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## Cortical bone deficit and fat infiltration of bone marrow and skeletal muscle in ambulatory children with mild spastic cerebral palsy

Daniel G. Whitney<sup>1</sup>, Harshvardhan Singh<sup>1</sup>, Freeman Miller<sup>2</sup>, Mary F. Barbe<sup>3</sup>, Jill M. Slade<sup>4</sup>, Ryan T. Pohlig<sup>5</sup>, and Christopher M. Modlesky<sup>1</sup>

<sup>1</sup>Department of Kinesiology and Applied Physiology, University of Delaware, Newark, DE

<sup>2</sup>Department of Orthopedics, Nemours Al duPont Hospital for Children, Wilmington, DE

<sup>3</sup>Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA

<sup>4</sup>Department of Radiology, Michigan State University, East Lansing, MI

<sup>5</sup>Biostatistics Core Facility, University of Delaware, Newark, DE

### Abstract

**Introduction**—Nonambulatory children with severe cerebral palsy (CP) have an underdeveloped bone architecture, low bone strength and a high degree of fat infiltration in the lower extremity musculature. The present study aims to determine if such a profile exists in ambulatory children with mild CP and if excess fat infiltration extends into the bone marrow.

**Materials and methods**—Ambulatory children with mild spastic CP and typically developing children (4 to 11 years; 12/group) were tested. Magnetic resonance imaging was used to estimate cortical, medullary and total bone volume and width, bone strength [i.e., section modulus ( $Z$ ) and polar moment of inertia ( $J$ )], and bone marrow fat concentration in the midtibia, and muscle volume, intermuscular, subfascial, and subcutaneous adipose tissue (AT) volume and intramuscular fat concentration in the midleg. Physical activity monitors worn on the ankle were used to assess physical activity.

**Results**—There were no group differences in age, height, body mass, body mass percentile, BMI, BMI percentile or tibia length, but children with CP had lower height percentile (19<sup>th</sup> vs. 50<sup>th</sup> percentile) and total physical activity counts (44 %) than controls (both  $p < 0.05$ ). Children with CP also had lower cortical volume (30 %), cortical width in the posterior (16 %) and medial (32 %) portion of the shaft, total bone width in the medial-lateral direction (15 %),  $Z$  in the

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Address of correspondence: Christopher M. Modlesky, PhD, Department of Kinesiology and Applied Physiology, University of Delaware, Newark, DE, Phone: 302-831-4185, Fax: 302-831-3693, modlesky@udel.edu.

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medial-lateral direction (34 %), J (39 %) and muscle volume (39 %), and higher bone marrow fat concentration ( $82.1 \pm 1.8$  % vs.  $80.5 \pm 1.9$  %), subfascial AT volume (3.3 fold) and intramuscular fat concentration ( $25.0 \pm 8.0$  % vs.  $16.1 \pm 3.3$  %) than controls (all  $p < 0.05$ ). When tibia length was statistically controlled, all group differences in bone architecture, bone strength, muscle volume and fat infiltration estimates, except posterior cortical width, were still present (all  $p < 0.05$ ). Furthermore, a higher intermuscular AT volume in children with CP compared to controls emerged ( $p < 0.05$ ).

**Conclusions**—Ambulatory children with mild CP exhibit an underdeveloped bone architecture and low bone strength in the midtibia and a greater infiltration of fat in the bone marrow and surrounding musculature compared to typically developing children. Whether the deficit in the musculoskeletal system of children with CP is associated with higher chronic disease risk and whether the deficit can be mitigated requires further investigation.

## Keywords

bone structure; bone strength; cerebral palsy; unloading; fat depots

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## 1. Introduction

Cerebral palsy (CP) is a neurological condition that is associated with dysfunctional gait and progressive decrements in physical activity from childhood to adulthood [1]. Nonambulatory children with more severe spastic CP present with low bone mass and underdeveloped bone architecture [2–6], as well as small, weak [7] and qualitatively-compromised musculature, as indicated by a high degree of fat infiltration [8]. It is not surprising that this musculoskeletal phenotype is associated with a high incidence of low-energy fractures, primarily occurring in the lower limbs [9, 10]. The latter complication may be due mainly to the lack of physical activity [4, 8] and mechanical loading, which reduces the stimulus for periosteal and endocortical expansion [4] in the lower extremities. To date, it is unclear if the adverse musculoskeletal profile exhibited in nonambulatory children with more severe CP is also present in ambulatory children with a milder form of the disorder.

In addition to possessing an underdeveloped musculoskeletal system, nonambulatory children with severe CP also have elevated adipose tissue (AT) surrounding bone and muscle [8]. Furthermore, human models of reduced mechanical loading show elevated levels of fat infiltration within the bone marrow cavity [11] and muscle [12], which are linked to osteoporosis [13–15], impaired glucose tolerance [12, 16, 17] and cardiometabolic disease [18]. The objective of the present study was to determine if ambulatory children with mild spastic CP have a deficit in bone architecture and elevated fat infiltration within the bone marrow cavity of the midtibia and the surrounding leg musculature. We hypothesized that ambulatory children with mild CP vs. typically developing children would have a thinner shaft with a thinner cortex and lower estimates of strength in the midtibia. We also hypothesized that children with CP vs. typically developing children would have an elevated fat infiltration within the bone marrow cavity and the surrounding leg musculature.

## 2. Materials and Methods

### 2.1. Participants and study design

Ambulatory children with mild spastic CP and between the ages of 4 and 11 y were recruited from the AI duPont Hospital for Children in Wilmington, DE and other pediatric hospitals in the Mid-Atlantic region of the U.S. Typically developing children that matched children with CP for age, sex and race were recruited from the Newark and Wilmington, DE areas using flyers and word of mouth. Additional inclusion criteria for controls included falling between the 5<sup>th</sup> and 95<sup>th</sup> percentile for height and body mass, no history of chronic medication use, no previous fracture in the nondominant lower extremity and no current or previous regular participation in an activity that involved high loading of the skeleton, such as artistic gymnastics. Participants were recruited from April 2012 through May 2016 and testing was conducted from November 2012 to May 2016. The Institutional Review Boards at the AI duPont Hospital for Children and the University of Delaware approved the study procedures. Prior to testing, written consent and assent was obtained by the parents and the participants, respectively.

### 2.2. Anthropometrics

Height and body mass were measured while the child was in a t-shirt and shorts. Height was measured to the nearest 0.1 cm using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER). Body mass was measured to the nearest 0.2 kg using a digital scale (Detecto 6550, Cardinal Scale, Webb City, MO). Height, body mass and BMI percentile were calculated from the normative graphs published by the Centers for Disease Control and Prevention [19].

### 2.3. Tanner Staging

Sexual maturity was assessed by a physician assistant using the Tanner staging technique [20]. The technique is based on a 5-point scale, with I indicating no development and V indicating full development. Pubic hair and breast development were assessed in girls. Pubic hair and testicular/penile development were assessed in boys.

### 2.4. Gross Motor Function

Gross motor function of children with CP was assessed by a physician assistant using the gross motor function classification system (GMFCS) [21]. Children who were GMFCS I or II were included in the study. In short, a child with the ability of walking indoors and outdoors and gross motor skills of running and jumping, but limited ability of speed, balance and coordination was classified as GMFCS level I. Limitations of walking on uneven surfaces and inclines and minimal gross motor skills of running and jumping was classified as GMFCS level II.

### 2.5. Physical Activity

Physical activity was estimated using accelerometer-based activity monitors (Actical; Respironics Inc., Bend, OR). The activity monitors contain an omnidirectional accelerometer that is most sensitive to movements in the vertical plane when worn on the

ankle and is sensitive to movements in the 0.5 to 3 Hz frequency range [22]. Physical activity counts were registered in 15 second epochs. Each participant wore two monitors on the lateral aspect of the ankle on the more affected side in children with CP and on the nondominant side in controls. Monitors were worn continuously (i.e., 24 hours per day) for four days (three week days and one weekend day). Participants and participant parents were instructed to take the monitors off only when swimming at a depth greater than 0.91 meters and during bathing/showering. This was confirmed by reviewing activity logs kept by the children with assistance from their parent and by visually examining the graphical output generated using software provided by the manufacturer. If participants did not wear the monitors on any of the days, they were asked to re-wear the monitors to make up for the lost day(s). The total physical activity counts per day averaged from the two monitors are reported. The reliability of the total physical activity counts was assessed in 8 ambulatory children with mild CP and 8 typically developing children between 4 and 11 years of age who wore the monitors for four days on two separate occasions approximately one month apart. The intraclass correlation was 0.935 for children with CP and 0.913 for typically developing children indicating excellent reliability.

## 2.6. Magnetic Resonance Imaging

Magnetic resonance imaging (MRI; GE, 1.5 T, Milwaukee, WI) was used to assess bone architecture and the degree of fat infiltration in the bone marrow at the level of the middle-third of the tibia (i.e., midtibia) and in the surrounding leg musculature (i.e., midleg). The more affected limb in children with CP and in the nondominant limb in controls were tested. Children were immobilized from the waist down using the BodyFIX (Medical Intelligence, Inc., Schwabm nchen, GER), as previously described [3]. A three plane localizer was used to identify the region of interest. Axial images were collected from the tibia plateau to the malleolar articular surface (0.5 cm thick separated by 0.5 cm of spacing) using a semiflex long bone array coil (ScanMed, Omaha, NE) and two different sequences. The first sequence (fast spin echo, TR = 650, TE = 14, FOV = 12, NEX = 3, BW = 15.63, frequency = 512, phase = 256) yielded T1-weighted images. The second sequence (IDEAL: fast-spin-echo, TR = 600, TE = min full, FOV = 12, NEX = 2, BW = 31.25, frequency = 320, phase = 224) yielded fat and water images.

All image collection was overseen by the senior author (CMM). Images at the level of the midtibia were processed blindly by the same technician using software developed with Interactive Data Language (Research Systems, Inc, Boulder, CO) and procedures previously described for the midtibia [8]. A general visual description of the image processing procedure used to quantify bone architecture and strength of the midtibia and the fat infiltration of the bone marrow and surrounding musculature is provided in Figure 1. A more specific description follows. Using an automated procedure, the T1-weighted images at the level of the middle-third of the tibia were filtered using a median filter and voxels were segmented and assigned to cortical bone, bone marrow, muscle and AT using a fuzzy clustering algorithm [23] and their volumes ( $\text{cm}^3$ ) were calculated. Widths (cm) of the cortical bone in the anterior, posterior, medial and lateral portions, widths of the medullary cavity and total bone in the anterior-posterior and medial-lateral directions and estimates of bone strength [i.e., polar moment of inertia (J) and section modulus] were also calculated

during the same procedure. As described previously [4], the parallel-axis theorem [24] was used to determine the cross-sectional moment of inertia of the midtibia in the anterior-posterior and medial-lateral directions. Polar moment of inertia ( $\text{cm}^4$ ) was calculated by averaging the cross-sectional moment of inertia in the two directions. Section modulus ( $Z$ ;  $\text{cm}^3$ ) was calculated by dividing cross-sectional moment of inertia measure by the furthest distance from the neutral axis in the anterior-posterior ( $Z_{\text{ap}}$ ) and medial-lateral ( $Z_{\text{ml}}$ ) directions. Tibia length was estimated by counting the number of images used to cover the entire midtibia and adjusting for image thickness and spacing. The test-retest reliability for measures of bone architecture, bone strength and muscle size measures in the lower extremity from MRI was assessed previously in a combined sample of children with CP and typically developing children tested twice on separate days or on the same day after repositioning [4, 8]. Intraclass correlation coefficients  $> 0.99$  and coefficients of variation between 0.6 and 2.6 % indicated excellent reliability.

Voxels representing muscle and bone marrow in the T1-weighted images were then used to identify the bone marrow and muscle voxels in the corresponding fat and water images. The fat concentration within the bone marrow of the midtibia and the muscles (i.e., intramuscular fat concentration) at the same level was determined by using the signal intensity (SI) from the fat image, the SI from the water image and the following equation: fat concentration =  $[\text{fat SI} / (\text{fat SI} + \text{water SI})] * 100$  [25]. Fat content estimated using MRI and the IDEAL sequence is highly correlated with fat content measured using magnetic resonance spectroscopy in phantoms ( $r^2 = 0.985$ ) [26] and human vertebrae ( $r^2 = 0.904$ ) [27].

Intermuscular, subfascial and subcutaneous AT volume were estimated using the same custom software and the T1-weighted images used to assess bone architecture, bone strength, muscle volume and AT volume. Using a procedure similar to the procedure used for the mid thigh [8], subcutaneous AT was separated from intermuscular and subfascial AT by manually tracing a line over the crural fascia. Intermuscular AT was separated from subfascial AT and bone marrow by manually tracing a line on the outside border of the cortical bone and another line on the inside border of skeletal muscle. In areas where muscles separated, the line was drawn midway between the muscle borders. Thus, subcutaneous AT was the volume of AT between the skin and the crural fascia. Subfascial AT was considered the volume of AT between the deep crural fascia and the skeletal muscle border. Intermuscular AT was considered the volume of AT between the subfascial AT and the bone marrow.

The test-retest reliability for AT measures in the lower extremity using MRI was assessed previously in a combined sample of children with CP and typically developing children tested twice on separate days or on the same day after repositioning [8]. Intraclass correlation coefficients  $> 0.99$  and coefficients of variation between 0.6 and 3.4 % indicated excellent reliability. The test-retest reliability of midtibia bone marrow fat and midleg intramuscular fat concentrations from MRI were assessed in four typically developing children and four children with CP (5 to 11 years of age) tested on two separate days, six months apart. The intraclass correlations were 0.96 and  $> 0.99$  and the coefficients of variation were 0.04 % and 0.3 % for bone marrow and intramuscular fat concentrations, respectively.

## 2.7. Statistical Analysis

Using an effect size of 1.2 and setting the alpha at 0.05 and power at 0.8, a minimum sample size of 10 participants per group was estimated to determine if there were group differences in measures of bone architecture and bone marrow and muscle fat infiltration. Data were analyzed by using SPSS version 24.0 (IBM Corp, Armonk, NY). Descriptive statistics were conducted for all variables to screen for outliers and to assess normality. Independent sample t-tests were used to determine if there were group differences. The Mann-Whitney U test was used to assess differences in Tanner stage. To adjust all outcome measures of bone, muscle, AT and fat concentration for tibia length, a one-way analysis of covariance was used with tibia length as a covariate. Due to the equal number of boys and girls in each group, and the small number of girls included in the study ( $n = 4/\text{group}$ ), sex differences were not considered in the statistical analysis. Mean  $\pm$  SD were reported for all data. The magnitude of the effects were determined using Cohen's  $d$  ( $d = \text{mean difference between groups/pooled SD}$ ), with 0.2, 0.5 and 0.8 representing small, moderate and large effect sizes, respectively [28].

## 3. Results

Twenty-three children with CP who met the inclusion criteria were invited to participate in the study. Eighteen children with CP enrolled in the study. Two children were unable to complete any MRI testing due to claustrophobia. One child was unable to remain still for the MRI testing in which the fat concentration of the bone marrow and muscle were determined due to attention deficit hyperactivity disorder and an inability to follow directions. Twelve typically developing children who met the inclusion criteria and matched a child with CP for age ( $\pm 1.2$  y), sex ( $n = 4$  girls and 8 boys per group) and race ( $n = 1$  white Hispanic, 10 white non-Hispanic and 1 Asian per group) were enrolled in the study as controls. All controls completed all testing. Data for the 12 matched pairs are presented. Two of the children with CP had a previous fracture (finger,  $n = 1$  and foot/ankle,  $n = 1$ ) and 3 were taking antiepileptic medication. None of the controls reported a previous fracture or chronic medication use. All data were normally distributed except subfascial and intermuscular AT volume, which were log transformed before statistical analyses were conducted.

Physical characteristics of the participants are shown in Table 1. Of the 12 children with CP, 8 children were considered GMFCS I and 4 children were considered GMFCS II. There were no group differences in age, pubic hair Tanner stage, testicular-penile/breast Tanner stage, height, body mass, body mass percentile, BMI, BMI percentile or tibia length (all  $p > 0.05$ ). However, children with CP compared to controls had lower height percentile ( $p < 0.05$ ). When compared to the 50<sup>th</sup> age- and sex-based percentiles, height percentile was lower in children with CP ( $p = 0.003$ ), but not different in controls ( $p = 0.531$ ). When compared to the 50<sup>th</sup> age- and sex-based percentiles for body mass and BMI, there were no differences in children with CP or controls (all  $p > 0.20$ ). Group comparisons of physical activity are also presented in Table 1. Children with CP had 44 % fewer physical activity counts at the ankle compared to controls ( $p < 0.05$ ).

Group comparisons of bone architecture at the level of the midtibia are shown in Table 2. Children with CP had 30 % lower cortical volume ( $p < 0.05$ ) than controls with no

differences observed in medullary volume ( $p > 0.05$ ). Although marginally insignificant ( $p = 0.063$ ), children with CP had 25 % lower total bone volume than controls. Cortical width was 16 % thinner in the posterior portion and 32 % thinner in the medial portion of the shaft in children with CP compared to controls ( $p < 0.05$ ). There were no group differences observed in the anterior or lateral portions of the bone ( $p > 0.05$ ). Although marginally insignificant ( $p = 0.058$ ), children with CP displayed 17 % lower medullary width in the medial-lateral direction compared to controls. No group difference in medullary width was observed in the anterior-posterior direction ( $p > 0.05$ ). Children with CP displayed 15 % lower total bone width in the medial-lateral direction compared to controls ( $p < 0.05$ ), but no difference was observed in the anterior-posterior direction ( $p > 0.05$ ). When tibia length was used as a covariate, the differences remained statistically significant for all bone architecture measures ( $p < 0.05$ ), except for cortical width in the posterior portion of the bone ( $p > 0.05$ ).

Group comparisons of bone strength estimates at the level of the midtibia are shown in Figures 2. Children with CP had 34 % lower  $Z_{ml}$  ( $d = 1.127$ ,  $p = 0.012$ ) and 39 % lower  $J$  ( $d = 1.001$ ,  $p = 0.023$ ) compared to controls. Although marginally insignificant, children with CP had 29 % lower  $Z_{ap}$  ( $d = 0.822$ ,  $p = 0.057$ ) compared to controls. When tibia length was used as a covariate, the significant differences in  $Z_{ml}$  and  $J$  remained statistically significant ( $p = 0.017$  and  $0.035$ , respectively). Figure 3 shows a higher bone marrow fat concentration in children with CP compared to controls ( $82.1 \pm 1.8$  % vs.  $80.5 \pm 1.9$  %;  $d = 0.909$ ,  $p = 0.037$ ), which remained significantly higher when tibia length was used as a covariate ( $p = 0.004$ ).

Group comparisons of muscle volume and AT volume in the midleg are shown in Table 3. Children with CP had 39 % lower muscle volume and 3.3 fold higher subfascial AT volume compared to controls (both  $p < 0.05$ ). There were no group differences in subcutaneous or total AT volume between groups ( $p > 0.05$ ). Although marginally insignificant ( $p = 0.077$ ), children with CP had 3.7 fold lower intermuscular AT than controls. The differences in muscle volume and subfascial AT volume remained statistically significant when tibia length was used as a covariate ( $p < 0.05$ ). Moreover, a significantly higher intermuscular AT in children with CP than controls emerged when tibia length was used as a covariate ( $p < 0.05$ ). Figure 4 shows a higher intramuscular fat concentration in children with CP compared to controls ( $25.0 \pm 8.0$  % vs.  $16.1 \pm 3.3$  %;  $d = 1.587$ ,  $p = 0.003$ ), which remained significantly higher when tibia length was used as a covariate ( $p = 0.002$ ).

A visual depiction of the bone, muscle and fat contrast between a boy with CP and a typically developing control boy of similar height and the same tibia length is shown in Figure 4.

## 4. Discussion

This is the first study to report that ambulatory children with mild spastic CP relative to typically developing children have markedly underdeveloped bone architecture and lower estimates of bone strength. It is also the first study to report that ambulatory children with mild spastic CP have elevated fat infiltration of bone marrow and skeletal muscle. The finding that bone marrow is infiltrated with fat in children with mild CP is particularly novel

because, to our knowledge, it has not been examined in individuals with CP, irrespective of level of involvement or age. Together the findings suggest that the underdevelopment of bone architecture and fat infiltration of the musculoskeletal system in nonambulatory children with more severe forms of CP is also present in ambulatory children with milder forms of the disorder. The findings are concerning because poor bone architecture and an elevated bone marrow adiposity are associated with an increased risk of fracture [13–15]. Moreover, elevated bone marrow and muscle adiposity are linked to impaired glucose tolerance [12, 16, 17] and cardiometabolic disease [18].

The finding that bone architecture is underdeveloped and bone strength is lower in ambulatory children with mild CP is consistent with previous studies in children [4, 6] and adults [29, 30] with reduced mechanical loading. However, the magnitude of the deficit is surprising. In the current study, cortical volume, Z in the medial-lateral plane and J were 30 % lower in the midtibia of ambulatory children with mild CP vs. controls. These estimated deficits are approximately half those observed in the midfemur of nonambulatory children with CP [4], but generally worse than the 15–31 % estimated bone deficits reported in the tibial [30] and femoral shaft [29, 30] of adults with complete spinal cord injury (SCI). The smaller deficits in the ambulatory children with CP observed in the present study compared to the deficits observed in a previous study of nonambulatory children with CP [4] is not surprising because ambulatory children with CP experience mechanical loading while they are walking or running. Moreover, although physical activity was 44% lower in ambulatory children with CP compared to typically developing children matched for age, sex and race in the present study, it was not as dramatic as the discrepancy (70 % lower) previously reported for nonambulatory children with CP [4]. The generally larger bone strength deficits observed in ambulatory children with CP than the deficits previously reported for adults with SCI is likely due to the onset of the condition. In children with CP, the restriction of mechanical loading begins at birth or shortly afterward and continues throughout life. Therefore, the mechanical loading needed for optimal cortical expansion at the subperiosteal surface is reduced or absent and the total width of the bone does not reach its capacity. On the other hand, because SCI usually occurs during adulthood, the restriction of mechanical loading usually begins after growth and cortical expansion is complete or nearly complete. Therefore, the total width of the bone reaches its capacity [29, 30]. The deficit in bone strength in adults with SCI results from increased bone remodeling and endocortical resorption, which has a smaller effect on bone strength than restricted subperiosteal expansion [31]. The difference in timing of physical activity restriction highlights the importance of mechanical loading during childhood to optimize cortical expansion and the strength of bone. Together, findings from the current study and previous studies suggest that ambulatory children with mild CP have considerable bone strength deficits that need clinical attention. A handful of studies suggest that treatments, such as high-frequency, low-magnitude vibration [33, 34], may aid in addressing such bone deficits.

The observation that ambulatory children with mild CP have elevated fat infiltration of muscle in the midleg, as indicated by elevated subfascial AT and intramuscular fat concentration, is consistent with the observation in nonambulatory children with moderate to severe CP [8] and young adults with mild to moderate CP [35]. Using magnetic resonance imaging, Johnson et al. [8] reported a 2.3-fold and 1.7-fold higher intermuscular and



subfascial AT area in the mid thigh of nonambulatory children with CP compared to typically developing children. These AT depots were negatively correlated with physical activity ( $r = -0.76$  and  $r = -0.63$ , respectively). Furthermore, Noble and colleagues [35] reported greater intramuscular fat in the leg muscles in young adults with CP compared to controls. In the present study, physical activity assessed using physical activity monitors was 44 % lower in children with mild CP than controls. There is also evidence that gait declines in 25 % of children with CP as they become adults [1]. The sedentary behavior and reduced mechanical loading observed with CP is also observed with SCI. Individuals with SCI, concomitant with the altered skeletal metabolism, experience vast muscular atrophy, increased muscular adiposity, insulin resistance and an increased prevalence of type 2 diabetes mellitus [12, 36]. On the other hand, the sedentary behavior and reduced mechanical loading in individuals with CP occurs at or near the time of birth, rather than it being acquired later in life. Therefore, the musculoskeletal metabolic dysregulation in children with CP, resulting in losses of normal muscle function, likely leads to an enhanced risk for cardiometabolic disease [18]. This idea is supported by the 2-to-3 fold greater mortality rate from coronary heart disease in individuals with CP compared to the general population [37]. Therefore, ameliorating regressive behavior in physical activity, which is apparent even in children with a mild form of the disorder, is imperative to the health of the musculature, and minimizing the risk of cardiometabolic disease in individuals with CP.

The novel finding of elevated fat concentration in the bone marrow of children with CP in the present study could be viewed as somewhat surprising because the children were ambulatory with a mild form of CP, the children averaged only 8.8 years of age and their BMI was not different from the 50<sup>th</sup> age-based percentile; however, the finding is consistent with their underdeveloped bone architecture and low estimates of bone strength relative to controls. Moreover, the finding is consistent with the elevated fat concentration in the bone marrow of other groups with limited mobility [11, 14, 38]. Bone marrow-derived mesenchymal progenitor cells have a multipotency to differentiate into an osteogenic or adipogenic lineage while concomitantly suppressing the opposed lineage [39]. Recently, it has been reported that bone marrow fat concentration is inversely related to cortical bone area in adolescents 15 to 20 years of age [40] and adults [40, 41]. There is evidence suggesting that physical activity governs the extent of bone marrow AT. Rantalainen et al. [42] reported that female athletes had significantly greater cortical area, strength strain index and bone marrow density (indicative of lower marrow adiposity) in the midtibia compared to healthy controls. On the other hand, it has been suggested that adults with SCI have a higher amount of yellow vs. red bone marrow AT compared to healthy controls [11]. The higher proportion of yellow marrow may be explained by the metabolic shift in mesenchymal progenitor cell differentiation away from osteogenesis and towards adipogenesis at the endocortical surface due to the reduced mechanical loading observed after an SCI. In the current study, children with mild CP had a deficit in cortical volume and bone strength, but they had no significant difference in the volume of the medullary cavity. Therefore, the connection between the higher concentration of bone marrow adiposity and the deficit in bone macroarchitecture in ambulatory children with mild CP relative to typically developing children and the clinical implications of the relationship requires further investigation.

The present study has several strengths. First, there was very careful attention paid to the selection of the research participants which limited the within group variability and the need for a large sample size. Specifically, all children with CP had spasticity, were ambulatory and had a mild form of the disorder. The typically developing children were not different from the children with CP in age, sex, race or sexual maturity. Furthermore, the typically developing children were not different from the 50<sup>th</sup> age- and sex-based percentiles for height, body mass and BMI. A second strength of the present study was that most of the deficits in bone architecture and bone strength and all of the elevated estimates of fat infiltration of bone marrow and muscle in the children with CP remained when small, insignificant differences in tibia length were statistically controlled. A third strength of the study was the use of MRI to assess different AT and fat depositions. Magnetic resonance imaging is considered the gold standard *in vivo* imaging modality for assessing soft tissue [44], it provides valid estimates of bone architecture [45] and fat concentration [26] and it does not expose participants to ionizing radiation, as does computed tomography. Furthermore, the reliability of the measures in the present study was excellent, with intraclass correlations for repeat testing 0.96 for all MRI measures of bone architecture, bone marrow and muscle fat concentration and muscle volume. Lastly, physical activity was estimated using accelerometer-based activity monitors that have been validated with a wide range of both gross and fine motor physical activities [43] and have good reliability for assessment in children with CP, as reported in the present study. The assessment of physical activity allowed for a broader mechanistic approach to understanding the underdeveloped musculoskeletal system and increased fat infiltration within muscle and bone in a group of ambulatory children with mild spastic CP.

The limitations of the study must also be discussed. One limitation is the small sample size. Therefore, the results should be interpreted with caution. Studies of children with CP using MRI are challenging because the children often have difficulty holding still due to spasticity and/or behavioral issues, and they often have noise sensitivity and cognitive issues. Moreover, the cost of MRI studies limits the number of participants that can be enrolled. However, even with these challenges, significant differences in cortical volume, bone widths, estimates of bone strength, bone marrow fat concentration, muscle volume, subfascial AT volume and intramuscular fat concentration were observed. This is attributed to the magnitude of the differences as well as the robustness of the measures. As previously noted, the reliability of the MRI assessment of bone architecture, muscle volume and fat infiltration measures used in the present study is extremely good. Furthermore, a set of identical twins was included in the study and their data reflect the group differences. For example, cortical volume, muscle volume, and bone marrow and intramuscular fat concentration were 30 % lower, 39 % lower, 2 percentage points higher, and 9 percentage points higher, respectively, in the children with CP compared to the typically developing children. The same measurements were 18 % lower, 39 % lower, 2 percentage points higher and 7 percentage points higher, respectively, in the twin with CP (5.5 years of age, GMFCS I) compared to the typically developing twin. A second study limitation is the absence of glucose tolerance, insulin sensitivity and inflammatory markers. It has been established that elevated AT surrounding muscle and fat within muscle are associated with a disturbance of euglycemic conditions [12] and insulin resistance [16], as well as promoting a pro-inflammatory

environment [46]; whether this relationship exists in children with mild CP and if elevated bone marrow fat is associated with a disturbance in these metabolic markers warrants further investigation.

Taken together, the findings from the present study suggest that ambulatory children with mild spastic CP have an underdeveloped bone architecture, low bone strength and a greater infiltration of fat within the bone marrow cavity of the midtibia and the surrounding leg musculature. The underdeveloped bone architecture and fat infiltration may be related to the low level of physical activity potentially promoting an intramuscular pro-inflammatory environment and blunting osteogenesis while favoring adipogenesis within the bone marrow cavity. Additional studies that determine whether the adverse musculoskeletal fat profile is associated with a disturbance in glucose homeostasis or insulin function are needed. Studies that examine whether increased physical activity or other interventions would enhance the bone architecture while reducing the observed infiltration of fat in the musculoskeletal system of children with mild CP are also needed.

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## Abbreviations

<b>CP</b>	cerebral palsy
<b>Z<sub>ap</sub></b>	section modulus in the anterior-posterior direction
<b>Z<sub>ml</sub></b>	section modulus in the medial-lateral direction
<b>J</b>	polar moment of inertia
<b>AT</b>	adipose tissue
<b>GMFCS</b>	gross motor function classification system
<b>SI</b>	signal intensity
<b>d</b>	Cohen's d

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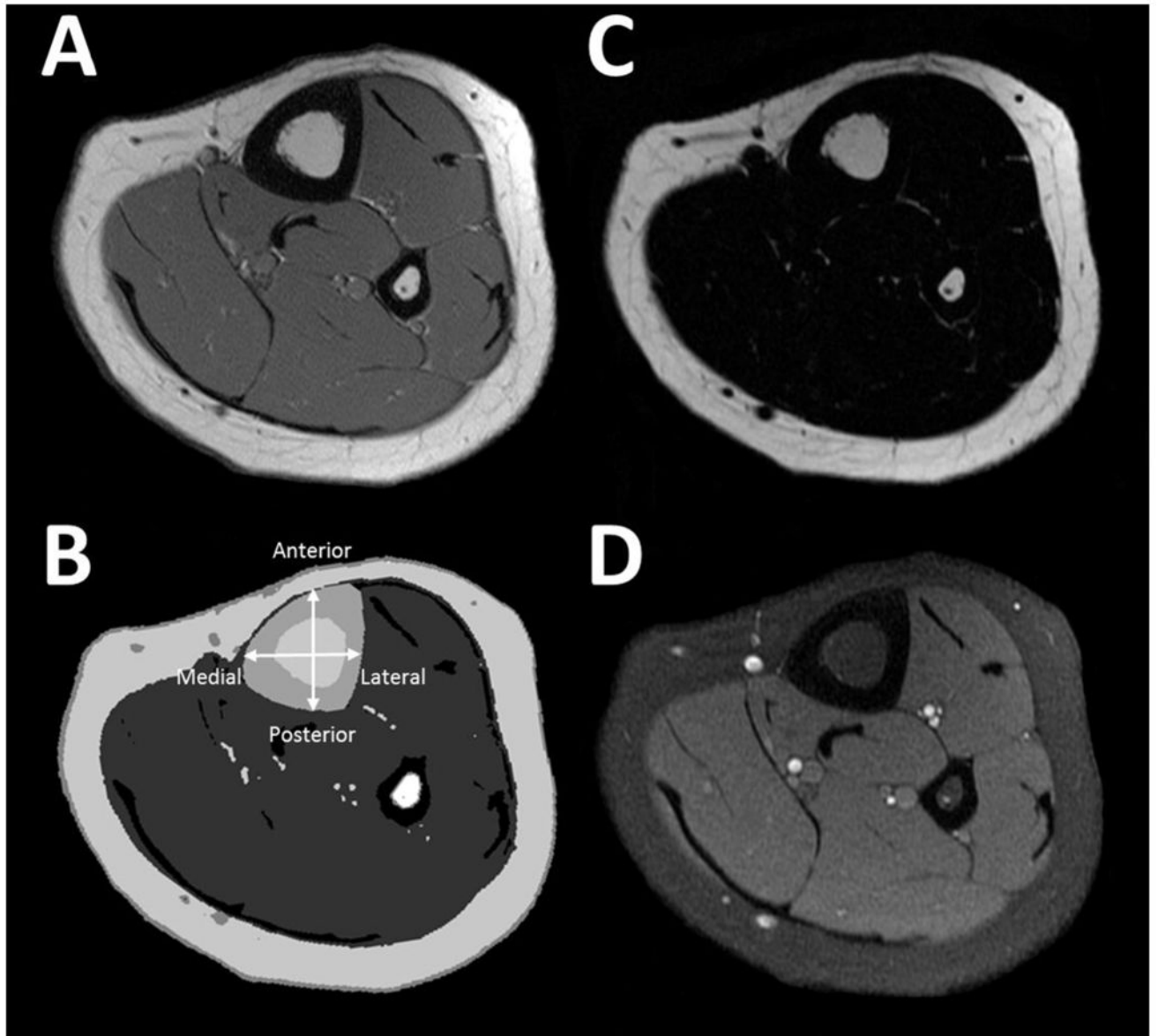
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**Highlights**

- Children with mild cerebral palsy (CP) vs. typically developing children had a marked deficit in cortical bone architecture and estimated bone strength.
- Bone marrow in the midtibia was more infiltrated with fat in children with mild CP vs. typically developing children.
- Muscle in the midleg was more infiltrated with fat in children with mild CP compared to typically developing children.



**Figure 1.**

A general description of the image processing procedure used to quantify bone architecture and strength of the midtibia and the fat infiltration of the bone marrow and surrounding musculature. Raw T1-weighted magnetic resonance images (A) were segmented (B) into tibia cortical bone (large black ring; fibula is the small black ring) and bone marrow (white region surrounded by the cortical bone), muscle (gray region surrounding the bone) and adipose tissue (AT; white ring surrounding the muscles and bones and the white voxels interspersed among the musculature). The bone, muscle and AT volumes, bone widths (cortical bone in the anterior, posterior, medial and lateral portions and the bone marrow and total bone in the anterior-posterior and medial-lateral directions) and estimates of bone strength (section modulus in the anterior-posterior and medial-lateral directions and polar moment of inertia) were calculated. Voxels identified as bone marrow and muscle were



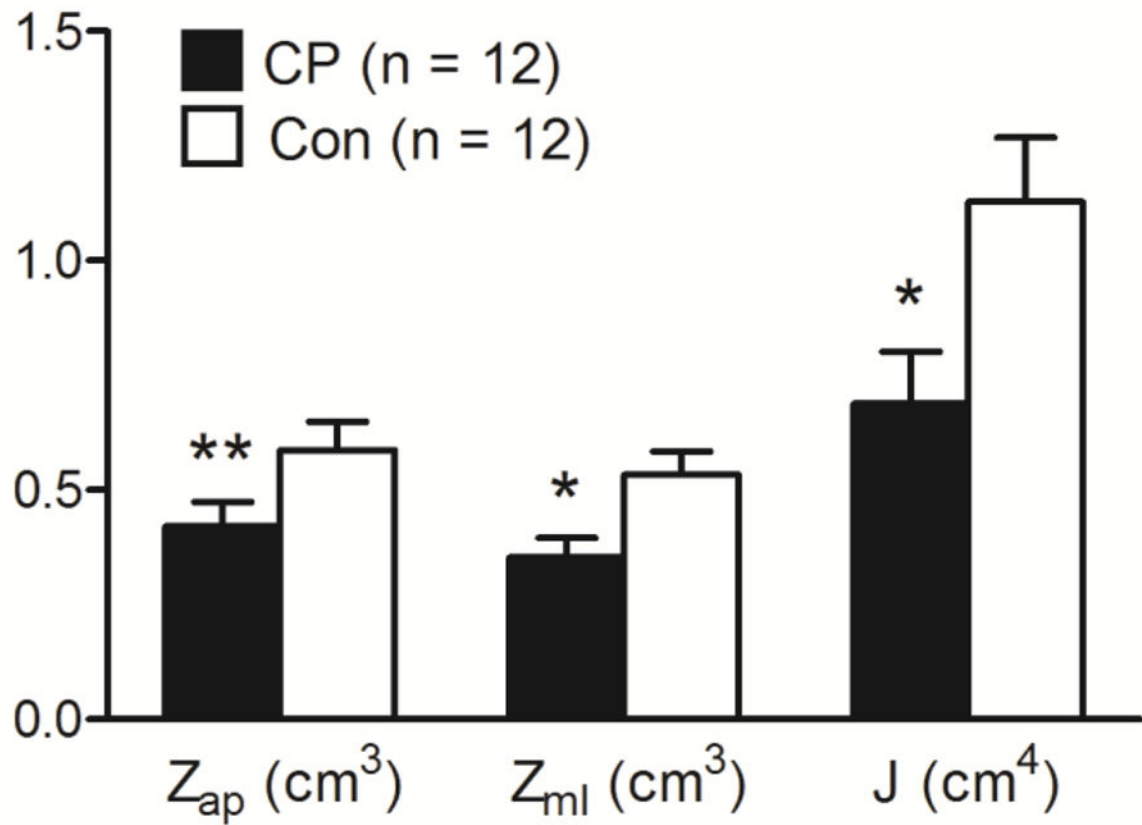
applied to fat (C) and water (D) images to determine bone marrow and intramuscular fat concentration [25].

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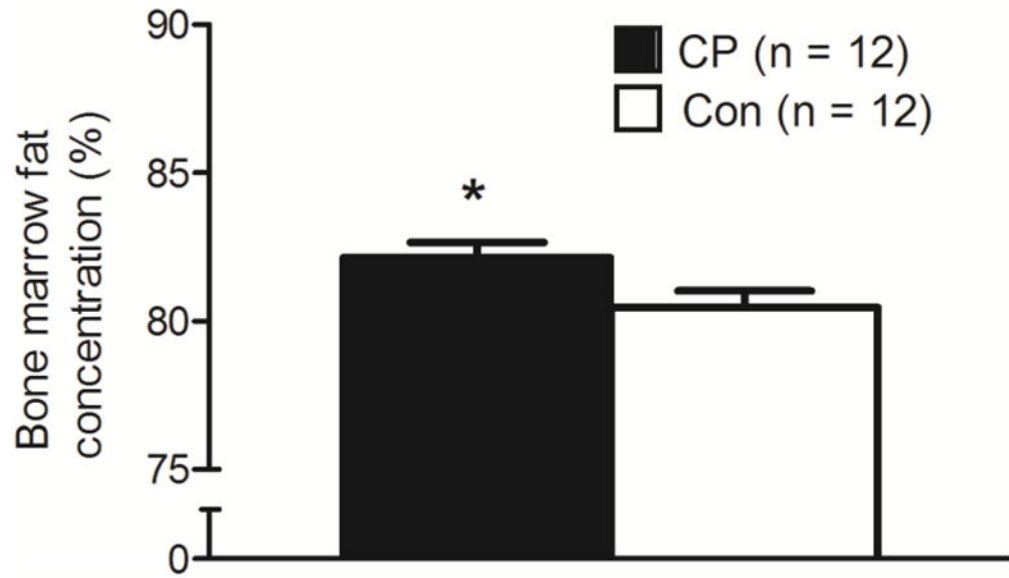
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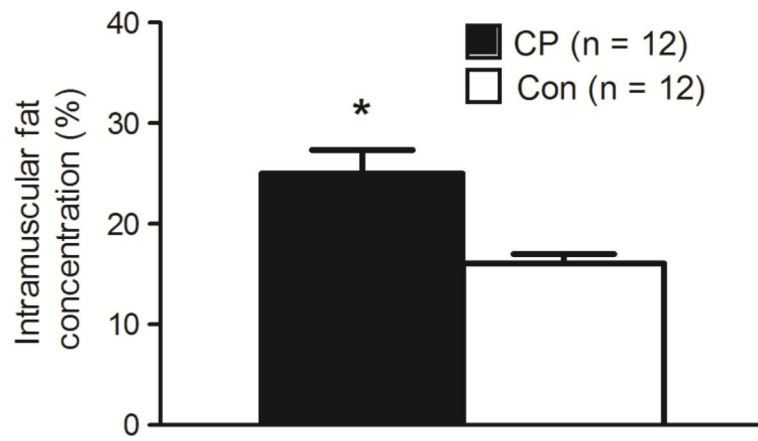
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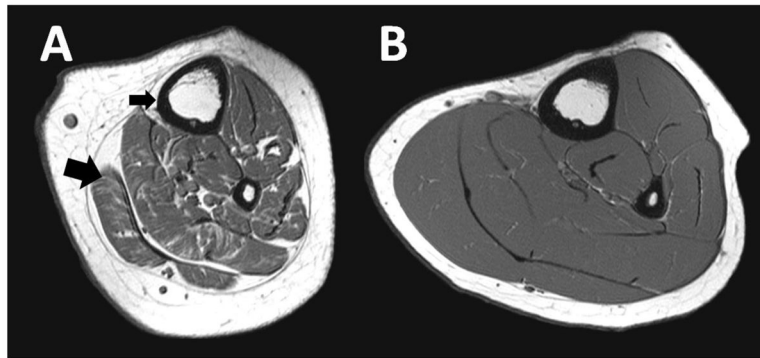
**Figure 2.** Estimates of bone strength [section modulus in the anterior-posterior ( $Z_{ap}$ ) and medial-lateral ( $Z_{ml}$ ) directions and polar moment of inertia ( $J$ )] in the midtibia of children with cerebral palsy (CP) and typically developing children (Con). \*Group difference,  $p < 0.05$ . \*\*Group difference,  $p = 0.057$ .



**Figure 3.** Bone marrow fat concentration (%) in the midtibia of children with cerebral palsy (CP) and typically developing children (Con). \*Group difference,  $p < 0.05$ .



**Figure 4.** Intramuscular fat concentration (%) in the midtibia of children with cerebral palsy (CP) and typically developing children (Con). \*Group difference,  $p < 0.05$ .



**Figure 5.**

Raw T1-weighted magnetic resonance images from the midtibia demonstrate the marked deficit in bone architecture and muscle volume and the high infiltration of fat within and around the musculature in an ambulatory boy with mild CP (A) compared to a typically developing boy with the same tibia length (B). In the image of the child with CP (A), the small black arrow highlights the thin cortical shell and the large arrow highlights the fat infiltration of muscle.

**Table 1**

Characteristics of children with cerebral palsy (CP) and typically developing children (Con)

	CP (n = 12)	Con (n = 12)	<i>p</i>	<i>d</i>
Age (y)	8.8 ± 2.1	8.8 ± 2.0	0.971	0.015
Tanner Stage (I/II/III)				
Pubic hair	8/2/2	11/1/0	0.291	0.767
Testicular-Penile/Breast	7/5/0	10/1/1	0.378	0.293
Height (m)	1.24 ± 0.11	1.32 ± 0.11	0.106	0.689
Height (%)	19 ± 23	50 ± 28	<b>0.006</b>	1.232
Body mass (kg)	26.6 ± 8.2	30.0 ± 7.4	0.293	0.441
Body mass (%)	34 ± 33	55 ± 25	0.094	0.720
BMI (kg/m <sup>2</sup> )	17.0 ± 3.4	17.0 ± 2.6	0.966	0.018
BMI (%)	50 ± 36	53 ± 28	0.872	0.067
Tibia length (m)	0.273 ± 0.036	0.293 ± 0.031	0.173	0.577
GMFCS (I/II)	8/4	-		
Physical activity (counts/d)	348,366 ± 197,001	628,044 ± 147,499	<b>0.001</b>	1.624

Values are means ± SD. % reflects the percentile relative to age- and sex-based norms; BMI = body mass index; GMFCS = Gross Motor Function Classification System

Bone architecture in the midtibia of children with cerebral palsy (CP) and typically developing children (Con)

Table 2

	CP (n = 12)	Con (n = 12)	P	d	P*
Cortical volume (cm <sup>3</sup> )	12.3 ± 4.7	17.6 ± 5.8	<b>0.022</b>	1.009	<b>0.012</b>
Medullary volume (cm <sup>3</sup> )	7.7 ± 3.7	9.0 ± 3.0	0.343	0.398	0.705
Total bone volume (cm <sup>3</sup> )	20.0 ± 8.2	26.6 ± 8.4	0.063	0.800	0.119
Cortical width (cm)					
Anterior	0.41 ± 0.10	0.43 ± 0.11	0.656	0.184	0.434
Posterior	0.32 ± 0.07	0.38 ± 0.06	<b>0.023</b>	1.004	0.070
Medial	0.26 ± 0.04	0.38 ± 0.10	<b>0.002</b>	1.770	<b>0.002</b>
Lateral	0.27 ± 0.06	0.25 ± 0.07	0.586	0.227	0.397
Medullary width (cm)					
Anterior-posterior	0.51 ± 0.10	0.51 ± 0.08	0.981	0.010	0.061
Medial-lateral	0.52 ± 0.07	0.59 ± 0.09	0.058	0.820	0.199
Total bone width (cm)					
Anterior-posterior	1.24 ± 0.22	1.32 ± 0.23	0.376	0.369	0.525
Medial-lateral	1.04 ± 0.11	1.22 ± 0.16	<b>0.006</b>	1.262	<b>0.015</b>

Values are means ± SD. *d* = the effect size of the group differences before tibia length was included as a covariate.

\* Group difference *d* value when statistically controlled for tibia length using analysis of covariance.

Muscle and adipose tissue (AT) volume in the midleg of children with cerebral palsy (CP) and typically developing children (Con)

**Table 3**

	CP (n = 12)	Con (n = 12)	<i>p</i>	<i>d</i>	<i>p</i> *
Muscle volume (cm <sup>3</sup> )	144.8 ± 56.4	237.1 ± 70.0	<b>0.002</b>	1.461	<b>0.001</b>
Intermuscular AT volume (cm <sup>3</sup> )**	5.1 ± 7.0	1.4 ± 1.1	0.077	0.787	<b>0.036</b>
Subfascial AT volume (cm <sup>3</sup> )**	4.2 ± 5.4	1.3 ± 0.7	<b>0.008</b>	1.229	<b>0.002</b>
Subcutaneous AT volume (cm <sup>3</sup> )	119.5 ± 58.3	140.6 ± 43.3	0.325	0.415	0.868
Total AT volume (cm <sup>3</sup> )	128.8 ± 66.2	143.3 ± 44.7	0.537	0.261	0.796

Values are means ± SD. *d* = the effect size of the group differences before tibia length was included as a covariate.

\* Group difference *p* value when statistically controlled for tibia length using analysis of covariance.

\*\* Group difference *p* value and *d* for the log transformed data are reported, but means ± SD for untransformed data are presented for easier comparisons among the adipose tissue depots.