Time Since Onset of Walking Predicts Tibial Bone Strength in Early Childhood

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Abstract:

Bone strength in adulthood is known to be affected by health at birth and early childhood. Habitual bone loading is a primary determinant of bone strength in later childhood and adulthood. However, the effects of physical activity in early childhood (*e.g.* crawling, standing and walking) on bone strength are unknown. Fifty-three children (twenty-seven males) were included in a longitudinal study in their early infancy. Shortly after birth (0.3±0.3 months), details of mass and height were obtained along with a pQCT scan at 20% distal-proximal tibia length. At 14.8±0.5 months of age the same data were collected, along with details of age at onset of standing, crawling, supported and unsupported walking. Time since onset of walking unsupported was associated with greater bone mass, cortical bone area, pericortical circumference and polar moment of inertia of both total and cortical bone (all $P < 0.05$). There were no significant associations between other physical activity timepoints and bone measures. Age at onset of walking was not significantly related to mass, length or bone measures at birth. The results suggest that time since attainment of independent walking – representing exposure of the tibia to the large reaction and muscular forces associated with locomotion - is a primary determinant of bone strength in early childhood. This finding raises the possible opportunity of physical activity interventions at young age in paediatric populations associated with low childhood bone strength and late walking (*e.g.* low birth weight, cerebral palsy and Down's Syndrome, *etc.*).

Key Words: BMD, pQCT, Physical Activity, Growth

Introduction

Fractures are highly prevalent in children, with annual incidence as high as 5% dependent on age and gender [1]. Childhood bone strength has been shown to be predictive of fracture risk, with an 89% increased fracture risk per SD decrease in bone mass (which indicates bone's strength in compression) [2]. In addition, health status at birth has a significant and persisting effect on bone strength, with preterm children having lower bone strength even in late childhood [3] and birth weight being related to bone mass more than 60 years later [4]. Given that low bone strength is also associated with increased fracture risk in later life independent of factors such as fall incidence and physical activity [5], it is important to understand factors that affect bone strength in early life. Gender [6], gestation length [7], birth weight [8, 9] and maternal nutrition [10, 11] all influence bone strength near birth and in infanthood. Alongside factors such as body size and pathological causes, physical activity is a known primary determinant of adult bone strength [12-14] – yet the effects of early habitual physical activity (*i.e.* crawling, standing and walking) on bone strength in infancy and early childhood have not been well explored. The few studies that are available suggest a minor effect of early physical activity on bone strength. Preterm children walk independently at a later age than term children [15], but walking age was not found to be a significant predictor of tibial bone strength, or able to explain bone differences in preterm and term children at 3-5 years old [16]. The interpretation of these studies is somewhat difficult because of the small variation in walking age (standard deviation of 2 months) and large variation in age at examination (and hence time since onset of walking). A longitudinal radiograph study revealed peaks in femoral bone accrual rate at 14-15 months (*i.e.* around typical onset of walking – although walking age was not

examined) which could not be explained by changes in height, body weight, bone length or muscle area [17]. Attainment of independent walking represents the first time that a child is required to support their entire mass on a single limb. Biomechanical studies have estimated muscular forces during adult walking at three times body mass (not considering reaction forces transmitted in response to body mass) [18]. Therefore despite some differences in gait between children and adults, it can be reasonably assumed that walking results in the highest bone stresses experienced in the lower limbs at this age.

In order to examine the effects of physical activity in early childhood, a novel analysis of existing data [10, 11] was performed in order to assess bone strength in the same children at birth and at ~15 months of age. Whilst the former reports on these data related to vitamin D status and bone strength, the present data analysis aims at the relationship between early exposure to independent postural and locomotory (crawling, standing and walking age) and lower limb bone strength examined at birth and in early childhood. It is hypothesized that bone strength at birth and follow-up will be related. In addition that greater body size and time since attainment of movement milestones (indicating the length of time the lower limb bones have been exposed to the loading regimes of these movements) will be positively related to bone strength in early childhood. Independent walking involves acceleration of whole body mass against gravity on only one limb, and bodyweight is supported on the forefoot (creating a long lever for the calf muscles to work against). These factors suggest that independent walking will likely result in the highest loading forces experienced by the limbs, and hence it is also hypothesized that timing of independent walking will prove to have the greatest influence on bone strength.

Materials and Methods

One hundred and twenty four families were recruited during their stay in hospital for their child's birth. This is a re-analysis of existing data, hence a priori sample size assessments were not performed. Details of sample size calculations for the cohort are detailed in the previous publication [11]. Cases were included when pregnancies were primiparious, single, full-term (37-42 weeks gestation), uneventful and resulted in a healthy birth. Absence of any of the inclusion criteria or birth weight outside Finnish sex-specific norms for gestational age [19] resulted in exclusion. In addition, mothers were Caucasian, healthy non-smokers aged between 20 and 40 years. The study adhered to Declaration of Helsinki guidelines, was approved by Helsinki University Hospital's Ethics Committee and written informed consent was obtained from all mothers prior to testing. Pregnancy follow-up records and the birth report were obtained in order to provide body mass at birth, length and pregnancy duration. Pregnancy duration was then used to assess both calendar age, and age adjusted for gestation length (*i.e.* to 40 weeks) – both ages were used in separate analyses. Sixty-seven (54%) of this original cohort agreed to participate in a follow-up visit at 14.8±0.5 months of age – there were no differences in newborn characteristics (*e.g.* body mass, length, *etc*.) between follow-up participants and non-participants. During the follow-up visit to the hospital, one of the researchers conducted an interview with the family about the child's development, recording age at onset of crawling, standing and walking both supported and unsupported. Further description of each milestone were provided according to WHO definitions [20] as required. Height (to the nearest mm) was measured in standing position using a wall-mounted stadiometer, and body mass in light clothing (to the nearest 0.1kg) was measured on a scale. Length and mass at birth, and height at follow-up

were transformed into Z-scores, and mass at follow was expressed as height-adjusted mass using sex-specific normative data for Finnish infants [19].

pQCT imaging:

pQCT images of tibia at 20% distal-proximal left tibia length (measured from medial malleolus to the palpated medial knee join cleft) were obtained 9.5±10.3 days after birth, and at 14.8±0.5 months of age. Childhood tibial shaft fractures are common, and occur most frequently in the distal third of the bone [21]. Total bone mass (indicating bone's compressive strength) is lowest at 15-20% distal-proximal length, whilst anterior-posterior and torsional strength (assessed by cross-sectional moments of inertia) are lowest at 15-35% and 20-25% distal proximal length respectively [22]. Therefore tibia is structurally weakest at 15-20% length. 15% distal-proximal length is metaphyseal bone, and therefore changes greatly during growth. Reference data for older children at 20% distal-proximal tibia [23], and positive effects of exercise interventions on bone strength in 3 to 5 year olds at this site have previously been reported [24]. Hence 20% distal-proximal length of tibia was examined. Normally, the dominant leg is scanned – however, as participants had not yet learned unilateral motor skills (*e.g.* hopping, kicking, etc.) limb dominance could not be determined. Little or no significant side difference in lower limb bone strength is found in children or adults [25, 26]. Where side differences are found they are in favour of the non-dominant limb, which is usually the left leg. Therefore for the majority of participants the eventual dominant leg was scanned. Even in the upper limbs where small side differences in bone strength develop, these asymmetries do not become evident until later in childhood (when – as with the legs – unilateral limb tasks are regularly practiced) [25]. Therefore it is

unlikely that leg selection would have significantly influenced results. In order to minimise scan time - and hence radiation exposure and possibility of movement artefact – no scout scan was performed. Instead the measured site was marked on the child's skin with a colour line. To stabilize the position of the leg during measurement, footwear was fixed to a holder with a firm Velcro fastening. As scans at birth were taken within a few days of delivery, babies were normally still very exhausted. If babies were active, investigators waited until the child had been fed and was asleep. Alternatively, some babies were given glucose to taste to keep them calm. At follow-up children sat on their parents' lap, and if necessary were given snacks to distract them during scanning. Images were taken using a Stratec XCT-2000 pQCT scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany), and all image analyses were done with the integrated XCT software in its version 6.00. Scan speed was set at 22mm.s^{-1}, and a voxel size of 0.2mm was used. Whilst successful pQCT scans were obtained from all participants (n = 67) at follow-up, for the present study only the highest quality scans *i.e.* where movement artefacts were absent or minimal were selected for analysis. This resulted in a final cohort of fifty-three participants (twenty-seven male), of which forty-one (twenty-two male) also had high-quality scans available at birth. There were no differences in baseline or follow-up characteristics between those selected and excluded from the final analysis.

Data processing and statistical analyses:

Figure 1 shows the effects of using different peeling thresholds on pQCT image analysis, whereby when a low threshold is used muscle area is also included in analysis – conversely when high thresholds are used some area of bone is filtered out from the analysed area. There was a large variation in BMD in baseline scans, and so a threshold (180 mg.cm^{-3}) was

used which filtered soft tissue without excluding areas of bone. Similarly, whilst a dense cortical shell was evident in follow-up scans, the outer and inner two or three pixels were of much lower density. Given that the partial volume effect would only affect the outermost pixels, this could not be the sole cause. Hence, – and in order for results to be comparable with baseline scan analysis – a peeling threshold of 180 $mg.cm^{-3}$ was used to analyse follow-up scans.

<Figure 1 about here>

The tibia did not contain a marrow cavity at baseline (as shown in Figure 1), hence only 'basic' bone variables – total bone mass (vBMC.tot, $mg.mm^{-1}$, total bone area (Ar.tot, mm^2) and total bone mineral density (vBMD.tot, $mg.mm^{-3}$) were examined. In follow-up scans, periosteal circumference (PsC, mm) and density-weighted moment of inertia (I_p, mm^4) were also measured. To calculate the appropriate cortical threshold for analysis of follow-up scans (where a cortical shell was visible and BMD was more uniform), a series of ten randomly selected scans were examined. These scans did not differ from cohort means for age, mass, height bone measures, *etc*. Using the 'Profile' function in Version 6.00 of the software supplied with the machine four profiles running anterior-posterior, medial-lateral and in both diagonal directions were drawn. The two bone mineral density maxima of each profile (corresponding to the peak BMD in the cortical portion) were recorded, and averaged across the subject and then between subjects, giving a value of 722±181$mg.cm^{-3}$. The same process was conducted in a series of adult scans (unpublished data) – the mean maxima (1186±87$mg.cm^{-3}$) was found to be almost exactly 10% less than the apparent density of cortical bone (1300$mg.cm^{-3}$) used for calculating the threshold for pQCT analysis (650$mg.cm^{-3}$). Hence, a similar relationship was assumed – the same adjustment was

applied to the child maxima (maxima + 10%, then divided by two) – therefore a threshold of 400mg.cm^{-3} was used as the cortical threshold.

All follow-up images were then analysed to yield cortical bone area (Ar.ct, mm^2) and cortical bone mineral density (vBMD.ct, mg·cm^{-3}). Pericortical circumference (PcC, mm), endocortical circumference (EcC, mm) and density-weighted moment of inertia of the cortical portion (I_{pCort}, mm^4) were also examined. Adjustments were made to the bone density values in follow-up images only (due to the thin cortex) to take into account the partial volume effect [27]. Gross muscle cross-sectional area (MuscA, mm^2) was also calculated using a threshold of 35mg.mm^{-3} from a hand-drawn ROI outlining the outer muscle border (total area of tibia and fibula bones was then subtracted). As the original study was not aimed at muscle assessment, in some cases the limb was supported under the calf area to minimize movement artefacts – this compressed the muscle and thereby would bias results. In addition, the scanning volume was minimized to reduce radiation exposure for participants – therefore in some cases muscle boundaries were not completely visible. Finally, in some baseline scans in particular muscle and fat X-ray attenuation was more homogenous and a clear border could not be identified. Therefore when scans affected by these issues were excluded MuscA could only be confidently identified in twenty-five baseline scans (61%) and twenty-eight follow-up scans (53%). These data were therefore not included in the main analysis and only effects of gender were considered. There were no significant differences in measured variables between children included or excluded from MuscA assessment. Duplicate measurements of five subjects were obtained to determine short-term precision – CVs for vBMD.tot were 4.4%, vBMC.tot 4.1% and Ar.tot 6.2%.

Statistical analyses were performed using the R statistical environment (version 2.14.0, www.r-project.org). Independent t-tests were used to examine gender effects on characteristics such as mass, height and age at attainment of movement milestones. Multiple linear regression models were used to examine effects of gestation length, gender, body mass and length on baseline bone variables and movement milestones. Similarly, models were also used to examine effects of gender and age, height, body mass and length of time since attainment of movement milestones at follow-up on bone strength variables obtained by pQCT at follow-up. Effects were considered significant at $P < 0.05$. If factors did not have a significant effect on bone measures, they were removed one by one on the basis of highest *P*-value (with models re-calculated after every factor removal. Data were also log transformed if required to ensure that models satisfied assumptions of normality. Effect sizes of significant predictors in regression models were measured as partial eta-squared (η^2_p) – values of 0.02, 0.13 and 0.26 are suggested to indicate small, medium and large effects respectively [28].

Results

<Table 1 about here>

Cohort characteristics:

At birth, boys were longer ($P < 0.05$) and had higher mass and length Z-scores (both $P < 0.01$) (Table 1). At follow up, boys were heavier and taller than girls ($P < 0.001$) – although height Z-scores were similar, as was mass adjusted for height. There was no effect of birth

mass, birth length, gestation length, gender or any baseline bone strength variable on any movement milestone attainment age (as measured at follow-up). There was a strong relationship between length and mass at baseline ($R^2 = 0.63$) and height and mass at follow-up ($R^2 = 0.65$) - both $P < 0.001$. Cortical polar moment of inertia and endocortical circumference were log-transformed to ensure models satisfied assumptions of normality – inverse logarithms of the regression coefficients were then used in further analysis.

Birth mass, length, gender and gestation-corrected age were not significantly associated with any baseline bone variable. When age was not gestation-adjusted, there was a negative association between age and total bone mineral content at baseline ($P = 0.03$). Age at attainment of movement milestones was not significantly associated with birth mass, length, gestation length or gender.

Bone strength at follow-up – baseline predictors:

Mass at birth was positively associated with total BMC ($P = 0.001$) and total bone CSA, periosteal circumference and polar moment of inertia (all $P < 0.001$) (Table 2). Analysis of cortical bone revealed positive associations between birth mass and cortical CSA, endocortical circumference (both $P < 0.01$), pericortical circumference and polar moment of inertia (both $P < 0.001$). In addition, males had greater total BMC ($P < 0.001$), total and cortical BMD ($P < 0.01$) and endocortical circumference ($P < 0.05$). Length at birth and gestation length were not significantly associated with any follow-up bone variable.

<Table 2 about here>

Bone strength at follow-up – follow-up predictors:

<Table 3 about here>

At follow-up, total BMC, cortical bone polar moment of inertia (both $P < 0.001$), cortical bone CSA, pericortical circumference (both $P < 0.01$) and total polar moment of inertia ($P = 0.04$) were all positively associated with time since onset of unsupported walking (Figure 2). The effect sizes of these relationships were large in terms of both correlation strength and the magnitude of the observed differences. Walking time explained ~40% variance in bone mass and cortical polar moment of inertia, and a standard deviation increase in walking time was associated with a 0.5 standard deviation increase in both bone variables. There were no significant associations between time since onset of other movement milestones and any bone variable. Mass at follow-up was positively associated with total BMC, cortical CSA, both polar moment of inertia measures (all $P < 0.01$) and endocortical circumference ($P = 0.002$) (Table 3). Height was positively associated with total CSA, periosteal ($P < 0.001$) and endocortical circumferences ($P < 0.01$). Total BMC, total and cortical BMD were higher (all $P < 0.01$) and endocortical circumference lower (both $P < 0.05$) in boys than girls. When gestation length was considered, there was no association between age at follow-up and any bone variable. Similarly, there were also no significant associations between baseline bone strength measures and their corresponding measure at follow-up.

<Figure 2 about here>

Muscle size

Muscle size was greater in males at follow-up ($P = 0.003$) but not baseline (Table 1) – follow-up sex differences could not be explained by differences in body size or age. There was a positive association between MuscA and cortical bone area at follow-up ($P < 0.001$, $R^2 = 0.70$) which remained highly significant even when mass, age and height were considered. In contrast, no significant association between muscle size and bone area at birth was observed ($P = 0.28$).

Discussion

The aim of this study was to investigate the effects of attainment of movement milestones on bone strength in early childhood. Movement milestones represent early life exposure of lower limb bones to the large reaction and muscular forces required for control and negotiation of body mass against gravity. The main findings are that time since onset of independent walking (but not crawling, standing and supported walking) has a strong association with tibia bone strength at ~15 months, even when gender and body size are taken into account.

<Figure 3 about here>

It is perhaps surprising that bone strength at birth was unrelated to that at follow-up, but this may be in part related to the bone site under examination. Bone growth during childhood and adolescence occurs by two different mechanisms (Figure 3) [29, 30]. Endochondral ossification increases long bone length, and as periosteal apposition exceeds endocortical resorption [31] cortical thickness and bone mass also increase. During growth, there is a negative bone density gradient running distal-proximal through the metaphysis due to the delay between the two phases of endochondral ossification [29]. As the metaphysis is fairly constant throughout growth and limb length was shorter in children at birth, it is likely that the measurement was taken closer to the growth plate in younger children, and also in longer children. A cortical shell is evident in the tibial midshaft of infants prior to birth [32] – that this was not evident in baseline scans suggest that diaphyseal bone was not examined. However, that length was not a significant predictor of baseline bone traits is not supportive of this having a large influence on results. It would be useful for these measurements to be repeated at a true diaphyseal site (*e.g.* mid-shaft) to confirm this.

Approximately 93% of the variation in DXA-derived bone mass is explained by total body mass at one year [33], and small-for-gestational-age infants have lower bone mass than normal-for-gestational-age children [34], although negative effects do not persist into adulthood [35]. Whilst body mass and height were related to some bone strength in this study, relationships – particularly with height - were not as strong as those found previously. This could be due in part to the use of pQCT – which examines a transverse cross-section rather than the bone as a whole – as opposed to DXA. Bone mass is a product of bone's

cross-sectional properties and its length – therefore the direct influence of height or limb length on bone mass would not be evident from the current results. Toddler body height and mass are closely related [33] – as both were initially included in regression models, it is likely that one or another factor would be removed because of this inter-dependency. There was no effect of age at follow-up on bone strength - covariance of age and body size may also have resulted in the lack of an independent age effect. Also, homogeneity of participant age at baseline (standard deviation 16 days) may have led to an inability to detect an effect. Similarly, the lack of variation in gestation length (standard deviation 1.1 weeks) likely explains the absence of associations with bone strength and motor development, contrary to previous findings [7, 36].

Timing of attainment of independent walking represents exposure to single-limb acceleration of body mass against gravity and the long lever arm that *e.g.* the calf muscles have to work against. Muscular forces acting on the tibia in adult walking have been estimated at three times body mass [18]. When body mass is also considered, it can be assumed that independent walking represents the greatest forces experienced by the tibia at this age. Accordingly, we notice that whilst muscle and bone size are unrelated at birth, by follow-up there is a strong relationship between muscle size (a surrogate for maximal muscular force) and bone size (a surrogate for bone strength). This suggests that the strong muscle-bone relationships observed in older children [37] and adults [38] are not merely anthropometric associations. Instead, it is supportive of a primarily functional relationship, which only manifests itself when muscle influences the bone mechanically via contraction. That physical activity (in this case walking) is positively associated with bone strength at early age is similar to findings in older children [39], adolescents [40] and adults [12, 41].

Diaphyseal bone (such as the site examined in this study) predominately experiences bending and torsional strains [42]. Bone strength in bending and torsion is a product of bone mass and distance from centre of mass – therefore, the most efficient way to increase bending and torsional strength is periosteal apposition [43, 44]. Accordingly we see that this is the mechanism (rather than changes in cortical BMD) by which diaphyseal bone adapts to physical activity [45, 46]. The same adaptation pattern was found in the current study - greater bone mass in early walkers was a result of greater cortical area due to a greater pericortical circumference and similar endocortical circumference, resulting in greater compression and bending/torsional strength. A previous study found no associations between age at onset of walking and bone strength indicators in a cohort of 3-5 year old children [16]. However, children in that study had walked for – on average – more than three years whilst the standard deviation of age at onset of walking was 2 months. Given the large variability in length of time walking, the influence of these small differences in start age would likely not be detected. The current results support previous speculation that a rapid increase in lower limb bone strength at around 1.4 years of age could be due to the influence of walking onset [17]. In fact, the influence of physical activity may arise even earlier. Newborns with intra-uterine onset neuromuscular diseases (hence reducing inter-uterine activity) have greater fracture risk and lower bone strength than age and bone length-matched controls [47, 48].

Unlike walking, bone strength was not related to crawling, standing, or supported walking age – this may relate to the level of skeletal loading caused by each movement. The lack of association of supported walking with bone strength is surprising, but it is unclear to what extent parents were supporting the child's bodyweight during this movement. Whilst

children's supported walking speed at walking onset is quicker than when unsupported the reverse is true in developed walkers [49] – therefore experienced walkers would likely experience greater loading during unsupported walking. During crawling and standing bodyweight is supported on two or more limbs, whereas a single limb supports the body during walking. During early walking the forefoot is predominately in contact with the ground [50], creating a long lever for the calf muscle to act against. Hence, the effect of walking may have 'over-ridden' any previous associations with the other activities.

The finding of greater bone mass in male toddlers (even when age, height, mass and walking time are controlled for) agrees with a previous DXA study [6]. The causes of gender differences at baseline and follow-up are unclear. Males were significantly taller at baseline and follow-up, and at follow-up only they were much heavier than females (although this was in proportion to the height difference). Males tended to have walked unsupported for 33% longer ($P = 0.10$) – however gender differences in bone mass were still significant when walking age was considered. Physiological effects of gender may have influenced results, particularly in view of gender disparity at birth. However males participate in play more frequently, and in more physical pursuits such as ball sports and wrestling than girls [51], and encouragement of sex-typed play activities is the only area in which sex differences in parental behavior are consistently evident [52] therefore environmental influences may well contribute. This is supported by greater muscle size observed in males at follow-up but not birth which may be related to post-natal physical activity.

Passive range of motion exercise improves bone mass gains in pre-term infants [53, 54] - however, children born pre-term have delayed onset of walking [15] and such exercises

would likely not replace the osteogenic stimulus of walking. In other clinical groups *e.g.* cerebral palsy [55-57] and Down Syndrome patients [58, 59], walking age is later than in unaffected children and accompanies bone strength deficits and increased fracture risk. Therefore the current findings suggest that interventions to hasten walking onset or replicate walking strains may therefore well be effective in these groups. It is not clear what the limiting factor is in onset of walking – certainly muscular strength and co-ordination are required. Daily supported and treadmill–based walking leads to earlier independent walking in both healthy children [60] and Down Syndrome children [58, 61] with the latter walking three months earlier than untrained controls. However, effects of these interventions on bone strength have not been examined. Conversely, use of baby walkers is associated with delayed onset of sitting, crawling and walking [62]. Whilst children in South American, African and South Asian societies walk, stand and sit earlier than European and American peers [63], behaviours such as crawling which were not regularly practiced occurred later or not at all [64] – suggesting a strong influence of specific practice.

Analysis methods for pQCT studies at diaphyseal sites in adult cohorts are well-established, with a threshold of 650mg.mm^{-1} resulting in accurate assessment of bone geometry [65]. As demonstrated in Figure 1, this threshold would be inappropriate for young child scans – therefore we used a novel method of assessing appropriate cortical bone threshold. In order for results of pQCT studies in infants and young children to be compared – and for reference data to be established – a common analysis method should be established. The authors welcome any contributions as to how the method used in the current study may be adapted.

There are some limitations to this study. First, not all the patients studied at baseline were included/willing to participate in the follow-up – however, there were no systemic differences in recruited and non-recruited families. This was an observational study rather than a controlled intervention and the authors are unable to confidently suggest explanatory mechanisms for variability in walking age – which may relate to genetic or environmental influences or perhaps nutrition. Physical activity data relied on parental recall of developmental milestones up to 10 months previously - however, parental recall has excellent reliability even 3 years after milestone attainment [66]. Physical activity was not assessed directly, although bipedal locomotion most likely results in the greatest forces experienced by the lower limbs in early childhood. Therefore time since onset of independent walking indicates the period since first postnatal exposure of the tibia to habitual loading far in excess of body mass (when muscular forces are considered). The number of cycles required for maximal bone formation is as low as 36 when strain is high [67] – therefore walking volume (which was not captured) may not have greatly influenced bone adaptation. Future studies incorporating accelerometry and/or motion capture data would help verify that attainment of independent walking leads to increased physical activity and bone loading. It is unclear whether this early life advantage in bone strength in early walkers will persist into later childhood and adulthood. It would be extremely interesting to follow this cohort into older childhood in order to be able to answer this question. Given the relatively small sample size, repetition of this study in a larger cohort or interventional model should be completed to further investigate the current findings. This is particularly important with regards to the strong observed muscle-bone relationships, which for technical reasons (detailed in Materials and Methods) were only examined in a subset of the cohort.

In summary, the results of this study suggest that physical activity (in particular independent walking) is a strong determinant of bone strength in early childhood alongside body mass, height and gender. Validation of these findings in a larger cohort or interventional model would raise the challenging but exciting possibility for physical activity interventions to be implemented even at early age in populations for which late walking and low bone strength are prevalent, in an attempt to prevent early life bone weakness continuing through childhood, and potentially into adulthood and older age.

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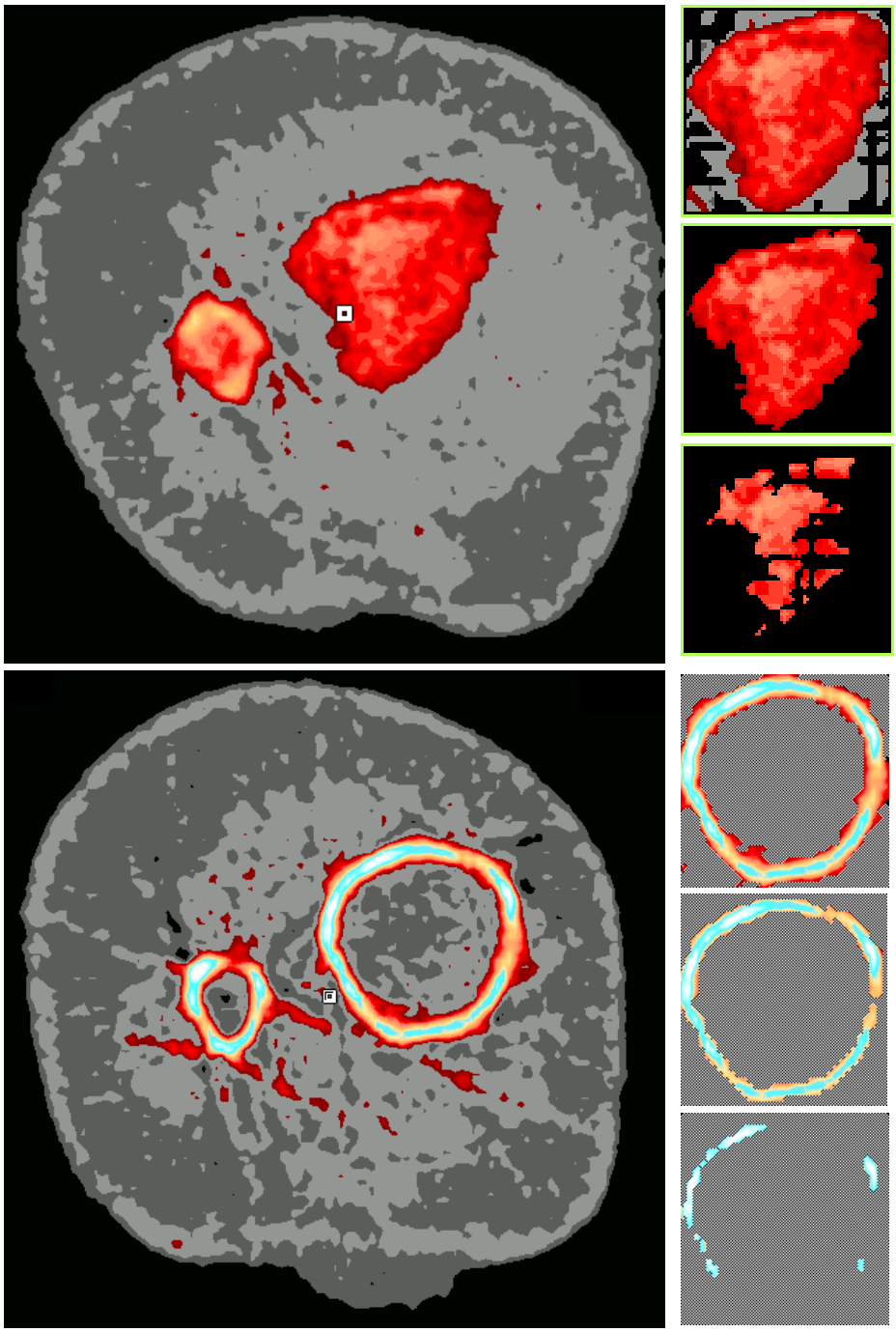

Figure 1. Effects of chosen threshold on bone analysis. Left panels shows original image (top image is a baseline image, bottom image is a follow-up image). Right panels alongside top image show (in descending order) the area excluded from analysis (in black) using thresholds of 80, 180 and 280 mg.cm^{-3} respectively. Right panels alongside bottom image show (in descending order) the area excluded from analysis (in grey) using thresholds of 180, 400 and 650 mg.cm^{-3} respectively .

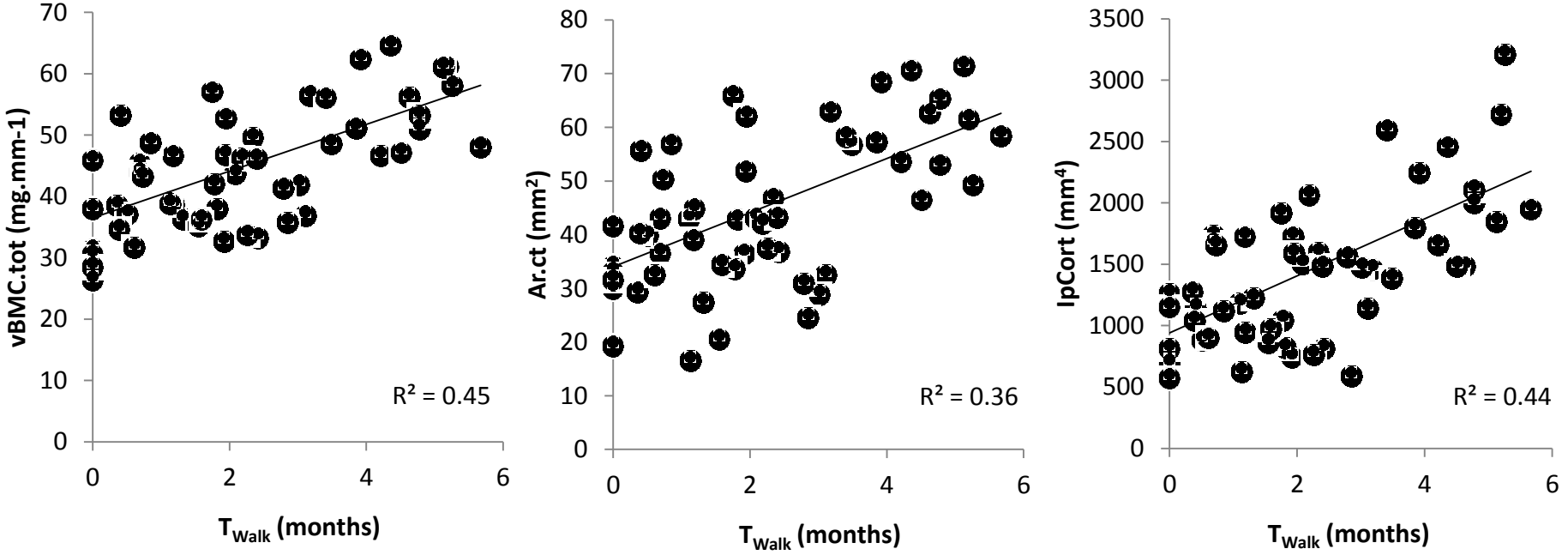

Figure 2. Relationships between time since onset of walking at follow-up (T_{Walk}) and unadjusted values of total bone mineral content(vBMC.tot), cortical bone CSA (Ar.ct) and polar moment of inertia (I_{pCort}) at follow-up. All regressions significant at $P < 0.001$.

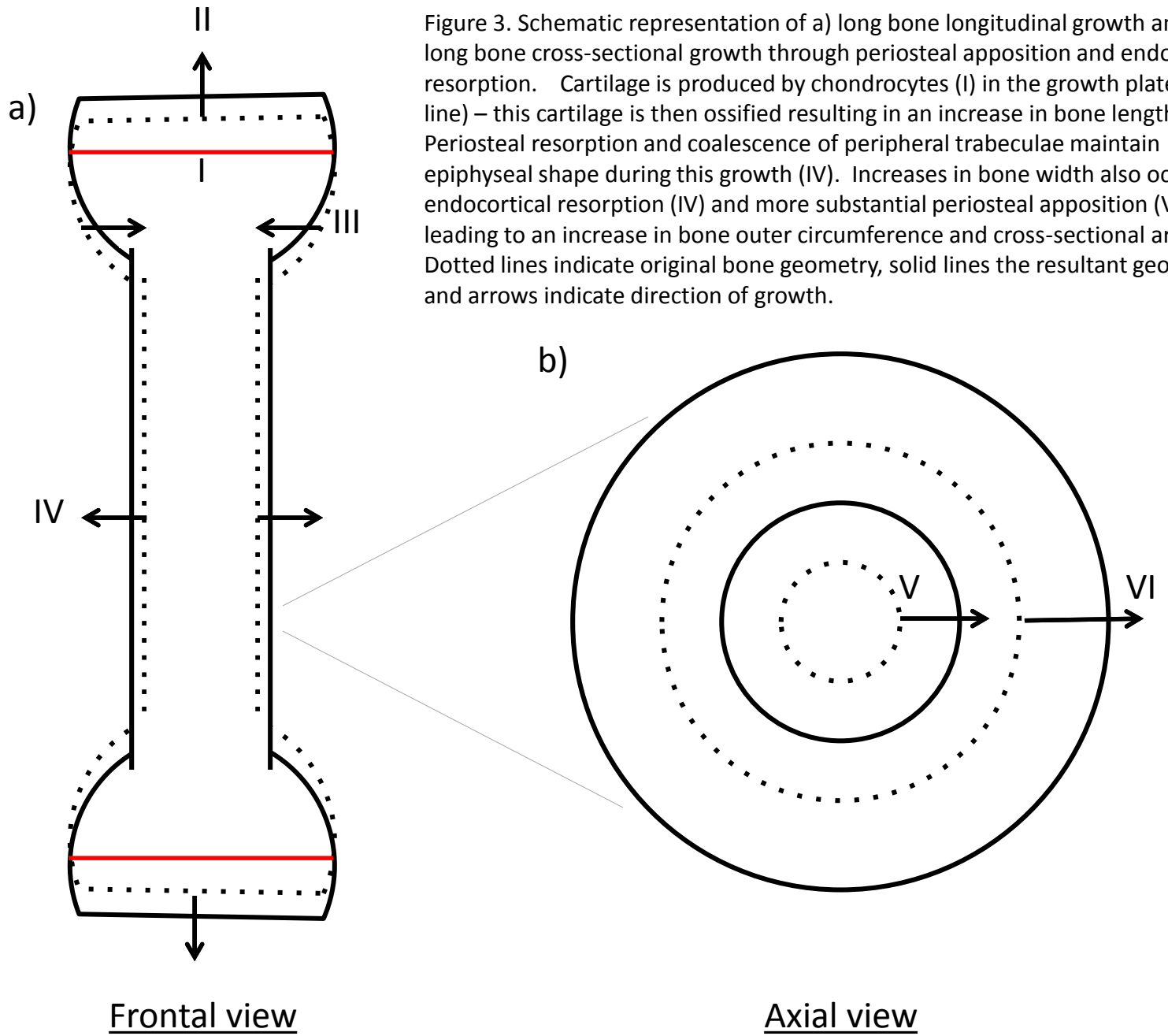

Figure 3. Schematic representation of a) long bone longitudinal growth and b) long bone cross-sectional growth through periosteal apposition and endocortical resorption. Cartilage is produced by chondrocytes (I) in the growth plate (red line) – this cartilage is then ossified resulting in an increase in bone length (II). Periosteal resorption and coalescence of peripheral trabeculae maintain epiphyseal shape during this growth (IV). Increases in bone width also occur (III) – endocortical resorption (IV) and more substantial periosteal apposition (V) leading to an increase in bone outer circumference and cross-sectional area. Dotted lines indicate original bone geometry, solid lines the resultant geometry and arrows indicate direction of growth.

Baseline measures			
Variable		Males	Females
N		22	19
Age (days)		11.5 (9.0)	10.3 (8.6)
Age range (days)		1-32	
Mass (kg)		3.66 (0.43)	3.51 (0.50)
Mass Z-score		0.31 (1.00)**	-0.52 (0.97)
Length (cm)		51.4 (1.8)*	50.0 (2.1)
Length Z-score		0.37 (0.91)***	-0.73(0.99)
Gestation length (wks)		40.6 (1.1)	40.9 (1.2)
Total BMC ($mg.mm^{-1}$)		37.1 (6.9)	36.3 (6.6)
Total bone CSA (mm^2)		80.0 (27.4)	80.0 (19.1)
Total BMD ($mg.mm^{-3}$)		487 (95)	466 (72)
Muscle CSA (mm^2) [a]		244 (59)	255 (85)
Follow-up measures			
Variable		Males	Females
N		27	26
Age (months)		14.9 (0.5)	14.7 (0.5)
Age range (months)		13.9-16.2	
Mass (kg)		11.3 (1.1)***	10.1 (1.4)
Relative mass (%)		0.3 (6.0)	-1.7 (9.5)
Height (cm)		79.8 (2.9)***	76.8 (2.9)
Height Z-score		0.34 (1.06)	-0.18 (1.12)
Crawling age (months)		8.3 (1.9)	8.7 (1.7)
Standing age (months)		8.7 (1.6)	8.9 (1.6)
Supported walking age (months)		9.8 (1.6)	9.8 (1.5)
Unsupported walking age (months)		12.2 (1.6)	12.5 (1.4)
Primary Analysis	Total BMC ($mg.mm^{-1}$)	49.9 (8.7)***	40.2 (7.7)
	Total bone CSA (mm^2)	146.6 (31.6)	145.5 (34.0)
	Total BMD ($mg.mm^{-3}$)	479 (77)**	411 (60)
	Periosteal circumference (mm)	42.7 (4.7)	42.5 (5.0)
	Polar moment of inertia (mm^4)	3262 (1180)	3036 (1349)
Secondary cortical analysis	Cortical bone CSA (mm^2)	52.8 (11.7)	37.9 (12.1)
	Cortical BMD ($mg.mm^{-3}$)	554 (67)**	505 (52)
	Pericortical circumference (mm)	36.7 (4.9)	34.3 (5.9)
	Endocortical circumference (mm)	33.8 (6.0)*	36.3 (6.3)
	Polar moment of inertia (mm^4)	1667 (546)	1249 (556)
Muscle CSA (mm^2) [b]		559 (59)**	402 (60)

Table 1. Cohort characteristics at baseline and follow-up as mean (SD), separated by gender. Asterisks indicate significant gender difference: *P – 0.05, ** - $P < 0.01$, *** - $P < 0.001$. Gender effects on bone and muscle variables were examined with multiple linear regression, for all other variables independent T-tests were used. Primary analysis was completed using a peeling threshold of $180mg.mm^{-3}$ – cortical analysis was completed using a threshold of $400mg.mm^{-3}$ as described in the 'Data processing and statistical analyses' section of *Materials and Methods.* [a]Data obtained in a subset of 12 males and 13 females. [b]Data obtained in a subset of 16 males and 12 females.'

Source	Variable	Prediction of variable by multiple regression							
		Gender			Mass			Final model	
		SRC	η^2_p	*P*	SRC	η^2_p	*P*	R^2	*P*
Primary analysis	Total BMC ($mg.mm^{-1}$)	0.42	0.22	<0.001	0.4	0.21	0.001	0.37	<0.001
	Total bone CSA (mm^2)				0.51	0.26	<0.001	0.24	<0.001
	Total BMD ($mg.mm^{-3}$)	0.43	0.22	0.001				0.17	0.001
	Periosteal circumference (mm)				0.52	0.27	<0.001	0.25	<0.001
	Polar moment of inertia (mm^4)				0.47	0.22	<0.001	0.2	<0.001
Secondary cortical analysis	Cortical bone CSA (mm^2)				0.41	0.17	0.003	0.15	0.003
	Cortical BMD ($mg.mm^{-3}$)	0.39	0.19	0.004				0.13	0.004
	Pericortical circumference (mm)				0.52	0.24	<0.001	0.22	<0.001
	Endocortical circumference (mm)	-0.28	0.08	0.04	0.4	0.16	0.005	0.15	0.007
	Polar moment of inertia (mm^4)				0.56	0.26	<0.001	0.3	<0.001

Table 2. Standardised regression coefficients (SRC), partial Eta-squared (η^2_p) and *P*-values for predictive models of bone strength at follow-up and baseline characteristics. Factor gender considers male traits with respect to female traits *i.e.* positive co-efficients indicate greater values in males and *vice versa*. Primary analysis was completed using a peeling threshold of $180mg.mm^{-3}$ – cortical analysis was completed using a threshold of $400mg.mm^{-3}$ as described in the 'Data processing and statistical analyses' section of *Materials and Methods.*

Source	Variable	Prediction of relevant variable by multiple regression													
		Gender			Mass			Height			Walk			Final model	
		SRC	η^2_p	*P*	SRC	η^2_p	*P*	SRC	η^2_p	*P*	SRC	η^2_p	*P*	R^2	*P*
Primary analysis	Total BMC ($mg.mm^{-1}$)	0.25	0.15	0.005	0.39	0.26	<0.001				0.48	0.42	<0.001	0.68	<0.001
	Total bone CSA (mm^2)							0.5	0.25	<0.001				0.23	<0.001
	Total BMD ($mg.mm^{-3}$)	0.43	0.22	0.001										0.17	0.001
	Periosteal circumference (mm)							0.51	0.26	<0.001				0.25	<0.001
	Polar moment of inertia (mm^4)				0.44	0.29	<0.001				0.25	0.07	0.04	0.29	<0.001
Secondary cortical analysis	Cortical bone CSA (mm^2)				0.47	0.12	<0.001				0.37	0.29	0.001	0.45	<0.001
	Cortical BMD ($mg.mm^{-3}$)	0.39	0.19	0.004										0.13	0.004
	Pericortical circumference (mm)							0.39	0.18	0.002	0.34	0.14	0.007	0.35	<0.001
	Endocortical circumference (mm)	-0.38	0.13	0.009	0.44	0.17	0.002							0.17	0.003
	Polar moment of inertia (mm^4)				0.45	0.33	<0.001				0.52	0.39	<0.001	0.6	<0.001

Table 3. Standardised regression co-efficients (SRC), partial Eta-squared (η^2_p) and *P*-values for predictive models of bone strength at follow-up using other follow-up characteristics. Primary analysis was completed using a peeling threshold of $180mg.mm^{-3}$ – cortical analysis was completed using a threshold of $400mg.mm^{-3}$ as described in the 'Data processing and statistical analyses' section of *Materials and Methods.*