TRABECULAR BONE MICROARCHITECTURE AND VIBRATION TRANSMISSION IN AMBULATORY CHILDREN WITH CEREBRAL PALSY

by

Harshvardhan

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Physiology

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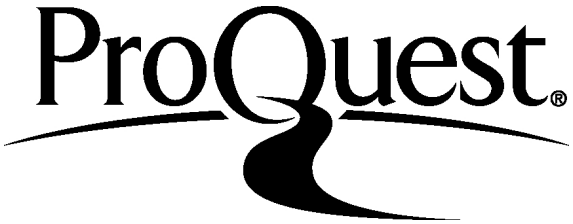

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LIST OF ABBREVIATIONS

Cerebral palsy = CP

High-frequency, low-magnitude vibration = HLV

Bone mineral content = BMC

Areal bone mineral density = aBMD

Gross motor function classification system = GMFCS

Magnetic resonance imaging = MRI

Apparent bone volume/total volume = appBV/TV

Apparent trabecular number = appTb.N

Apparent trabecular thickness = appTb.Th

Apparent trabecular separation = appTb.Sp

ABSTRACT

Children with physical disabilities, such as cerebral palsy (CP), have reduced bone mass and quality, especially in the lower extremities. It is known that low bone mass is present across all levels of CP, including nonambulatory and ambulatory children. It is also known that trabecular bone microarchitecture, an important feature of the skeleton that is an independent predictor of fracture risk, is markedly underdeveloped in nonambulatory children with more severe forms of CP. However, the level of underdevelopment in trabecular bone microarchitecture in ambulatory children with milder forms of CP has not been explored. In addition to gaining a better understanding of their bone deficit, identifying treatment strategies that increase bone mass, enhance trabecular bone microarchitecture and reduce fracture risk in children with CP is imperative.

An obvious approach is to increase their participation in physical activity because it is lower than typical, even in ambulatory children with milder forms of CP. However, because of their poor balance and coordination, alternative activities or treatments for enhancing bone development for children with CP are sought. One treatment that has received considerable attention during the past 10 years is high-frequency, low-magnitude vibration (HLV). A handful of studies have shown that HLV can increase bone mass and enhance bone architecture in children with CP. However, the effect of HLV at specific bone sites is likely dictated by the degree to which the HLV stimulus is transmitted. Unfortunately, no studies have examined the transmission of HLV in children with CP.

The first aim of this dissertation was to determine the degree of underdevelopment in trabecular bone microarchitecture at the distal tibia and the distal

femur in ambulatory children with CP. The primary finding was that the degree of underdevelopment in trabecular bone microarchitecture at the distal femur in ambulatory children with CP was not as marked as the underdevelopment observed in nonambulatory children with CP ($p < 0.05$). However, measures of trabecular bone microarchitecture deviated from control values by ≥ 1 SD ($p < 0.05$) in both groups. Furthermore, the degree of underdevelopment in trabecular bone microarchitecture was more pronounced in the distal tibia than in the distal femur in ambulatory children with CP compared to typically developing controls with measures of apparent trabecular thickness lower and apparent trabecular separation higher by $\geq$ 2 SD ($p < 0.05$).

The second aim of this dissertation was to determine the degree to which the HLV signal emitted by a floor-based platform transmits across the distal tibia and the distal femur in ambulatory children with spastic CP during standing. The primary finding was that relative to the HLV signal emitted at the platform, the signal was amplified at the distal tibia but dampened at the distal femur in children with CP ($p < 0.05$). The damping of HLV was related to the degree of spasticity, with greater spasticity associated with less signal transmission to the distal tibia ($r = -0.547$, $p < 0.05$) and distal femur ($r = -0.566$, $p < 0.05$).

The third aim of the dissertation was to determine the effect of tissue composition on HLV transmission in ambulatory children with spastic CP. The primary finding was that measures of trabecular bone microarchitecture were not related to HLV transmission in children with CP or typically developing children $p > 0.05$). Muscle volume in the midleg was inversely related to HLV transmission to the

distal femur in typically developing children ($r = -0.626$, $p \leq 0.05$), but not in children with CP.

The overall findings from this dissertation provide a greater understanding of the level of skeletal compromise in ambulatory children with mild CP. They also provide insight into the degree to which HLV transmits to the primary fracture sites of children with CP. The findings will help guide future studies aimed at determining the usefulness of HLV as a treatment for poor bone development in children with CP.

Chapter 1

INTRODUCTION

Cerebral palsy (CP) refers to a group of neurological disorders resulting in a loss in the ability to control movement, posture and balance (1). In the US, the prevalence rate of CP is 3.1 per 1000 live births (2). Almost 80% of children with CP are diagnosed with spastic CP (3). Nonambulatory children with moderate to severe CP show underdeveloped trabecular bone microarchitecture (4) and a substantial adipose tissue infiltration of skeletal muscle in their lower limbs (5). This musculoskeletal deficiency in children with CP is associated with less force generating capacity of the muscles (6,7) and a higher incidence of low-energy fractures in the lower extremity (8-10). Although, it is clear that bone mass is low in children with ambulatory CP (11), the level of underdevelopment in trabecular bone microarchitecture has not been established. Furthermore, the levels of deficit in the distal femur and the distal tibia, which are the two common fracture sites in children with CP (8-10), has not been determined.

One treatment that has been proposed to counteract the deficit in bone development in children with CP is a floor-based high-frequency, low-magnitude vibration (HLV). Earlier studies have shown that an HLV stimulus has an anabolic effect on bone in children with CP (12-14). Moreover, it is plausible that the effectiveness of HLV as a treatment for musculoskeletal health is dictated by the degree to which the HLV signal is transmitted to a particular bone site, similar to the

site-specific effects of exercise (15-18). It should be noted that the tibia and femur are of major interest in children with CP because the highest number of fractures are reported in the lower extremity in children with CP and the distal femur is the most commonly fractured site (8). An amplification of vibration at the ankle and attenuation at the knee has been observed in typically developing children (19). However, no studies have investigated the transmission of a floor based HLV platform in children with CP. It becomes imperative to know if ambulatory children with CP show site-specific HLV transmission which could help understand effects of HLV on the distal tibia versus the distal femur in ambulatory children with CP. Furthermore, a number of factors may influence the degree to which HLV is transmitted across the lower extremities such as composition of the soft tissue and the trabecular bone microarchitecture which might be different between ambulatory children with CP and typically developing children.

It has been established that children with moderate-to-severe CP, who are unable to ambulate independently, have severely compromised bone density (10,20) and bone microarchitecture compared to typically developing children (21). Although bone mineral content (BMC) has been found to be lower in children with mild CP than typically developing children; no significant difference has been reported in areal bone mineral density (aBMD) (22,23), suggesting that bone deficits in ambulatory children with mild CP are not captured by aBMD. Although aBMD is the primary measure used to evaluate fracture risk, it has limited utility in children as it is strongly influenced by bone size (24). Moreover, it fails to provide information about the structural properties of bone. An important structural property of bone, that is trabecular bone microarchitecture, is a robust predictor of bone strength and fracture

(25,26). Unfortunately, the level of underdevelopment in trabecular bone microarchitecture in ambulatory children with CP is unknown.

Soft tissue such as muscle can modulate vibrations produced during various activities such as walking, running, and isometric or isotonic force production (27,28). A recent study (29) suggested that a bone with enhanced trabecular properties could lead to amplification of vibration at a bone site. However, we do not know the effect of soft tissues such as muscle volume and trabecular bone on HLV transmission in children with CP. Thus, we see that based on current knowledge, there are some important gaps that need to filled to understand the nature of HLV transmission, underdevelopment in trabecular bone microarchitecture, and the effect of tissues on HLV transmission in children with CP.

1.1 Literature Review

1.2 Bone and CP

Skeletal system is adversely affected by chronic unloading as is evident in humans exposed to bed rest (30) or in population with movement disorders, such as CP (21). A lack of mechanical loading can lead to weak bones which are susceptible to experience fractures.

1.2.1 Fractures in CP

In one of the seminal studies, McIvor et al. (31) concluded that the femur and tibia were the most fracture-susceptible sites in individuals with CP. They reported a total of 134 fractures in a group of 92 patients, out of which 89 fractures occurred at the femur or tibia (31). Most of the patients in their study were spastic and most of the fractures occurred due to fall or convulsive seizures. By 1994, cases of spontaneous

fractures were reported in children and adolescents with CP (32). Lingam and Joester (32) reported demineralization of the bone matrix in adolescents with CP (n = 5; 10-19 years old) suggesting a markedly reduced accretion of bone in this population. Spontaneous fractures which healed quickly were seen at the femur and the tibia. They also noticed thinning of bones. Low-energy fractures (n = 54) were reported in patients with CP (n = 37) by Brunner and Doderlein. Out of 54 fractures, 40 fractures occurred in the femoral shaft and the supracondylar region. The major causes of these fractures were fragile lever arms and stiffness in major joints.

Almost 10,000 adults and children with CP suffer fractures every year costing ~$150 million (33). There have been few studies in the past investigating the prevalence of fracture in children with CP (8,9). While one study reported a fracture prevalence of 12% (34), another study reported a fracture prevalence of 6% in children with CP (8). Previous studies and review of fractures in children with CP provide evidence that most of the fractures (70 - 80%) occur in the lower limbs in children with CP (8,9,35). Furthermore, over 15% of children with CP develop secondary complications such as malunion, nonunion, and infections including pneumonia after a fracture (8).

1.2.2 Assessment of bone status using dual-energy X-ray absorptiometry in CP

If the terms ‘bone and cerebral palsy’, ‘bone density and cerebral palsy’, ‘bone strength and cerebral palsy’, and ‘trabecular bone and cerebral palsy’ are searched in PubMed, we get a total of 1379, 119, 41, and 5 hits, respectively. This suggests that there is a lot of work that needs to be done with respect to understanding bone microarchitecture in children with different types of CP.

The first documented study ever in CP was by Palmer et al. (36) where he documented various clinical techniques for the treatment of mandibular facet slip in patients with CP. Most of the early studies examined the efficacy of various treatment techniques for a variety of conditions, chiefly orthopedic, in patients with CP. Another earlier study (37) was able to identify delayed bone age on the affected side in children with CP. Additionally, epiphyseal development was found to be retarded on the affected side in children with CP, more so in hemiplegia than diplegia or quadriplegia (37).

In an editorial piece in 1995 (38), it was emphasized how important it was to pay attention to the orthopedic conditions in individuals with CP and how little was known until that time. The editorial emphasized on a specific area of interest which was to gain knowledge about the prevailing musculoskeletal abnormality and coming up with optimal treatment options. In 1994, Roberts et al. (39) reported the effects of hemiplegia on skeletal maturation and growth, with the less affected side used as the control. The main aim of their study was to determine if delayed skeletal maturation seen in children with CP is related to their underlying brain pathology or concomitant malnutrition. They calculated skeletal index (SI) which was equal to skeletal age divided by chronological age multiplied by 100. They found that SI was significantly lower for the affected versus unaffected side in their population ($p < 0.0001$, $n = 19$; 3 - 18 years). Furthermore, they reported a significant positive relationship between skeletal age ratio (affected skeletal age/unaffected skeletal age) and the chronological age ($r = 0.72$, $p < 0.05$), suggesting that with increasing age, there was less difference in skeletal age in between the affected and unaffected sides. Moreover, the skeletal age ratio was positively related to the functional severity score, as measured by QUEST (r

= 0.77, $p < 0.05$). Their findings suggested that there was an effect of the central nervous system on skeletal maturation; however, it was unclear if it was a direct effect on the bone or if it was an indirect effect due to a lack of physical activity? It should be noted that these authors calculated skeletal index from the bones of the upper extremity which primarily are non-weight bearing sites.

In a study by Lin and Henderson (40), bone mineralization was determined in the affected versus non-affected extremities of children with spastic CP with a special emphasis on the bones of the lower limb (n = 19; 3 - 15 years). The researchers reported 21% lower BMC and 5.6% lower areal bone mineral density (aBMD) using dual energy X-ray absorptiometry in the affected side versus unaffected side. A separate analysis showed that the BMC deficit was greater in the affected upper extremities compared to affected lower extremities (26.5% versus 15.6%). It was postulated that a combination of neurotrophic and mechanical factors was responsible for decreased aBMD and that weight bearing, in part, counteract the effect of neurological involvement on bone. However, the potential role of neurotrophic factors, if any, on bone was not examined in this study.

Henderson et al. (10) examined bone density at the distal femur and metabolism in non-ambulant children with CP (n = 117, 2 - 19 years). They reported a severely lower deteriorated aBMD at the distal femur (aBMD z score: -3.5 ± 0.2) and a high number of fractures (15% of their subjects) with more fractures (28%) seen in children older than 10 years of age. Also, the deficit in aBMD was greater at the distal femur than the lumbar spine ($p < 0.05$, aBMD z score: -3.5 ± 0.2 versus -2.0 ± 0.1, respectively). Interestingly, this study reported a significant lower aBMD z score in GMFC level 5 children compared to level 4 children, although both the groups are

non-ambulatory. This suggested that other factors besides mechanical loading play a role in imparting strength to a bone.

The growing years are presumed to be a vital period for increasing bone mass (41). Previous studies have suggested that mechanical loading in growing years lead to an increase in peak bone mass and enhancement of bone strength (42,43). Also, these changes in skeleton are maintained in adulthood even after the cessation of physical activity.

Kontulainen et al. (42) in their 5-year prospective study showed a good maintenance of the interlimb BMC differences between playing versus non-playing upper extremity in female tennis players, even after a decreased training frequency and hours of training. They divided their study population in two groups: young starters, who started training before menarche (n = 36) and old starters, who started training 1 year after menarche (n = 28), and compared them with controls (n = 27). During a 5-year follow up, researchers noted that the interlimb difference in BMC was 22% in the humeral shaft in young starters versus 10% in the old starters and 3.5% in controls. Similarly, the Iowa Bone Development Study (44) reported that physical activity is critical for enhancing bone strength during childhood. Janz et al. (44) examined longitudinal relationship between physical activity and femoral neck strength by dual energy X-ray absorptiometry in children (n = 581, 8 - 11 years). Bone scans and accelerometry based physical activity measures were done at 5, 8, and 11 years of age. They reported that physical activity was an independent predictor of femoral neck cross-sectional area (CSA) and polar moment of inertia (all $p < 0.05$). They also found that 10 minute increases in daily activity had similar effects on CSA in girls and polar moment of inertia in boys as would an additional 1 kg of body weight.

In their next study, Janz et al. (45) reported a longitudinal follow up of relationship between physical activity and BMC of whole body, spine and hip in 333 children (age = 5 - 11 years, boys = 148, girls = 185). They reported that physical activity at age 5 was a significant predictor of BMC at ages 8 and 11 in boys and girls ($p < 0.05$). Furthermore, most active children at age 5 had the highest BMC at ages 8 and 11 than least active children. In an another study, Erlandson et al. (46) compared bone geometric and densitometric properties in the forearm and lower leg in former gymnasts 10 years after their retirement. Their peripheral quantitative computed tomography results revealed that retired gymnasts had significantly greater adjusted total BMC, total bone area, trabecular number and trabecular BMC than controls at the distal radius (all $p < 0.05$). Retired gymnast also showed significantly greater adjusted total BMC, total bone area, cortical BMC, cortical bone area and medullary area than controls at 30% and 65% radial shaft (all $p < 0.05$).

1.2.3 Importance of bone architecture in the assessment of bone status

In a study by Tasdemir et al. (47), authors examined volumetric BMD of lumbar vertebrae (L1-L3) in ambulant and non-ambulant children with CP (Total CP = 24, ambulant CP = 9, non-ambulant CP = 15; 10 months - 12 years) and compared it to age and sex- matched typically developing children (n = 19). They found that volumetric BMD was significantly lower in non-ambulant children with CP than in the control group ($p < 0.01$). No difference was noted in volumetric BMD between ambulatory children with CP and controls, suggesting a critical role of mechanical loading in bone accretion during childhood. Although ambulatory children with CP showed higher volumetric BMD than non-ambulatory children with CP and lower volumetric BMD than controls, the differences did not reach statistical significance

level statistically. The lack of a difference may be due to the small and unequal sample sizes between these two groups as well as wide age range of the samples studied.

Wilmshurst et al. (48) reported that calcaneal broadband ultrasound attenuation (BUA) is lower in immobile children with CP than the most mobile group of children with CP ($F = 8.958$, $p < 0.001$). It should be noted that broadband ultrasound attenuation reflects the structural properties of bone.

Binkley et al. (49) investigated the differences in bone geometry and strength in children with CP ($n = 13$, 3 - 20 years) by peripheral quantitative computed tomography. Periosteal circumference, endosteal circumference, cortical thickness, cortical BMC, and cortical area were lower in children with CP ($n = 13$) compared to a group of matched controls (2 matched per child with CP, $n = 26$) (all $p < 0.001$). Polar strength-strain index was lower in the CP group compared to the control group ($p < 0.001$). It was also shown that participants in the CP group with higher weight had higher cortical volumetric BMD. Also, children with CP showed greater cortical volumetric BMD than control group at higher cortical thickness suggesting that the lower polar strength-strain index in children with CP is due to smaller and thinner bones and not due to cortical volumetric BMD.

Bone strength is a critical factor that can predict the risk of fracture; it becomes imperative to gain the knowledge about the status of bone strength and various factors affecting it in children with CP. Recently, magnetic resonance imaging (MRI) studies have gained popularity in research with children, chiefly because it can yield valid and reliable estimates of bone geometry and strength without exposing children to any ionizing radiation. Also, a robust prediction of bone strength and fracture can be obtained by trabecular bone microarchitecture (26).

Using MRI, Modlesky et al. (50) investigated bone structure and strength in the femoral midshaft in children with quadriplegic cerebral palsy (n = 10). The CP group was matched by age, gender, and BMI with a control group of typically developing children (n = 10). Children with quadriplegic CP vs. controls showed lower total bone volume (54%), cortical volume (55%), medullary volume (51%), lower bone width in anterior-posterior direction (29%), lower bone width in medial-lateral direction (28%) and a thinner cortical wall by, and in the anterior (28%), posterior (32%), and lateral (43%) directions (all $p < 0.001$). These architectural differences were consistent with a markedly lower cross-sectional moment of inertia, section modulus, and polar moment of inertia (60-71%) which are estimates of resistance to bending and torsion. This study provided evidence that non-ambulatory children with quadriplegic CP have a drastically deteriorated bone structure and strength in the midfemur. Moreover, Modlesky et al (48) study provided the parameters to test the efficacy of any treatment protocol aimed at ameliorating reduced bone strength. Additionally, the findings from this study could explain the high number of midshaft femur fractures in children with CP.

Modlesky et al. (4) also used MRI to determine if trabecular bone microarchitecture is underdeveloped in the distal femur of children with moderate-to-severe CP. The distal femur was studied because it is the most common fracture site in children with CP. Markers of trabecular bone microarchitecture such as apparent bone volume/total volume (appBV/TV), apparent trabecular number (appTb.N), apparent trabecular thickness (appTb.Th) were found to be significantly lower by 30%, 21%, and 12% respectively in children with CP than controls and apparent trabecular separation (appTb.Sp) was significantly higher by 48% in children with CP than

controls (all $p < 0.05$) suggesting a marked deterioration in trabecular bone microarchitecture in children with CP (4). This finding could explain the reason for high number of fractures seen clinically at the distal femur in children with CP. Additionally, this study reported a very good reproducibility for all measures of trabecular bone microarchitecture (coefficient of variation% < 4%).

Carrying their work forward, Modlesky et al. (21) recently published an article that examined the pattern of underdevelopment of trabecular bone microarchitecture in distal femur in non-ambulatory children with quadriplegic cerebral palsy ($n = 12$). It becomes important to know the level of underdevelopment in trabecular bone microarchitecture to provide us the insight into the sites that are most susceptible to fracture and thus, can be used as a diagnostic tool to rate the usefulness of any rehabilitation protocol. In children with quadriplegic cerebral palsy versus controls, appBV/TV and appTb.N was 24 and 17% lower in the region closer to the growth plate, respectively but 34 and 27% lower in a subregion further from the growth plate suggesting trabecular bone microarchitecture gets progressively worse in children with CP with growing expanse from the growth plate. This suggests that the modeling-remodeling cycle is impaired in children with CP where less bone is produced in the first place at the growth plate and then due to lack of loading, less bone is conserved.

All of these studies point out the importance of physical activity which modulates skeletal health in a positive way by means of mechanical loading contributing toward attainment of peak skeletal mass later in adulthood. Also, the positive changes in bone are well maintained in adulthood, long after the activity has decreased. This becomes important in children with CP who show lower levels of physical activity.

A recent review article by Carlon et al. (51) directly compared the levels of physical activity and sedentary time of ambulatory children and adolescents with CP to controls. Irrespective of the outcome measures, ambulatory children and adolescents with CP showed significantly less levels of physical activity than their healthy peers. Additionally, ambulatory children and adolescents with CP demonstrated significantly less levels of physical activity than recommended exercise guidelines. A previous study by Modlesky et al. had shown similar findings where children with CP showed significant low level of physical activity compared to age and gender matched controls. It can be related to research findings where BMC of the total body and total proximal femur was found to be more than 1 SD lower and of the femoral neck by more than 2 SD lower in ambulatory children with CP than typically developing children (23). However, no significant differences have been reported in aBMD in ambulatory children with CP versus controls (22,23), suggesting aBMD might not be a sensitive measure to capture the true deteriorated skeletal status in this population.

It can be postulated that due to less mechanical loading, skeletal mass in children with CP never attains peak bone mass in presence of a severely reduced accretion of bone, which can clinically be related to development of osteopenia/osteoporosis over time (20) and in huge number of fractures that these children report.

1.3 Effect of High-Frequency, Low-Intensity Vibration/Acceleration on Bone

With the knowledge that children with CP participate in lower levels of physical activity (51,52), which can lead to underdeveloped bone, identifying alternate nonpharmacologic treatments which could attenuate adverse effects of reduced

physical activity becomes very important (53). Recently, HLV has been gaining lot of attention as some studies showed that HLV has anabolic potential for bone and muscle in children with disabling conditions such as CP.

1.3.1 Longitudinal studies

The first study that reported anabolic effects of HLV was published by Rubin et al. (54). In this study, authors investigated anabolic potential of a low intensity 0.3 *g* (where g is the earth's gravitational field) and high frequency (30 *Hz*) acceleration on hindlimbs of adult female sheep. The animals were subjected to HLV for 20 min/day for 5 days a week while they were allowed to do their normal physical activity with the controls (i.e., untreated sheep). After 1 year, trabecular bone density was found to be ~35% greater in the treated animals than the controls. Additionally, trabecular bone volume and trabecular number increased by 32% and 45% respectively in the treated groups compared to the controls.

The first clinical trial investigating the effect of HLV on bone in children with CP was done by Ward et al. (14). In their study, the researchers found an increase of 6.3% in trabecular volumetric bone mineral density at the proximal tibia in response to a daily HLV treatment (90 *Hz*, 0.3 *g*; n = 9) for 6 months, while a decrease of 11.9% was seen in controls (n = 10; $p < 0.05$); thus, showing a net benefit ~18% on volumetric bone mineral density in the intervention group (who stood on the HLV platform for 10 minutes daily for 5 days/week for 6 months) than controls (who stood on the placebo platform for 10 minutes daily for 5 days/week for 6 months). It should be noted that these positive changes in the intervention group were seen with a very low compliance which was ~44%. No effects were noted in the spine.

A 1 year randomized, double-blind, and placebo-controlled trial of 70 postmenopausal women investigating the effects of HLV on postmenopausal related bone loss found that women who were in the highest quartile for compliance in the HLV group (86% compliant) showed a 2.17% relative benefit of treatment at the femoral neck compared to the placebo group who lost 2.13% at the femoral neck (55). Additionally, there was a 1.5% treatment benefit at the lumbar spine compared to a decrease of 1.6% at the lumbar spine in the placebo group. Interestingly, women who weighed less and were in the highest quartile for compliance in the HLV group showed the maximum benefit, of treatment (i.e., 3.35%) at the spine.

In another study (56), the prospect for HLV (0.3 *g*, 30 *Hz*) to increase bone density in 48 young women (15 - 20 years) with low aBMD and a history of at least 1 skeletal fracture was explored. Researchers found that a 1 year intervention in the HLV group ($n = 24$) showed a 3.4% increase in cortical bone mineral density in the femoral shaft ($p < 0.001$) and a 2.1% increase in lumbar spine trabecular bone mineral density ($p = 0.0250$) when compared to controls ($n = 24$). Additionally, lowest quartile in the compliance group (0 - 25%) failed to respond to HLV stimulus. Trabecular density in the spine was found to be 3.9% greater ($p = 0.007$) in the 3 highest quartiles combined group versus controls and lowest quartiles combined group.

In a separate study (12), a regional effect of HLV stimulus on aBMD was noted. Children with various motor disabilities ($n = 57$; 6 - 9 years) completed this study. A regional HLV (0.3 *g*, 60 *Hz* or 0.3 *g*, 90 *Hz*, 10 minutes daily for 6 months) was provided either at elbows or knees. 0.3 *g*, 60 *Hz* group showed significantly higher aBMD at distal radius than placebo or 90 *Hz* group ($p = 0.011$) signifying a

regional effect of HLV stimulus on the enhancement of aBMD. However, no data were reported on the knee.

In a recent clinical trial, Wren et al. (13) examined the effects of HLV in 31 children with CP (6 - 12 years). The children were randomized either to standing on a floor or on HLV platform for 10 minutes daily for 6 months. The results showed beneficial effects of HLV on the cortical bone. The cortical bone area in the tibia midshaft showed an increase by 8.5% in the HLV group compared to 4.9% in the standing group ($p = 0.02$). These changes were seen across all the GMFC's from I-IV. Additionally, moment of inertia in the tibia midshaft was enhanced in the HLV group than controls ($p < 0.03$). All of these studies discussed above provide evidence that HLV is anabolic to bone in populations such as children with CP. It can help in making bone denser and stronger via the mechanical stimulus; although, the exact mechanism by which HLV acts is unclear.

1.3.2 Mechanisms of HLV

Various factors like intramedullary fluid flow shear stress, fluid pressure, neuromuscular feedback, stochastic resonance could play a role in anabolic response to HLV stimulus. Various animal studies have been done to elucidate the anabolic potential of HLV stimulus (26,29,57,58).

A study by Tanaka et al. (58) suggested that stochastic resonance has an important role in the osteogenic response of bone to HLV. They reported new bone formation on the ulnar periosteal surface in mice by sine wave and HLV loading but not with HLV loading only. Also, sine wave and HLV loading led to a ~4 fold increase in bone formation on the periosteal surface compared to the sine wave loading only. Their data suggested the importance of HLV in the preservation of

bone's anabolic response to stimuli such as of daily activities. One of the limitations of their study was that they did not examine trabecular bone response to their loading protocol.

A recent study (59) suggested that nucleus within the cytoplasm of bone cells oscillates providing the physical source of cellular loading in response to the HLV. This suggests that HLV acts on the bone cells itself by providing a mechanical loading on the bone cells. Another study (60) provided evidence that 3 weeks of HLV (0.3 *g*, 45 *Hz*) decreases osteoclastic activity in the trabecular metaphysis and epiphysis of the tibia in HLV treated mice (n = 8) by 33 and 31% respectively. Also, bone formation rate on the endocortical surface of the tibia metaphysis was 30% greater than age matched control (n = 10). It should be noted that mice used in this study were young and represented model of a growing skeleton. The data from this study suggested that HLV may provide a means for providing a nonpharmocological way to increase bone mass in a growing skeleton.

Also, HLV has also been shown to arrest a decline in bending and compressive strength and increase in bone formation rate in ovariectomized rats (61). The mechanism of HLV loading was further explained by a study (62) in which osteocytes showed a reduction in soluble RANKL by 53% ($p < 0.01$) and PGE_2 by 61% ($p < 0.01$) in the HLV group (0.3 *g*, 30,60, and 90 *Hz*). In this study, authors also showed that vibrated cells attenuated the formation of large osteoclasts by 36% ($p < 0.05$) and the amount of osteoclastic resorption by 20% ($p = 0.07$). This suggests that osteocytes can sense HLV signals in such a way that it leads to inhibition of osteoclast formation.

HLV (0.3 *g*, 35 *Hz*) has also been shown to augment callus formation, mineralization, and remodeling by 25-30% during fracture healing in osteoporotic

ovariectomized rats (57). Additionally, HLV resulted in 70% increase in energy to failure of the healed bone compared to ovariectomized controls rats. Furthermore, HLV (0.3 *g*, 45 *Hz*) has been shown to diminish bone resorption and increase mechanical strength in young mice with osteogenesis imperfecta (63).

All of these studies show that HLV has both, anti-catabolic and anabolic effect on bone. It is known that mechanical loading, such as exercise, have a site-specific influence on bone with the greatest effects at the site that experiences the load (15, 17, 18). However, it remains to be examined if adaptation of bone to HLV is related to the amount of acceleration transmitted.

1.4 Factors Influencing the Effectiveness of HLV

Factors affecting vibration transmission in standing can be grouped in three categories: 1) source of acceleration, 2) the human body, and 3) the interaction between human body and acceleration source (64). Body posture is one of the critical factors affecting acceleration transmission in standing (64). Different postures can impact the position of the bony site receiving acceleration, the level of muscular tension or intramuscular pressure in different muscle groups, and contact area of feet with the accelerating source. Additionally, different postures can influence local site-specific resonance leading to a variable response in acceleration transmission in unique frequency bands (29,64,65)

1.4.1 Posture and acceleration transmission in various populations

Factors affecting vibration transmission in standing can be grouped in three categories: 1) source of acceleration, 2) the human body, and 3) the interaction between human body and acceleration source (64). Body posture is one of the critical

factors affecting acceleration transmission in standing (64). Different postures can impact the position of the bony site receiving acceleration, the level of muscular tension or intramuscular pressure in different muscle groups, and contact area of feet with the accelerating source. Additionally, different postures can influence local site-specific resonance leading to a variable response in acceleration transmission in unique frequency bands (29,64,65).

Although, acceleration transmission in seated position has been established (66), not much is known about acceleration transmission in standing position, especially with respect to HLV transmission at different skeletal sites such as the distal femur and the distal tibia. Most of the earlier work investigated acceleration transmission to head. One of the earliest studies (67) measured acceleration transmissibility to head in the frequency range of 1 - 50 *Hz*. and 0.003 - 0.02 mm in amplitude. In this study, 12 healthy adult subjects (average 23.42 years) were tested and individual plots were obtained for each subject. Resonance peaks around 2, 6, 20, and 40 *Hz* were noted in all the 12 plots. A study by Matsumoto et al. (65) investigated acceleration transmission at various sites such as T1, T8, L4 spines, iliac crest, and knee (i.e., patella) in 12 healthy adult males (24 - 35 years) in response to acceleration over a frequency range of 0.5 - 30 *Hz* and an amplitude of 0.125 - 2 m/s^2 r.m.s. (0.17 - 0.3 *g*). Accelerometers were used to measure acceleration transmission in various postures – normal standing, legs bent, and standing on one leg. In this study, the researchers reported an amplification of acceleration at knee at frequencies greater than 10 *Hz* in the normal standing posture and at greater than15 *Hz* in legs bent posture, whereas, attenuation was noted with one leg standing posture at frequencies less than 20 *Hz*. Additionally, vertical transmission to the knee (i.e., patella) increased

with the increasing frequency and increasing magnitude ($p < 0.05$). The findings from this study implied that the human body responds dynamically to the acceleration stimulus.

Harazin et al. (64) examined the dissemination of vertical acceleration transmission ($4 m/s^2$ r.m.s or 0.57*g* and 4 - 300 *Hz*) to different parts of the body in various postures in college-aged population (n = 10; mean age = 23.1 years). They measured acceleration transmission by accelerometer at various skeletal sites such as medial malleolus, lateral epicondyle, iliac crest, metatarsus and acromion process. The transmissibility curve at the medial malleolus showed resonance at three bands: 4 - 8, 12.5, and 25 - 63 *Hz*. It was shown that around 31.5 *Hz*, transmissibility at the ankle was more than the unity for all postures except standing on toes posture. On the other hand, resonance at two bands: 4 - 8 and 12.5 - 25 *Hz* were noted at the lateral epicondyle. Also, lateral epicondyle displayed transmissibility near unity at 31.5 *Hz* in step standing posture. At frequencies above 31.5 *Hz*, lateral epicondyle demonstrated an attenuated acceleration transmission in all postures. It should be noted that Harazin et al. (64) reported inter-subject variations in transmissibility to be as high as 6:1 for the vibration transmission from the vibrating source to the ankle and the hip. Also, a set of ten transfer functions corresponding to 10 postures was obtained and was used for correction of measure of transmission. Previously, it has been shown by Kim et al. (68) that without transfer functions, skin mounted accelerometer can overvalue the actual peak acceleration by 10 - 15%.

Kiiski et al. (69) explored transmissibility in healthy male participants (n = 4; age = 24 - 47 years) over a wide range of vibration amplitude (0.04 *g* - 19 *g*) and frequencies (10 - 90 *Hz*) using skin mounted accelerometers (20 grams) at the ankle

(medial malleolus) and knee (tibial tuberosity). Every participant in their study reported discomfort between 20 - 25 *Hz* frequency at amplitude of 0.5 mm or greater (i.e., 0.8 *g* and 7.5 *g*). Sizeable amplification of peak acceleration between 10 - 40 *Hz* and 10 - 25 *Hz* was observed at the ankle and knee, respectively. Also, waveforms emitted by the vibrating platform appeared distorted and lost their sinusoidal character with higher magnitude acceleration. The results from previous studies (19,64,69) suggested that accelerations greater than being emitted by the platform can occur at specific skeletal sites due to resonance phenomenon

So far, only one study (19) has examined vibration transmission in children. Bressel et al. (19) investigated the nature of vibration transmission in children (n = 11) versus adults (n = 10). Motion analysis system was used to measure vibration transmission when subjects stood on the vibrating platform (28/33/42 *Hz*). Although, transmissibility at the head was not different between children and adults (p = 0.92), transmissibility in children was 42% and 62% greater than adults for the ankle (lateral malleolus) and the hip (anterior superior iliac spine) (p = 0.03), respectively. It was noted that accelerations at all the sites increased with frequency and magnitude. At the ankle, transmissibility was found to be two times higher than the acceleration at the platform. However, at the knee (tibial tuberosity), acceleration was attenuated in children at all the frequencies. A novel finding of this study was amplified values of acceleration at 33 *Hz* setting which was higher than 28 *Hz*. Researchers credited this amplification of vibration transmission at the distal tibia to the resonance frequency of the distal tibia which is ~30 *Hz.*

It should be noted that human body responds dynamically to any external stimulus. Also, the physiological response to any external stimulus usually is non-

linear. Mansfield et al. (70) showed that human body exhibits a non-linear biodynamic response to vertical vibration. In their study, 12 subjects were exposed to vibration stimulus (0.25 - 2.5 ms^{-2}) in the frequency range of 0.2 - 20 *Hz* in a seated position. Acceleration transmission was measured at upper and lower abdominal wall, L3 spine, posterior superior iliac spine and iliac crest. Resonance frequency for the apparent mass decreased with increased vibration magnitude in all the subjects and apparent mass non-linearities were displayed in the frequency range of 3 - 16 *Hz*. Furthermore, frequency of the first resonance decreased with increase in vibration magnitude and the shape of graphs depicting vertical transmission from seat to the spine and pelvis showed a general non-linear response.

An interesting study showed the importance of joint position in vibration transmission in a temporomandibular joint (71). In this study, the relative orientation of two contributing bones that constitute a joint was found to affect vibration transmission. Furthermore, ligaments which bind one bone to another bone also changed their length with different joint positions such as open (when the ligaments became tight) versus closed jaw (when the ligaments became slack) which might have contributed to different patterns of vibration transmission with different positions (high vibration transmission with the tight ligaments and lower vibration transmission with slack ligaments). Since the ankle and the knee joint can be considered hinge joints (72), similar to the temporomandibular joint, it can be postulated that joint position can play an important role in HLV transmission in this populations. Interestingly, different level of spasticity can lead to unique joint positions in individuals with CP.

There are two main techniques of measuring acceleration transmission: invasive (i.e., bone mounted accelerometer), and non-invasive (i.e. skin-mounted accelerometer). Bone mounted accelerometers are considered the ideal technique to assess acceleration transmission. Previous studies have used accelerometers attached to a pin which was glued to the bone of interest (73). However, skin mounted accelerometers have also been shown to yield reasonable estimates of acceleration transmission (64,68,69,74). The biggest advantage with skin mounted accelerometers is its non-invasive nature and ease to use with any human populations. With populations like disabled children, elderly individuals, or other clinical populations, use of bone mounted accelerometer could be considered unethical.

In one of the earlier studies, Ziegert et al. (74) showed that acceleration output values obtained from a low weight skin mounted accelerometers at the tibia are similar to measures obtained by bone mounted accelerometer. Later, Kim et al. (68) showed a loss of signal at high frequency component (> 110 *Hz*) with skin mounted accelerometers compared to bone mounted accelerometers. Also, between 15 - 30 *Hz* frequency spectrum, without the transfer-function correction, skin mounted accelerometer can overestimate the bone mounted accelerometer values by 12% (68). However, it should be noted that the nature of vibration used in the study by Ziegert et al. (74) and Kim et al. (68) was not sinusoidal; rather it was an impact force. Previous studies have utilized skin mounted accelerometers, ranging from 2 grams to 150 grams (64,74); while some studies used transfer function correction (64), some did not (69). Interestingly, the findings of these studies have been similar with respect to transmissibility at various skeletal sites such as the tibial tuberosity and the

medial/.lateral malleolus, suggesting the wider application of skin mounted accelerometers in research settings.

1.4.2 Tissue composition and acceleration transmission

It is plausible that tissue composition could play a critical role in the vibration transmission. It is known that resonance can lead to amplification of the signals when the signal frequency is close to the natural frequency of the tissue. On the other hand, tissues such as fat, muscle and bone can act as low pass filters to dampen the acceleration signal (27). One of the seminal studies found that a skin mounted accelerometer of 1.5 *g* gave the same value of accelerations as a bone mounted accelerometer at the tibia suggesting no effect of skin on accelerations at frequencies less than 3000 *Hz* (74). The data from their study suggested that a tight coupling of low mass accelerometers to the skeletal site yields a value which is very similar to bone mounted accelerometer. The finding is not entirely unexpected because factors that could affect acceleration transmission such as muscle, adiposity, and other soft tissues such as ligament are not present in appreciable amount between the ankle and the foot. It is only at the sites above the medial/lateral malleolus that the effect of soft tissues on acceleration transmission can be postulated to work upon.

Previous studies have emphasized the role of muscles in damping of the acceleration signals (27,75). Wakeling et al. (27) determined the role of muscle length, muscle contraction velocity, and muscle force production in damping the vibration signals. Accelerometers (15.9 grams) were used to measure acceleration at various muscles, such as gastrocnemius, tibialis anterior, and vastus lateralis in 14 young adults (male = 7, female = 7). Isometric muscle torques, joint angle and joint angular velocity were recorded by a Biodex apparatus. Vibration stimulus was provided to

muscle during each contraction by a strike with a wooden mallet. Increased damping was observed with an increase in torque for any angle and increasing joint power for all directions (x, y, and z). Results from this study suggested that damping coefficient of the oscillations increased with increased muscular force production. An activated muscle generates tension which in turn depends on the number of recruited motor units eventually resulting in the generation of intermuscular pressure. Additionally, fascia can also contribute toward the enhancement of intermuscular pressure. An individual difference in muscle geometry, muscle-tendon compliance and the pattern of recruitment between motor units can affect intramuscular pressure which in turn can affect vibration propagation.

Reduced number of motor units (76), and reduced motor unit recruitment (77) in the affected versus the unaffected side in children with CP can adversely affect the capacity of muscle to dampen the vibration stimulus. It can be postulated that an interplay of various factors such as muscle mass, muscle quality, neuromuscular feedback, adiposity, and blood pressure would be the driving force in determining intermuscular pressure, the level of which can affect damping of accelerations. Wakeling et al. [19] also showed that damping coefficient depends on absolute mass of soft tissue such as muscle. An increased muscle mass was found to decrease the damping coefficient. Additionally, an independent effect of joint angle was reported on damping of acceleration signals. An increased damping coefficient was reported with an increased muscle contraction velocity.

Currently, the response of muscle tuning to HLV transmission remains unknown. We have reported before (unpublished data) that in individuals with unilateral CP, there is an interlimb difference in muscle work suggesting decreased

muscular force production in the affected limb. It can be postulated that HLV will be transmitted more with a decreased lower limb muscular force production as muscle would fail to dampen the vibration stimulus. A previous study has shown that there is an increased adipose infiltration of skeletal mass in the lower extremities in children with CP. Furthermore, children with spastic CP have compromised muscle length, and reduced musculo-tendinous compliance (78). All of these factors could affect HLV transmission in children with spastic CP.

In addition to muscle, the collagen content of bone can be a critical factor that can affect vibration transmission. In one of the seminal studies, the collagen content of the femur bone was estimated across different species, such as ox, guinea pig, and human (79). The study utilized samples of freshly excised bones from oxen and guinea pigs, while in humans, bone samples were taken from the mid-femur from children and adults across a wide-spectrum of age after autopsies (9 months to 90 years). The collagen content was found to be ~90% - 95% of the total organic matrix across different species. Mineralization was found to increase with age in humans. Since collagen provides the ductility and ability to absorb external energy/shock, its role in HLV transmission could be critical.

This is further supported by findings from another study (80) where no relationship was observed between vibration transmission and changes in the bone mineral density during various stages of fracture healing of the mid-tibial shaft. The authors in that study (80) measured vibration transmission produced by an impact hammer on mid-shaft of the tibia. They found no relationship between changes in vibration transmission characteristics and mineral content of the callus. It was

suggested that a greater role of the collagen compared to the mineral content led to these results.

In another study done on human trabecular bone (81), authors provided evidence of increasing collagen content with increasing age, increasing porosity, and decreasing bone mass. Authors studied the collagen content in trabecular bone from the iliac crest that was obtained after autopsy in the age range of 23-83 years. The organic matrix represented 28% by weight of the fat-free dry bone, and collagen 23%. Thus, the collagen content constituted 80% of the organic matrix. A tendency of increased collagen content was noted in the more porotic samples. A significant amount of collagen becomes insoluble with increasing age and increasing porosity was a novel finding of this study. A change in the nature of collagen cross-links can be hypothesized to affect vibration transmission. These findings can be related to a work by Currey et al. (82) where the authors found a decrease in impact absorption energy by more than three-fold between the ages of three and ninety. Furthermore, mineralization was higher in older bone. Interestingly, higher porosity in young bones did not materially affect its energy absorption but the higher porosity of older bones produced a deleterious effect on impact energy absorption.

Shape and mineral distribution of bone can also be a critical factor affecting vibration transmission. A recent study (83) based on finite element modeling reported that shape and mineral distribution in human femur can induce large variations in the calculated natural frequency/resonance frequency. Twenty-two different modes of femoral shape were created to describe 95% of the variance in the femoral appearance. In this study (83), changing the femoral shape from the mean shape by up to three standard deviations led to 7-16% variation in the natural frequencies of the vibration.

Overall, natural frequencies varied between 25 – 38% from their mean values for all the modes that were tested. This implies that vibration of a bone to induce its maximum apposition by choosing a specific frequency as a resonance frequency as suggested by Zhao et al (29) is not recommended.

Patient-specificity plays a critical role in imparting resonance frequency to the femur. A recent study by Dodge et al. (75) examined damping capacity of bone, joint tissue, muscle, and skin in the lower hindlimb of mice (n = 25) in response to a 5 N peak–to–peak force applied at 0.5 *Hz* increments from 1-20 *Hz*. Two damping parameters (i.e., phase sift angle and dissipative energy) were determined in 5 different samples: 1) intact lower hindlimb, 2) limb without the skin, 3) limb without the skin and foot, 4) the tibia and the fibula alone without an intact joint at either end or 5) muscle removed. A significant difference was noted in phase shift angle and dissipative energy in sample IV and V than the others ($p < 0.001$). Data from their study showed that muscle, bone, and knee joint were the largest contributors to the energy loss in response to axial loading while skin had no effect on damping at any the frequencies. Interestingly, normal bone curvature enhanced the damping capacity of bone by 40%. The findings from this study suggested that the bone curvature, the bone's damping capacity, and the muscle surrounding the bone are the biggest contributors to the external mechanical damping. However, the researchers did not investigate the effect of leg adiposity on mechanical damping.

Recently, Zhao et al. (29) showed that Young's modulus can affect the resonance frequency of a skeletal site suggesting factors such as age, gender, and bone structure could modulate a skeletal site's response to loading at specific frequencies. The study by Zhao et al. [54] also suggested that an enhanced quality of trabecular

bone microarchitecture will be related with better acceleration transmission and vice versa suggesting an important role of bone quality in the acceleration transmission. Interestingly, bones in children have low Young's modulus and yet absorb more energy before getting a fracture (84). Until now, no one has looked at the role of adiposity, muscle volume, and trabecular bone microarchitecture in acceleration transmission in children with CP. also, with the knowledge that children with CP show an infiltration of adipose tissue in the muscles of their lower extremity, and have underdeveloped trabecular bone microarchitecture, it becomes pertinent to know how tissue composition can affect HLV transmission, and if that affects the adaptation of bone to HLV transmission.

1.5 Specific Aims

My long term goal is to reduce the underdevelopment of bone in children with CP. The overall objective of my study was to determine the extent of underdevelopment in trabecular bone microarchitecture in ambulatory children with CP and to examine the degree to which the signal from a floor-based HLV platform transmits to different bone sites. My central hypothesis was that ambulatory children with mild CP will have lower estimates of trabecular bone microarchitecture in the distal tibia and the distal femur than typically developing children (controls); however, the underdevelopment will not be to the same degree as previously reported for nonambulatory children with moderate-to-severe CP. Moreover, the acceleration from a floor-based HLV platform will amplify at the distal tibia and attenuate at the distal femur in children with CP and in controls. The rationale for this pilot study was that it will allow me to understand the differences in trabecular bone microarchitecture between ambulatory children with CP and controls and gain an insight into the nature of HLV transmission at different bone sites in ambulatory children with CP. I plan to test my central hypothesis by using the following specific aims:

Aim 1. To determine the level of underdevelopment in trabecular bone microarchitecture at the distal tibia and the distal femur in ambulatory children with spastic CP.

Hypothesis 1: Trabecular bone microarchitecture at the distal tibia and the distal femur will be lower in ambulatory children with spastic CP than controls.

Aim 2. To determine the degree to which an HLV signal emitted by a floor-based platform transmits across the distal tibia and the distal femur in ambulatory children with spastic CP.

Hypothesis 2: The stimulus from the HLV platform will amplify at the distal tibia and attenuate at the distal femur in children with spastic CP and controls.

Aim 3. To determine if the HLV signal transmission to the distal tibia and the distal femur in children with spastic CP and typically developing children is affected by tissue composition.

Hypothesis 3: The transmission of an HLV signal to the distal tibia and the distal femur will be negatively related to the muscle volume in the leg and positively related to trabecular bone microarchitecture at the distal tibia and the distal femur in children with CP and controls.

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Chapter 2

UNDERDEVELOPMENT OF TRABECULAR BONE MICROARCHITECTURE IS RELATED TO THE LEVEL OF MOTOR FUNCTION IN CHILDREN WITH CEREBRAL PALSY

Abstract

Nonambulatory children with cerebral palsy (CP) have severely underdeveloped trabecular bone microarchitecture; however, whether the degree of underdevelopment is related to motor function has not been determined. Twenty-six children with spastic CP (13 ambulatory and 13 nonambulatory) and 50 typically developing children between 5 and 13 years of age participated in the study. Twenty-six axial magnetic resonance images (175 x 175 x 700 μm^3) of the distal femur were collected from the more affected limb in children with CP and the nondominant limb in typically developing children. Measures of trabecular bone microarchitecture [apparent trabecular bone volume to total volume (appBV/TV), trabecular number (appTb.N), trabecular thickness (appTb.Th) and trabecular separation (appTb.Sp)] were estimated using the 20 most central images. Level of gross motor function was assessed using the gross motor function classification system (GMFCS) with levels I and II representing children able to ambulate independently, and levels III to V representing children unable to ambulate independently. There was a significant difference in measures of trabecular bone microarchitecture [appBV/TV, appTb.N, appTb.Th, and appTb.Sp] between the total sample of children with CP and typically developing children (Cohen's d (d) = 1.0 - 2.1, all $p < 0.001$). When separated based on ambulatory status, nonambulatory and ambulatory children with CP had lower appBV/TV by 28% ($d = 2.4$, $p < 0.001$) and 11% ($d = 1.0$, $p = 0.007$), lower appTb.N by 18% ($d = 2.5$, $p < 0.001$) and 9% ($d = 1.3$, $p = 0.001$), and higher appTb.Sp by 31%

(d = 2.6, p < 0.001) and 15% (d = 1.2, p = 0.004), respectively. Additionally, compared to typically developing children, nonambulatory children with CP had lower appTb.Th by 11% (d = 1.6, p < 0.001). GMFCS was negatively correlated with appBV/TV, appTb.N, and appTb.Th (r = -0.45 to -0.56, p < 0.05) in children with CP; on the other hand, GMFCS was positively related to appTb.Sp (r = 0.48, p = 0.014). The findings suggest that the degree of underdevelopment of trabecular bone microarchitecture in children CP is related to the level of compromise in motor function with more profound deficits in nonambulatory children with CP. However, even children with milder forms of CP had deficits in trabecular bone microarchitecture deviating from control values by ≥ 1 SD.

2.1 Introduction

The growing years are recognized as a vital period for optimizing bone strength (1). A primary stimulus of bone development in children is physical activity (2). This is demonstrated by cross-sectional studies showing higher areal bone mineral density (aBMD) (3) and estimates of bone strength (4) in athletes vs. non-athlete controls, as well as longitudinal studies demonstrating increases in bone mineral accrual (5) and bone strength (6) in children exposed to interventions that increase mechanical loading. On the other hand, children with limited mobility, such as children with moderate-to-severe CP, who are unable to ambulate have aBMD in the lower extremity bones that is ≥ 3 SD lower than observed in typically developing children (7). Interestingly, ambulatory children with mild CP have a BMC in the proximal femur that is ≥ 1 SD lower than typically developing children but no difference in aBMD (8). This suggests that ambulatory children with mild CP have bone deficits that are not captured by aBMD. Although aBMD is the primary measure used to assess fracture risk, it has considerable limitations, especially in children as it is strongly influenced by bone size. Moreover, it does not provide information about the structural properties of bone.

In addition to having very low aBMD, nonambulatory children with moderate-to-severe CP have markedly underdeveloped trabecular bone microarchitecture in the distal femur (9). Furthermore, the level of underdevelopment in trabecular bone microarchitecture becomes more pronounced with distance from the growth plate (9). These observations are important because the distal femur is the primary fracture site in children with CP (10,11). Moreover, trabecular bone microarchitecture is an important feature that is an independent predictor of bone strength (12). Although it is clear that nonambulatory children with moderate-to-severe CP have a marked deficit

in trabecular bone microarchitecture, whether there is a deficit in ambulatory children with CP is unknown. It is plausible that there is an underdevelopment in trabecular bone microarchitecture across all levels of motor function in children with CP, which includes children with mildest forms of the disorder.

The primary aim of this study was to determine if the underdevelopment of trabecular bone microarchitecture in the distal femur is associated with the level of motor function in children with CP. We hypothesized that trabecular bone microarchitecture will be underdeveloped in ambulatory and nonambulatory children with CP relative to typically developing children and the level of underdevelopment will be directly related to their motor function. A secondary aim of this study was to examine if the pattern of underdevelopment in trabecular bone microarchitecture is consistent across the distal femur in children with CP with different levels of motor function. We hypothesized that the underdevelopment in trabecular bone microarchitecture will get worse with increasing distance from the growth plate.

2.2 Methods

2.2.1 Participants

Twenty-six children with CP and 50 typically developing children aged 5-13 years participated in this study. Children with CP (16 boys and 10 girls) were recruited from the AI duPont Hospital for Children, Wilmington, DE and surrounding hospitals in the Mid-Atlantic region of the U.S.A. All participants had the maturity and cognitive ability to complete the study. Children were excluded if they had a baclofen pump, any chemodenervation surgery within the past year, any musculoskeletal surgery, or if any metal rods were placed in their thigh or legs.

Typically developing children (28 boys and 22 girls), comparable in age, BMI, and sexual maturity to children with CP, between the 5^{th} and 95^{th} percentiles for height and body mass, no history of chronic medication use, and no history of fracture within the past one year were recruited as controls. The study was approved by the institutional review boards at the University of Delaware and the AI duPont Hospital for Children, Wilmington, DE. Parents provided written consent, and the participating children gave written assent before any testing was performed.

2.2.2 Study design and procedures

A between group comparison design was used in this study. Anthropometrics, Tanner staging, and gross motor function classification were assessed at the University of Delaware. Magnetic resonance imaging (MRI) was done at the AI duPont Hospital for Children, Wilmington, DE.

2.2.3 Anthropometrics

In ambulatory children with CP and typically developing children, height was measured to the nearest centimeter using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER). In nonambulatory children with CP, height was estimated from forearm length (13). Body mass to the nearest kilogram was measured using an electronic wheelchair scale (Detecto 6550, Cardinal Scale, Webb City, MO, USA). All measurements were taken while the children were in minimal clothing and without any shoes or bracing devices.

2.2.4 Tanner Staging

A physician assistant assessed sexual maturity of each participant using Tanner staging technique. Pubic hair growth and testicular/penile development were evaluated

in boys and pubic hair growth and breast development were evaluated in girls. A rating of I indicates no sign of sexual maturity while a rating of V indicates full sexual maturity (14,15).

2.2.5 GMFCS

A physician assistant evaluated gross motor function in children with CP using the GMFCS which ranges from I - V. Level I indicates mobility without limitations, level II indicates independent ambulation with minimal ability to perform gross motor skills, level III indicates ambulation with an assistive mobility device, level IV indicates powered wheeled mobility, and level V indicates a complete lack of independent mobility (16).

2.2.6 Magnetic Resonance Imaging

A 1.5 T MRI machine (GE, Milwaukee, WI) was used to collect magnetic resonance images of the distal femur of the more affected limb and non-dominant limb, respectively in children with CP and controls, to assess measures of trabecular bone microarchitecture [apparent bone volume/total volume (appBV/TV), apparent trabecular number (appTb.N), apparent trabecular thickness (appTb.Th), and apparent trabecular spacing (appTb.Sp)]. Before the start of imaging, a VacFIX system (PAR Scientific A/S; Sivlandvaenge, Denmark) was used to hold one of two connected phased array coils (USA Instruments; Aurora, OH) to the lateral portion of the distal femur of the more affected lower limb in children with CP and of the nondominant lower limb in controls. The BodyFIX (Medical Intelligence, Inc., Schwabműnchen, GER) was used to immobilize children from the waist down to help limit motion during the MRI scan, as described previously (17). After immobilizing children, a

three-plane localizer was used to identify the distal femur. High resolution images of the distal femur were obtained using a 3D fast gradient echo sequence with a partial echo acquisition (echo time = 4.5 ms, repetition time = 30 ms, 30° flip angle, 13.89 kHz bandwidth), a 9 cm field of view, and a reconstructed imaging matrix of 512 × 512. Twenty-six axial images (700 μm thick) of the metaphysis were collected immediately above the growth plate. Measures of trabecular bone microarchitecture were estimated in the lateral half of the 20 most central images of the distal femur using custom software created with Interactive Data Language (IDL, Research Systems Inc., Boulder, CO) and an analysis procedure patterned after the procedure outlined by Majumdar et al. (18). The average values of appBV/TV, appTb.N, appTb.Th and appTb.Sp from the 20 images are reported. The CV% for appBV/TV, appTb.N, appTb.Th, and appTb.Sp in our laboratory ranges from 2% - 3% (17). The procedure used has been described in detail previously (17,19).

2.2.7 Statistical analysis

SPSS Statistics 22.0 (IBM Corp., Armonk, NY) was used to analyze all the data. Normality of the data was calculated using skewness, kurtosis, and the Shapiro-Wilk test. If data were normally distributed, group differences in physical characteristics were assessed using a one-way ANOVA. If data were not normally distributed, group differences were assessed using a Kruskal-Wallis test. The Chi-square test of independence was used to determine if there were group differences in Tanner stage. A one-sample t-test was used to determine the differences between the sex and age-based percentiles for height, body mass, and body mass index (BMI) relative to the sex and age-based 50^{th} percentile. A one-way ANOVA was used to analyze group differences in measures of trabecular bone microarchitecture [i.e.

appBV/TV, appTb.N, appTb.Th, and appTb.Sp] in the distal femur. Data are reported as means ± SD in the text and in Table 2.1.

Table 2.1. Physical characteristics of study participants

	CP (n =26)	Nonambulatory CP (n =13)	Ambulatory CP (n =13)	Control (n =50)
Age (years)	9.6 ± 1.9	9.4 ± 1.8	9.7 ± 2.0	9.6 ± 1.9
Tanner stage (1/2/3)				
Pubic hair	18/7/1	10/3/0	8/4/1	41/7/2
Breast/testicular-penile	18/8/0	11/2/0	7/6/0	40/7/3
Height (m)	1.27 ± 0.1[a]	1.24 ± 0.1[a]	1.31 ± 0.13	1.37 ± 0.1
Height (percentile)	20 ± 23[a,b]	17 ± 21[a,b]	24 ± 26[a,b]	50 ± 26
Body mass (kg)	28.8 ± 10.3	26.3 ± 9.5[a]	31.4 ± 10.8	32.5 ± 8.1
Body mass (percentile)	34 ± 34[a,b]	25 ± 32[a,b]	42 ± 36	52 ± 26
BMI (kg/m2)	17.2 ± 4.3	16.6 ± 4.1	17.8 ± 4.6	17 ± 2.4
BMI (percentile)	47 ± 35	42 ± 35	52 ± 36	48 ± 30
GMFCS (I/II/III/IV/V)	7/6/4/1/8	4/1/8	7/6	-N/A

Values are means ± SD
[a]Different from control, $p < 0.05$
[b]Different from the 50th age-based percentile, $p < 0.05$
Gross motor function classification system (GMFCS)

Linear regression analysis was used to determine if the pattern of trabecular bone microarchitecture measures relative to distance from the growth plate in the distal femur was different in ambulatory children with CP, nonambulatory children with CP and controls. A one-way ANOVA was conducted to determine if there were group differences in the slopes of the regression lines representing the relationship between distance from the growth plate and measures of trabecular bone microarchitecture at the same distance in the distal femur. Spearman correlation analysis was used to determine if there were relationships between GMFCS and

measures of trabecular bone microarchitecture. In addition, spearman correlation analysis was used to examine relationship between slopes of distance from the growth plate versus measures of trabecular bone microarchitecture. Values are reported as mean ± SD. We have reported the median and ranges if data were not normally distributed. The alpha level was set at $p < 0.05$ for all the significance tests. The magnitude of effects was measured by using Cohen's *d*, with values of 0.2, 0.5, and 0.8 demonstrating small, medium, and large effects (20), respectively, and by comparing percent differences.

2.3 Results

Of the 26 children with CP who participated in the study, 13 (5 boys and 8 girls) could not ambulate independently and were classified between level III - V on the GMFCS.-The remaining 13 children with CP (11 boys and 2 girls) could ambulate independently and classified as level I/II on the GMFCS. Physical characteristics of the participants are reported in Table 2.1. All data were normally distributed except for BMI and height percentile in ambulatory children with CP. There were no group differences in age ($p = 0.921$), body mass ($p = 0.068$), BMI ($p = 0.784$, nonambulatory CP, median = 15.5, range = 11.8 - 24.4; ambulatory CP, median = 16.8, range = 12.5 - 30.4; control, median = 16.4, range = 13.6 - 22.4), BMI percentile ($p = 0.672$), pubic hair Tanner stage ($p = 0.296$), and breast/penile-testicular Tanner stage ($p = 0.149$). The total sample of children with CP and the subsample of nonambulatory children with CP had lower height ($p < 0.05$ for both), height percentile ($p < 0.001$ for both), and body mass percentile ($p < 0.05$ for both) compared to controls. Nonambulatory children with CP also had lower body mass ($p = 0.021$) than controls. Ambulatory children with CP had lower height percentile ($p = 0.001$, median = 12, range = 3 - 91)

than controls. Additionally, the total sample of children with CP and nonambulatory children with CP had lower height ($p < 0.001$ for both) and body mass ($p < 0.05$ for both) compared to 50th age- and sex-based percentiles. Ambulatory children with CP had lower height ($p = 0.003$) than 50th age- and sex-based percentiles. Height, body mass and BMI were not different than 50th age- and sex-based percentiles in controls ($p > 0.596$).

Compared to controls, the total sample of children with CP showed lower appBV/TV by 18% ($d = 1.8$, $p < 0.001$), lower appTb.N by 13% ($d = 2.1$, $p < .001$), lower appTb.Th by 6%, ($d = 1.0$, $p < 0.001$) and higher appTb.Th by 23% ($d = 2.1$, $p < 0.001$) in the distal femur as shown in Figure 2.1. When examined based on ambulatory status, nonambulatory children with CP and ambulatory children with CP versus controls had lower appBV/TV by 28 % ($d = 2.4$, $p < 0.001$) and 11% ($d = 1.0$, $p = 0.007$), lower appTb.N by 18% ($d = 2.5$, $p < 0.001$) and 9% ($d = 1.3$, $p = 0.001$), and higher appTb.Sp by 31% ($d = 2.6$, $p < 0.001$) and 15% ($d = 1.2$, $p = 0.004$), respectively, in the distal femur. Moreover, nonambulatory children with CP had lower appTb.Th by 11% ($d = 1.6$, $p < 0.001$) relative to controls. Nonambulatory children with CP versus ambulatory children with CP had lower appBV/TV by 17% ($d = 1.4$, $p = 0.002$), appTb.N by 9% ($d = 1.2$, $p = 0.018$), appTb.Th by 9% ($d = 1.2$, $p = 0.009$), and appTb.Sp by 16% ($d = 1.5$, $p = 0.005$), as depicted in Figure 2.2.

Scatter plots of distance from the growth plate and measures of trabecular bone microarchitecture are presented in Figure 2.3. Distance from the growth plate was strongly and inversely related to appBV/TV and appTb.N in nonambulatory children with CP, ambulatory children with CP, and controls ($r^2 = 0.68$ - 0.92, all $p < 0.001$). A negative relationship between distance from the growth plate and appTb.Th was noted

in nonambulatory children with CP (r^2 = 0.24, p = 0.03); however no such relationship was noted in ambulatory children with CP or controls. Also, distance from the growth plate was strongly and positively related to appTb.Sp in nonambulatory children with CP, ambulatory children with CP, and controls (r^2 = 0.62 - 0.90, all p < 0.001).

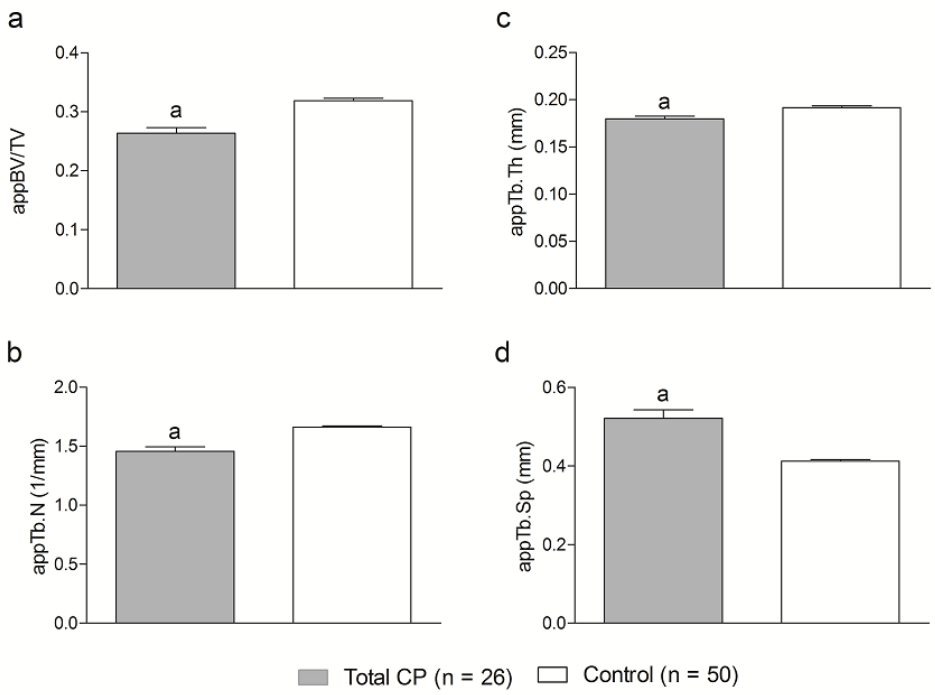

Figure 2.1 Comparison of a) apparent bone volume/total volume (appBV/TV), b) apparent trabecular number (appTb.N), c) apparent trabecular thickness (appTb.Th), and d) apparent trabecular separation (appTb.Sp) in the distal femur of all children with CP (n = 26) and controls (n = 50). Values are expressed as means ± SE. [a]Different from control, p < 0.05

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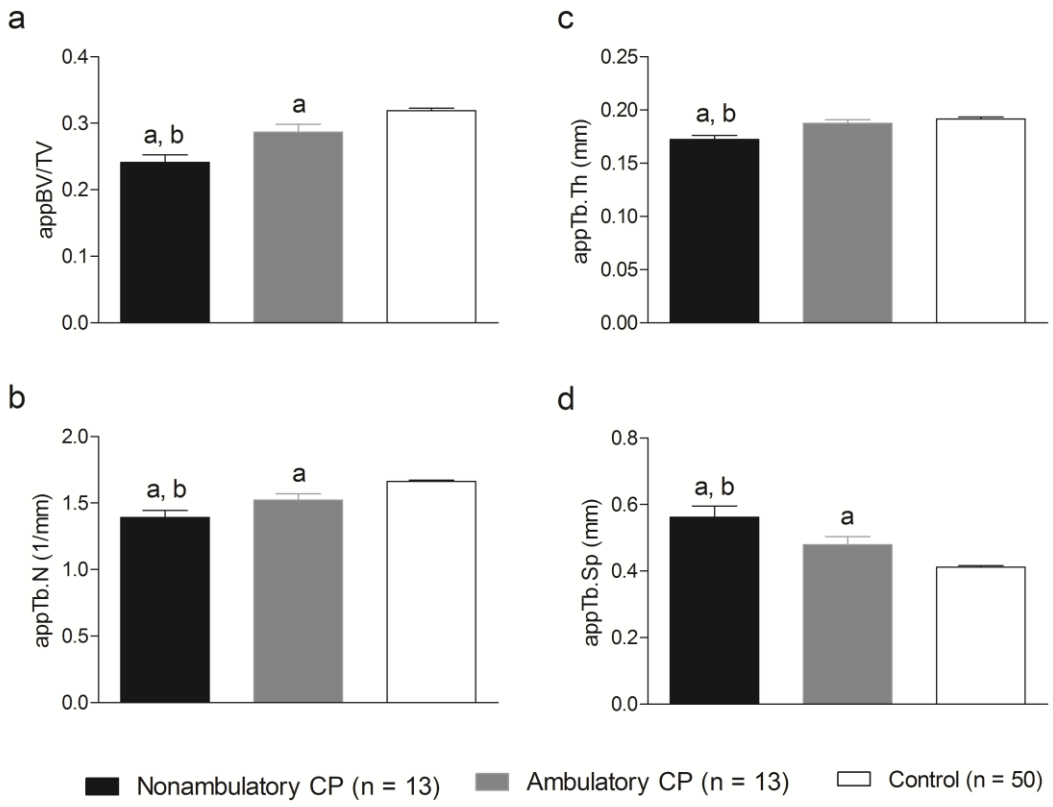

Figure 2.2 Comparison of a) apparent bone volume/total volume (appBV/TV), b) apparent trabecular number (appTb.N), c) apparent trabecular thickness (appTb.Th), and d) apparent trabecular separation (appTb.Sp) in the distal femur of nonambulatory children with CP (n = 13), ambulatory children with CP (n = 13) and controls (n = 50). Values are expressed as means ± SE. [a]Different from control, $p < 0.05$; [b]Different from ambulatory children with CP, $p < 0.05$

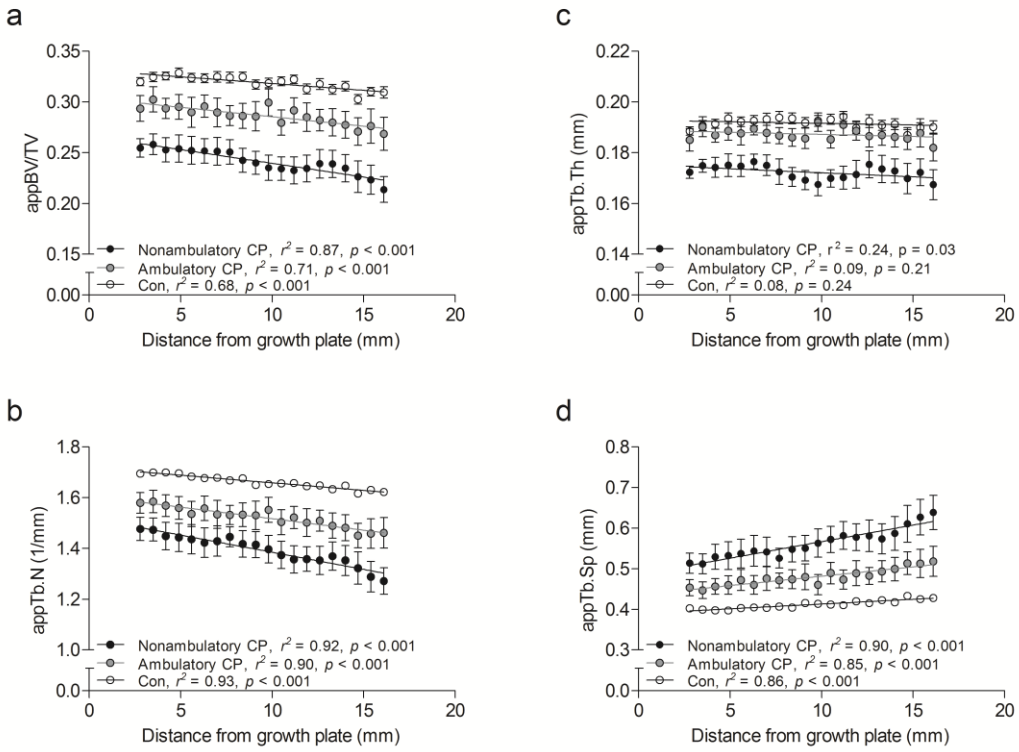

Figure 2.3 Scatter plots show the relationships between distance from the growth plate in the metaphysis of the distal femur and mean ± SE of apparent bone volume/total volume (appBV/TV) (a), apparent trabecular number (appTb.N) (b), apparent trabecular thickness (appTb.Th) (c), and apparent trabecular separation (appTb.Sp) (c) at the same distance in the distal femur of nonambulatory children with CP (n = 13), ambulatory children with CP (n = 13) relative to controls (n = 50).

Group differences in slopes of the regression lines (reported in Figure 2.3) are illustrated in Figure 2.4. Slopes were steeper for nonambulatory children with CP than for control children for appBV/TV (d = 0.91, p = 0.014), appTb.N (d = 1.80, p < 0.001) and appTb.Sp (d = 2.20, p < 0.001). In addition, ambulatory children with CP had a steeper appTb.Sp slope (d = 0.86, p = 0.039) than controls. Steeper slopes were

noted for nonambulatory children with CP than for ambulatory children with CP for appTb.N (d = 1.05, p = 0.026) and appTb.Sp (d = 1.35, p = 0.007). A moderate effect size (d = 0.74) suggested ambulatory children with CP had a steeper slope for appTb.N in the distal femur than controls; however, the difference did not reach statistical significance (p = 0.06).

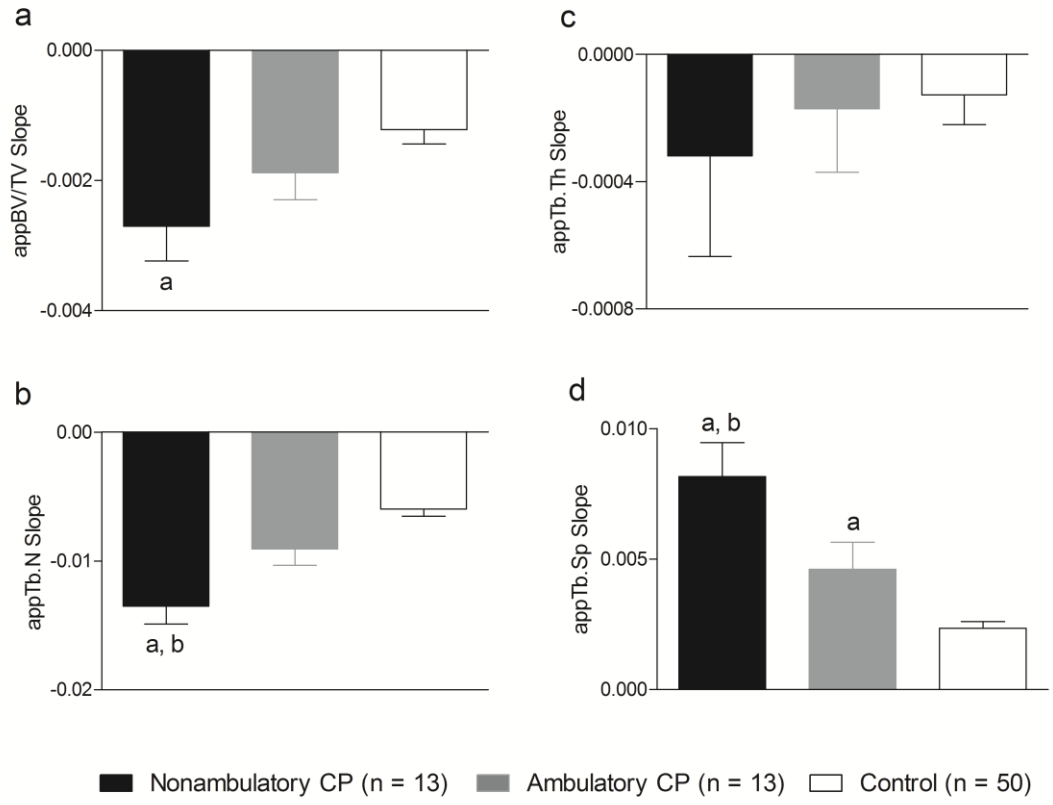

Figure 2.4 Comparison of slopes of distance from the growth plate versus a) apparent bone volume/total volume (appBV/TV), b) apparent trabecular number (appTb.N), c) apparent trabecular thickness (appTb.Th), and d) apparent trabecular separation (appTb.Sp) in the distal femur of nonambulatory children with CP (n = 13), ambulatory children with CP (n = 13) and controls (n = 50). [a]Different from control, $p < 0.05$; [b]Different from ambulatory children with CP, $p < 0.05$.

GMFCS was significantly and negatively correlated with appBV/TV, appTb.N, and appTb.Th (r = -0.45 to -0.56, $p < 0.05$) in children with CP; in contrast, GMFCS was positively related to appTb.Sp (r = 0.48, p = 0.014) as illustrated in Figure 2.5. GMFCS was also negatively related to the slope of distance from the growth plate versus appTb.N (r = -0.50, p = 0.009) and positively related to the slope of distance from the growth plate versus appTb.Sp (r = 0.49, p = 0.012) as depicted in Figure 2.6.

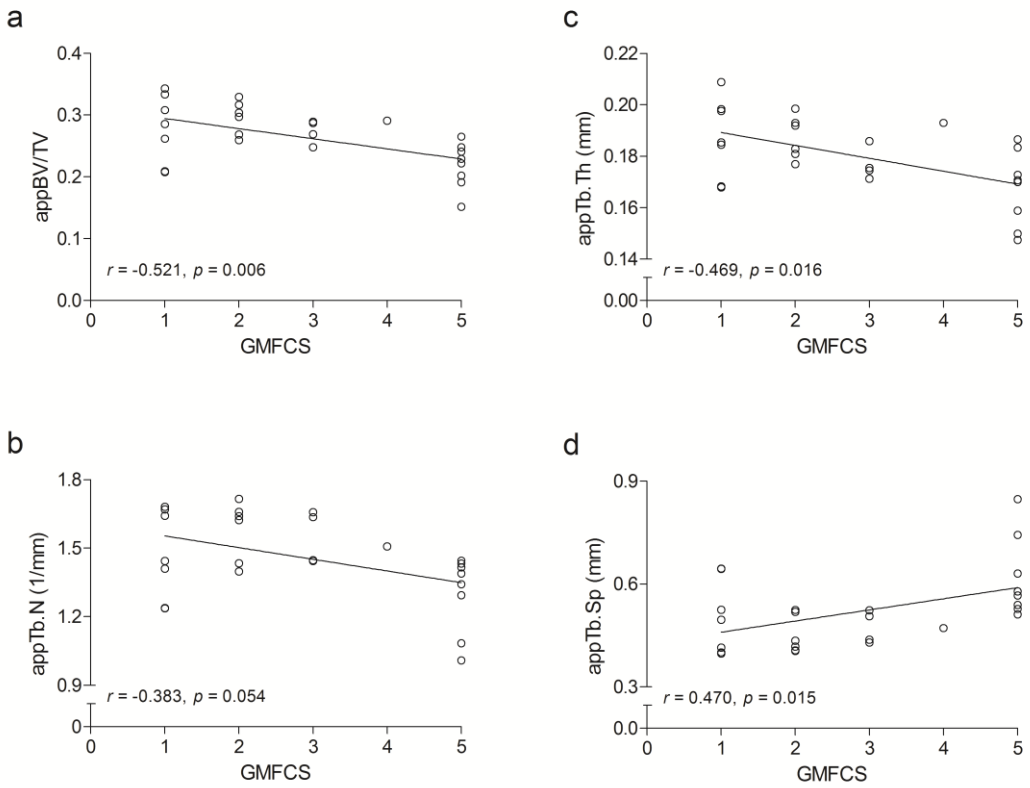

Figure 2.5 Scatter plots show the relationships between GMFCS and a) apparent bone volume/total volume (appBV/TV), b) apparent trabecular number (appTb.N), c) apparent trabecular thickness (appTb.Th), and d) apparent trabecular separation (appTb.Sp) in the distal femur of nonambulatory children with CP (n = 13) and ambulatory children with CP (n = 13).

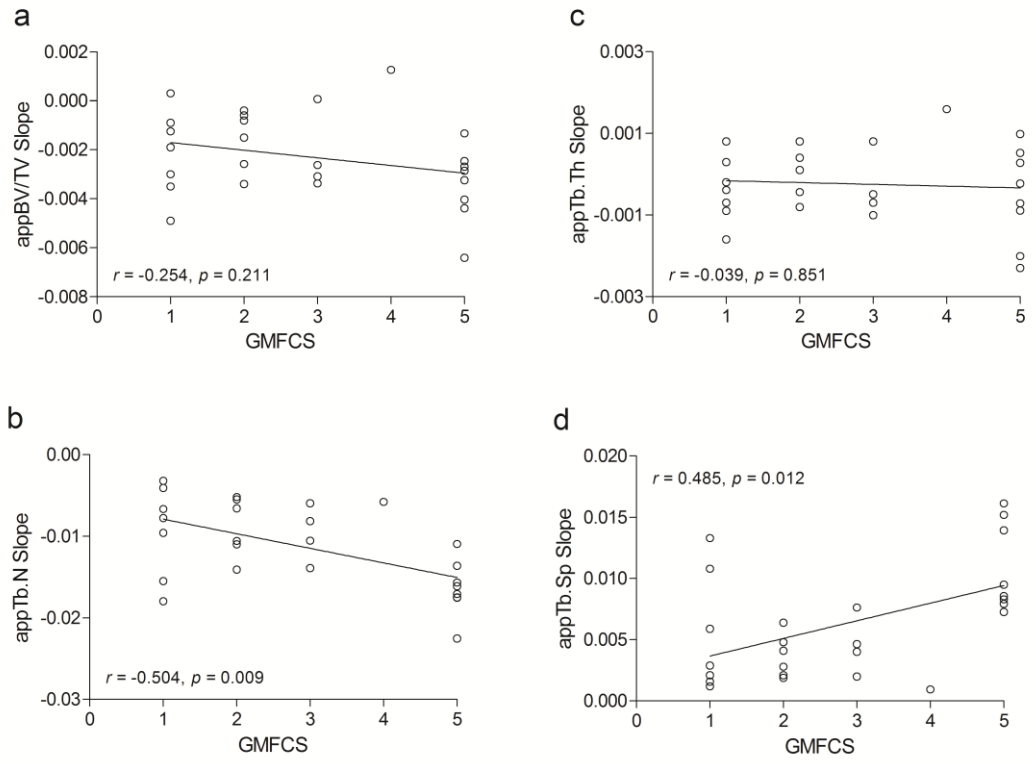

Figure 2.6 Scatter plots show the relationships between GMFCS and slopes of distance from the growth plate versus a) apparent bone volume/total volume (appBV/TV), b) apparent trabecular number (appTb.N), c) apparent trabecular thickness (appTb.Th), and d) apparent trabecular separation (appTb.Sp) in the distal femur of all children with CP (n = 26).

Representative binarized magnetic resonance images showing the distal femur from nonambulatory child with CP, ambulatory child with CP, and a typically developing child near the 50th percentile for height and body mass is represented in Figure 2.7.

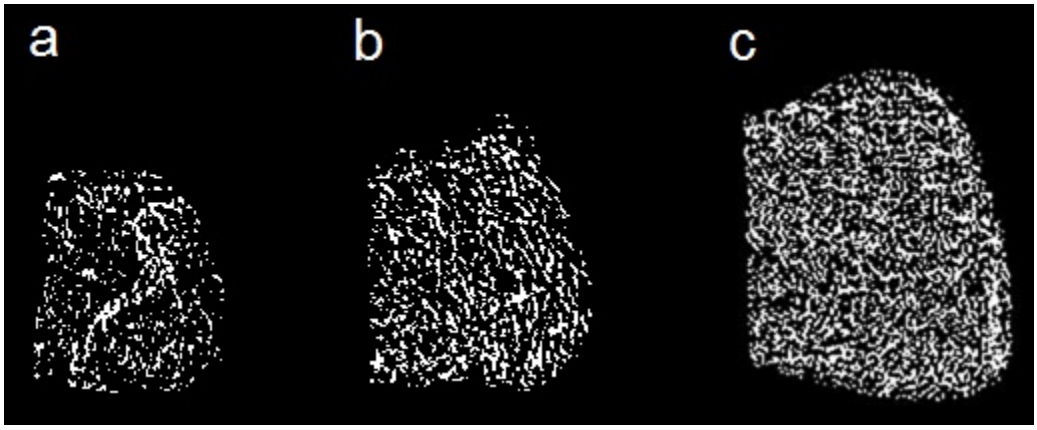

Figure 2.7 Binarized magnetic resonance images of the distal femur from a nonambulatory child with CP (a), ambulatory child with CP (b), and a typically developing child near the 50th percentile for height and body mass (c), show that the underdevelopment of the distal femur is dictated by the level of severity in children with CP. The children were matched for age, gender, and race and each child represents the mean height of their group.

2.4 Discussion

The present study is the first to demonstrate an inverse relationship between motor function, as assessed by GMFCS level, and trabecular bone microarchitecture in children with CP. The finding is consistent with previous studies that reported an inverse relationship between GMFCS level and bone volumetric density in the metaphysis of the distal tibia (21) and between GMFCS and areal bone mineral density (22) in children with CP. The present study is also the first to show that ambulatory children with mild CP had significantly underdeveloped trabecular bone microarchitecture in the distal femur compared to controls (≥ -1 SD. Moreover, we also found that with reduced motor function, the underdevelopment in trabecular bone microarchitecture in the distal femur gets progressively worse with distance from the growth plate. It should be noted that the underdeveloped trabecular bone

microarchitecture in the distal femur is consistent with the very high-incidence of low-energy fractures at this site in this population (10,11).

Previous studies examining bone architecture in children with CP have focused on children with moderate-to-severe forms of the disorder (23,24). We present evidence for the first time that children with mild CP have reduced measures of trabecular bone microarchitecture relative to controls in the distal femur. This can be attributed to several factors such as insufficient mechanical loading due to decreased levels of physical activity (25), reduced accretion of bone, delayed skeletal maturation (26), and the degree of the neuromotor impairment (27). All ambulatory children with CP in this study had spasticity in the lower extremities. A previous study has demonstrated a negative relationship between muscle spasticity and bone strength in the distal tibia in stroke patients (28). Evidently, there is a remarkable reduction of muscle growth (29) and increased muscle fibrosis in spastic muscles (30) leading to reduced muscle strength. It can be predicated, therefore, that ambulatory children with mild CP experience insufficient mechanical strains on the bones of the lower extremity due to reduced muscular strength which can lead to compromised bone architecture and decreased bone strength. Previous studies found that physical activity in ambulatory children with mild CP is significantly reduced relative to their healthy peers (25,31,32). Specifically, moderate-to-vigorous physical activity was found to be significantly reduced in ambulatory children with mild CP relative to controls (32). A reduced quantity and intensity of physical activity can lead to insufficient mechanical loading on the bones in the lower extremities which can, in turn, restrict peak skeletal mass in this population. It has been suggested that non-attainment of peak skeletal mass is related to the development of secondary osteoporosis later in life (1,33). We

observed a deviation of ≥ 1 SD in measures of trabecular bone microarchitecture in the distal femur in ambulatory children with CP compared to controls implying an increased susceptibility of this group to develop secondary osteoporosis later in their life. This idea is bolstered by another study (34) where children with moderate-to-severe CP developed clinically significant osteopenia over a span of 3 years.

We also report that the underdevelopment in trabecular bone microarchitecture in the distal femur becomes progressively worse with distance from the growth plate in ambulatory children with CP. Our previous work (9) had demonstrated the same pattern in nonambulatory children with CP and controls. The steeper slope in appTb.Sp in ambulatory children with CP compared to controls was unanticipated. A greater compromise in trabecular bone microarchitecture with increased distance from the growth plate in ambulatory children with CP compared to controls can be accredited to several factors such as reduced accretion of bone, neuromotor impairment and a decoupling of modeling-remodeling. A mismatch between modeling-remodeling processes, with lower modeling, higher remodeling or both, will lead to reduced accretion of bone (that is, less formation of trabeculae) and less preservation of bone (that is, marked deterioration of trabeculae) as the bone grows in size. This idea is supported by animal studies (35,36) where short-term unloading led to marked deficits in BV/TV, Tb.N, and an increase in Tb.Sp in the the distal femur. Interestingly, trabeculae were reinstated on introduction of a high strain mechanical loading.

Diminished measures of trabecular bone microarchitecture in the distal femur in nonambulatory children with CP are in line with findings from previous studies (17). Furthermore, thin cortical walls and drastically reduced bone strength in the

midfemur in nonambulatory children with CP has also been reported before (23). Seemingly, the reduced measures of trabecular bone microarchitecture can impart a weak architecture to the distal femur in nonambulatory children with CP which can be clinically associated with the maximum number of fractures at the distal femur in this population. It is verified that the incidence of fracture in children with moderate-to-severe CP can approach 4% per year with the highest number of fractures occurring at the distal femur (37). Recently, aBMD at the distal femur was shown to be related to the level of involvement in ambulatory children with CP (27). It aids our finding that the level of underdevelopment in the trabecular bone microarchitecture is associated with the degree of involvement in CP.

One of the strengths of the present study was that children with CP covered the full range of GMFCS. Both groups of children with CP and controls were not different in age, BMI or sexual maturity. Moreover, controls were not different from the 50^{th} age-based percentile for height, body mass and BMI. Another strength of this study is that the measures of trabecular bone microarchitecture were evaluated at the distal femur, which is the most vulnerable site for fracture in children with CP. All magnetic resonance images were analyzed by a single research assistant who was blind to the participant group. A limitation of this study is the susceptibility of magnetic resonance images to partial volume effects due to limited resolution. To counter the effects, the lower resolution of the slice (700 μm^3 in this study) was placed in line with the principal direction of the trabecular structures (38). Additionally, we have addressed all surrogate measures of trabecular bone microarchitecture as ‘apparent’.

2.5 Conclusions

In conclusion, the results from this study suggest that the degree of the underdevelopment in trabecular bone microarchitecture across all levels of motor function in children with CP is related to the level of involvement in children with CP. Future studies are needed to explore treatment paradigms to reduce the underdevelopment of bone in children with CP and to examine effects of intervention on the pattern of underdevelopment in trabecular bone microarchitecture in the distal femur.

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Chapter 3

BONE-SPECIFIC UNDERDEVELOPMENT OF TRABECULAR BONE MICROARCHITECTURE IN AMBULATORY CHILDREN WITH CEREBRAL PALSY

Abstract

The level of underdevelopment in trabecular bone microarchitecture in ambulatory children with cerebral palsy (CP) has not been determined. Furthermore, the level of deficit in the distal femur and the distal tibia which are the common fracture sites in children with CP is unknown. Ten ambulatory children with spastic CP and 10 typically developing children between 5 and 13 years of age participated in this study. Twenty-six axial magnetic resonance images (175 x 175 x 700 μm^3) of the distal tibia and the distal femur were collected from the more affected limb in children with CP and the nondominant limb in controls. Measures of trabecular bone microarchitecture [apparent trabecular bone volume to total volume (appBV/TV), trabecular number (appTb.N), trabecular thickness (appTb.Th) and trabecular separation (appTbSp)] were estimated using the 20 most central images and custom software (Interactive Data Language, Boulder, CO). Compared to controls, the distal tibia and the distal femur in ambulatory children with CP had lower appBV/TV by 26% ($p < 0.001$, $d = 2.32$) and 14% ($p < 0.05$, $d = 1.15$), lower appTb.N by 18% ($p < 0.01$, $d = 2.32$) and 11% ($p < 0.001$, $d = 1.66$), and higher appTb.Sp by 29% ($p < 0.01$, $d = 2.10$) and 19% ($p < 0.001$, $d = 1.5$), respectively. Additionally, compared to controls, children with CP had lower lower appTb.Th by 9% ($p < 0.05$, $d = 1.36$) at the distal tibia The lower appTb.Th ($p < 0.05$) and higher appTb.Sp ($p < 0.05$) was demonstrated more in the distal tibia than the distal femur in ambulatory children with CP versus controls. The degree of underdevelopment in trabecular bone

microarchitecture is more pronounced in the distal tibia than the distal femur in ambulatory children with CP with deviation of > 2 SD below the controls.

3.1 Introduction

Cerebral palsy (CP) is the most common physical disability of childhood (1) which refers to a group of permanent disorders of the development of movement and posture, causing activity limitations that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain (2). Children with CP can vary in their level of disorder based on gross motor function classification system (GMFCS) rating (3), from mild (Grade I and II) reflecting independent ambulation to moderate (Grade III) reflecting ambulation with assistive mobility devices to severe (Grade IV and V) indicating severely limited self-mobility or complete lack of independent ambulation. Nonambulatory children with moderate-to-severe CP have severely reduced areal bone mineral density (aBMD) (4) and underdeveloped bone microarchitecture (5) in the distal femur compared to typically developing children. Also, there is evidence that these children have severely deteriorated bone strength in the femur (6) and the distal tibia (7). Previous studies have shown that ambulatory children with mild CP also have diminished aBMD in the distal femur (8). It can be clinically related to the highest number of fractures reported in the lower extremity in children with CP (9,10). Apparently, the primary variable utilized to determine fracture risk is aBMD but its use in children is substantially limited, primarily because it is strongly influenced by bone size. In addition, it fails to provide information about the architectural properties of bone.

Studies have shown that trabecular bone microarchitecture, which is an important feature of bone, is a robust predictor of bone strength (11) and fracture (12). It can identify individuals who are at a greater risk for fracture who otherwise have normal aBMD (13). Although there is a lot of evidence on underdeveloped bone microarchitecture in children with moderate-to-severe CP, the state of bone in

ambulatory children with mild CP remains elusive due to limited research. Previous studies have shown that bone mass is low in ambulatory children with CP (8), however, the level of underdevelopment in trabecular bone microarchitecture has not been established. Furthermore, the level of deficit in the distal femur and the distal tibia which are the common fracture sites in children with CP (10,14) is unknown. An attractive research tool to examine skeletal properties in children is magnetic resonance imaging (MRI). It does not expose them to ionizing radiations and can be utilized to assess trabecular bone microarchitecture.

The primary aim of this study was to determine the level of underdevelopment of trabecular bone microarchitecture in the distal tibia in ambulatory children with spastic CP. We hypothesized that the trabecular bone microarchitecture in the distal tibia will be lower in ambulatory children with CP than controls. We also sought to compare the level of deficits in trabecular bone microarchitecture in the distal tibia versus the distal femur in ambulatory children with mild CP.

3.2 Methods

3.2.1 Participants

Ten ambulatory children with spastic CP (n = 7 boys and 3 girls), between 5 to 12 years of age, who could ambulate independently and classified as level I/II on GMFCS were recruited from the AI duPont Hospital for Children, Wilmington, DE and surrounding hospitals in the Mid-Atlantic region of the U.S.A. All the participants had the maturity and cognitive ability to complete the study. Exclusion criteria included children with baclofen pump, any chemodenervation surgery within the past year, any musculoskeletal surgery, or presence of any metal rods in their thigh or legs.

Ten typically developing children (7 boys and 3 girls) in the same age range and between the 5th and 95th percentiles for height and body mass, no history of chronic medication use, and no history of fracture within one year prior to the onset of participation year were recruited as controls. Our study was approved by the institutional review boards at the University of Delaware and the AI duPont Hospital for Children, Wilmington, DE. Written consent from parents, and the written assent forms from the participating children were obtained before any testing was performed.

3.2.2 Study design and procedures

A within-subject and between group comparison design was used in this study. Anthropometrics, pubertal development, degree of spasticity, and gross motor function classification were evaluated by a physician assistant and trained research assistants at the University of Delaware. Magnetic resonance imaging (MRI) was done by trained technicians at the AI duPont Hospital for Children, Wilmington, DE.

3.2.3 Anthropometrics

Height was measured to the nearest centimeter using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER) while participants stood without any shoes or bracing devices. Body mass was measured to the nearest kilogram using a weighing scale (Detecto D1130; Detecto, Webb City, MO) while the children stood in minimal clothing.

3.2.4 Tanner Staging

Tanner staging was conducted by a physician assistant to assess pubertal maturity of each participant. Pubic hair growth and testicular/penile development in boys and breast development in girls were assessed to grade sexual maturity. The

Tanner stage rating scale ranges from I to V, with I indicating no sign of sexual maturity and V indicating full sexual maturity (15,16).

3.2.5 Modified Ashworth Scale (MAS)

MAS was used to assess spasticity of plantar flexor muscles. The grading system in MAS ranges from 0 to 4, with 0 indicating presence of normal tone and 4 indicating muscle rigidity in flexion/extension (17).

3.2.6 GMFCS

Gross motor function was assessed using the GMFCS, which ranges from I - V with level I and II indicating independent ambulation, level III indicating walking with assistive devices, and level IV and V indicating a complete lack of independent ambulation (3). Only those children who were GMFC level I or II participated in our study, meaning all of the participants in the CP group could ambulate independently.

3.2.7 Magnetic Resonance Imaging

A 1.5 T MRI machine (GE, Milwaukee, WI) was used to collect magnetic resonance images of the distal femur and the distal tibia of the more affected limb and non-dominant limb, respectively in children with CP and controls to assess measures of trabecular bone microarchitecture [apparent bone volume/total volume (appBV/TV), apparent trabecular number (appTb.N), apparent trabecular thickness (appTb.Th), and apparent trabecular spacing (appTb.Sp)]. Before the start of imaging, a VacFIX system (PAR Scientific A/S; Sivlandvaenge, Denmark) was used to hold one of two connected phased array coils (USA Instruments; Aurora, OH) to the lateral side of the distal femur and medial side of the distal tibia of the more affected lower limb in children with CP and of the nondominant lower limb in controls. The

BodyFIX (Medical Intelligence, Inc., Schwabműnchen, GER) was used to immobilize children from the waist down to help limit motion during the MRI scan, as described previously (18). After immobilizing children, a three-plane localizer was used to identify the distal femur and distal tibia. High resolution images of the distal femur and distal tibia were obtained using a 3D fast gradient echo sequence with a partial echo acquisition (echo time = 4.5 ms, repetition time = 30 ms, 30° flip angle, 13.89 kHz bandwidth), a 9 cm field of view, and a reconstructed imaging matrix of 512 × 512. Twenty-six axial images (700 μm thick) of the metaphysis were collected immediately above the growth plate in the distal femur and the distal tibia. Measures of trabecular bone microarchitecture were estimated in the lateral half of the 20 most central images of the distal femur and medial half of the 20 most central images of the distal tibia using custom software created with Interactive Data Language (IDL, Research Systems Inc., Boulder, CO) and an analysis procedure patterned after the procedure outlined by Majumdar et al. (19). The average values of appBV/TV, appTb.N, appTb.Th and appTb.Sp from the 20 images are reported. The CV% for appBV/TV, appTb.N, appTb.Th, and appTb.Sp in our laboratory ranges from 2% - 3% (18). The procedure used has been described in detail previously (18).

3.3 Statistical Analyses

All data were analyzed by SPSS 22.0 (IBM Corp, Armonk, NY). Normality of the data was calculated using skewness, kurtosis, and the Shapiro-Wilk test. If data were normally distributed, group differences in physical characteristics were assessed using an independent sample t-test. If data were not normally distributed, group differences were assessed using a Mann-Whitney U test. The Chi-square test of independence was used to determine if there group differences in Tanner stage.

Differences between the sex and age-based percentiles for height, body mass, and body mass index (BMI) relative to the sex and age-based 50th percentile were calculated using a one-sample t-test. A two-way ANOVA (group x site) with repeated measures on site was used to determine if there were group differences and an interaction effect in measures of trabecular bone microarchitecture [i.e. appBV/TV, appTb.N, appTb.Th, and appTb.Sp] at the 2 sites (distal femur and distal tibia). If a group by site interaction was detected, simple contrasts were used to identify specific differences. Independent T-tests were utilized to compute group differences in measures of trabecular bone microarchitecture [i.e. appBV/TV, appTb.N, appTb.Th, and appTb.Sp] at the distal tibia and the distal femur. A Bonferroni adjustment was made for multiple comparisons. Values are reported as mean ± SD. The median and ranges are reported if data were not normally distributed. The alpha level was set at 0.05 for all the significance tests. The magnitude of effects were measured by using Cohen's *d* (*d*), with values of 0.2, 0.5, and 0.8 demonstrating small, medium, and large effects respectively, and by comparing percent differences.

3.4 Results

Physical characteristics of the participants are reported in Table 3.1. All data were normally distributed except for Tanner stage measures in control children and height percentile and BMI in children with CP. There were no group differences in age (p = 0.949, d = 0.03), height (p = 0.171, d = 0.64), height percentile p = 0.067, d = 0.87), body mass (p = 0.798, d = 0.12), body mass percentile (p = 0.186, d = 0.62), BMI (p = 0.940, d = 0.23; CP, median = 16, range = 12.5 - 30.6; control, median = 16, range = 13.6 - 21.6), BMI percentile (p = 0.961, d = 0.02), pubic hair Tanner stage (p = 0.232, d = 0.59), and breast/penile-testicular Tanner stage (p = 0.714, d = 0.16).

Children with CP had lower height compared to 50th age-based percentiles but did not approach significance level (p = 0.06) whereas body mass and BMI were not different than 50th age-based percentiles (p > 0.332). Height, body mass and BMI were not different than 50th age-based percentiles in controls (p > 0.380).

Table 3.1. Physical characteristics of study participants

	Ambulatory CP (n = 10)	Control (n = 10)
Age (years)	8.8 ± 2.2	8.7 ± 1.7
Tanner stage (1/2/3)		
Pubic hair	7/2/1	9/1/0
Breast/testicular penile	9/1/0	8/1/1
Height (m)	1.27 ± 0.01	1.32 ± 0.07
Height (percentile)	29 ± 31[a,b]	56 ± 31
Body mass (kg)	28.9 ± 9.4	29.8 ± 6.7
Body mass (percentile)	39 ± 35	58 ± 27
BMI (kg/m2)	17.9 ± 5.3	16.9 ± 2.9
BMI (percentile)	49 ± 39	48 ± 33
MAS (1/1.5/2/3)	6/1/1/2	N/A
GMFCS (I/II)	6/4	N/A

Values are means ± SD
[a]Different from control, p < 0.05
[b]Different from the 50th age-based percentile, p < 0.05
Gross motor function classification system (GMFCS)
Modified Ashworth Scale (MAS)

A group comparison of trabecular bone microarchitecture measures in the distal femur and the distal tibia are depicted in Figure 3.1. Compared to controls, children with CP had lower appBV/TV by 26% (p < 0.001, d = 2.3) and 14% (p <

0.05, d = 1.15) and lower appTb.N by 18% ($p < 0.01$, d = 2.3) and 11% ($p < 0.001$, d = 1.67) in the distal tibia and the distal femur, respectively. Compared to controls, children with CP had lower appTb.Th by 9% ($p < 0.05$, d = 1.36) at the distal tibia but there was no group difference at the distal femur ($p > 0.05$, d = 0.52). Compared to controls, children with CP had higher appTb.Sp by 29% ($p < 0.01$, d = 2.10) and 19% ($p < 0.001$, d = 1.51) in the distal tibia and the distal femur, however, the group differences were greater at the distal tibia.

Representative binarized magnetic resonance images showing the distal tibia and the distal femur from ambulatory child with CP, and a typically developing child near the 50th percentile for height and body mass is represented in Figure 3.2. The children were matched for age, gender, and race and each child represents the mean age and height of their group.

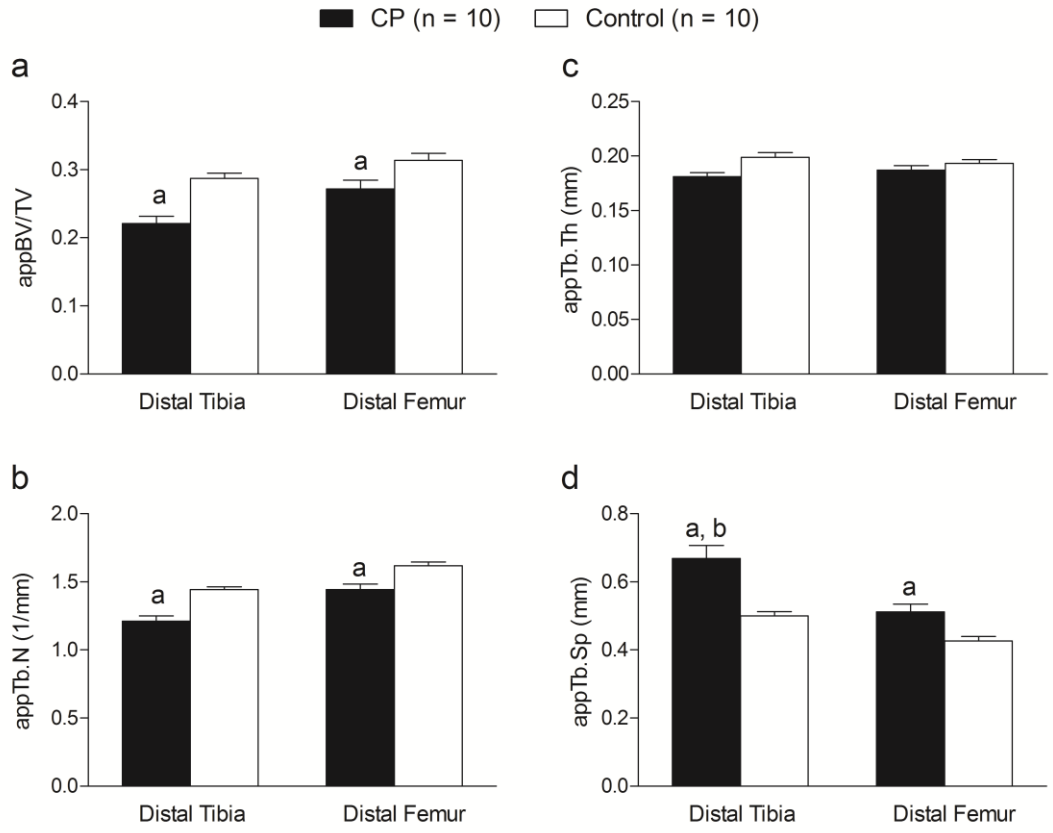

Figure 3.1. Comparison of a) apparent bone volume/total volume (appBV/TV), b) apparent trabecular number (appTb.N), c) apparent trabecular thickness (appTb.Th), and d) apparent trabecular separation (appTb.Sp) in the distal tibia and the distal femur of ambulatory children with CP (n = 10) and controls (n = 10). Values are expressed as means ± SE. [a]Different from control, $p < 0.05$, [b]Group x Site interaction, $p < 0.05$

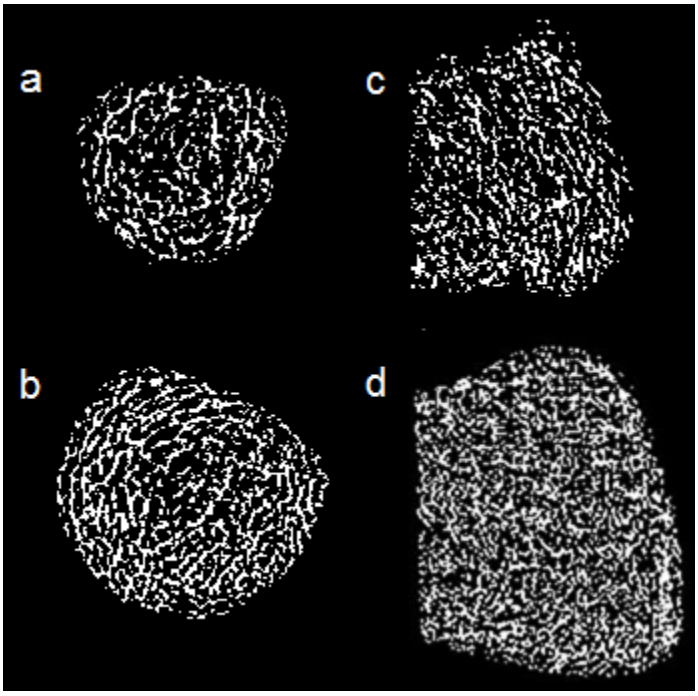

Figure 3.2 Binarized magnetic resonance images of the distal tibia (a and b) and the distal femur (c and d) from ambulatory child with CP and a typically developing child, respectively. Figures show the underdevelopment of trabecular bone microarchitecture in the distal tibia and the distal femur in ambulatory children with CP. The children were matched for age, gender, and race and each child represents the mean age and height of their group.

3.5 Discussion

The architectural properties of bones in the lower extremity of ambulatory children with mild CP are poorly studied. To our knowledge, this is the first study to evaluate whether ambulatory children with spastic CP have underdeveloped trabecular bone microarchitecture in the distal tibia. Furthermore, we are also the first to report the level of bone deficit in the distal tibia versus the distal femur in ambulatory children with mild CP compared to typically developing children. Results from our study provide preliminary evidence that ambulatory children with mild CP have

underdeveloped trabecular bone microarchitecture in the distal tibia (≥ 1.8 SD lower than typically developing children) and that the degree of underdevelopment in the distal tibia is greater than the distal femur as demonstrated by effect sizes that are 2-fold larger and %differences that are 1.5-fold larger. This is a novel finding because most studies suggest that the distal femur is the most vulnerable site to get fracture in children with CP. Furthermore, the magnitude of the differences in trabecular bone microarchitecture irrespective of the site, is larger than aBMD and bone mineral content differences between ambulatory children with CP and typically developing children (*z* scores no more than 0.6 SD lower in children with CP) previously reported (20).

A recent study (8) showed that strength of antigravity muscles such as knee extensor muscles and not motor function was related to aBMD in the distal femur in ambulatory children with mild CP. Evidence suggests that muscles with spasticity show muscle fibrosis (21) which can reduce muscle strength. Furthermore, ambulatory children with CP have smaller and weaker muscles in the lower extremity compared to typically developing children (22). Overall, these past findings indicate that a weaker muscle in ambulatory children with CP will produce insufficient mechanical strains on bone, thus, compromising bone microarchitecture and integrity.

One of the factors that may have contributed to the underdeveloped bone microarchitecture could be the muscle spasticity in ambulatory children with CP. A previous study (23) in individuals with stroke showed a negative relationship between spasticity in the affected leg and bone strength in the distal tibia ($r = -0.415$, $p < 0.05$). In addition, reduced bone strength in the distal femur in individuals with spastic CP compared to individuals with dyskinetic CP has been reported before (24), further

suggesting that muscle spasticity has a negative effect on bone strength. Since ambulatory children with CP in our study had muscle spasticity in the plantarflexor group of muscles of the lower leg, underdeveloped trabecular bone microarchitecture in the distal tibia and the distal femur is not unanticipated. However, we did not find any relationship between MAS and measures of trabecular bone microarchitecture. It should be noted that the use of MAS as a sensitive measure of muscle spasticity is debatable (25). Since thigh muscles are less affected than the leg muscles in children with CP, these muscle probably help minimize the level of underdevelopment in the distal femur resulting in a greater level of deficit in trabecular bone microarchitecture in the distal tibia versus the distal femur. Additionally, muscle volume can also affect bone strength. A reduced upper leg muscle volume in ambulatory children with CP can result in diminished bone strength in the distal femur (26). Muscle volume has also been shown to be a strong predictor of muscle work in children with CP (22). A greater amount of muscle volume in the upper leg than the lower leg can further contribute to discrepancy in mechanical strain on the distal femur versus the distal tibia. Overall, an interaction of diminished muscle volume, reduced muscle strength, and degree of spasticity in the lower leg can interact to result in underdeveloped trabecular microarchitecture more in the distal tibia than the distal femur in ambulatory children with CP.

Recent studies (27,28) have revealed that ambulatory children with CP show depressed habitual physical activity compared to typically developing children and that participation in moderate-to-vigorous physical activity is significantly reduced in ambulatory children with spastic CP (28). Per the Frost model (29), abated physical activity can lead to a decline in sufficient mechanical strain on bone which can bring a

failure in achieving mechanical bone competence via mismatched modeling-remodeling mechanism in ambulatory children with CP.

Evidence suggests that altered muscle composition and fat infiltration in muscles of lower limb is linked with reduced physical activity in nonambulatory children with moderate-to-severe CP (30). Therefore, it can be postulated that a decreased level of physical activity in ambulatory children with mild CP can expedite fat infiltration of muscles of the lower limb diminishing the mechanical strain on bone and thus further compromising its microarchitecture. An early presence of significant underdevelopment of bone microarchitecture in key skeletal sites in our study further confirms this theory. Other factors such as diminished accretion of bone, delayed skeletal maturation, and neuromotor impairment can also interact to adversely affect bone microarchitecture in ambulatory children with spastic CP. Decreased physical activity can also be related to non-attainment of peak skeletal mass in this population which is a known risk factor for the development of secondary osteoporosis later in life (31). It is not surprising that adults with CP can face the problem of secondary osteoporosis at a younger age and with more severity (32). Interestingly, obesity has been reported to be high in ambulatory children with CP (33) which might be related to a compromised skeletal status (34); however, our participants were not obese.

Advanced medical practices are leading to survival of low-birthweight infants precipitating a global increase in the incidence rate of CP (35). Compared to typically developing children, children with CP grow slowly and also display a reduced accretion of bone which accentuates with advancing age (36). This can be related to observations where children with CP were reported to develop clinically significant osteopenia with advancing age (36). Per the 2013 International Society of Clinical

Densitometry pediatric position statement, a aBMD Z-score less than -2.0 adjusted for age, gender and body size and a history of fracture would classify as osteoporosis in the pediatric population (37). In our study, we found that appBV/TV, appTb.N, appTb.Th, and appTb.Sp in ambulatory children with CP deviated from controls by more than 2 SD, signifying a need to closely monitor their skeletal status. Various pharmacological and nonpharmacological interventions are under investigation to ameliorate low bone mass in children with CP; however, more knowledge about bone accrual at clinically significant fracture sites in children with CP is required to determine the efficacy of treatment. Our findings imply that trabecular bone microarchitecture in the distal tibia and the distal femur can act as diagnostic sites to examine effectiveness of various treatment paradigms focusing on mitigating fracture risk in ambulatory children with mild CP.

One of the strengths of the present study was homogeneous nature of participants. All children with CP had a mild form of the disorder and had muscle spasticity in their lower leg. Furthermore, typically developing children were matched to children with CP for age, sex, and race. In addition, there was no difference in age, BMI or sexual maturity between children with CP and controls, and controls were not different from the 50^{th} age- and sex- based percentiles for height, body mass, and BMI. Moreover, we examined trabecular bone microarchitecture at the distal tibia and the distal femur which are clinically relevant sites as indicated by the high number of fractures reported at these two sites (9, 10). One of the limitations of this study is susceptibility of magnetic resonance images to partial volume effects due to limited resolution. To counter these effects, the lower resolution of the slice (700 μm^3 in this study) was placed in line with the principal direction of the trabecular structure as

described earlier (38). We have addressed all surrogate measures of trabecular bone microarchitecture as ‘apparent’. In addition, sample sizes are small. However, despite this limitation, significant site-specific differences in trabecular bone microarchitecture were detected in children with CP versus typically developing children.

3.6 Conclusions

In conclusion, results from this study suggest that the degree of underdevelopment in trabecular bone microarchitecture in the distal tibia is more pronounced compared to the distal femur in ambulatory children with CP compared to typically developing children. Measures of trabecular bone microarchitecture in the distal tibia and the distal femur could act as a diagnostic tool to examine the efficacy of various treatment paradigms focusing on decreasing fracture risk in ambulatory children with spastic CP.

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Chapter 4

SITE-SPECIFIC TRANSMISSION OF A HIGH-FREQUENCY, LOW-MAGNITUDE VIBRATION SIGNAL IN CHILDREN WITH CEREBRAL PALSY

Abstract

The primary objective of this study was to determine the transmission of a high-frequency, low-magnitude vibration (HLV) signal from a floor-based platform to the distal tibia and the distal femur of children with spastic cerebral palsy (CP). It was also determined if the HLV transmission is related to the degree of spasticity in children with CP. This study was a cross-sectional study. All testing was done at the musculoskeletal physiology research laboratory at the University of Delaware. Children between 4- to 13-year-old with spastic CP who could stand independently (n = 18) and typically developing children (n = 10) participated in this study. The vibration signal at the HLV platform (~30 *Hz* and 0.3 *g*), distal tibia and distal femur was measured using accelerometers. The degree of plantar flexor spasticity was assessed using the Modified Ashworth Scale. The HLV signal was greater ($p < 0.001$) at the distal tibia than at the platform in children with CP (0.36 ± 0.06 vs. 0.29 ± 0.05 *g*) and controls (0.40 ± 0.09 vs. 0.24 ± 0.07 *g*). Although the HLV signal was also higher at the distal femur (0.35 ± 0.09 *g*, $p < 0.001$) than at the platform in controls, it was lower in children with CP (0.20 ± 0.07 *g*, $p < 0.001$). The degree of spasticity was negatively related to the HLV signal transmitted to the distal tibia ($r = -0.547$) and distal femur ($r = -0.566$) in children with CP (both $p < 0.05$). Results from this study suggest that an HLV signal from a floor-based platform was amplified at the distal tibia, attenuated at the distal femur and inversely related to the degree of muscle

spasticity in children with spastic CP. Whether this transmission pattern affects the adaptation of their bones to HLV requires further investigation.

4.1 Introduction

Children with physical disabilities such as cerebral palsy (CP) have reduced muscle (1) and bone (2,3) mass and quality, especially in the lower extremities. This musculoskeletal deficiency in children with CP is associated with less force generating capacity of the muscles (4) and a higher incidence of low-energy fractures in the lower extremities (5). Because children with CP have difficulty participating in physical activities(6) leading to reduced mechanical loading on their skeletal system, identifying alternate nonpharmacologic treatments is of interest (7).

Studies have shown that a floor based, high-frequency, low-magnitude vibration (HLV) signal has an anabolic effect on bone in various populations(8,9) including children with disabling conditions, such as CP(10,11). There are also studies showing no effect of HLV on bone(12,13) or an inconsistent effect across sites(14,15). It is plausible that the effectiveness of HLV as a treatment for musculoskeletal health is dictated by the degree to which the HLV signal is transmitted to a particular bone site. Other types of mechanical loading, such as exercise, have a site-specific influence on bone with the greatest effects at the site that experiences the load(16). The tibia and femur are of interest in children with CP because they are the most commonly fractured bones and the distal femur is the most commonly fractured site(5). Unfortunately, the transmission of HLV to key bone sites in children with CP has not been studied.

The primary aim of this study was to determine the degree to which an HLV signal emitted by a floor-based platform transmits to the distal tibia and distal femur of

children with spastic CP during standing. An amplification of vibration at the ankle and an attenuation at the knee has been observed in typically developing children(17). We hypothesized that a similar profile would be exhibited in children with CP. A secondary aim was to determine the relationship between spasticity and the transmission of an HLV signal at the distal tibia and the distal femur in children with CP. Since, children with CP in our study had spasticity in their lower leg and were unable to completely rest their heel on the platform, we hypothesized an inverse relationship between the degree of spasticity and HLV transmission at both the sites in children with CP.

4.2 Materials and Method

4.2.1 Participants

Ambulatory children with spastic CP, 4 to 13 years of age, at the level of I or II on the gross motor function classification system (GMFCS), and deemed to have the maturity and cognitive ability to complete the study were recruited from the AI duPont Hospital for Children, Wilmington, DE. Children were excluded if they had any treatment with botulinum toxin in the lower extremities within the past year or if they had any metal rods in their thigh or leg. Typically developing children were in the same age range and between the 5^{th} and 95^{th} percentiles for height and body mass and were without a history of chronic medication use were recruited from the Newark, DE community. This study was approved by the AI duPont Hospital for Children and the University of Delaware Institutional Review Boards. The participating children and the parents of participating children, respectively, gave written assent and consent before any testing was performed.

4.2.2 Study Design and Procedures

A within-subject and between group comparison design was used. Anthropometrics, pubertal development, degree of spasticity, gross motor function, and vibration transmission were assessed during a single visit by trained research assistants. All testing took place at the University of Delaware.

4.2.3 Anthropometrics

Height and body mass were measured while the children were wearing minimal clothing and were without shoes or braces. Height was measured to the nearest centimeter using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER) while participants were standing. Body mass was measured to the nearest kilogram using a weighing scale (Detecto D1130; Detecto, Webb City, MO).

4.2.4 Tanner Staging

Tanner staging was conducted by a physician assistant to assess sexual maturity of each participant. The Tanner stage rating scale ranges from I to V, with I indicating no sign of sexual maturity and V indicating full sexual maturity(18,19).

4.2.5 Modified Ashworth Scale (MAS)

Ankle plantar flexor tightness was assessed in children with CP while the participant was lying on a table in a supine position using the MAS. The grading system ranges from 0 to 4, with 0 indicating presence of normal tone and 4 indicating muscle rigidity in flexion/extension(20). The grade for each limb was based on an average grade of three trials.

4.2.6 GMFCS

Gross motor function was assessed using the GMFCS, which ranges from I - V with I indicating walking without limitations and V indicating wheelchair empowered mobility(21). Only those children who were GMFCS level I or II participated in our study, meaning all of the participants in the CP group could ambulate independently.

4.2.7 Vibration Transmission

Participants stood on an HLV platform (Juvent 1000 Motion Therapy system, Juvent Inc., Riviera Beach, FL) for three consecutive conditions (pre-HLV, HLV and post-HLV) of 30 seconds per condition. The HLV platform delivered a sinusoidal vertical vibration signal of approximately 0.3 *g* at a frequency of 30-37 *Hz*. No HLV was transmitted during the pre-HLV and post-HLV conditions, which were immediately before and immediately after the HLV condition, respectively. They stood on the platform without shoes, socks or braces while their feet were inside a 16 x 16 cm square region outlined at the center of the HLV platform. Participants were instructed to stand on the platform as still as possible in a relaxed positon and were encouraged to stand without support. However, if support was needed for balance, they were allowed to lean on a standard wooden chair which was placed in front of the HLV platform. A spotter stood on either side of the participant to prevent falls. Only data collected during independent standing was used for the analysis. The same HLV platform was used for all participants.

Prior to the participant standing on the HLV platform, a uniaxial accelerometer (Model: 3711B1110G, PCB Piezoelectronics Inc, Depew, NY) was secured to the platform at its central point using double-side tape placed between the accelerometer and the platform as per recommendations by Rauch et al.(22). The accelerometer was

further secured to the platform using single sided tape over the top of the accelerometer. The uniaxial accelerometer was used to quantify the amount of vibration emitted by the HLV platform. To quantify the transmission of HLV signal to the distal tibia and the distal femur, triaxial accelerometers (Model: 3713B1113G, PCB Piezoelectronics Inc, Depew, NY) were secured to the skin immediately above the medial malleolus of the distal tibia and at the lateral condyle of the distal femur to the limit of the participant's comfort using a self-adhesive elastic bandage. The placement of all accelerometers was done by a single research assistant for all participants. All accelerometers were calibrated prior to each data collection session. The same accelerometers were used at the same sites for all participants. The data collection setup is displayed in Figure 4.1.

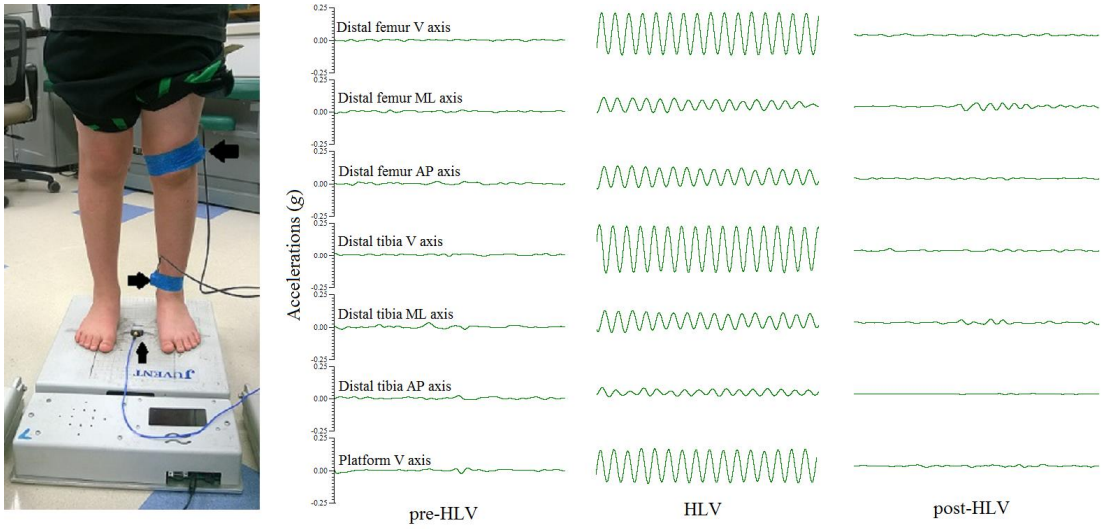

Figure 4.1. On the left (A) is a participant with triaxial accelerometers secured to the distal femur (large arrow) and distal tibia (medium arrow) and a uniaxial accelerometer secured to a platform that emits a high-frequency, low-magnitude vibration (HLV) signal when turned on (small arrow). On the right (B) are sinusoidal waveforms showing signals at the HLV platform, distal tibia, and distal femur in the vertical (V), mediolateral (ML) and anteroposterior (AP) axis while standing before the HLV platform is turned on (pre-HLV), while it is on (HLV) and after it is turned off (post-HLV).

A 12-bit AD converter data acquisition device (Model: USB-1208FS, Measurement Computing Corp., Norton, MA) was used to collect the vibration data at a sampling frequency of 2 kHz using DASYLab® (software version 11.0, Measurement Computing Corportaion, Norton, MA). Spike 2 (version 7.10, Cambridge Electronic Design, Cambridge, UK) was used to analyze the data after it was filtered using custom MATLAB coding. Peak-to-peak voltage values (mV) were converted to *g* force per the manufacturer specified sensitivity for each respective axis. The resultant *g* was calculated for the triaxial accelerometers at the distal tibia and the

distal femur using the following equation: $R = \sqrt{(V_g^2 + ML_g^2 + AP_g^2)}$ with V_g, ML_g, AP_g representing *g* forces in the vertical, mediolateral, and anteroposterior directions.

A 5 second time period within each 30 second condition (pre-HLV, HLV and post-HLV) was chosen to analyze and calculate amplitude and frequency of HLV signals because all participants were able to stand independently without leaning on the chair or creating any movement for a minimum of 5 seconds. HLV transmission was defined as the difference in HLV signals at the platform vs. the distal tibia and the distal femur.

4.2.8 Statistical analysis

Using an effect size of 0.25 and setting the alpha at 0.05 and power at 0.8, a minimum sample size of 9 participants per group was estimated to determine if the HLV signal transmission was significantly different across the sites within participants (i.e., HLV platform vs. distal tibia and distal femur) and between groups (CP vs. control). Data were analyzed using SPSS Statistics 22.0 (IBM Corp., Armonk, NY). Data were assessed for normality using skewness, kurtosis, and the Shapiro-Wilk test. If data were normally distributed, group differences in physical characteristics were assessed using an independent sample t-test. If data were not normally distributed, group differences were assessed using a Mann-Whitney U test. A paired t-test was used to determine if pre-HLV and post-HLV signals were different. If they were not different, the pre-HLV condition was used for comparison to the HLV condition. A two-way ANOVA (group x site) with repeated measures on site was used to determine if there were group differences and an interaction effect in HLV transmission at the 3 sites (platform, distal tibia, and distal femur). If a group by site interaction was detected, simple contrasts were used to identify specific differences. A Bonferroni

adjustment was made for multiple comparisons. Spearman correlation analysis was used to determine if there were relationships between MAS and HLV signal measured at the platform, the distal tibia and the distal femur. Values are reported as mean ± SD. The median and ranges are also reported if data were not normally distributed. The magnitude of effects was measured by using Cohen's *d* (*d*), with values of 0.2, 0.5, and 0.8 demonstrating small, medium, and large effects (23)

4.3 Results

Physical characteristics of the participants are reported in Table 4.1. All data were normally distributed except pubic hair and breast/testicular-penile Tanner stage. There were no group differences in age ($d = 0.33$, $p = 0.413$), body mass ($d = 0.48$, $p = 0.231$), pubic hair Tanner stage ($d = 0.47$, $p = 0.261$; CP, median = 1, range = 1 to 3; control, median = 1, range = 1 to 2), breast/penile-testicular Tanner stage ($d = 0.42$, $p = 0.323$; CP, median = 1, range = 1 to 2; control, median = 1, range = 1 to 3), BMI ($d = 0.11$, $p = 0.786$), or BMI percentile ($d = 0.11$, $p = 0.791$). Children with CP had lower height ($d = 1.1$, $p = 0.011$) and height percentile ($d = 1.3$, $p = 0.003$) than controls. In addition, height in children with CP was lower than the 50th age-based percentile ($p < 0.001$). Body mass percentile ($d = 0.76$, $p = 0.064$) was lower in children with CP than controls and body mass in children with CP was also lower than the 50th age-based percentile ($p = 0.059$), although the differences did not reach statistical significance. Height of children with CP was lower than the 50th age-based percentile ($p < 0.001$). Height, body mass and BMI in controls were not different from the 50th age- and sex-based percentiles ($p > 0.380$).

Table 4.1. Physical characteristics of children with cerebral palsy (CP) and typically developing children (Control)

	CP (n = 18)	Control (n = 10)
Age (y)	8.0 ± 2.5	8.7 ± 1.7
Tanner stage (1/2/3)		
Pubic hair	13/4/1	9/1/0
Breast/testicular-penile	16/2/0	8/1/1
Height (m)	1.2 ± 0.12[a]	1.32 ± 0.07
Height (percentile)	21 ± 24[a,b]	56 ± 32
Body mass (kg)	25.7 ± 9.3	29.8 ± 6.7
Body mass (percentile)	35 ± 32	58 ± 27
BMI (kg/m^2)	17.3 ± 3.8	16.9 ± 2.9
BMI (percentile)	52 ± 37	48 ± 33
GMFCS (I/II)	12/6	N/A
MAS (1/1.5/2/3)	6/7/1/4	N/A

Values are means ± SD
[a]Group difference, $p < 0.05$
[b]Different from the 50th age-based percentile, $p < 0.05$
Gross motor function classification system (GMFCS)
Modified Ashworth Scale (MAS)

HLV transmission data are reported in Figure 4.2. No differences in pre-HLV and post-HLV measures were observed at the platform ($d = 0.18$, $p = 0.850$), distal tibia ($d = 0.19$, $p = 0.805$), or distal femur ($d = 0.01$, $p = 0.450$) so the pre-HLV was used for comparison against the HLV condition. There was a significant group by site effect ($d = 1.49$, $p < 0.001$). Specifically, in children with CP, the HLV signal was significantly higher at the distal tibia ($d = 1.3$, $p < 0.001$) and lower at the distal femur ($d = 1.6$, $p < 0.001$) than at the platform. In controls, the HLV signal was significantly

higher at the distal tibia (d = 2.1, p < 0.001) and higher at the distal femur (d = 1.5, p < 0.001) than at the platform.

MAS was moderately and negatively related to the transmission of HLV from the platform to the distal tibia (r_s = -0.547, p = 0.019) and from the platform to the distal femur (r_s = -0.566, p = 0.014) in children with CP, as depicted in Figure 4.3.

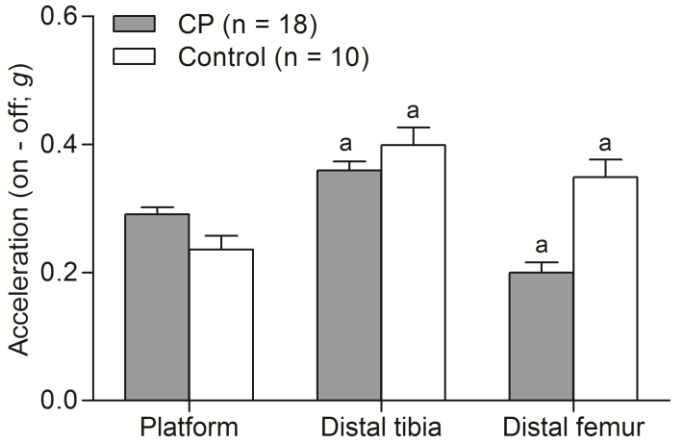

Figure 4.2 Bar graphs showing transmission of the high-frequency, low-magnitude vibration (HLV) signal from the HLV platform to the distal tibia and the distal femur. The HLV signals are presented as values in the on condition (HLV) minus the off conditions (average of pre-HLV and post-HLV). [a]Different from the HLV signal values at the platform, p < 0.05.

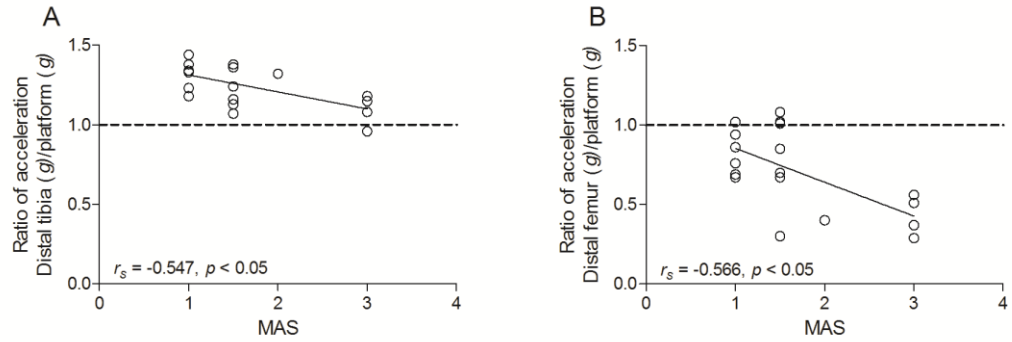

Figure 4.3. Scatter plots show the relationship between Modified Ashworth Scale (MAS) and the transmission of high-frequency, low-magnitude vibration (HLV) with CP (n = 18). The transmission of HLV signal was expressed as the ratio of acceleration measured at the bone site divided the acceleration measured at the HLV platform. A value of 1.0 (dotted line) indicates 100 % transmission. Values above 1.0 indicate an amplification of HLV signal and values below 1.0 indicate a loss of HLV signal.

4.4 Discussion

This is the first study to investigate the transmission of a floor-based HLV platform (i.e., transmits < 1.0 *g*) across the lower extremity of ambulatory children with spastic CP. Relative to the signal generated at the HLV platform, the signal at the distal tibia was amplified in children with CP and in typically developing children. The signal was also amplified at the distal femur in typically developing children but it was dampened in children with CP to ~65% of the signal emitted at the platform.

One factor that may have contributed to the different pattern of HLV signal transmission in children with CP vs. typically developing children is the spasticity in children with CP. There was an inverse relationship between MAS and the degree of HLV transmission at the distal femur. The reason for this relationship is unclear but it may be related to its effect on posture. Some of the children with CP had difficulty

limiting knee and ankle flexion and had difficulty or were unable to place their feet completely flat on the HLV platform, which are common postures in children with spastic CP (24). Previous studies have shown that increased knee flexion and toe standing leads to attenuation of a vibration signal at the knee (25,26). Another factor that may have contributed to the dampened transmission of HLV to the distal femur is unequal weight distribution in the lower extremities creating variable degrees of muscle tension or muscular force production. Increased muscular force production has been shown to dampen vibration (27). The loss of HLV signal at the distal femur may also be related to mechanical filtering through altered soft tissue (27). Children with CP have a high concentration of adipose tissue within and surrounding their musculature (28), which might lead to attenuated HLV signals at the distal femur. However, the effect of leg fat concentration on HLV transmission has not been investigated. Body position on the HLV platform is another element that could affect vibration transmission and local site-specific resonance frequency (22). For example, standing in the step position (i.e. standing with one foot behind the other) has been shown to give rise to local resonance at 12.5 - 25 *Hz* leading to amplified vibration transmission at the lateral epicondyle of the femur (25). On the other hand, an attenuation rather than resonance has been observed in a relaxed standing position (25).

The amplified HLV signal at the distal tibia in children with CP and in typically developing children is consistent with previous findings in adults (17,25,29). It is also consistent with a study by Bressel et al.(17) who reported an amplification of vibration signal to the distal tibia in typically developing children during standing; however, the magnitude of their vibration signal (~2.15 - 5.15 *g*) was much greater

than used in the present study and not considered an HLV (i.e., not < 1 *g*). The amplification of HLV signal observed in the present study and in previous studies (17,25,29) can be attributed to many factors, chief being the resonant frequency. Resonance is the propensity of a structure to oscillate at greater amplitude at some specific frequencies or over a range of frequencies. Previous studies have determined that resonance frequency of the ankle lies between 10 - 63 *Hz* in healthy adults (25,29) and at ~28 - 33 *Hz* in children (17). In the current study, a mean frequency of 33 *Hz* was used. Another factor that may contribute to the amplified HLV signal at the distal tibia is the lack of natural shock absorbers to attenuate the vibration signal at the foot (30). The effect of an amplified signal on bone is unknown and requires further investigation.

The HLV signal at the distal femur of typically developing children was higher than the signal measured at the HLV platform (Figure 2). This is inconsistent with previous studies that have examined HLV transmission to the knee and showed a dampening of the signal (17,25,29). However, previous studies have assessed vibration signals at different sites, such as the lateral epicondyle (25) and the tibial tuberosity (17,29). In the present study, we assessed the HLV signal at the lateral femoral condyle. There is evidence that the resonance frequency is different along the length of a bone (31), which may, partially account for our different results.

One of the strengths of our study was the homogeneous nature of the participants. All the children with CP were able to stand independently (i.e., GMFCS I, II, or III) and all had spasticity in their leg muscles. The pattern of findings remained the same when typically developing children were matched to children with CP for age, sex, and race. Moreover, typically developing children were not different from

the 50th age-based percentile for height, body mass and BMI. Another strength of this study is that the transmission of HLV was evaluated at key bone sites. More than 80 % of all fractures in children with CP occur in the lower extremities, with almost half of all fractures occurring at the distal femur (5).

4.5 Study Limitations

One of the limitations of this study is the lack of kinematic data. We did not collect any data related to posture of our participants. However, children with CP were able to stand still and without any support. Skin-mounted accelerometers can overestimate the acceleration signal by ~10% (32). However, even accounting for that, the pattern of the findings remains the same.

4.6 Conclusion

The results from this study suggest that the vibration signal from a floor-based HLV platform is amplified at the distal tibia but dampened at the distal femur in children with CP. The damping of HLV at the distal femur is related to the degree of spasticity, with greater spasticity associated with less signal transmission. Future studies are needed to determine if the potential benefits to bone mass and architecture at the distal tibia, the distal femur and other bone sites in children with CP, are influenced by the degree of HLV transmission.

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Chapter 5

INSIGHT INTO VIBRATION TRANSMISSION IN CHILDREN WITH SPASTIC CEREBRAL PALSY: ROLE OF SOFT TISSUES AND BONE MICROARCHITECTURE

Abstract

Children with cerebral palsy (CP) have underdeveloped muscles and bones and a high incidence of low-energy fractures. There is evidence that a high-frequency, low-magnitude vibration (HLV) stimulus has an anabolic effect on bone in children with CP; however, the degree of bone adaptation may be related to HLV transmission. Whether the composition of the tissues in the lower extremities affect the transmission of HLV in children with CP is unknown. 4- to 12-year-old children with spastic CP who could stand independently (n = 16) and typically developing children (n = 10) participated in this study. The vibration signal at the HLV platform (~30 *Hz* and 0.3 *g*), the distal tibia and the distal femur was measured using accelerometers. Twenty-six axial magnetic resonance images (175 x 175 x 700 μm^3) of the distal femur and the distal tibia were collected from the more affected limb in children with CP and the nondominant limb in controls. Muscle volume from the middle third of the leg, and measures of trabecular bone microarchitecture [apparent trabecular bone volume to total volume (appBV/TV), trabecular number (appTb.N), trabecular thickness (appTb.Th) and trabecular separation (appTb.Sp)] in the distal femur and the distal tibia were estimated using the 20 most central images and custom software (Interactive Data Language, Boulder, CO). Compared to the HLV signal at the platform, HLV transmission to the distal tibia was higher by 27% vs. 66% in children with CP and controls, respectively. Moreover, relative to HLV at the platform, HLV transmission to the distal femur was higher by 46% in controls and lower by 33% in

children with CP. Muscle volume was lower by 54% in children with CP compared to controls ($p < 0.001$). The primary finding was that measures of trabecular bone microarchitecture were not related to HLV transmission in children with CP or typically developing children ($p > 0.05$). Muscle volume in the midleg was inversely related to HLV transmission to the distal femur in typically developing children ($r = -0.626$, $p \leq 0.05$), but not in children with CP. The findings suggest that muscle volume can attenuate HLV transmission in typically developing children but not in ambulatory children with CP. There is no relationship between trabecular bone microarchitecture and HLV transmission in ambulatory children with CP.

5.1 Introduction

Children with cerebral palsy (CP) show underdeveloped trabecular bone microarchitecture (1) and a substantial adipose tissue infiltration of skeletal muscle in their lower limbs (2). The compromised quality of muscle in this population is associated with less force-generating capability of the muscles (3) and an increased incidence of low-energy fractures in the lower extremities (4,5). There is evidence that a daily high-frequency, low-magnitude vibration (HLV) stimulus increases bone mineral density and improves bone structure in children with CP (6,7). Despite the potential of HLV as a treatment to promote musculoskeletal health, the degree to which the HLV signal is transmitted to a particular bone site may dictate its effectiveness.

Previous studies have shown that muscle surrounding the bone in the lower extremity acts as a dampener to attenuate the vibrations during various activities such as walking, running, (8) or isometric/isotonic force production (9). Computational studies (10) also point toward the role of muscle as one of the critical dampener of vibration. On the other hand, a recent study (11) suggested that vibration transmission in the distal tibia is greater than normal if the trabecular bone microarchitecture is more developed. Resonance frequency of a bone is also a critical factor that can affect vibration transmission (11). Moreover, it has been suggested that the resonance frequency of the tibia differs along its length (11). Studies have also shown that vibration transmission can predict bone strength of the tibia in animals (12) and humans (13).

The aim of this study was to determine if the transmission of an HLV signal to the distal tibia and the distal femur is related to the muscle volume of the lower limb or trabecular bone microarchitecture in the leg in children with spastic CP and

typically developing children. We hypothesized that vibration transmission to the distal femur will be inversely related to the muscle volume in the leg and vibration transmission to the distal femur and the distal tibia will be positively related to their trabecular bone microarchitecture.

5.2 Methods

5.2.1 Participants

Sixteen independent ambulatory children with spastic CP (n = 10 boys and 6 girls), between 4 to 12 years of age, categorized as level I/II/III using the gross motor function classification system (GMFCS) were recruited from the AI duPont Hospital for Children, Wilmington, DE and surrounding hospitals in the Mid-Atlantic region of the U.S.A. All participants possessed the maturity and cognitive ability to complete the study. Exclusion criteria included children with a baclofen pump, any chemodenervation surgery within the past year, any musculoskeletal surgery, or presence of any metal rods in their thigh or legs. Ten typically developing children (n = 7 boys and 3 girls) in the same age range and between the 5^{th} and 95^{th} percentiles for height and body mass, no history of chronic medication use, and no history of fracture within the past 1 year were recruited as controls. The study was approved by the institutional review boards at the AI duPont Hospital for Children, Wilmington, DE, and the University of Delaware, Newark, DE. Written consent from parents and the written assent forms from the participating children were obtained before any testing was performed.

5.2.2 Study design and procedures

A correlation study design was used in this study. Anthropometrics, pubertal development, degree of spasticity, and gross motor function classification were evaluated by a physician assistant and trained research assistants at the University of Delaware. Magnetic resonance imaging (MRI) was done by trained technicians at the AI duPont Hospital for Children, Wilmington, DE.

5.2.3 Anthropometrics

In ambulatory children with CP and typically developing children, height was measured to the nearest centimeter using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER). All measurements were taken while the children were in minimal clothing and without any shoes or bracing devices.

5.2.4 Tanner Staging

A physician assistant conducted Tanner staging to evaluate pubertal maturity of each participant. Pubic hair growth and testicular/penile development in boys and breast development in girls were assessed to grade sexual maturity. The Tanner stage rating scale ranges from I to V, with I indicating no sign of sexual maturity and V indicating full sexual maturity (14,15).

5.2.5 Modified Ashworth Scale (MAS)

Spasticity of plantar flexor muscles was rated using the MAS. The grading system of the MAS ranges from 0 to 4, with 0 indicating presence of normal tone and 4 indicating muscle rigidity in flexion/extension (16).

5.2.6 Gross Motor Function Classification System (GMFCS)

Gross motor function was assessed using the GMFCS, which ranges from I - V with level I and II reflecting independent ambulation, level III reflecting walking with assistive devices and levels IV reflecting and V reflecting electrically powered wheelchair mobility (17). Only those children who were GMFC level I, II or III participated in our study, meaning all of the participants in the CP group were able to stand independently.

5.2.7 Vibration Transmission

Participants stood on an HLV platform (Juvent 1000 Motion Therapy system, Juvent Inc., Riviera Beach, FL) for three consecutive conditions (pre-HLV, HLV and post-HLV) of 30 seconds per condition. A sinusoidal vertical vibration signal of 0.3 *g* at a frequency of 30-37 *Hz* was emitted by the HLV platform. There was no transmission of HLV during the pre-HLV and post-HLV conditions, which were immediately before and immediately after the HLV condition, respectively. Participants stood on the platform without shoes, socks or braces while their feet were inside a 16 x 16 cm square region outlined at the center of the HLV platform. Participants stood as still as possible in a relaxed positon and without any external support on the HLV platform. However, if support was needed for balance, they were allowed to lean on a standard wooden chair which was placed in front of the HLV platform. A spotter stood on either side of the participant to prevent falls. Only data collected during independent standing was used for the analysis. The same HLV platform was used for all participants.

Prior to the participant standing on the HLV platform, a uniaxial accelerometer (Model: 3711B1110G, PCB Piezoelectronics Inc, Depew, NY) was secured to the

platform at its central point using double-side tape placed between the accelerometer and the platform as per recommendations by Rauch et al.(18). The accelerometer was further secured to the platform using single sided tape over the top of the accelerometer. The uniaxial accelerometer was used to quantify the amount of vibration emitted by the HLV platform. To quantify the transmission of HLV signal to the distal tibia and the distal femur, triaxial accelerometers (Model: 3713B1113G, PCB Piezoelectronics Inc, Depew, NY) were secured to the skin immediately above the medial malleolus of the distal tibia and at the lateral condyle of the distal femur to the limit of the participant's comfort using a self-adhesive elastic bandage. The placement of all accelerometers was done by a single research assistant for all participants. All accelerometers were calibrated prior to each data collection session. The same accelerometers were used at the same sites for all participants. The data collection setup is displayed in Figure 5.1.

A 12-bit AD converter data acquisition device (Model: USB-1208FS, Measurement Computing Corp., Norton, MA) was used to collect the vibration data at a sampling frequency of 2 kHz using DASYLab® (software version 11.0, Measurement Computing Corportaion, Norton, MA). Spike 2 (version 7.10, Cambridge Electronic Design, Cambridge, UK) was used to analyze the data after it was filtered using custom MATLAB coding. Peak-to-peak voltage values (mV) were converted to *g* force per the sensitivity for each respective axis. The resultant *g* was calculated for the triaxial accelerometers at the distal tibia and the distal femur using the following equation: $R = \sqrt{(V_g^2 + ML_g^2 + AP_g^2)}$ with V_g, ML_g, AP_g representing *g* forces in the vertical, mediolateral, and anteroposterior directions.

A 5 second time period within each 30 second condition (pre-HLV, HLV and post-HLV) was chosen to analyze and calculate amplitude and frequency of HLV signals because all participants were able to stand independently without leaning on the chair or creating any movement for a minimum of 5 seconds. HLV transmission was defined as the difference in HLV signals at the platform vs. the distal tibia and the distal femur.

5.2.8 Magnetic Resonance Imaging

A 1.5 T MRI machine (GE, Milwaukee, WI) was used to collect magnetic resonance images of the distal femur and the distal tibia of the more affected limb and non-dominant limb, respectively in children with CP and controls to assess measures of trabecular bone microarchitecture [apparent bone volume/total volume (appBV/TV), apparent trabecular number (appTb.N), apparent trabecular thickness (appTb.Th), and apparent trabecular separation (appTb.Sp)]. Before the start of imaging, a VacFIX system (PAR Scientific A/S; Sivlandvaenge, Denmark) was used to hold one of two connected phased array coils (USA Instruments; Aurora, OH) to the lateral side of the distal femur and medial side of the distal tibia of the more affected lower limb in children with CP and of the nondominant lower limb in controls. The BodyFIX (Medical Intelligence, Inc., Schwabműnchen, GER) was used to immobilize children from the waist down to help limit motion during the MRI scan, as described previously (1). After immobilizing children, a three-plane localizer was used to identify the distal femur and the distal tibia. High resolution images of the distal femur and the distal tibia were obtained using a 3D fast gradient echo sequence with a partial echo acquisition (echo time = 4.5 ms, repetition time = 30 ms, 30° flip angle, 13.89 kHz bandwidth), a 9 cm field of view, and a reconstructed imaging matrix of 512 ×

512. Twenty-six axial images (700 μm thick) of the metaphysis were collected immediately above the growth plate in the distal femur and the distal tibia. Measures of trabecular bone microarchitecture were estimated from the lateral half of the 20 most central images of the distal femur and medial half of the 20 most central images of the distal tibia using custom software created with Interactive Data Language (IDL, Research Systems Inc., Boulder, CO) and an analysis procedure patterned after the procedure outlined by Majumdar et al. (19). The average values of appBV/TV, appTb.N, appTb.Th and appTb.Sp from the 20 images are reported. The CV% for appBV/TV, app.TbN, app.TbTh, and app.TbSp in our laboratory ranges from 2% - 3% (1). The procedure used has been described in detail previously (20).

Axial T1 weighted images (0.5 cm thick separated by 0.5 cm) were collected along the entire length of the more affected limb and non-dominant limb, respectively in children with CP and controls, with a torso PA coil (repetition time = 750, echo time = 14, field of view = 12, NEX = 1) and a reconstructed imaging matrix of 512 X 512 to calculate volume of the middle third of the total muscle volume. The procedure has been described in detail previously (2).

5.3 Statistical Analysis

All data were analyzed by SPSS 22.0 (IBM Corp, Armonk, NY). Normality of the data was calculated using skewness, kurtosis, and the Shapiro-Wilk test. If data were normally distributed, group differences in physical characteristics were assessed using an independent t test. If data were not normally distributed, group differences were assessed using a Mann Whitney U test. The Chi-square test of independence was used to determine if there were group differences in Tanner stage. A one-sample t test was used to determine the differences between the sex- and age-based percentiles for

height, body mass, and body mass index (BMI) relative to the sex and age-based 50^{th} percentile. A two-way ANOVA (group x site) with repeated measures on site was used to determine if there were group differences and an interaction effect in %HLV transmission to the 2 sites (distal tibia and distal femur). If a group by site interaction was detected, simple contrasts were used to identify specific differences. An independent T-test was used to calculate group differences in age-adjusted muscle volume and measures of trabecular bone microarchitecture [apparent bone volume/total volume (appBV/TV), apparent trabecular number (appTb.N), apparent trabecular thickness (appTb.Th), and apparent trabecular spacing (appTb.Sp)] in the distal femur and the distal tibia. A Bonferroni adjustment was made for multiple comparisons. Spearman correlation coefficients were used to determine the relationships between measures of soft tissue normalized to age (such as, muscle volume) and trabecular bone microarchitecture at the distal tibia and the distal femur to HLV transmission to the distal tibia and the distal femur. Values are reported as mean ± SD. We have reported the median and ranges if data were not normally distributed. The alpha level was set at $p < 0.05$ for all the significance tests. With a power (1-β) of 0.8 and $r \geq 0.58$, a bivariate correlation analysis model yielded a minimum of 10 participants per group.

5.4 Results

Physical characteristics of the participants are reported in Table 5.1. All data were normally distributed except BMI. There were no group differences in age (p = 0.32), body mass (p = 0.25), body mass percentile (p = 0.08), BMI percentile (p = 0.80), and BMI (p = 0.87; CP, median = 15.9, range = 13.3 to 30.6; control, median = 16.1, range = 13.6 to 21.6). Children with CP had lower height (p = 0.003) and height

percentile (p = 0.006) than controls. In addition, height in children with CP was lower than the 50th age-based percentile (p = 0.001). Height, body mass, and BMI in controls and body mass and BMI in children with CP were not different from the 50th age- and sex-based percentiles (p > 0.094).

Table 5.1. Physical characteristics of study participants

	Ambulatory CP (n = 16)	Control (n = 10)
Age (years)	8.0 ± 2.3	8.7 ± 1.7
Tanner stage (1/2/3)		
Pubic hair	12/3/1	9/1/0
Breast/testicular penile	13/3/0	8/1/1
Height (m)	1.19 ± 0.11	1.32 ± 0.07
Height (percentile)	21 ± 27[a,b]	56 ± 31
Body mass (kg)	25.6 ± 9.7	29.8 ± 6.7
Body mass (percentile)	35 ± 34	58 ± 27
BMI (kg/m2)	17.5 ± 4.6	16.9 ± 2.9
BMI (percentile)	52± 37	48 ± 33
GMFCS (I/II/III)	9/6/1	N/A

Values are means ± SD
[a]Different from control, p < 0.05
[b]Different from the 50th age-based percentile, p < 0.05
Gross motor function classification system (GMFCS)
Modified Ashworth Scale (MAS)

Group differences in soft tissues are depicted in Figure 5.1. Age-adjusted muscle volume was lower by 54% in children with CP compared to controls (p < 0.001).

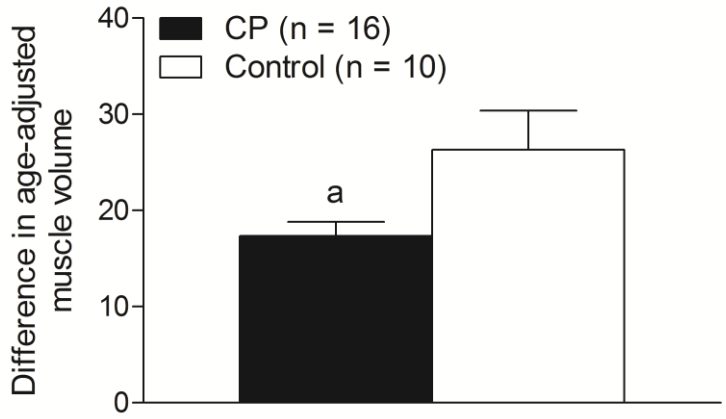

Figure 5.1 Comparison of muscle volume in ambulatory children with CP (n = 16) and controls (n = 10). Values are expressed as means ± SE. [a]Different from control, $p < 0.05$

Group differences in measures of trabecular bone microarchitecture at the distal tibia and the distal femur are shown in Figure 5.2. Compared to typically developing children, the distal femur and the distal tibia had lower appBV/TV by 8% (p = 0.011) and 18% ($p < 0.001$), lower appTb.N by 6% and 10% (p = 0.001 for both), lower appTb.Th by 2% (p = 0.384) and 9% (p = 0.003), and higher appTb.Sp by 12% (p = 0.002) and 22% ($p < 0.001$), respectively, in children with CP. Group differences in HLV transmission to the distal tibia and the distal femur versus the HLV platform is represented in Figure 5.3. HLV transmission to the distal tibia vs the HLV platform was higher ($p < 0.001$) by 27% and 66% in children with CP vs. controls, respectively. No group difference was noted between HLV transmission to the distal tibia between children with CP and typically developing children (p = 0.517). HLV transmission was higher by 46% ($p < 0.001$) to the distal femur in controls whereas it was lower by

33% ($p < 0.001$) in children with CP. A group difference ($p < 0.001$) was evident in HLV transmission to the distal femur but not at the distal tibia.

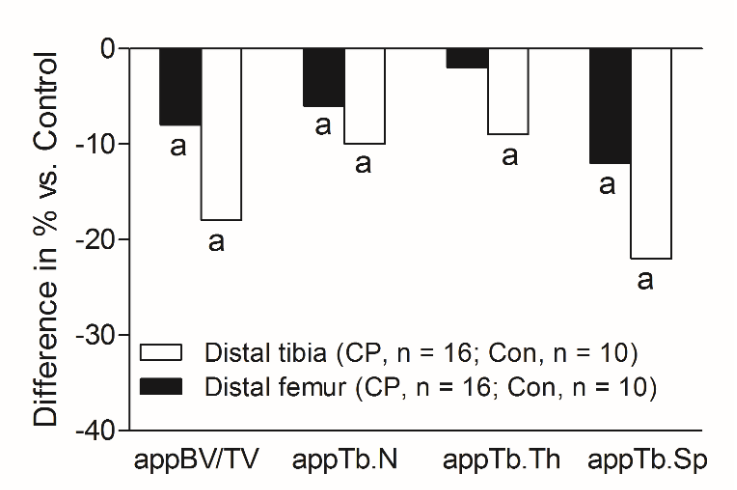

Figure 5.2 Comparison of a) apparent bone volume/total volume (appBV/TV), b) apparent trabecular number (appTb.N), c) apparent trabecular thickness (appTb.Th), and d) apparent trabecular separation (appTb.Sp) in the distal femur and the distal tibia of ambulatory children with CP ($n = 16$) compared to controls ($n = 10$). Values are expressed as percentage change relative to controls. [a]Different from control, $p < 0.05$.

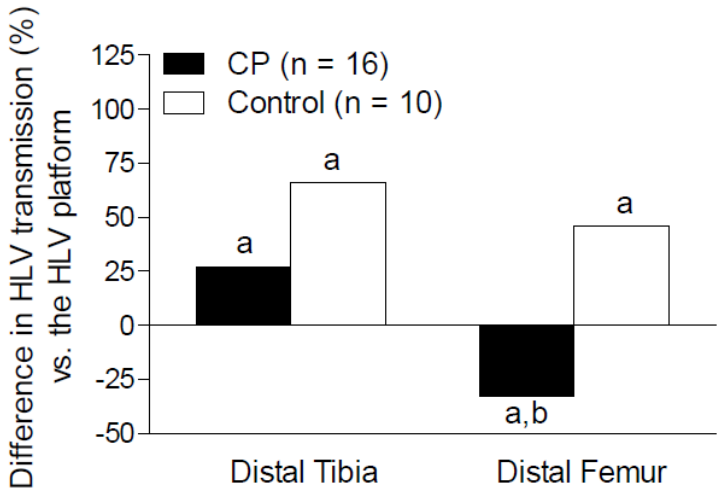

Figure 5.3 Comparison of high-frequency, low-magnitude vibration (HLV) in the distal tibia and the distal femur relative to the platform of ambulatory children with CP (n = 16) and controls (n = 10). Values are expressed as percentage change from the HLV at the platform. [a]Different from HLV at the platform, $p < 0.05$, [b]Different from controls, $p < 0.05$.

A negative relationship was noted between HLV transmission to the distal femur and muscle volume in typically developing children (r = -0.626, $p \leq 0.05$). Furthermore, children with CP showed no relationships between HLV transmission to the distal femur and muscle volume as illustrated in Figure 5.4. Also, no relationships were noted between HLV transmission to the distal tibia and the distal femur to measures of trabecular bone microarchitecture at the distal tibia and the distal femur in both the groups as depicted in Figure 5.5 and 5.6.

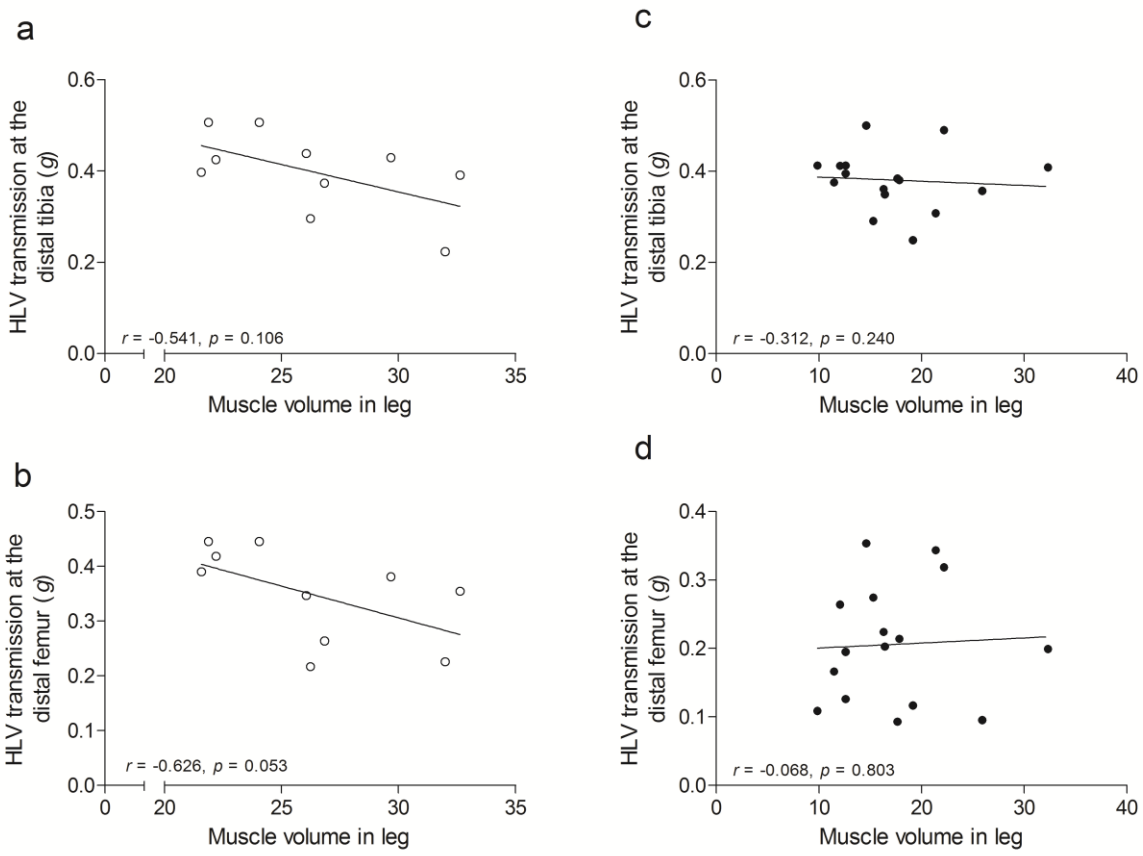

Figure 5.4 Scatter plot show the relationships between high-frequency, low-magnitude vibration transmission (HLV) to the distal tibia and the distal femur with muscle volume in typically developing children (a and b) (n = 10), and children with CP (c and d) (n = 16).

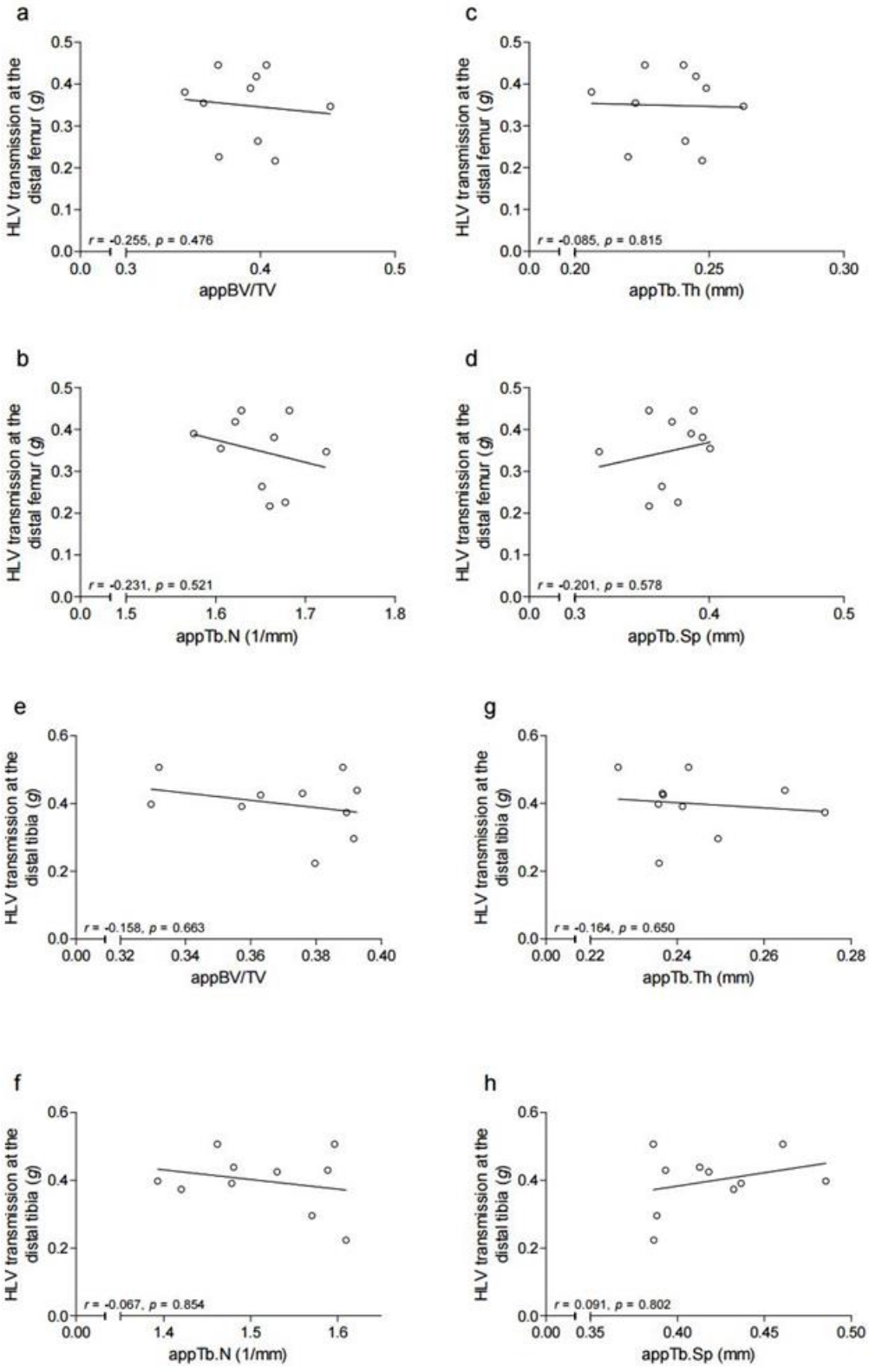
a
HLV transmission at the distal femur (g)
r = -0.255, p = 0.476
appBV/TV
c
HLV transmission at the distal femur (g)
r = -0.085, p = 0.815
appTb.Th (mm)
b
HLV transmission at the distal femur (g)
r = -0.231, p = 0.521
appTb.N (1/mm)
d
HLV transmission at the distal femur (g)
r = -0.201, p = 0.578
appTb.Sp (mm)
e
HLV transmission at the distal tibia (g)
r = -0.158, p = 0.663
appBV/TV
g
HLV transmission at the distal tibia (g)
r = -0.164, p = 0.650
appTb.Th (mm)
f
HLV transmission at the distal tibia (g)
r = -0.067, p = 0.854
appTb.N (1/mm)
h
HLV transmission at the distal tibia (g)
r = 0.091, p = 0.802
appTb.Sp (mm)

Figure 5.5 Scatter plot show the relationships between high-frequency, low-magnitude vibration transmission (HLV) to the distal tibia and the distal femur with measures of trabecular bone microarchitecture at the distal femur (a - d) and the distal tibia (e - h) in typically developing children (n = 10).

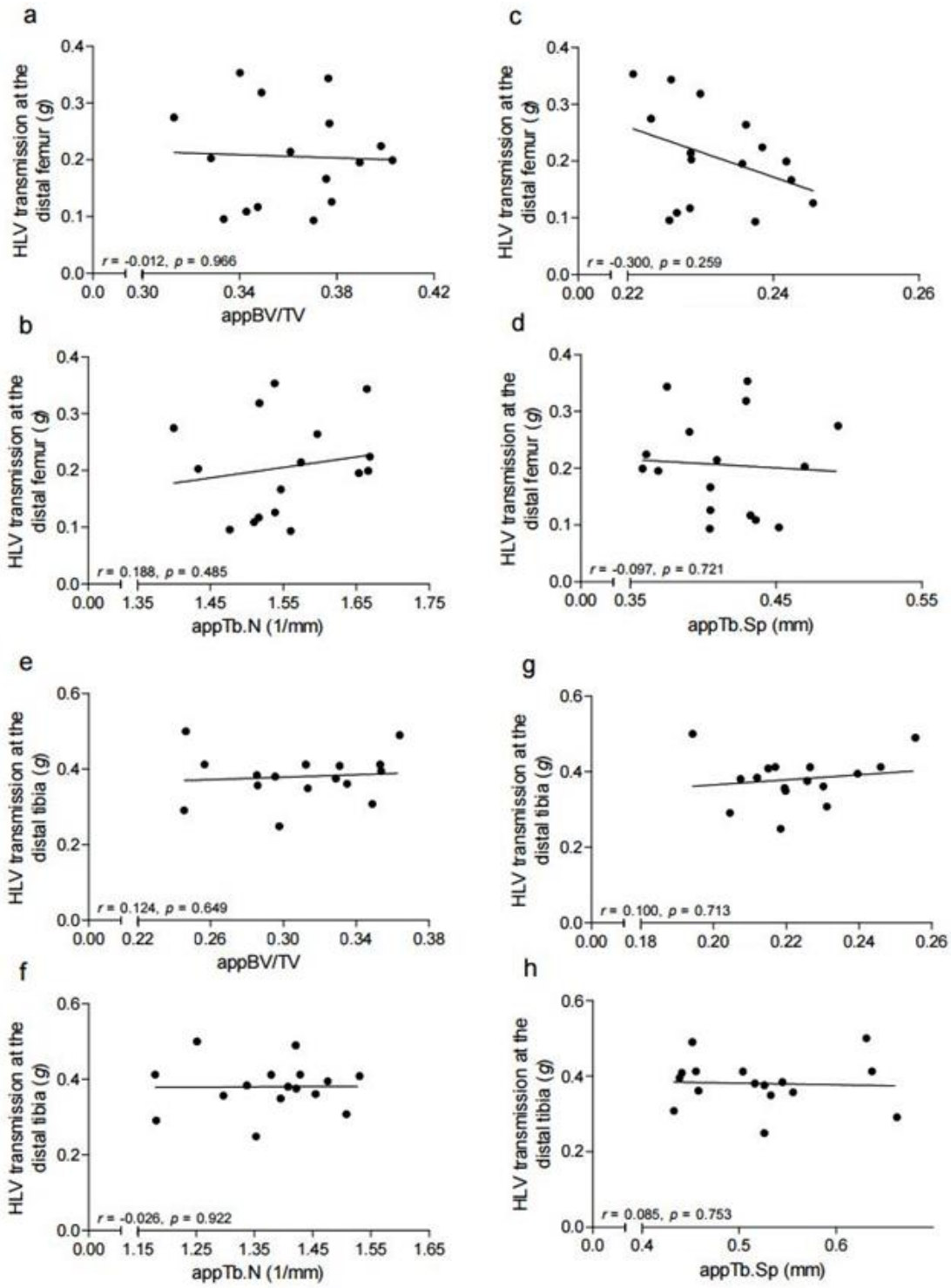
a
HLV transmission at the distal femur (g)
r = -0.012, p = 0.966
appBV/TV
c
HLV transmission at the distal femur (g)
r = -0.300, p = 0.259
b
HLV transmission at the distal femur (g)
r = 0.188, p = 0.485
appTb.N (1/mm)
d
HLV transmission at the distal femur (g)
r = -0.097, p = 0.721
appTb.Sp (mm)
e
HLV transmission at the distal tibia (g)
r = 0.124, p = 0.649
appBV/TV
g
HLV transmission at the distal tibia (g)
r = 0.100, p = 0.713
f
HLV transmission at the distal tibia (g)
r = -0.026, p = 0.922
appTb.N (1/mm)
h
HLV transmission at the distal tibia (g)
r = 0.085, p = 0.753
appTb.Sp (mm)

Figure 5.6 Scatter plot show the relationships between high-frequency, low-magnitude vibration transmission (HLV) to the distal tibia and the distal femur with measures of trabecular bone microarchitecture at the distal femur (a - d) and the distal tibia (e - h) in children with cerebral palsy (CP) (n = 16).

5.5 Discussion

We report for the first time that HLV transmission to the distal femur is inversely related to soft tissues such as the leg muscle volume in typically developing children. However, there is no relationship between muscle volume and the HLV transmission to these two sites in children with CP. We are also the first to report that there is no relationship between trabecular bone microarchitecture and HLV transmission to the distal tibia and the distal femur in children with mild CP.

In line with the previous studies done in adults (8,9), we found that muscle acts as a damping tissue to the HLV transmission to the distal femur in typically developing children. This is established by a negative relationship between muscle volume and HLV transmission to the distal femur in typically developing children in our study. A contracted muscle produces muscular force which in turn depends on number of recruited motor units eventually resulting in generation of intramuscular and intermuscular pressure. Fascia can also contribute toward enhancement of inter- and intramuscular pressure. Thus, a heightened inter- and intramuscular pressure can attenuate HLV stimulus. We did not find muscle as a dampener of HLV in children with CP. This can be due to less muscle volume in children with CP as evident in our study.

There is evidence that muscle work is lower in ambulatory individuals with CP (3) suggesting a reduced recruitment of motor units. Recent work has shown a presence of less number of motor units (21) and reduced motor unit recruitment (22)

in the affected versus the unaffected side in children with CP. A reduced number and decreased recruitment of motor units can result in diminished contraction of muscles, thus, compromising the production of inter- and intramuscular pressure, which can further contribute to a non-significant role of muscle in damping the HLV transmission in children with CP. It is also known that muscle fibers are stiffer in children with spastic CP due to ongoing fibrosis (23) and changes in extracellular matrix (24). These changes can amplify the diminished production of inter- and intramuscular pressure due to adverse effects on muscle contraction. Overall, all these factors can dynamically interact to decrease the ability of muscle to act as a dampener of HLV transmission in children with CP. Overall, these findings suggest that the muscle condition is not related to the HLV transmission in children with CP. However, there are other parameters such as testing with activated muscle and over a greater range of muscle sizes and postures that are still unknown.

The relationships between trabecular bone microarchitecture and HLV transmission to the distal tibia and the distal femur were not as expected. One of the main factors that may have contributed is collagen content of bone (25). Bone mainly has two components: a) the mineral component, which provides the stiffness and b) the collagen component, which provides the ductility and the capacity to absorb impact (26). Interestingly, even with a lower modulus of elasticity, bones in children tend to absorb more energy than adults before a fracture (27). It can be postulated that a higher collagen content of bone in children helps in absorbing external impact force, such as vibration and as a result leading to the same degree of HLV transmission to the distal tibia in children with CP and typically developing children. Thus, it can mask the response of a deteriorated bone to external impact forces.

A previous study (25) found that impact energy absorption was higher at young age compared to old age and that, with increased mineralization, there was a decrease in impact energy absorption which supports this postulation. Furthermore, that same study showed that absorption of impact energy did not attenuate with the increased porosity at younger ages. Interestingly, in an animal study, no relationship between vibration transmission and mineral content of a healing bone was found (28). It was suggested that a pronounced effect of the collagen content of bone over the mineral content of bone was responsible for that. Since, collagen constitutes almost 90% of the bone matrix (29) and there is an increase of collagen with decreasing trabecular bone mass in humans (30), it can be inferred that collagen content of the distal tibia in children with CP is relatively enhanced imparting the same level of absorption capacity of HLV stimulus similar to typically developing children; even in the presence of deteriorated trabecular microarchitecture in the distal tibia in children with CP.

The other main factor that can affect a bone's response to external impact stimulus is its shape. Computational studies show that increased curvature leads to a non-linear rise in damping capacity in the mouse tibia (10). Moreover, a sinusoidal load can lead to an asymmetrical response in the viscoelastic properties of bone (10). It should be noted that in our study we applied a sinusoidal HLV stimulus. There is evidence that the natural frequency of femur is affected by its shape and density distribution (31). Therefore, it can also hold true with the tibia. Although, resonance is a chief factor for amplification of vibration stimulus at ~ 30 *Hz* at the tibia (32), shape-specificity and unique density distribution can infer unique resonance frequency to the tibia. Therefore, use of a single value as the resonance frequency of the tibia or

femur to increase its strength as suggested in a recent study (11) cannot be proposed. Interestingly, we do not know if the flow of intramedullary fluid in bone can affect HLV transmission. This can be further explored.

One of the strengths of our study was the homogeneous nature of the participants. All the children with CP were mild-to-moderate (i.e., GMFCS I, II, or III), able to stand independently, and had spasticity in their leg muscles. Furthermore, the typically developing children were matched to children with CP for age, sex and race and were not different in pubertal development. Moreover, the typically developing children were not different from the 50^{th} sex- and age-based percentiles for height, body mass and BMI. Another strength of this study is that the transmission of HLV and trabecular bone microarchitecture was evaluated at key bone sites. More than 80 % of all fractures in children with CP occur in the lower extremities, with almost half of all fractures occurring at the distal femur and the distal tibia. All magnetic resonance images were analyzed by a single research assistant who was blind to the participant group.

5.6 Study Limitations

One of the limitations of this study is the lack of kinematic data. We did not collect any data related to posture of our participants. However, children with CP were able to stand still and without any support. Furthermore, skin-mounted accelerometers can overestimate the acceleration signal by ~10% (33). However, even if the vibration signal assessed in the present study using skin-mounted accelerometers is corrected for a 10 %, the pattern of the findings remains the same. Another limitation of this study is the susceptibility of magnetic resonance images to partial volume effects due to limited resolution. To counter the effects, the lower resolution of the slice (700 μm^3 in

this study) was placed in line with the principal direction of the trabecular structures (34). Additionally, we have addressed all surrogate measures of trabecular bone microarchitecture as ‘apparent’.

5.7 Conclusion

The findings suggest that muscle volume is inversely related to the HLV transmission at the distal femur in typically developing children but not in children with mild CP suggesting the use of HLV should not be negatively impacted by the muscle size or volume in children with CP. Additionally, no relationship was noted between trabecular bone microarchitecture and HLV transmission to the distal tibia and the distal femur in typically developing children and in children with mild CP.

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SUMMARY

The overall findings from this dissertation provide a greater understanding of the level of skeletal compromise in ambulatory children with mild CP. The findings show that the level of deficit in the bone microarchitecture is greater in the distal tibia versus the distal femur in ambulatory children with CP calling for a need to closely monitor the skeletal status in ambulatory children with CP. The findings also provide an insight into the degree to which HLV transmits to the primary fracture sites of children with CP. It is plausible that the effectiveness of HLV as a treatment for musculoskeletal health is dictated by the degree to which the HLV signal is transmitted to a particular bone site, similar to the site-specific effects of exercise. The findings will help guide future studies aimed at determining the relationship between site-specific HLV transmission and site-specific effects of HLV as a treatment for poor bone development in children with CP. Furthermore, findings from this dissertation suggest that the use of HLV should not be negatively impacted by the muscle condition or size in ambulatory children with CP.

Appendix A

PARTICIPANT'S BROCHURE APPROVED BY THE AI duPONT HOSPITAL FOR CHILDREN, WILMINGTON, DE

BECOMING A RESEARCH VOLUNTEER:

IT'S *YOUR* DECISION

What is Research?

- Research is a study that is done to answer a question.
- Scientists do research because they don't know for sure what works best to help you.
- Some other words that describe research are clinical trial, protocol, survey, or experiment.
- Research is not the same as treatment.

Why is Research Important?

Research has led to important discoveries that make our lives better. Some examples are:

- New drugs to treat cancer, diabetes, and other diseases
- Ultrasound, X-ray machines, and diagnostic tests
- Vaccines
- Ways to stop smoking
- Improved medical procedures

Points to Consider

- A research study may or may not help you personally.
- In the future, the results could help others who have a health problem.
- Taking part in research is voluntary.

Someday, you or a family member may want to take part in a research study. If this happens, the information here may help you make the right decision.

Questions to Ask

- What exactly will happen to me in the research?
- Will there be any unpleasant side effects?
- Will the research help me personally?
- What other options do I have?
- Can I leave the study at any time?
- Will it cost me anything personally?

Research discoveries can improve people's health.

Before you decide to become a research volunteer, get the facts:

- Know what you're getting into.
- Ask questions.
- Learn as much as you can.
- Know the pros and cons.

It's Your Decision

For more information call:

The Nemours Office of Human Subjects Protection: 904-697-4023
Toll Free: 1-800-SOS-KIDS
Email: NOHSP@nemours.org

Office for Human Research Protections
Toll-Free (866) 447-4777
1101 Wootton Parkway
Suite 200
Rockville, MD 20852
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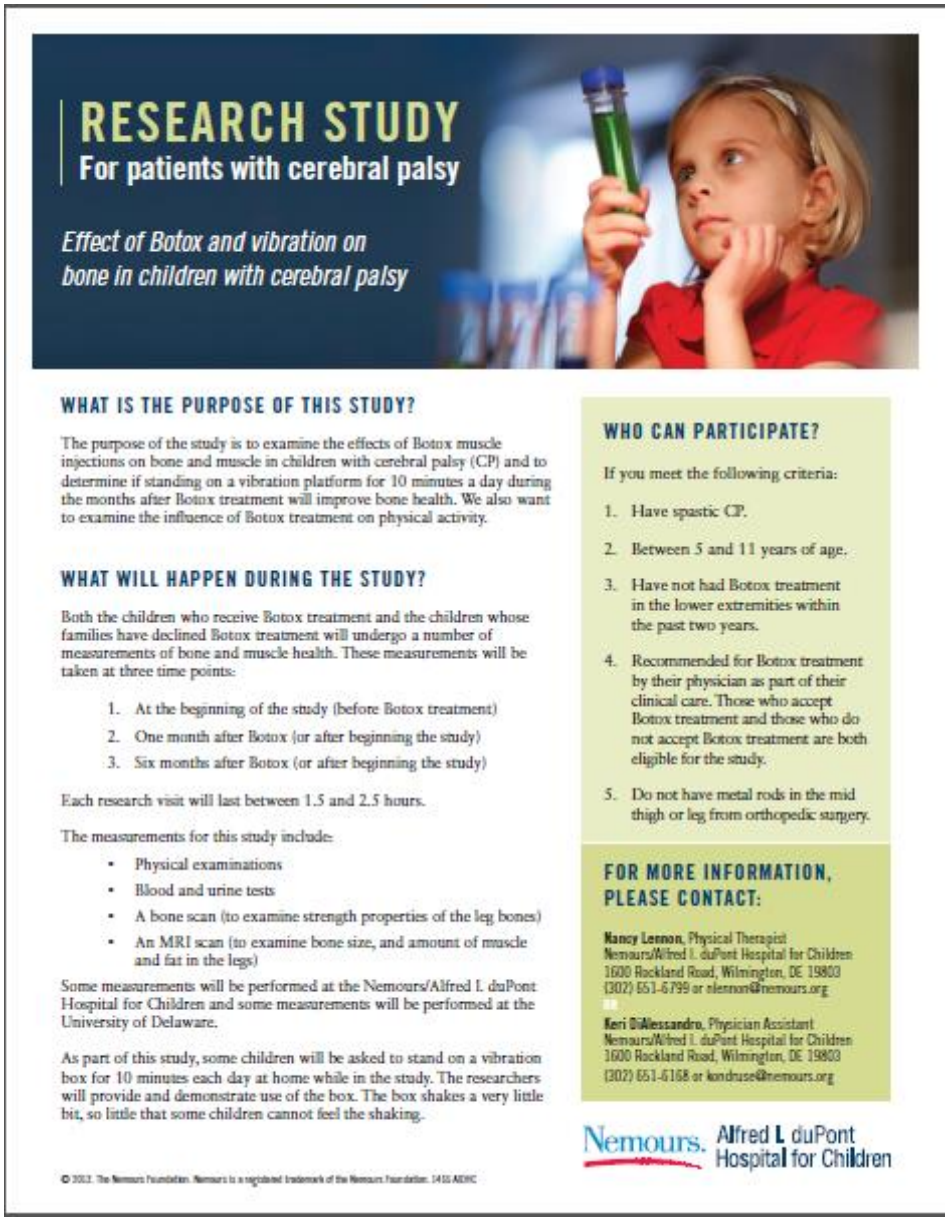
E-mail: ohrp@osophs.dhhs.gov

Appendix B

FLYER APPROVED BY THE AI duPONT HOSPITAL FOR CHILDREN, WILMINGTON, DE



RESEARCH STUDY

For patients with cerebral palsy

Effect of Botox and vibration on bone in children with cerebral palsy

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of the study is to examine the effects of Botox muscle injections on bone and muscle in children with cerebral palsy (CP) and to determine if standing on a vibration platform for 10 minutes a day during the months after Botox treatment will improve bone health. We also want to examine the influence of Botox treatment on physical activity.

WHAT WILL HAPPEN DURING THE STUDY?

Both the children who receive Botox treatment and the children whose families have declined Botox treatment will undergo a number of measurements of bone and muscle health. These measurements will be taken at three time points:

1. At the beginning of the study (before Botox treatment)
2. One month after Botox (or after beginning the study)
3. Six months after Botox (or after beginning the study)

Each research visit will last between 1.5 and 2.5 hours.

The measurements for this study include:

- Physical examinations
- Blood and urine tests
- A bone scan (to examine strength properties of the leg bones)
- An MRI scan (to examine bone size, and amount of muscle and fat in the legs)

Some measurements will be performed at the Nemours/Alfred I. duPont Hospital for Children and some measurements will be performed at the University of Delaware.

As part of this study, some children will be asked to stand on a vibration box for 10 minutes each day at home while in the study. The researchers will provide and demonstrate use of the box. The box shakes a very little bit, so little that some children cannot feel the shaking.

WHO CAN PARTICIPATE?

If you meet the following criteria:

1. Have spastic CP.
2. Between 5 and 11 years of age.
3. Have not had Botox treatment in the lower extremities within the past two years.
4. Recommended for Botox treatment by their physician as part of their clinical care. Those who accept Botox treatment and those who do not accept Botox treatment are both eligible for the study.
5. Do not have metal rods in the mid thigh or leg from orthopedic surgery.

FOR MORE INFORMATION, PLEASE CONTACT:

Nancy Lennon, Physical Therapist
Nemours/Alfred I. duPont Hospital for Children
1600 Rockland Road, Wilmington, DE 19803
(302) 651-6799 or nlennon@nemours.org

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Nemours/Alfred I. duPont Hospital for Children
1600 Rockland Road, Wilmington, DE 19803
(302) 651-6168 or kondruse@nemours.org

Nemours. Alfred I. duPont Hospital for Children

Appendix C

IRB APPROVED BY THE AI duPONT HOSPITAL FOR CHILDREN, WILMINGTON, DE

Nemours Office of Human Subjects Protection
10140 Centurion Parkway North
Jacksonville, FL 32256
Phone: 904-697-4023 Fax: 904-697-4024

MEMORANDUM

DATE:	February 27, 2012
TO:	Freeman Miller, MD
FROM:	Nemours Delaware IRB
STUDY TITLE:	[115648-2] Effect of Botox and vibration on bone in children with cerebral palsy
IRB #:	115648
SUBMISSION TYPE:	Response/Follow-Up
ACTION:	APPROVED
APPROVAL DATE:	January 26, 2012
EXPIRATION DATE:	January 25, 2013

Thank you for your submission of Response/Follow-Up materials for this research study. Your initial submission received full review at the January 26, 2012 meeting and met all DHHS [and FDA] criteria for approval. The approval was contingent on the response to minor stipulations. Your response has received Expedited Review and is accepted. The above-referenced research study is approved.

The IRB has determined that:

- This is "Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects per 45CFR46.405 and 21CFR50.52".
- Parental Permission is required prior to initiation of any research procedures using only the most current IRB approved form(s) posted as a Board Document in IRBNet. All protocol documents, including Board approved documents are found in the "Study Designer" for each study in IRBNet.
- The IRB approved telephone script is required prior to initiation of any research procedure.
- The permission of one parent is sufficient. A person who is not a parent may not give permission without prior IRB review and approval.
- Assent of minors is required prior to initiation of any research procedures, using only the most current assent form(s) posted as a Board Document in IRBNet. If the investigator chooses to obtain assent, the form(s) listed below must be used and the minor's dissent must be honored.
- A signed copy of the Parental Permission/Informed Consent form must be included in the Nemours' medical record. Research data may also be included into the Nemours medical record.
- To continue, the research requires IRB review and approval on an annual basis. Otherwise, January 25, 2013 is the last day that research may be conducted. The Principal Investigator is responsible for the timely submission of the continuing review application. Please post this date on your research calendar.

Reviewed/approved documents in this submission:

- Application Form - Revised Application Form (UPDATED: 02/14/2012)
- Child Assent - Revised child assent (UPDATED: 02/14/2012)

Appendix D

IRBAPPROVED BY THE UNIVERSITY OF DELAWARE

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE:	July 23, 2012
TO:	Christopher Modlesky, PhD
FROM:	University of Delaware IRB
STUDY TITLE:	[359767-1] Effect of botox and vibration on bone in children with cerebral palsy UD
SUBMISSION TYPE:	New Project
ACTION:	APPROVED
APPROVAL DATE:	January 26, 2012
EXPIRATION DATE:	January 25, 2013
REVIEW TYPE:	Administrative Review

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on Nemours IRB review and approval. This submission has received Administrative Review based on the applicable federal regulation and the University of Delaware IAA with Nemours Foundation.

This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.