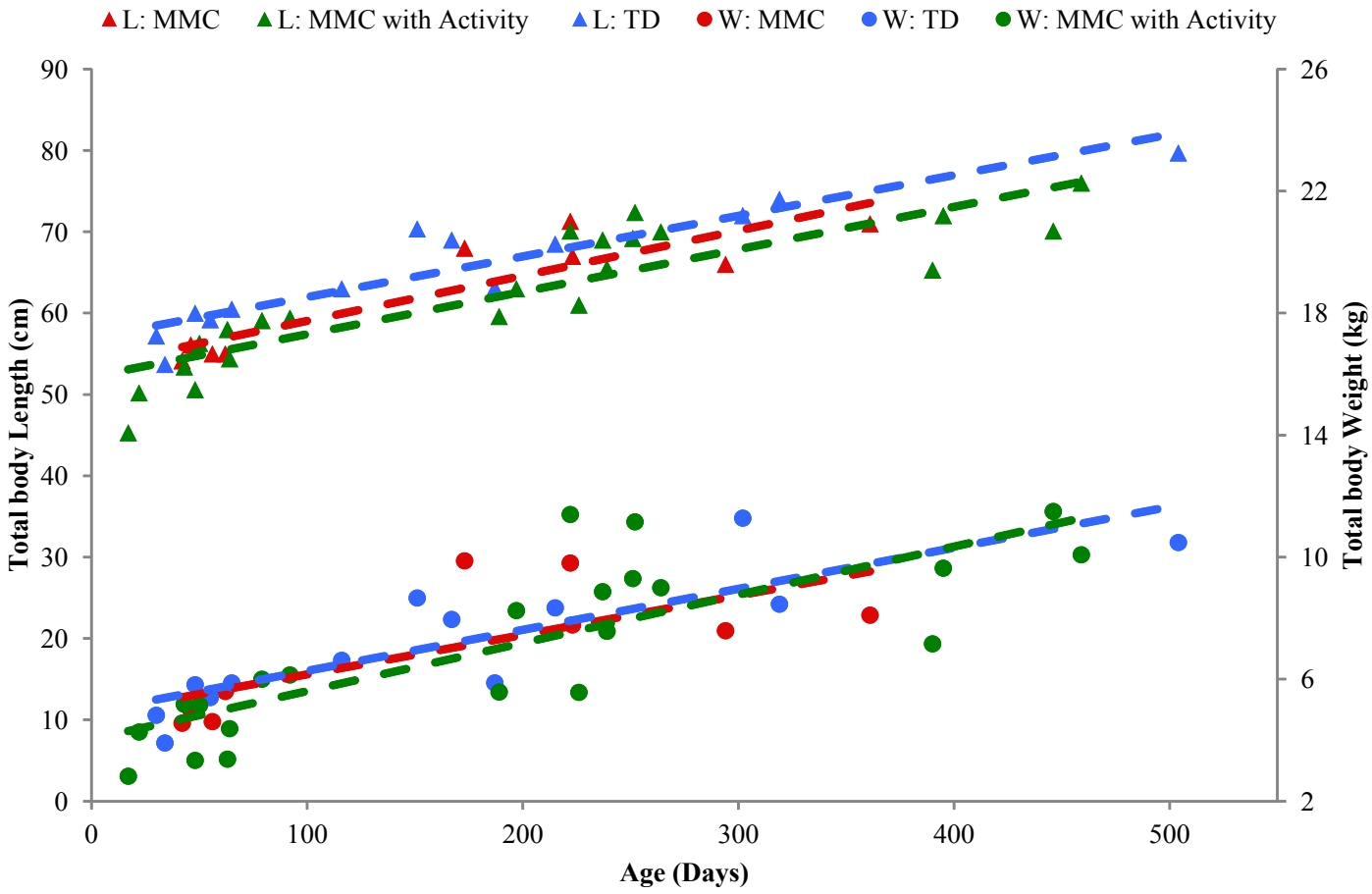



Figure D. 1. Growth changes – length and weight

L: length; W: weight.