BONE MINERAL ACCRUAL DURING CHILDHOOD AND ADOLESCENCE: A CRITICAL ANALYSIS OF SIZE-CORRECTION TECHNIQUES

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Abstract

The true pattern of bone mineral density changes during childhood and adolescence is unclear owing to a lack of longitudinal investigations, a lack of adequate control for maturational differences, and size-mediated errors in the measurement of areal bone mineral density (aBMD). This investigation incorporated mixed-longitudinal (distance data) and longitudinal (velocity data) designs to describe pediatric changes in bone density at the total body (TB), femoral neck (FN) and lumbar spine (LS). Maturational differences were controlled by aligning participants on their age at peak height velocity (PHV). Changes in areal BMD (aBMD) were compared with densities obtained from two methods of size correction: bone mineral apparent density (BMAD) based on geometric assumptions, and statistically-corrected BMD (sBMD), which utilizes linear regression. Correlations between bone projected area (PA) and density were strongest with aBMD, intermediate with BMAD, and generally insignificant with sBMD, supporting sBMD's size independence. With the distance data aBMD increased over the entire growth period at all sites. Contrastingly, TB BMAD declined initially and then stabilized after PHV, and FN and LS BMAD were generally stable until PHV, increasing afterward. Similarly, TB and LS sBMD decreased until PHV, increasing afterward, and FN sBMD was stable until PHV, increasing thereafter. aBMD velocity was positive at all sites and all ages. In contrast, TB and FN BMAD had a negative velocity until after PHV, and LS BMAD velocity was generally stable until near PHV, with a positive velocity afterwards. sBMD velocity was negative at the TB and LS until PHV and there was a stabilization (males) or decrease in velocity (females) in FN sBMD until PHV. Velocity curves for PA and bone mineral content displayed a consistent dissociation at all sites with bones increasing in area first and later consolidating when rapid growth ceased or slowed. The point of minimal density suggested from the corrected data coincided with PHV and is supported by epidemiological data that reports the highest rates of fracture in adolescents during this time. These results highlight the size-dependence of aBMD and cautions against its use in the pediatric population. Physiologically, sBMD appeared the more appropriate size-correction technique.

Acknowledgements

This extraordinary investigation is the result of many people's work. I would like to first thank my committee members, Dr. Robert Faulkner, Dr. Don Bailey, Dr. Don Drinkwater and Dr. Paul Peloso for their continual support and guidance. I would also like to acknowledge the wise contributions made to this thesis by my external examiner, Dr. Thomas Beck, of Johns Hopkins Hospital. Further, I would like to particularly thank Dr. Heather McKay for all of her hard work in the early years of the Saskatchewan Pediatric Bone Mineral Accrual Study and to my brother, Brian Davison, for all his computer support. Lastly, a hearty thanks to all the children who gave unselfishly of themselves for this investigation, for the advancement of our knowledge in how bone changes with growth.

Dedication

This thesis is dedicated to the two women who have inspired me to be a better person.

To my mother, Marion Davison, who instilled in me the passion for learning, demonstrated to me

the meaning of sacrifice for those you love,

and taught me early that "might doesn't mean right."

I love you, Mom.

To my angel, Joan Heimbecker, who taught me to love life,

to laugh heartily, and

to appreciate every day.

I still miss your smile, Joan.

The woods are lovely, dark, and deep,
But I have promises to keep,
And miles to go before I sleep,
And miles to go before I sleep.

- R. Frost

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List of Abbreviations

- BMD: bone mineral density
- aBMD: areal bone mineral density
- sBMD: statistically corrected bone mineral density
- BMAD: bone mineral apparent density
- PA: bone projected area
- BMC: bone mineral content
- BMDvol: volumetric bone mineral density at proposed by Kroger et al.

- SPA: single photon absorptiometer
- SXA: single x-ray absorptiometry
- DPA: dual-energy photon absorptiometer
- DXA: dual-energy x-ray absorptiometer
- QCT: quantitative computerized tomographer
- pQCT: peripheral quantitative computerized tomographer

- LS: lumbar spine (L1-L4)
- FN: femoral neck
- PF: proximal femur
- TB: total body
- GT: greater trochanter
- FS: femoral shaft

- PHV: peak height velocity in cm/yr
- PBMAV: peak bone mineral accrual velocity in g/yr
- PBM: peak bone mass
- SPBMAS: Saskatchewan Pediatric Bone Mineral Accrual Study
- Yrs: Years

1 Introduction

1.1 *Bone, more than a framework for locomotion*

The skeleton is a series of interconnecting levers, articulated by muscles, which aid in the movement of an organism around its environment and provide scaffolding for the body's tissues. However, bone, a specialized connective tissue, plays additional crucial roles in haemostases (most significantly calcium and phosphate concentration), erythropoesis, vital organ protection (i.e. brain, heart, lungs), sound wave conduction (inner ear), and immune function.

Despite the integral roles bone plays in the body, there is a paucity of information regarding its normal physiology and response to stimuli at the tissue and cellular levels, especially when compared to other more metabolically active tissues such as muscle. There are numerous arguments as to why bone research has comparatively lagged behind in scientific understanding. Firstly, bone was previously erroneously believed to be a relatively static tissue with limited ability to adapt; in reality, bone is a dynamic tissue that is responsive to both mechanical and hormonal stimuli via pathways still incompletely understood. Secondly, bone's comparatively slow rate of turnover, and adaptation, make its investigation time-consuming and expensive. Lastly, the relatively central position of bone within the body makes its study difficult, often necessitating undesirable invasive or radiographic techniques. Invasive techniques commonly elicit an acute reparative response which overshadows any chronic adaption to the introduced stimulus, and accurate and valid radiographic techniques often emit unsafe levels of radiation to make their use widespread except in those instances where trauma or pathology necessitates it.

1.2 *Osteoporosis*

Osteoporosis is a debilitating disease defined by pathologically low bone mass and microarchitectural deterioration resulting in skeletal fragility and an associated increased risk of fracture (1).

In 1992, a 50 year-old Caucasian woman had an approximate 40% risk of at least a hip, spine or wrist fracture occurring during her remaining lifespan (2) and it was estimated that in North American Caucasians one-third of women and one-sixth of men over the age of 65 yrs experienced osteoporosis-attributable hip fractures (3). World-wide projections predict that by the year 2050 there will be a 310% increase in men and a 240% increase in women who experience hip fractures (4). In Canada, the prevalence of osteoporosis-related fractures is expected to increase by 73% between the years 1987 and 2006; at least partially a consequence of an aging population (5). Similarly, it is predicted that the rate of hip fracture over the next 40 years in Canada will increase exponentially, further taxing an already overburdened medical system and resulting in tragic losses of personal independence (6).

The economic and personal implications of osteoporosis and its associated fractures are staggering. In 1993 the annual hospitalization, treatment and rehabilitation costs of osteoporosis in Canada was estimated to be $1.3 billion (7). More importantly, osteoporotic fracture is associated with a decrease in personal independence and quality of life for the effected individual, particularly in the case of hip fracture (8). Following a fracture of the hip, individuals experience significant morbidity and loss of independence, and approximately a quarter face mortality in the year following the fracture due to fracture-related complications (8). Vertebral fractures also significantly impact quality of life (9-11), whether diagnosed or undiagnosed (12), and mortality after five years is approximately the same as that experienced with hip fracture (8;13;14). These statistics illustrate the importance of increased investigation of methods to treat, and more importantly, prevent osteoporosis.

Preventative strategies to avoid the premature onset of osteoporosis include the maximization of bone mass accrual during growth, the maintenance of skeletal mass through adulthood, and the reduction of loss associated with old age and menopause (in females).

Genetics account for approximately 50-80% of the variability in bone mass, with the remaining being attributable to modifiable environmental factors (15;16-24); consequently, 20-50% of the variability in bone mass can be modified through choices in health behaviour or through the administration of bone active pharmaceuticals. In light of the relative plasticity of bone mass levels with regards to modifiable factors, numerous investigators have studied the effects of interventions to maximize, maintain, or slow the losses of bone during different phases of life. The most commonly investigated interventions include dietary modification, physical activity, and bone-active pharmaceuticals.

1.2.1 Treatment of osteoporosis – geriatric

The bulk of bone research has focused on interventions that maintain or increase bone mass through adulthood and arrest or slow bone loss in the elderly. Outside of pharmaceutical interventions, the preponderance of research suggests that after peak bone mass (PBM) has been realized the most that can be expected is maintenance (25).

The treatment of osteoporosis is difficult due to the pathogenesis of the disease, particularly at the histomorphometric level. While many pharmaceutical therapies are efficacious in slowing accelerated levels of bone resorptive activity (i.e. hormone replacement therapy, bisphosphonates, selective estrogen receptor modulators) (26), there is no proven effective anabolic therapy. hPTH (1-34) therapy is promising for the future, displaying anabolic action in a preliminary trial in humans, but requires further investigation (27). The absolute losses in bone mass alone do not adequately account for the decrease in mechanical integrity that result from the disintegration of cancellous bone structure in severe osteoporosis (28-35). In advanced osteoporosis, elevated bone turnover and imbalance between osteoblast and osteoclast activity cause trabecular struts within the medullary cavity to become irreparably disjointed from extensive osteoclastic resorption and no longer contribute to mechanical integrity of the bone as a whole (35-37). Following trabecular malunion even radical treatment is largely ineffective and fragility fractures almost invariably result (38). Even the anabolic activity of PTH cannot repair disjointed trabeculae. Considering the relative irreversibility of osteoporosis, prevention is of paramount significance (39).

1.2.2 Prevention of osteoporosis– paediatric

Osteoporosis has prominent pediatric antecedents (40-44). Attainment of an optimal PBM is crucial for lifelong skeletal adequacy, and has been suggested to be the best indicator of susceptibility to osteoporosis in later life (45-51). Approximately half of the variability in elderly bone mass is a product of the bone mass that was acquired during childhood and adolescence (52), and many investigations have demonstrated that at least 90% of the total adult bone mineral has been deposited by the end of adolescence (48;53-59). Puberty is an especially critical period of bone growth considering that in the four years surrounding peak height velocity (PHV) there is as much bone mineral laid down as is lost during the whole of adult life (59). The adolescent years may be the optimal time for gains in bone mass and also the most sensitive time to any perturbations that decelerate or reverse accrual (40;48;54). To prevent osteoporosis it is essential to delineate and encourage those interventions that maximize bone accrual during adolescence and to avoid those that slow or reverse normal accrual (60).

1.3 *Development of normative bone density values in pediatrics*

When investigating bone mass during growth prudence must be taken to ensure that the effects ascribed to interventions are not those that would be observed from normal growth and maturation alone; that is, during periods of rapid growth positive accrual rates could be wrongly attributed to the intervention. Before the impact of interventions, or perturbations, on pediatric bone can be established it is imperative to first describe bone accrual and density in normal children and adolescents through the development of normative growth curves. The development of normative bone mineral values are further necessary to identify children and adolescents who may have bone mass low enough to be deemed clinically relevant (56;61).

While a moderate number of investigations have attempted to describe the changes in bone density through childhood and adolescence, few to date have done so in a methodologically sound manner. Problems in study design, the methods for grouping of individuals, and the tools used to estimate bone density are the foremost sources of error leading to the creation of misleading standards.

The preponderance of normative research in pediatric bone mineral accrual has been cross-sectional in design. Growth data derived from cross-sectional investigations tend to be smoothed and mask the individual variation between individuals, leading to the development of incorrect or imprecise standards (62). Longitudinal data must be used to develop the shape of the growth curve around the time of puberty, otherwise serious error can result (63). Further, longitudinal standards are required when a child is observed on more than one occasion, particularly during adolescence (63). Longitudinal investigations are rare owing to the tremendous financial and personnel commitment, as well as problems with attrition (64). Once normal growth curves are developed, effective interventions can be identified and utilized to promote appropriate behaviour to augment PBM.

Secondly, the bulk of normative bone research in children and adolescents has grouped individuals by chronological age. By organizing growth data based on chronological age and not maturational age the true magnitude and shape of the group growth curve is attenuated and not representative of individual growth (the dashed line in the upper panel of Figure 1. demonstrates the average growth curve that would be produced if subjects were aligned on chronological age, and the bottom panel displays the same data but grouped on maturational age). This attenuation occurs as a consequence of the tremendous variability in the timing of puberty in both males and females. As is obvious from Figure 1., any attempt to describe growth through puberty based on chronological groupings is flawed. Individuals should be grouped on a maturationally-based benchmark to properly compare changes with growth and development, particularly during adolescence.

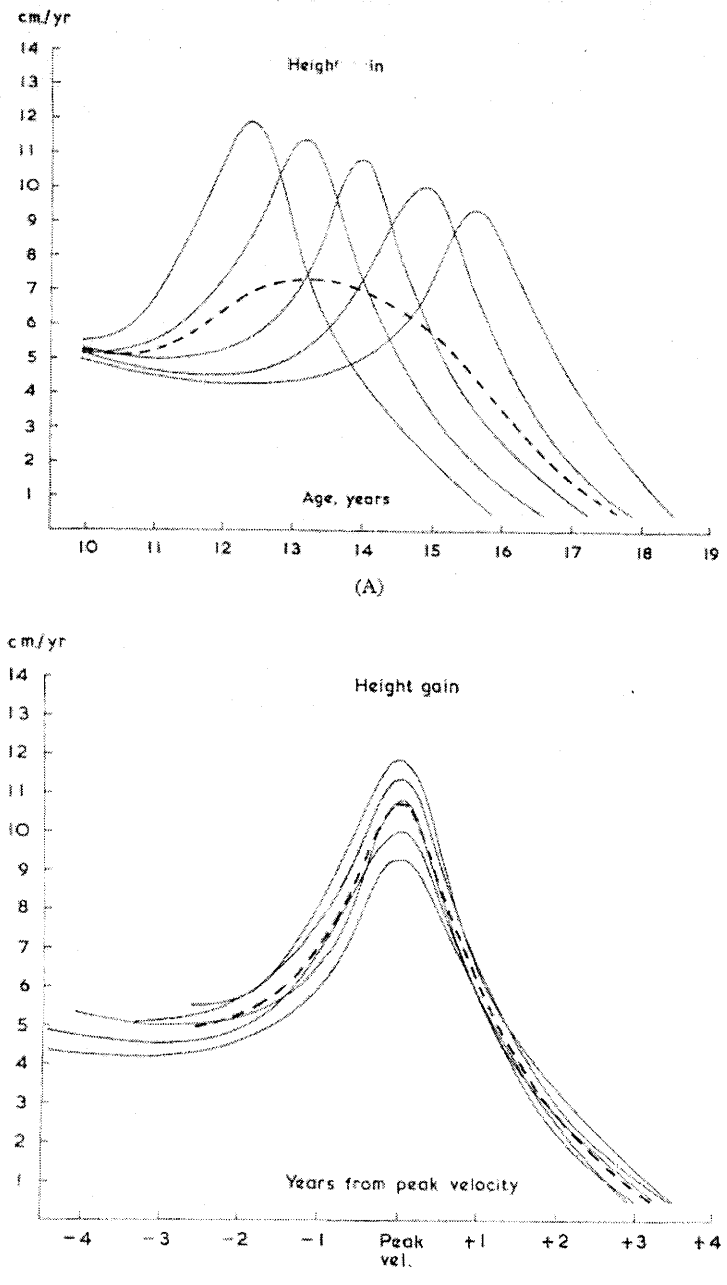


Figure 1.1. Methodological problems with using chronologically-based data for normative growth curve development during adolescence. From Tanner et al. 1966 (65).

Lastly, almost all of the pediatric normative bone research in the past decade has utilized dual-energy x-ray absorptiometry (DXA) to estimate areal bone mineral density (aBMD). Confounding the already limited understanding of bone growth in children, DXA-derived aBMD provides only an estimate of volumetric bone density. Measurement of aBMD by DXA is flawed as it inadequately corrects for bone size, leading to consistent overestimation of density with larger

and underestimation with smaller bones (56;66-69). This error in the estimation of aBMD is particularly misleading in longitudinal study of the pediatric population where rapid growth is punctuated with dramatic changes in bone size, and in cross-sectional investigations where children of similar chronological age can have vastly different bone sizes. Observed longitudinal changes in aBMD as measured by DXA in pediatric investigations can be ascribed to either increases in density, bone size, or a combination of both. With current DXA technology it is impossible to discern which combination of these factors account for the change in estimated bone density observed.

To conclude, longitudinal normative bone density values, independent of bone size and based on a maturationally-grouped cohort, need to be generated before the affects of interventions on pediatric BMD can be determined and to allow for the identification of children and adolescents who have clinically low levels of bone mass.

This investigation compared DXA-derived aBMD with two hypothetical size-corrected BMD values over adolescence in an investigation of longitudinal (and mixed-longitudinal) design with maturationally-controlled comparisons.

2 Review of Literature

The following reviews the general trends in bone growth through childhood and adolescence and discusses how these trends may be erroneous as a consequence of the dependence of DXA-derived aBMD on bone size. First, the different measurement techniques and tools utilized to establish bone mineral mass and/or density will be introduced and their strengths and weaknesses will be briefly discussed. Second, the use of DXA for the measurement of aBMD, and its inherent strengths and problems, will be addressed. Third, the scaling of biological entities for differences in body size is discussed, with a particular emphasis on skeletal structure. Fourth, changes in bone size, mass and density with growth and maturation are presented at the three most frequently assessed areas: the total body (TB), femoral neck (FN), and lumbar spine (LS). Fifth, techniques that have been utilized for the correction of DXA-derived aBMD for bone size will be examined, along with a comparison of corrected and uncorrected BMD trends during growth. Lastly, the underlying physiological mechanisms that may be responsible for the oft-reported phenomenon of an increased period of skeletal fragility during adolescence are discussed.

2.1 *Measurement of bone mass*

The measurement of bone mineral by radiographic techniques is based on the principle that a direct correlation exists between the amount of bone present and the absorption of ionizing radiation. Conventional radiography is inadequate to evaluate bone objectively as bone mass may decrease 30-40% before changes in bone density are optically apparent as osteopenia (70).

Bone densitometry is founded on the supposition that the amount of bone mass present at a given skeletal site is directly correlated with the strength of that site (71). While this relationship is well established in osteoporotic bone, it is not as well defined in non-osteoporotic bone. Bone density as assessed by densitometric methods has been consistently demonstrated to have significant correlations with bone strength and therefore, fracture risk (72-78). While BMD is an

important determinant of skeletal integrity, other factors, such as bone geometry and architecture, also impact bone strength (28;71;79-82).

In the contemporary measurement of bone mass the mostly commonly reported variable is bone mineral density (BMD). However, the method of determining BMD is inconsistent between instruments making it difficult to ascertain what each respective measurement of BMD is portraying without a working knowledge of the instrument and its limitations and assumptions. For example, aBMD by DXA provides an areal density, single photon absorptiometry (SPA) estimates a density derived from the length of the region of interest, and a true volumetric density is provided by quantitative computerized tomography (QCT), however they are all often reported simply as BMD.

QCT is the only instrument available that is able to provide a true, volumetric measure of BMD in vivo (83). Volumetric bone density refers to the amount of mineralized tissue per unit volume of tissue (osseus and non-osseus). Volumetric bone density must be distinguished from the intrinsic material density of bone, which is expressed as the ash weight per unit volume of bone (osseus only). The material density reflects the degree with which the bone matrix is mineralized and is variable over any particular bone, although the variability is typically small (84). BMD measures from SPA, dual photon absorptiometry (DPA) and DXA are not true measures of density, but often a poor estimate based on the most available linear or areal measures of the region of interest. aBMD does not take into consideration the nonmineralized spaces within bone, such as the medullary cavity, or intracortical remodeling sites or for channels to allow for the passage of extracellular fluids or vasculation, when determining bone volume. These assumptions lead to a density that is assumed to be homogeneous across the bone's volume (84).

Table 1. presents the precision, accuracy, scanning time and radiation dose for the most commonly used densitometric techniques (71;85). Errors in the noninvasive measurement of bone include both technical and biologic factors including source variability, radiation scatter, differing marrow composition, fat content both within and surrounding (overlying/underlying) the bone, fractures, osteophyte formation, extraosseus calcification, and subject positioning (85).

Table 2.1. Comparison of precision error, accuracy error, and radiation dose of techniques for bone mineral measurement.

Technique	*Cortical/ Trabecular Ratio*	*Precision in Vivo (%)*	*Accuracy Error (%)*	*Scanning time (min)*	*Effective Dose Equivalent (μSv)*
SPA					
Distal third radius	95/5	1-2	4-6	10	<1
Ultradistal radius	60/40	1-2	4-6	10	<1
Os calcis	5/95	1-2	4-6	15	<1
DXA					
PA spine	50/50	1-1.5	4-10	5-10	~1
Lat spine	10/90	2-3	5-15	15-20	~3
Proximal femur	60/40	1.5-3	6	5-10	~1
Forearm		~1	5		<1
Whole body	80/20	~1	3	20	~3
QCT					
Spine trabecular	0/100	2-4	5-15	20	~50
Spine integral	?	2-4	4-8	25	~50
pQCT					
radius trabecular	0/100	1-2	?	3	~1
radius total	site dependent	1-2	2-8	3	~1
US					
SOS calcaneus/tibia	?	0.3-1.2	?	1	0
BUA calcaneus	?	1.3-3.8	?	1	0

Adapted from Genant, 1996 (85) and Faulkner, 1991 (71).

2.1.1 Single photon absorptiometry (SPA) and single x-ray absorptiometry (SXA)

SPA and SXA measure appendicular sites (usually the radius) where variations in soft tissue are minimal (86). SXA has improved precision and spatial resolution and has a reduced exposure time as compared to SPA (85). A highly collimated photon beam from a radioactive source (SPA), or an x-ray tube (SXA), is directed towards the region of interest to measure radiation attenuation, and from that the estimation of bone mineral content (BMC, in g) (85). BMD is calculated by BMC divided by the length (cm) of the region of interest (g/cm). Since neither the shape nor the depth of the radius can be assessed and the size and shape of the medullary cavity is unknown, precise volumetric or intrinsic bone mineral density cannot be established from SPA (87). Further, since SXA and SPA are based on an areal measure they are unable to ascertain measurements of density for the different bone envelopes. However, evidence has suggested that SPA and SXA can predict fracture risk, particularly in non-spinal sites with predominant cortical bone composition (45;88-90).

2.1.2 Dual photon absorptiometry (DPA)

Single-energy measurements, such as SPA, cannot assess BMD at sites with variable tissue thickness and composition (ie. axial skeleton, hip, whole body, lumbar spine) (85). DPA, a predecessor of DXA, is primarily used to evaluate axial sites where there is both bone and substantial soft tissues present. The low radiation associated with this technique and the ability to non-invasively assess axial sites made it a popular choice in the 1980's (86). With DPA, BMC (g) is measured by the attenuation of two differing energies of photons, which is then divided by the region of interest surface area (cm^2) to estimate aBMD (g/cm^2). DPA uses a radioactive isotope (^{153}Gd) which decays over time necessitating constant adjustments in the attenuation values and making measurements of mass and density variable. This variability introduces error that is often larger than the effect of the intervention making its use for research limited.

2.1.3 Dual-energy x-ray absorptiometry (DXA)

DXAs are the tool most widely utilized for the assessment of appendicular and axial bone mineral density (91) owing to their high speed, low radiation density, accuracy, reliability and relatively inexpensive capital costs (86;92). DXA measures bone mass by posteriorly emitting two energies of x-rays that pass though the body's hard and soft tissues and are collected by a collimator after passing out the anterior surface of the subject. The differential absorption of these two energies of x-rays within the body allow for DXA, after comparing the absorption to a calibration phantom, to estimate bone mass (as well as lean and fat mass). The use of an x-ray tube instead of an isotopic source decreases radiation exposure, reduces scanning times, improves both short and long term precision, and enhances image resolution (71;85). DXA provides an areal density (g/cm^2), based on the same calculation as the DPA. Unfortunately, areal BMD is unable to distinguish between osseus and non-osseus areas within the bone envelope, rather, assuming it to be a homogenous substance.

Recent modifications to DXA have included the ability to perform lateral lumbar spine scans. Lateral DXA may have increased sensitivity in the diagnosis of osteoporosis since it eliminates the posterior elements of the vertebrae, which are primarily of cortical bone (losses in cortical bone are typically observed following dramatic losses in trabecular bone). By the removal of the posterior elements from the LS measurement, a greater proportion of the vertebral body, and it's primarily trabecular bone, is measured (85). However, the precision of lateral DXA is lower (6.4%) and often L2 or L4 cannot be measured due to the presence of an overlying rib or the iliac crest, respectively (93). Some DXAs are able to estimate volumetric density by taking both lateral and PA scans of the LS region and calculating volumetric density (g/cm^3). While these lateral DXA-derived vertebral volumetric densities theoretically are robust, they combine the errors from both the posterior-anterior and lateral scans, making them highly variable and questionable for scientific investigation.

2.1.4 Ultrasound

Ultrasound is an attractive option for the measurement of bone mass due to its low relative cost, portability, ease of use and lack of ionizing radiation (85). Two forms of ultrasound have been developed: speed of sound and broadband ultrasound attenuation. The speed of sound ultrasound is postulated to produce a signal influenced by the elastic properties of bone, while the broadband ultrasound attenuation is believed to provide an estimate of physical density (94). Most ultrasounds now provide both of these measurements within their protocol. Common measurement sites include the patella and the os calcis. While ultrasound is a promising research tool, the technology is still in its infancy and needs further validation (94), and to date no commercial ultrasound has the capability to measure the elastic modulus of a bone.

2.1.5 Quantitative computerized tomography (QCT)

2.1.5.1 Axial quantitative computerized tomography

The only way to assess true volumetric density (g/cm^3) *in vivo* is through QCT, since it is capable of obtaining an accurate and valid measure of both bone volume and mass (85). Further, it is able to discern between trabecular and cortical bone mass. Accordingly, the best sensitivity and specificity for diagnosing patients with spinal osteoporosis is provided by QCT, followed by DXA, DPA and then SPA (71). However, even with QCT there are sources of error: error in the measurement of vertebral cortical bone owing to the thinness of the cortical shell and differing levels of intravertebral marrow fat lead to errors in the estimation of trabecular density (85;95). Unfortunately, regardless of the diagnostic accuracy of QCT, the high doses of radiation associated with QCT make it restricted to only those individuals that have undergone some form of traumatic accident or require clinical investigation for pathology, and is not justifiable for serial scientific research into children's bone density.

2.1.5.2 Peripheral quantitative computerized tomography (pQCT)

Recent advancements in QCT design have allowed for the development of peripheral QCT (pQCT). The pQCT is used to evaluate bone density at appendicular sites, primarily the distal radius and ulna. pQCT has an advantage over the SPA as it allows for separate measurements of

the cortical and trabecular bone compartments and provides a true volumetric density. Radiation doses associated with the pQCT are far below that of the QCT since the diameter of tissue to be scanned is diminished (85), allowing its use in descriptive and interventional research. The CV associated with pQCT is high (~5%), which may limit its use except in those cases where dramatic changes (>5%) are observed (85).

2.2 *Measurement of bone mineral density by DXA*

For the calculation of areal bone mineral density, DXA estimates both the surface area and the bone mineral found in the region of interest. The bone projected area is assumed to be a valid estimate of bone volume, but since it is not an actual volume measure, but rather a measure of area, the BMD supplied by the DXA is not a true volumetric density and should be designated as an areal BMD (aBMD).

2.2.1 Assessment of bone projected area by DXA

The measure of bone projected area (BA), the denominator in the areal bone density equation, by DXA is quite accurate, with little measurement error associated with its estimate; the algorithm which determines bone edge detection leads to limited error in the estimation of PA (96). Pencil beam versions of DXA (typically the older versions) allow for slightly better estimates of bone projected area, whereas the fan-beam models (typically the newer versions) are more variable with error introduced as a function of the distance of the subject from the scan aperture.

2.2.2 Assessment of bone mass by DXA

The determination of bone mineral content (BMC) with DXA has been repeatedly found to be highly valid, accurate, and reliable (96). However, BMC is highly dependent on bone size (69), limiting its use for comparison of bone mass between individuals of differing sizes. Similar to the measurement of bone projected area, errors associated with bone edge detection in the software of DXA can lead to variability in the measurement of BMC (96). However, this error introduced by systematic magnification error affects bone projected area and BMC in the same direction, thereby decreasing the absolute error in aBMD assessment as compared to either measure alone (67).

2.2.3 Estimation of areal bone mineral density (aBMD)

To estimate aBMD, DXA divides BMC by the respective BA. The estimation of aBMD is advantageous when compared to BMC for several reasons: it reduces the influence of skeletal size (by dividing for area) and decreases accuracy error (since errors in BMC and PA are in the same direction and somewhat cancel out one another). Utilizing aBMD has been demonstrated to halve the affect of size as compared to uncorrected measures of BMC (92). In fact, the use of aBMD partially removes some of the 20-30% sex difference witnessed with BMC in adolescents and young adults, allowing for better direct comparison between the two (92;96).

2.2.3.1 Positive implications of bone mineral assessment by DXA

There are a number of situations where the measurement of aBMD by DXA is valuable. In investigations where repeat testing (i.e. longitudinal investigations) is performed over a period of time without a change in bone size, aBMD may be beneficial since differences due to slight movements or differences in bone edge detection tend to affect PA and BMC in the same direction, making aBMD more reproducible, and therefore clinically accurate, as compared to BMC (67). However, there are few instances, if ever, over the lifespan when bones are unchanging in terms of size.

Foremost, measurement of aBMD by DXA is a useful clinical tool for the management of osteoporosis and the prediction of future fracture risk in adults (72;73;92;93). With no change in bone size over the period of interest, aBMD can be considered a good clinical measure for assessing change in an individual's density. Similarly, the comparison of aBMD in individuals of the same bone size is a valid procedure for comparing bone mass and density among individuals (97); however, it is exceedingly difficult to match individuals on PA prospectively.

Because aBMD possesses a strong dependence on bone size, it may be a sensitive predictor of osteoporotic fracture. Bone size itself, particularly cross-sectional area of bone, correlates strongly with architectural strength just as increasing the radius of a pipe increases its strength by increasing the moment of inertia (see Figure 2.1).

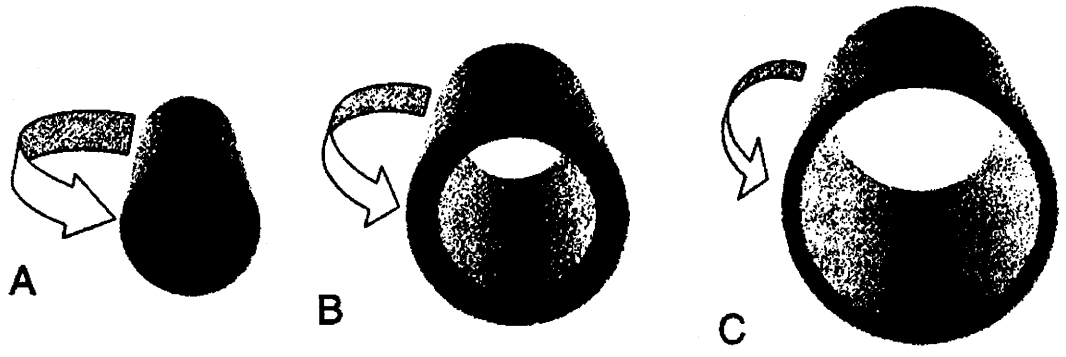


Figure 2.1. Moment of inertia and bone strength

The cross-sectional area of these three cylinders are identical ($A_A = A_B = A_C$). The elastic modulus of the material that makes up these cylinders is also identical ($E_A = E_B = E_C$). Therefore for an axial force (ie. Pushing or pulling at the ends of the bar), the stress is identical ($\sigma_A = \sigma_B = \sigma_C$). However, because the geometry of the cross sections is different, the ability of each of these cylinders to resist bending and or torsion is strongly dependent on the distance of the material relative to the centre of the cylinder. The relative resistance to bending of these cylinders is I_A = 100%, I_B = 400% and I_C = 700%. Subtle changes in a bone's cross-sectional geometry will contribute heavily to the bone's structural properties. From Rubin and Rubin, 1999 (98).

Since aBMD combines both a rough measure of density and a gross measure of skeletal size, correction for size may actually decrease the predictive value of aBMD on future fracture risk (99). In the Study of Osteoporotic Fractures, an extensive investigation of hip fracture risk in Caucasian postmenopausal women (n=7963), it was concluded that at the femoral neck aBMD and aBMD adjusted for bone size had a similarly strong predictive value for future hip fracture and that despite the theoretical appeal, size correction had no advantage over standard aBMD for prediction of hip fracture (99). In a similar investigation (93), geometric information from PA and lateral DXA scans were combined to estimate the volume of L3 in 26 postmenopausal women with osteoporotic vertebral fractures and in 114 control women with no fractures; no improvement in diagnostic accuracy for fracture risk was found. Jergas et al (100) concluded that the best diagnostic sensitivity for vertebral fracture is from QCT, followed by a volumetric estimate of LS BMD based on paired PA and lateral LS scans; less diagnostic sensitivity was achieved with DXA aBMD or estimates of volumetric density from only PA DXA LS scans. Therefore, assuming there

is no change in the size of site during the trial, the use of aBMD is probably merited for use in clinical fracture studies and for assessment of osteoporotic fracture risk (101).

2.2.3.2 Limitations of bone mineral assessment by DXA

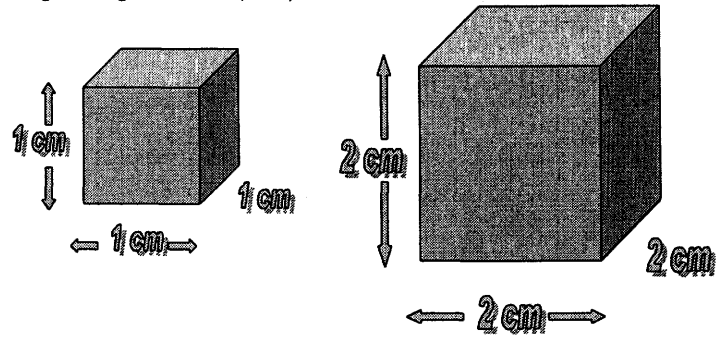
Areal BMD is highly dependent on bone size, as well as on overall mass and body height (67-69). While DXA supplies reliable and accurate estimates of aBMD, these measures are not valid measures of true volumetric density and are subject to error introduced by differences in bone size serially. In the comparison of individuals of different sizes or of a given individual through a period of rapid growth, areal density may be misleading and lead to incorrect measures as changes in bone thickness confound the estimate of areal density (69;102). Prentice et al. (67) concluded that the use of aBMD in research should be discontinued in epidemiologic investigations as it often leads to spurious associations between factors that are themselves related to body size, such as calcium intake.

Density is defined as the mass of an object divided by its respective volume. DXA is unable to measure volume since its scan path travels in a posterior-anterior manner, precluding a measurement of depth, and without a depth, volume ascertainment is impossible (height x width x depth). DXA software makes the assumption that surface area and volume increase in a linear fashion, which is a highly erroneous assumption as there is a disproportionately greater increase in volume as compared to surface area (67). If the theory of a linear relationship between volume and surface area were true, a 1% change in volume will lead to a concomitant 1% change in BA. When scaling based on the laws of geometric similarity, it is a basic tenet that surface area varies to volume by a factor of 0.67, not the 1:1 ratio that the DXA software assumes. In biological tissue this assumed relationship is rarely, if ever, realized and is used in the estimation of DXA-based measurement for lack of better-developed methodologies. The result of the volume:surface area discrepancy with DXA is that larger people will have an overestimate of their true volumetric bone density and smaller people an underestimate.

A definitive test of the supposed linear relationship between surface area and volume of bone is the regression of the natural logs of BMC and BA. The resulting power coefficient should not be significantly different from unity if this theory utilized by DXA is true. If it is significantly different

then the assumption of the 1:1 relationship between BMC and PA is not met and the calculation of aBMD is an inaccurate adjustment for size. Consequently, any variation in aBMD may be due to differences in size rather than differences in true mineralization (67).

The effect of bone size on aBMD is diagrammatically illustrated in Figure 3. In this example both bones are assumed to be of an identical true volumetric density (1g/cm^3). For the ease of calculation the two bones are assumed to be isometric and exactly cubic (as in the assumption by Katzman et al. of the LS (69). The lumbar vertebra of the older boy is assumed to be exactly twice the physical size of the younger boy's lumbar vertebra. After a DXA scan the PA and BMC are estimated and the aBMD calculated. When the aBMDs are compared between the two individuals it becomes apparent that for two individuals with identical volumetric densities, but different physical sizes, there are vast differences in the aBMD, a supposed adequate estimate of volumetric density. This example illustrates the effect of changing bone size on the measure of aBMD by DXA. It comes as no surprise that the dependency of areal BMD values on bone size is seen most dramatically in growing children (101).



True volumetric density =	1 g./cm.^{3}	1 g./cm.^{3}
DXA BMC =	1 g.	8 g.
DXA PA =	1 cm.^{2}	4 cm.^{2}
DXA aBMD	1 g./cm.^{2}	2 g./cm.^{2}

Figure 2.2. Size dependence of DXA-derived areal bone mineral density. Adapted from Katzman et al. 1991 (69).

2.2.4 Implications of areal BMD measures in studies of growth and maturation

One of the problems with monitoring aBMD over a period of years, such as in a longitudinal investigation over adolescence, is whether the changes in aBMD are a function of volumetric density, bone size, or a combination of the two. In the study of children over a period of growth, changes in bone size may mask the biological phenomenon occurring in terms of bone density. In the recognition that the estimation of aBMD is most likely flawed by bone size, many growth and maturation investigations through adolescence have simply relied on DXA-attained measures of BMC. BMC measures are highly correlated to body and bone size and are not adequate for comparing individuals of different sizes or for comparing an individual through a period of rapid growth, such as adolescence (67;101). Perhaps more alarming is the widespread use of uncorrected DXA aBMD measures in the pediatric literature, despite the widespread knowledge of its inappropriateness during this period.

It is assumed, based on data from largely cross-sectional investigations of aBMD, that during growth and maturation bone density increases, but during this period there are also concomitant increases in bone size. Schonau et al. (103) concluded that BMC and aBMD determined by DXA were closely related to age, height, and mass and that in growing children the observed increase may be simply an artifact of increased bone size. Schonau et al. further postulated that if aBMD values were corrected for bone size, the values would become stable across childhood and adolescence. Until adequate controls for the confounding effect of bone size on aBMD have been developed and proven valid it is difficult to ascertain the true changes in bone density that are associated with growth and maturation apart from those solely attributable to measurement artifact. Only after the changes in bone density over growth are fully understood longitudinally can the true effect of interventions on bone mass during the pediatric years be established.

To date, the true pattern of bone formation over childhood and adolescence is largely unknown owing to several methodological constraints including primarily cross-sectional designs (very few longitudinal), frequent grouping based on chronological age (few maturationally-based), and DXA-based, size-dependent, measures of aBMD (very few size-corrected). No study before this one has eliminated these three constraints simultaneously.

2.3 *Scaling in biological tissues*

2.3.1 Basic scaling theory

From the most pragmatic point of view, the skeleton can be thought of as the body's scaffolding, and hence should obey the laws of engineering physics. In engineering when increasing the size, and therefore the mass, of a structure, three different parameters of the supporting structure may be altered to adapt to the increased forces:

1.) By increasing the physical dimensions of a supporting structure, larger applied loads can be supported;

2.) By altering the material composition of a structure to include materials that are of greater material strength larger loads can be tolerated (i.e. from pine to oak), and;

3.) By altering the design of a structure to better resist the applied loads upon it, either through more functional distribution of materials (i.e. buttressing stress points), or through a change in the type of forces resisted (i.e. changing from compression to tension elements, such as is seen with bridges), a more judicious use of materials and architecture results in an increased ability to bear loads.

In humans there is evidence that all three of these adaptations are used by the body to provide a skeleton that possesses greater mechanical integrity. As body mass increases the size of a number of long bones increase, thereby increasing their moment of inertia and ultimately their resistance to the greater bending moments brought about through a greater diameter (98). Further, it has been demonstrated that the vertebrae in elderly men combat the aged-associated persistent decreases in bone mass and trabecular thinning by increasing the CSA of the vertebral cortices in an attempt to preserve mechanical integrity (79;104); this phenomenon of increasing the second moment of area has also been described in the midshaft of long bones (105;106). A recent investigation concluded that fracture risk was significantly associated with bone size in a cohort of men (107). In essence, the further that the building material is from the neutral axis, the better placed it will be to resist bending; this strategy is an economical way to rapidly increase flexural stability for a small investment in material (98;108).

Functional strength is constantly augmented in bone by qualitative material changes. As primary osteons are turned over into secondary osteons there is an increase in the actual material density of the bone and, as a consequence, the compressive strength (109). As bone ages within the body the material density increases, barring newly formed remodeling spaces.

Lastly, numerous investigations have elegantly displayed the ability of bone architecture to adapt to applied strains in order to better match the strains engendered (110;111); for example, in the greater trochanter region of the femur the trabecular architecture matches the strain patterns applied to it (81).

Surprisingly, when bone material strength and internal architectural patterns are surveyed across species, these parameters remain relatively constant, despite vast differences in body mass (112). The major dissimilarity observed among species is differences in bone size; different species and sizes of animals adapt to changes in their respective mass primarily by altering the dimensions of their bone to better match the loads placed upon them (for an example see Figure 2.3.).

Figure 2.3. Two extinct mammals. Neohipparion (right) and Mastodon (left).
Drawn to the same scale, yet in reality the Neohipparion is the size of a deer and the Mastodon an elephant. Intuitively, it seems natural to assume that the Mastodon is a larger animal based on the underlying premise that skeletons must be scaled to support the weight of the body as its bulk increases with the third power of the linear dimensions. Adapted from Gregory, 1912.

2.3.1.1 Geometric Similarity

Geometric similarity assumes that any two geometrically similar bodies will have their corresponding linear dimensions related to one another in the same constant proportion. Any two bodies or objects that share geometric similarity are called isometric. The following equation

demonstrates the relationship between corresponding linear measurements in two isometric structures:

Formula 1: $L_2=k_L\ L_1$ **or** $L_2/L_1=k_L$

where L_1 is the length of one linear dimension on object 1 and L_2 is the corresponding linear dimension on object 2. Solving for k_L provides the similarity ratio. This similarity ratio allows for the proportional relation of all other linear measures between the two objects (113).

The surface areas of one side of isometric structures vary to the square of the similarity ratio, as shown in the following equation:

Formula 2: $(L_2)^2 = (k_L)^2 \times (L_1)^2$

Further, the volume of two isometric objects changes in proportion to the cube of the linear measurement:

Formula 3: $(L_2)^3 = (k_L)^3 \times (L_1)^3$

These relationships are well established and are foundational in the study of geometric similarity and can be summarized as:

if Projected area α (length)^2 and Volume α (length)^3, then

Formula 4: Projected area α (volume)$^{2/3}$

Formula 4 provides a power function to describe the relationship between the projected area and volume of two isometric objects (113), which is of particular importance in the case of normalization of aBMD for bone size. The surfaces of any two isometric objects are related to their volumes by a power of 0.67. As a consequence of this equation, a smaller object will have a larger surface area relative to its volume than a larger one. Extrapolating these observations to the measurement of aBMD it can be observed that this power function between area (projected area) and mass has vast implications on the assumptions made by DXA in its estimation of bone density. DXA assumes bone density to be a ratio of bone mass divided by projected area, and it incorrectly assumes projected area and volume change linearly. A smaller bone will have a correspondingly projected area and a larger bone a larger area. However, when taken as a ratio of their volume, the smaller bone will have a greater projected area as compared to its volume. This will result in a corresponding lower aBMD value for the smaller individual and a larger aBMD value

for the larger person, even with identical true volumetric densities. These observations are based solely upon the laws of geometric similarity.

2.3.1.2 Allometric Scaling

It is rare that organisms are isometric. Instead, certain proportions change in a non-isometric regular fashion (length, mass, height, etc.). Non-isometric scaling is referred to as allometric scaling (113). In biology numerous physiologic and morphological variables are scaled, relative to body size, according to allometric equations in the form:

$y = a x^b$ **or** $\log y = \log a + b \log x$.

When two variables (y and x) are plotted on logarithmic coordinates, the result is a straight line with the slope b. Isometric structures have a slope of one (b=1). If structures are not isometric they exhibit slopes other than one, with the slope dependent upon how much one variable changes out of proportion with respect to the other (67).

In animals, as body mass increases the mass of the bones increase disproportionately faster, resulting in a positive slope greater than one. Conversely, if the dependent variable increases at a slower rate than would be estimated by proportionality, the regression line would have a slope less than one. Variables that do not increase with body size have a slope of zero (i.e. brain) and functions that decrease with body size have a negative slope (i.e. heart rate) (113).

2.3.1.3 Scaling the bones of animals

The extremities of animals support body mass, in both stationary and dynamic activity. Logically, as the mass of the structure increases and the strains engendered upon it increase so must the strength of the support.

Based on isometric scaling:

Formula 5: CSA $\propto$ (length)^2 and length $\propto$ (volume)$^{1/3}$, and strength $\propto$ (volume)$^{0.67}$.

Given proportional increases in bone mass with body mass, bone CSA would only increase at two-thirds the rate of body mass, leading to structural failure. Therefore, in terrestrial vertebrates there is disproportionate scaling between body mass and bone mass. The relative size of the bones must be scaled out of proportion in order to supply the required strength to support

increasing body mass. Because of this, large bone is disproportionately thicker than small bone (113).

2.3.1.4 Scaling mammalian bones

Terrestrial mammals are scaled with mass increasing to the cube of the linear measure. The material properties of bone are relatively homogeneous across terrestrial mammal sizes, thus increases in body mass, and the additional compressive force it creates, must be resisted by an increasingly large supportive structure. To increase the strength of a bone in proportion to the load, the bone CSA must increase in proportion to that load, since the supportive strength of a columnar structure is directly dependent upon its CSA (113).

Assigning the load (mass) as being M_b, the length of the supporting column increases in proportion to a characteristic linear dimension (i.e. in proportion to $M_b^{0.33}$; inverse cube of body mass). As CSA must increase proportionally to mass, the volume, or mass, of the supporting column will then be a product of the CSA ($M_b^{1.0}$) and its length ($M_b^{0.33}$). Therefore, the mass of the bone must scale proportionally to $M_b^{1.33}$. In other words, if the height of the column is scaled in proportion to the linear dimensions of the load, the volume or mass of the supporting column should be scaled as the load raised to the power of 1.33. Unfortunately, scaling animal bones is not this simplistic as they experience extreme ranges of dynamic strain, which changes the loading environment; empirical evidence has shown that the supportive bones do not scale according to this function. Static stresses are not the primary factor in the scaling of mammalian skeletons since dynamic loading can invoke stresses on the bone a full magnitude higher than while stationary. If bones were scaled for static support, they would fail (113). Bones must scale in relation to the dynamic strains placed upon them, or be susceptible to failure.

2.3.1.5 Scaling the bones of real mammals

Selker & Cater (114) observed that as animals get larger, their bone lengths get proportionally shorter. Increasing the stoutness of the bones with increased mass reduces the magnitude of the bending and torsional moments created as bone is loaded. Empirical data shows that the volume of supporting bone in a vast range of animal sizes scales to the volume of the body by 1.08

(±0.04), which is far from the theoretical slope of 1.33 derived from the consideration of static loads and the slope of 1.0 subscribed to by proportionality (115).

Based on empirical evidence modeling mammalian bone in proportion to body mass based on static loads is not valid as the mammalian skeleton is not scaled primarily to support stationary gravitational loads. Alexander et al. (116) and Biewener (117) measured the forces encountered during active locomotion across a number of mammalian groups (25,000-fold size range). They concluded that the estimated bone stresses, regardless of body mass, were all within a small window (50-150 MN/m^2). The maximum stresses from this group of animals in the most vigorous forms of locomotion approach the calculated yield strength for bone in both tension and compression (118).

Scaling bones to support body mass through geometric similarity will lead to fracture failure with locomotion because the compressive strength of the supporting column (i.e. legs) increases only with the square of the diameter (the CSA, which is a measure of surface area; surface area α $volume^{2/3}$), whereas the load increases with the third power of the diameter (the diameter being a linear measure; volume α $length^3$). Bones of heavier animals would most certainly fail during active locomotion if they were scaled according to this assumption. Selker & Carter (114) observed that in real animals the strength of the bone was proportional to $mass^{0.82}$, rather than the expected proportion of $mass^{0.67}$.

McMahon (119) concluded that if a structure is too slender for its respective load, any small lateral displacement of force will cause the structure to fail past the elastic region (on the stress-strain curve), and fracture would result. The critical variables in the strength of a column are the length and the diameter (assuming that the elastic modulus remains constant). Accordingly, the length of a bone should scale one-quarter the body mass and that the diameter should scale with three-eighths the body mass. In an investigation of a wide range of ungulates, McMahon (120) later concluded that bone length should scale with the bone diameter to the power of 0.67.

Formula 6: $l \propto M_b^{1/4}$ and $d \propto M_b^{3/8}$

To test his hypothesis McMahon (120) measured the limb bones of a number of adult ungulates and found that the length of their limb bones differed by a factor of seven. When

investigating the difference in body masses, he found that the difference was close to the accepted range to support his elastic load hypothesis. In McMahon's model, limb bone length should scale with bone diameter by a factor of 0.67, and body mass by a factor of 0.25.

Alexander et al. (116) concluded after measuring the bones of mammals ranging from shrews to elephants that limb bone lengths tend to be proportional to (body mass)$^{0.35}$ and diameters to (body mass)$^{0.36}$. Interestingly, for the animals that were in the family Bovidae, the exponents for length were much closer to the 0.25 predicted by McMahon (120). Alexander suggested that this may be reflective of the stiff-legged stance of the bovids, which is much like a column that was used as the model for this procedure. Other animals rarely have straight, vertical legs.

When looking at the results of Alexander's study (116) the volume of the bones (length multiplied by diameter squared) is proportional to body mass by the power 1.07, similar to the prediction of Prange et al. (115).

Observing the following equation:

Formula 7: Volume α $l \cdot d^2 = M_b^{0.35} \cdot M_b^{0.36 \cdot 2} = M_b^{1.07}$

The exponent 1.07 for the bone volume is exactly that for skeletal mass in mammals described earlier, which suggests there must be similar principles in scaling leg bones and the entire skeleton. The findings of Alexander et al. (116) have been supported in that measurements across several magnitudes of animal mass in adult mammals have indicated that bones scale more closely to geometric similarity than to static strain similarity. Recently, it has been shown that scaling characteristics may not be a result of intrinsic genetic factors, but rather the consequence of highly conserved, extrinsic biophysical processes where bone strain is the primary modulator of skeletal morphogenesis (121). In fact, the supposition that bone width is primarily determined by the engendered strains makes sense when one looks at the variation even in human humeri (122). Tennis athletes' dramatically hypertrophied playing-arm humeri is evidence of the variation that can occur naturally from different loading patterns (122). Rubin & Lanyon (123) similarly concluded that the safety factors in bone were maintained by the allometric scaling the magnitude of the peak forces applied to them during vigorous locomotion, instead of by scaling by bone dimensions. Therefore, bones in weight-bearing areas are scaled in proportion to dynamic strains

encountered with active locomotion. It can be seen that none of the theories (geometric similarity, static, elastic) can explain how to adequately scale bone volume for changes in body volume in mammals. Further investigation needs to be performed in order to elucidate the stresses involved in determining the scaling factors in this group.

Larger animals, particularly those with columnar-type legs structure (i.e. elephants), are very careful about the way they locomote; for example, an elephant will not full gallop. There appears to be some deeper evolutionary safeguard that helps ensure that the safety levels of certain bones are not exceeded, to ensure safety and longevity in the wild (124).

2.4 *Specific techniques used in attempting to adjust for bone size in humans*

Despite the well-acknowledged limitations of aBMD measurement when comparing individuals of different size or for following an individual through a period of rapid growth and change in bone size (56;68;69;93;97;101-103), aBMD has been widely used in both the pediatric and adult literature. However, in light of the problems associated with the estimation of aBMD and the radiation concerns with QCT, a number of investigators have tried to utilize the information gathered from the DXA, along with basic anthropometric measures, to further correct for bone size.

There have been two main streams in the attempt to control for bone size: 1.) Those that use a model of scaling to estimate the third unmeasured dimension (geometric correction) or; 2.) Those that use sophisticated statistical techniques (statistical correction) to control for the effects of bone size. Each of these methods has strengths and weaknesses and valid applications.

2.4.1 Geometric normalization procedures

Geometric normalization procedures are rooted in the principles of geometric scaling. In these procedures size and volume are assumed to have well defined relationships that can be utilized to estimate the third dimension (depth). Estimation of the third dimension allows for BMC to be corrected for an estimated volume.

2.4.1.1 Bone mineral apparent density (BMAD)

Katzman et al. (69) was the first group to publish the pitfalls of measuring aBMD in subjects of differing sizes. To overcome the problem with bone size they proposed a number of equations for calculating the volume of the LS, FN and TB based on basic anthropometric information and DXA output. They proposed an "apparent" volume by assuming a constant relationship between PA and other skeletal measurements to volume. This apparent volume was then used in the calculation of a volumetric bone density (BMC/apparent volume), which they termed bone mineral apparent density (BMAD) in grams per cm cubed (g/cm^3). It was believed that since the calculated reference volume (apparent volume) was approximately proportional to the actual volume, BMAD could more accurately reflect changes in volumetric bone density than aBMD (69). Indeed, in the investigation by Katzman et al. (69) BMAD was shown to be less dependent on size measures than aBMD as assessed by correlation.

Katzman et al. (69) used a log-log linear regression model to examine the interaction of whole body area and body height. In this equation PA increased disproportionately faster than height. Another log-log linear regression with average skeletal width (whole body BA/height) and height found that height and average width did not change proportionately either. For the whole body, the volume was therefore estimated to be proportional to both the PA and the height of the individual being examined, with an emphasis on PA (in proportion to bone depth) over that of height:

Formula 8: TB BMAD = $BMC/(BA^2/height)$

In the estimate of LS BMAD the lumbar spine was assumed to be a perfect cube. The area of the front surface of the cube was estimated by the DXA and the depth was assumed proportional to the square root of the area. Therefore, BMAD at the LS was assumed to be:

Formula 9: LS BMAD = $BMC/BA^{1.5}$

The estimation of volumetric BMD with the BMAD formula is likely an overestimate as in the scanning of the vertebrae the posterior processes of the spine are invariably included in the quantification of LS BMC.

The femoral neck was assumed to be a cylinder. Since the DXA software imposes a 1.5 cm limit to the length of the femoral neck scanning region:

Formula 10: FN BMAD = BMC/(BA^2/1.5)

While BMAD removes some of the dependence of bone density on bone projected area, it has a number of flaws, primarily because of its stringent geometric assumptions. The authors stressed "this reference volume is only an approximation of the true bone volume, and that it can be estimated only by employing certain assumptions about the geometric proportionality or lack thereof for different bones." (69). Unfortunately, rarely do bones scale in exact geometry with one another; errors results in the estimation of true volumetric density if bones deviate from perfect geometry. Additionally, by forcing the data to fit a predefined relationship the removal of some of the deleterious effects of bone size on the density measure is attained, but it is impossible to estimate exactly how much variability has been accounted for (67). While BMAD adjustment will decrease dependence on size, it is impossible to ascertain how much error is corrected.

2.4.1.2 BMDvol

Kroger et al. (68) similarly proposed normalization procedures for aBMD based on geometric assumptions of skeletal isometry, stating that the normalization of BMD values for the size of bones is necessary when the aBMD changes of an individual child are followed; otherwise, bone growth, and therefore bone size, affects the results.

Unlike the equation of LS BMAD by Katzman et al. (69) the vertebra was assumed to be cylindrical, like the femoral neck. Accordingly, the BMDvol of the spine is calculated as a cylinder of a known length and height:

Formula 11: LS BMDvol = BMC/[pi * (width/2)^2 * (BA/width)].

Similar to Katzman et al. (69) the femoral neck is assumed to be cylindrical:

Formula 12: FN BMDvol = BMC/[pi * (BA^2/(4 * height))].

Kroger et al. (125) utilized MRI and DXA to test whether their equation of BMDvol for the LS was valid. They recruited 16 pairs of monozygotic twins (24 men, 8 women) aged 25-69. MRI based volume was positively correlated with age (r=0.56), height (r=0.73) and mass (r=0.68). aBMD and BMC were also correlated with height (r=0.47,0.76) and mass (r=0.65,0.76). After correction for the size of the vertebral body as assessed by MRI, the correlations between aBMD and size measures diminished or disappeared, illustrating the dependence of aBMD on size.

Interestingly, BMDvol and BMDmri decreased with age, while no such trend was observed with aBMD. Based on these findings the investigators concluded that volume-corrected aBMD measurements removed part, but not all, of the dependence of aBMD on body or bone size.

2.4.1.3 Others

In newer models of DXA, lateral scanning of the LS is possible. With the information garnered from both the PA scan (height and width) and the lateral scan (depth) it is possible to make an estimate of volumetric density; unfortunately, the lateral scan is associated with large degrees of error which make its use limited (93). Decreased utility of the lateral exam is a consequence of poor LS access, thick tissue masses to pass through (necessitating increased radiation), and poor software design.

Static procedures that attempt to control for bone size have conclusively demonstrated that they decrease some of the variability in density measures that can be attributed to bone size alone. However, static procedures force data to fit predefined relationships; consequently, it is not possible to judge whether any residual effects of bone and body size remain once the correction has been applied.

2.4.2 Dynamic normalization procedures

2.4.2.1 Linear regression analyses

Dynamic correction of bone mass for bone volume makes no assumptions of the underlying bone geometry and thus is not influenced to any great extent to variations in bone shape as is static approaches. Prentice et al. (67) was the first group to formally control for differences in bone size through a dynamic correction technique using linear regression as the basis. In recognizing the problems with uncorrected aBMD measures they proposed that aBMD provides a simple and crude adjustment for bone size, and strongly urged that its use in population-based research be abandoned (67).

In the Prentice et al. approach, the PA and BMC are first converted into their natural logarithms for two reasons. First, the transformation is performed to strengthen the linear relationship between the variables, a necessary tenet when utilizing linear regression analyses (in

the untransformed state the variables of PA and BMC typically share a slightly curvilinear relationship). Secondly, the regression coefficient value supplied from the linear regression analysis allows for direct quantification of the relationship of the variables as percentages of one another. For instance, a regression coefficient of 1.5 suggests that for a 1% change in one of the variables there would be a corresponding 1.5% change in the other (67). The primary assumption of aBMD is that for every 1% increase in BMC there will be a corresponding 1% increase in BA. If this assumption were true then in the linear regression of the natural logs of PA on BMC the resulting regression coefficient should not be significantly different from unity (1). However, if there is a significant difference between unity and the regression coefficient then the DXA aBMD is likely erroneous.

After the regression coefficient (lambda) has been determined, a new equation for dynamically-adjusted BMD (sBMD) based on the relationship between PA and BMC can be generated. The equation takes the form:

Formula 13: $sBMD = BMC/BA^{lambda}$

2.4.2.1.1 *Use in population-based studies*

Dynamic BMD normalization is useful in population-based investigations because it creates equations specific to the population and the measurement site and is not confounded by errors in the assumptions of geometry. Further, since factors such as calcium intake, grip strength, and energy expenditure are all dependent on both body size, only after control of body size can the relationship between these variables and BMD be ascertained (67). Prentice et al. (67) concluded that the simplest way to avoid the possibility of size-related artifacts in the analysis of bone mineral data is to use BMC as the dependent variable and to include PA, weight, and height as independent variables in all multiple regression models.

One of the inherent problems with the dynamic correction of bone mass is its dependence on sample size. Since it is a statistically driven technique, there is a need to collect a relatively large population for the formulation of the regression equations and subsequent sBMD. With small samples, increasing probability of error is introduced due to lack of power. Thus, this technique cannot be used on an individual basis until standardized equations are made available for specific

populations. The static equations, which are based on geometric similarity, can be used on an individual basis without prior establishment of normative values.

2.4.2.1.2 *Use in clinical-based studies*

Bone mineral content is dependent on both the absolute size and the density of the bone (126); therefore, differences in BMC may be due to either differences in size, tissue density, or both. In children with diseases that affect normal growth and maturation of the skeletal tissue it is often difficult for the clinician to determine the cause of the low bone mass. Confounding this is the vastly different sizes of children and their often rapid rates of growth. Since DXA has been shown to be highly size-dependent in its assessment of aBMD it is essential to control further for the effect of size. A low BMC may be due to low stature, narrow bones (low PA for height), or low size-adjusted BMC (corrected for PA and height) (126).

The investigation of the linear relationship between BMC and PA is clinically applicable for individuals who are afflicted with bone growth or mineralization disorders. Recently, a number of investigations have utilized a version of the Prentice et al. (67) technique to estimate the amount of bone mass patients should have for their PA, height, weight and age or pubertal status (126-130). These investigations endeavored to create specific regression equations that can be utilized to establish normative BMC values based on given anthropometric and age values. This BMC estimate is then compared to the actual value of BMC to acquire a percentage value of normal compartment density. Unfortunately, many subjects are needed to decrease the error associated with the generation of these equations. Most recently, Warner et al (128) published norms for children based on sample sizes that were likely inadequate.

The use of size-adjusted normative density measures promise to provide a substantial tool for the assessment of growth-delayed children in clinical practice but more research with larger sample sizes in specific populations needs completion.

2.5 *Changes in bone with growth and maturation*

2.5.1 Changes in bone size with growth and maturation

Since bone size is infrequently reported in the literature in its raw form, but rather as a component of density, it is difficult to establish how it changes with growth and maturation.

Bone size increases both longitudinally and laterally during the growing years, with bone apposition occurring at both the endosteal and periosteal surfaces (131). Once linear growth has ceased with skeletal maturity, many bones continue to increase their total cross-sectional area until death through periosteal apposition at the expense of endosteal resorption (79). This increase of total cross-sectional area, as witnessed frequently in the vertebrae, is thought to be an attempt of the body to maintain skeletal integrity during a time of absolute bone losses; by increasing cross-sectional area the cross-sectional moment of inertia increases, perhaps partially compensating for the absolute losses in mass (104).

2.5.1.1 Stature: A gross measure of changes in bone size

While there are few direct measures of bone size in the literature, there are many reports on changes of stature with growth and maturation. Stature can be used as a general, but imperfect, surrogate of bone size since height is closely associated with bone size in healthy individuals (129), however there are gender and race differences in bone lengths after correcting for stature.

Yearly gains in stature are typically observed to decelerate from the one year of age and slow until the adolescent growth spurt, when a dramatic acceleration in statural growth is observed (132). In some instances, there is a smaller magnitude, transient growth spurt around the age of 6.5-8.5 years, termed the mid-growth spurt (65). The adolescent growth spurt begins at about 10-12 years in females and 12-14 years in males, on average. A recent Canadian longitudinal investigation, with a primarily Caucasian cohort, concluded that the age of peak linear growth was at 13.5 years for males and 11.6 years for the females (59). These maximal rates of growth velocity can be surmised to comprise the time of maximal bone envelope expansion. At PHV males had attained 90% and females 92% of their respective adult statures (59). After the peak of the adolescent growth spurt, longitudinal growth decelerates into adulthood and with the closure of

the epiphyseal growth plates increases in stature halt. It seems that by early in the life span the majority of linear bone growth has occurred and that further observed increases in bone mass must be either a consequence of periosteal expansion, endosteal contraction, and/or increased material density.

Accordingly, before puberty the association between bone mass and stature is strong (53;133), but loses strength as adolescents experience puberty, and is weakest in adults (133).

2.5.1.2 Changes in bone projected area

2.5.1.2.1 *Bone projected area and chronological age*

Bone projected area (PA) increases with chronological age, with dramatic acceleration around the adolescent growth spurt, followed by deceleration to adult values. Molgaard et al. (129) investigated 201 girls and 142 boys (6 to 19 yrs. of age) and concluded that TB PA was similar between sexes from 6 to 11 yrs. TB PA between sexes was identical until 15 yrs. of age after which gains in females plateaued and the males continued gaining PA, slowing at 17 yrs. PA was more highly related to height than age in both sexes (129). Bonjour et al. (133) reported that LS PA increased with chronological age from 9-18 yrs. in both males and females, with a dramatic acceleration in LS PA in females from 12 through 15 yrs. and in males from 13 to 17 yrs. Therefore, changes in PA most closely approximate the changes in statural growth, at least until the arrest of linear growth with the fusion of the epiphyseal growth plates.

A study by Gilsanz et al. (108) utilizing QCT to measure the cross sectional areas of both the femur and vertebrae concluded males had an 11% larger vertebral body cross-sectional area, while there was no gender difference for femoral cross-sectional area. Both gender and body mass were significant predictors of vertebral CSA. When chronological age, skeletal age, height, muscle and fat masses were entered into a linear regression model along with total body mass, none of the other variables significantly accounted for variance in the femoral measures. They concluded that in children, regardless of gender, body mass was the primary determinant of vertebral cross-sectional area and the area of cortical bone in the midshaft of the femur. Similarly, an investigation by Moro et al. (134) concluded that body mass was the primary determinant of femoral mid-diaphyseal bone mass and structure in adolescents.

2.5.1.2.2 *Bone projected area and pubertal stage*

Data concerning the relationship between bone projected area and pubertal status is sparse. Goulding et al. (135) investigated 200 girls aged 3-16 yrs. to examine changes in femoral neck geometry with aging and to establish at what age adult hip geometry was achieved. At 12.5 yrs. (average age of menarche) the girls had attained 91% of their femoral axis length (FAL), 92% of femoral neck width (FW), and 93% of femoral aBMD, but only 72% of adult BMC. By 15 yrs. of age the girls had adult dimensions of hip geometry and 85% of their total BMC (135). FAL was highly dependent on height and pubertal stage. Both FAL and FW were significantly associated with TB BMC and FN aBMD, height and weight. However, adjustment for both age and height eliminated all significant relationships between bone mineral accrual and femoral geometry. In a similar investigation of hip geometry in adolescent females, it was concluded that full adult hip geometry is achieved by age 15 yrs. (136). In females, it appears that the majority of growth in the bone envelope occurs largely by the time of peak height velocity, slowing thereafter. Bass et al. (137) reported that in a large cohort of girls 70% of the predicted adult peak in femoral cortical width was achieved prepubertally. Through puberty, periosteal diameter increased with little change in endocortical diameter, resulting in an increased cortical width, particularly after the endocortical diameter contracted following menarche. There is a paucity of data concerning the expansion of the bone envelope in males with respect to puberty.

2.5.1.2.3 *Bone projected area and gender differences*

In a recent cross-sectional investigation no gender difference was observed in TB PA between the ages of 6 and 15 yrs. (129). While there was a general increase in TB PA with age, with a slight acceleration with the onset of puberty, gains plateaued in females by 15 yrs. of age and in males by 17 yrs. of age. In both sexes TB PA was highly related to height. The same trends were observed at the LS with no apparent sex difference until 14 yrs. when the females ceased significant growth in size whereas the LS PA in the males continued to expand until 18 yrs. of age. As a consequence of this discrepancy in growth duration males possessed significantly greater LS PA as compared to females from 17 yrs. onward (129). In another investigation, it was

concluded that when males and females were aligned on pubertal status, males consistently displayed greater LS PA (133).

Seemingly, the differences between males and females in terms of bone projected area largely are a consequence of a longer period of bone expansion in males as compared to females during the adolescent growth spurt and the years immediately thereafter.

2.5.2 Changes in bone mass with growth and maturation

Bone mass, or bone mineral content (BMC), increases throughout childhood and adolescence, peaking sometime shortly after the peak height velocity (61;138). A recent longitudinal analysis of calcium accretion from the cohort used in this investigation suggested that the age of peak BMC accrual for the TB occurred at approximately 14.0 yrs. in males and 12.5 yrs. in females, after PHV (138). The rapid change in bone mass with growth and maturation in males and females is illustrated by the approximate tripling of BMC that occurs between the ages of 8 and 17 yrs. (61). Depending on the skeletal site, at least 90% of the adult BMC is deposited by the end of adolescence (48;53;55;57;60;139).

2.5.2.1 BMC and Stature

BMC is intimately associated with height in children up until the point of linear growth cessation (53;133;140), after which there is dissociation of BMC with height as the bones continue to consolidate and accrue bone mineral (59;141-143). In a recent longitudinal investigation both girls and boys accrued approximately 90% of their final adult height by peak height velocity (PHV), but had only achieved about 57% of their adult total body BMC values, illustrating the dissociation of these two measures following prepubescence (59). Additionally, peak velocity in BMC occurred approximately a year later than PHV in both girls and boys at the TB, LS and FN (40;59). Matkovic et al. (144) reported that in girls skeletal height reached a maximum 1 to 7 years earlier than maximum values in BMC and aBMD (depending on site) further demonstrating the dissociation between BMC accrual and linear growth after linear growth has ceased. In females, after longitudinal bone growth ceases and the epiphyses fuse, skeletal consolidation continues as the shoulders and hips broaden to their mature state (145). Clearly, stature and BMC are closely

related so long as there is moderate-rate longitudinal bone growth occurring at the epiphyseal growth plates, but once the adolescent growth spurt is realized, longitudinal growth may simply outpace the body's ability to supply adequate calcium supplies, leading to dissociation between stature and BMC (138;146). After the epiphyseal growth plates become fused, growth of bone length is arrested while BMC consolidation continues leading to further dissociation.

2.5.2.2 BMC and Chronological Age

Numerous investigations have reported a significant association between chronological age and BMC, particularly before puberty. While growth occurs somewhat steadily throughout childhood there is an acceleration around the years of puberty with the peak gains in BMC at the lumbar spine, femoral neck and total body occurring at approximately age 13.0 for girls and 14.5 yrs. for boys, approximately 1 year after PHV (56;141;147-150).

Hannan et al. (151) measured LS and TB BMC in 216 females between the ages of 11 and 17.9 yrs. and concluded that chronological age was a significant predictor of LS and TB BMC. Another cross-sectional evaluation determined that chronological age was significantly ($p<0.001$) associated with BMC for the TB and all it's subregions (152). In another investigation by Faulkner et al. (61) a significant association was found at all sites between BMC and age in both males and females. A recent cross-sectional study displayed that by 12.5 years of age females had attained 72% of adult TB BMC and by 15 years of age 85% of their total BMC had been accrued (135).

Sugimoto et al. (87) investigated radial BMC with SPA in 229 prepubertal Japanese children from the ages of 3 days to 11 yrs. to establish normative curves. BMC of the radius increased from birth until age 12 (end of study group), whereas the bone width (BW) rapidly increased until age two after which there was a slower increase. Consequently, BMD did not change significantly until age two after which there was a significant increase throughout childhood. Therefore, after two years of age the bone formation accelerated as compared to the bony expansion processes. Radial BMC, BW and BMD were all highly correlated with chronological age ($r = 0.955$, 0.783, 0.937, respectively), stature ($r = 0.957$, 0.871, 0.907, respectively) and body mass ($r = 0.966$, 0.832, 0.916, respectively).

2.5.2.3 BMC and pubertal stage

Comparison of adolescent bone accrual rates with reference to chronological age is misleading as individual timing and tempo of growth and development is variable at similar chronological ages; thus, comparison based on maturational benchmarks, such as pubertal stage or time of peak height velocity (PHV), are necessary to determine true accrual rates. Following childhood, gains in BMC are more a function of pubertal stage than chronological age (68;153-155), as is supported by biochemical bone marker studies (156;157).

Children accrue tremendous amounts of bone tissue during childhood and early adolescence, with a maximum rate, the peak bone mineral accrual velocity (PBMAV), occurring approximately one year after PHV (59;138;146). Before puberty, there is a direct relation between chronological age and BMC, as described in detail above. Numerous investigations have reported a progressive increase in bone mineral mass through childhood (53;152;158;159) with an acceleration of BMC accrual during puberty (53;133;150;160;161). During puberty, the strong prepubertal relationship between BMC and chronological age is typically observed to diminish as the influence and different timing of puberty comes into play. Postpubertally, the association between BMC and age is diminished further as skeletal maturity is realized and rapid rates of accrual are ceased. De Schepper et al. (162) reported BMC to be relatively stable over Tanner stages 2 and 3, with a rapid accrual between stages 3 and 4, with a subsequent stabilization into stage 5.

The results from a recent longitudinal investigation concluded that in males peak TB bone mineral accrual occurred approximately 0.6 yrs. after PHV (TB PBMAV= 14.0 yrs. and PHV = 13.4 yrs.) in males (138). In an earlier investigation with the same cohort analyzed in a cross-sectionally, by PHV males attained approximately 59% of adult TB BMC and in the four years surrounding PHV, 36% of the adult TB BMC was laid down, demonstrating the importance of this time for bone mineral accrual (59). Similarly, females peak TB bone mineral accrual occurred approximately 0.7 yrs. after PHV (TB PBMAV = 12.5 yrs. and PHV = 11.8 yrs.) (138). At PHV 60% total adult value of the females TB BMC had already been laid down and in the four years surrounding PHV 36% of the adult TB BMC was accrued (59). Cadogan et al. (143) estimated, from a short-term longitudinal study, that in females during the transition from Tanner stage II to

Tanner stage IV, the annual increase in TB BMC was 15.5 ±4.0%. They further concluded that height velocity peaked more than 2 years prior to menarche and at the time of menarche the greatest accrual of bone mass was observed.

In the study by Bailey et al. (59) males LS PBMAV was at 14.6 yrs. and by the time of PHV 58% of the total adult LS BMC had been laid down. Astoundingly, in the four years surrounding PHV 39% of the total male adult LS BMC had been accrued. In females LS PBMAV was a 13.1yrs. and at PHV 60% of the total adult LS BMC had been laid down. Similar to the males, in the four years surrounding PHV 35% of adult LS BMC was accrued. At the FN, the PBMAV occurred at 14.4 yrs. in the males and by the time of PHV 71% of the FN BMC had been accrued. Further, 28% of the total male adult FN BMC was laid down in the four years surrounding PHV. In the females FN PBMAV occurred at 12.4 yrs. and 72% of the total adult value of FN BMC was present at PHV (59). In the four years surrounding the PHV the females accrued 27% of their total adult FN BMC. Similarly, in another investigation it was concluded that 27% of the female total adult FN BMC was laid down in four years surrounding PHV (163).

In females, the onset of menarche is closely related to the accrual of bone mass. In an investigation by Bonjour et al. (133) in the two years following menarche a substantial increase in LS BMC in females was observed and after four to eight years postmenarche there were general losses in LS BMC with maintenance of bone projected area, displaying the importance of adequate calcium intake, eumenorrhea and physical activity in the years immediately following menarche. Bass et al. (137) reported between 7-11 years of age (prepuberty), 11-14 years of age (puberty) and 14-17 years of age (postpuberty) the increases in spine BMC in healthy females was 38, 72, and 46 g, respectively. The corresponding increases in leg BMC were 240, 241 and 149 g, respectively. While appendicular accrual is relatively constant prepubertally and pubertally, there is a dramatic increase in accrual at the spine pubertally as compared to prepubertally. In a longitudinal sample, McKay et al. (164) concluded that menarche and the peak rate of BMC accrual occurred at approximately the same time, with PHV occurring about 1 year before these events, similar to the findings of Cadogan et al. (143). Further, earlier maturing girls were found to

possess greater peak BMC velocity and PHV as compared with later maturing girls in the McKay et al. study (164).

In summary, bone accrual occurs in step with increases in statural growth or chronological age until the adolescent growth spurt. However, after and during the growth spurt this association disappears with tremendous accrual occurring at all sites, particularly during a period of four years surrounding PHV. After the closure of the epiphyseal plates there is little association between BMC and stature. At the onset of menarche in females there typically is a maximal rate of BMC accrual, suggesting an effect of elevated sex hormone on bone accrual.

2.5.2.4 Gender Differences in BMC

Before puberty, there is little gender difference in BMC. There were no significant differences between genders for TB BMC in a large cohort (n=773) children who were examined once in third grade (mean age 8.9 yrs.) and later in fourth grade (mean age = 9.9 yrs.) (165). During this stage of life the variance in BMC found was a factor of age and mass, not gender (165). Numerous other investigations have concluded almost unequivocally that there are no gender differences in BMC prepubertally at either axial or appendicular sites (40;57;129;140;150;154;166-169). In contrast, a cross-sectional investigation of males and females (n=27 and 43, respectively) aged 5.3 to 14.4 yrs. concluded that before puberty females had significantly lower BMC at the radius, LS and hip (170). The preponderance of evidence, however, suggests a relative equality of BMC between the genders before puberty.

Since females typically experience puberty earlier than males, they exhibit initially higher levels of BMC in early adolescence, but this relationship is reversed as skeletal maturity approaches. Chronologically, females have slightly greater BMC at all sites from approximately 11-14 years of age when compared to males; however after this period males typically exhibit higher levels of BMC as seen in adults (53;57;87;133;141;142;154;166;168;171). However, when aligned on PHV, males tend to have only slightly greater BMC following the onset of puberty (59). In a mixed-longitudinal investigation of BMC during adolescence, males had consistently greater BMC at all sites, due to their larger skeletal size, but when taken from the standpoint of percent of adult values there was little gender difference (59).

In a group of 234 children between the ages of 8 and 16 yrs., there were no differences in TB BMC at any chronological age (152). In a later investigation of the same cohort with further data, males were seen to have greater TB BMC than the females after 14 yrs. of age (61). Molgaard et al. (129) observed TB BMC in a cohort of males and females (n=142 and 201, respectively) aged 6 to 18 yrs. and concluded that between the ages of 6 through 11 yrs. there were no gender differences in TB BMC. They further reported that from 11 through 15 yrs. females possessed slightly higher TB BMC than the males. At 15 yrs. of age BMC gains arrested in the females and continued in the males until 18 yrs. Consequently, after the age of 16 yrs. males possessed higher TB BMC than the females. Similarly, results from the most comprehensive mixed-longitudinal database to date demonstrated that TB BMC is similar between the genders until about age 13 yrs. after which BMC gains are greater in males (59). These data are consistent with others that have reported no differences in TB BMC values in prepubertal boys and girls, but report boys to have a more pronounced increase in BMC during puberty and at skeletal maturity possess greater bone mass than their female counterparts (57;140;154;166-169).

There are inconsistencies in the data comparing gender BMC differences at the LS. Although some studies report small or no gender differences in BMC during childhood or adolescence (147;153;155;170), others report greater values in females (56;158), while still others have reported greater values in females until about age 15-18 yrs. when males surpass the female values (53;140;161;166;169). In a recent longitudinal investigation LS BMC was found to be equal for males and females until the age of 12 and 13 yrs. after which females had significantly higher BMC until 17 yrs. of age when the males had significantly higher LS BMC (61). In a study by Bonjour et al. (133) LS BMC was equal between genders until 12 yrs. of age after which females had greater LS BMC until the age of 15 yrs. In this investigation, the males continued to accrue bone until 16 yrs. after which their gains plateaued similar to what was seen with the females at 14 yrs. (133). The varying results using DXA between investigations may be a consequence of inadequate controls for maturity and the inclusion of numerous cross-sectional investigations. For example, when males and females are aligned on pubertal status, males consistently display greater LS BMC than their female counterparts at all time points (133;150).

Several studies have reported greater BMC values in males at the FN at some stage in childhood and certainly by late adolescence (56;68;133;150;158;169). Others, although reporting no statistically significant gender differences in FN BMC, have found a trend toward greater values in males (153;170;172). Mixed-longitudinal data suggest that boys have greater FN BMC at all ages, but the differences are clearly accentuated after age 13 yrs. (40). In another investigation of the same mixed-longitudinal dataset, there were no gender difference in FN BMC before the age of 14 yrs., but after this there was significantly higher FN BMC in the males (61).

Lastly, at the radius, there were no significant differences observed between a large group of prepubertal (<12 yrs.) males and females for radial BMC (87), supporting the concept of relative equality before puberty.

In general, at skeletal maturation males have greater BMC than females, theoretically due to their larger skeletal size. Women generally have a smaller skeleton than men, accounting for much of the gender differences in BMC (173). Additionally, the postpubertal gender difference at skeletal sites containing relatively greater amounts of cortical bone may be a result of the greater skeletal size and greater cortical shell mass in males (158;166;174). There are smaller gender differences at sites containing relatively more cancellous bone (148;166;175). Supporting the theories that gender differences may be a consequence of size, a group using QCT demonstrated that the greater lumbar spine BMC found postpubertally in males is primarily a function of their larger vertebral bodies (160).

2.5.3 Changes in bone mineral density with growth and maturation

Studies have demonstrated that aBMD increases are gradual throughout childhood (53;160;176) and accelerate during adolescence until skeletal consolidation is attained (53;61;160). After this consolidation, decreasing sex-steroid levels and almost imperceptible losses due to bone turnover imbalance are hypothesized to be responsible for the gradual bone loss witnessed until death.

2.5.3.1 Changes in areal bone mineral density

The relative importance of chronological age, stature and body mass on aBMD was demonstrated in a cross-sectional investigation of 216 girls between 11 and 17.9 yrs. (151) where a considerable proportion of the variation in LS aBMD was attributable to stature. Surprisingly, when linear regression analyses were completed, the inclusion of Tanner stage did not significantly contribute to the regression equation after age, mass and stature were entered. In the regression equation a second degree polynomial (age squared) always led to the best fit while stature, body mass and shoulder width consistently entered significantly into the equations (151). Similarly, LS aBMD was analyzed by DPA in a multiracial group of 184 (n=101 boys) children aged 5 to 11.99 years of age and it was concluded that LS aBMD had significant correlations with body mass (r=0.746), lean body mass (r=0.725), stature (r=0.693) and chronological age (r=0.580) (159). The relationship of lean body mass (LBM) on bone density was further demonstrated by Faulkner et al. (152) with a significant association between TB aBMD and LBM in males (r^2=0.75) and females (r^2=0.80) (152). These results demonstrate the significant relationship between aBMD and anthropometric measures such as stature and body mass.

2.5.3.1.1 *Areal bone mineral density and chronological age*

aBMD increases with age in children, with a peak or plateau typically occurring some time in the third decade of life (69;141;147;150;158); however, increases in height and body mass are thought to account for most of the age-dependent increase in aBMD (158). In one mixed-longitudinal and one cross-sectional investigation of changes of bone density with growth and maturation, Faulkner et al. (61;152) observed a significant effect of chronological age on aBMD at all sites in both males and females. However, after the age of 17 yrs. aBMD in females stablized, suggesting PBM may have been attained (152). Further, there were no significant differences between either aBMD or BMC for any site between groups of 17 and 21 yrs. females, indicating a relative attainment of PBM (61).

In a group of 205 males and 295 females aged 4-20 yrs. of age it was shown that TB aBMD increased significantly with chronological age (177). There was an accelerated increase in bone

density after 13 yrs. of age in males and 11 yrs. in females, with age-dependent increases continuing to 20 yrs. Stature was significantly associated with TB aBMD throughout the age span.

Theintz et al. (150) conducted a longitudinal investigation of changes in LS aBMD for approximately a year in a cohort of adolescents (n=198; 100 boys). LS aBMD increased rapidly through 11-14 yrs. in females, decelerating to 16 yrs. with the gains becoming insignificant by 17-20 yrs. A pubertal-associated rapid accumulation in LS aBMD in females from 11-12 to 13-14 yrs. was observed, as was a rapid accumulation in LS aBMD in males from 13-14 to 16-17 yrs. Male LS aBMD and BMC continued to increase significantly through 17-20 yrs. (150). In a cohort of 207 boys and girls aged 9-18 yrs. LS aBMD was found to be highly dependent on age throughout the entire age range (133). Boot et al. (177) confirmed a positive relationship between LS aBMD and chronological age in a group of 205 males and 295 females aged 4-20 yrs. of age. There was an accelerated increase in bone density after 13 yrs. of age in the males and 11 yrs. in the females and the age-dependent increase continued to 20 yrs. (end of trial). Stature accounted for most of the variance in LS aBMD, again displaying the relative dependency of aBMD on size measures. Additionally, body mass was significantly associated with LS aBMD measures after adjustment for age (177).

Rubin et al. (178) assessed 299 children (163 girls and 136 boys) between 6 and 18 yrs. for changes in distal radial aBMD and LS aBMD. Gains in LS aBMD were generally low in the prepubertal years for both sexes. Females had greater LS aBMD from 11-15 yrs. as compared to the males. In general, there was a rapid increase in girls from 10 to 15 yrs. and in males from 13 to 17 yrs., with most rapid increase during 15-16 yrs. in the males. In both sexes the increments for bone size growth slowed before significant increments in BMC and BMD were observed.

Theintz et al. (150) reported that FN aBMD increased rapidly through 11-14 yrs. in females, decelerating to 16 yrs. with the gains becoming insignificant by the 17-20 yrs. period. There was a rapid accumulation of FN aBMD in females from 11-12 to 13-14 yrs. An important and significant gain in FN aBMD was observed in the two years postmenarche, displaying the importance of estrogen on bone consolidation. In general, there was rapid accrual in FN aBMD for males from

13-17 yrs. with a decline in aBMD velocity with further age (150). In a similar investigation, FN aBMD was highly dependent on chronological age throughout the entire age range (133).

Moreira et al. (179) investigated changes in radial density with growth and maturation in 121 children (52 girls, 69 boys) aged 3-18 yrs. Three radial measures were acquired: the one-third distal radius, middle distal radius and ultra-distal radius. Radial aBMD increased slowly, but significantly, with age in both genders and the only gender difference observed was in the 10-11 yrs. group in which the boys had denser radii than the girls (179).

In summary, aBMD seems to increase at all sites until approximately 17 yrs. in females, and up to 19 yrs. of age in males. The dramatic acceleration of bone mass accumulation during the pubertal years as well as the distinct age-patterning in maximal gain between males and females clearly underline the influence of sex steroids on bone mass.

2.5.3.1.2 *Areal bone mineral density and pubertal stage*

In a cross-sectional investigation of 500 children (177), Tanner stage was a significant predictor of TB aBMD in both boys and girls. After adjustment for age, Tanner stage was still significantly correlated with TB aBMD only in the females (177). Body mass was also significantly associated with TB aBMD after adjustment for age in both genders. Variance of aBMD increased during puberty for all sites displaying the sensitivity of bone during this period to interventions or perturbations as compared to prepubertal values (177). In a cross-sectional investigation of 216 girls between 11 and 17.9 yrs. (mean = 13.4 yrs.) it was concluded that a considerable proportion of the variation in TB aBMD was attributable to body stature, but in contrast to the above study, inclusion of Tanner stage did not significantly contribute to the regression equation. (151).

In the investigation by Boot et al. (177), Tanner stage was a significant predictor of LS aBMD in both genders and after adjustment for age, Tanner stage was still significantly correlated with LS aBMD. An increase in LS aBMD with age occurred until puberty after which there was acceleration in aBMD gains and bone mass became more associated with pubertal status than age. From Tanner stage II to Tanner stage IV, the annual increase in LS aBMD was 5.5 ±1.8%. Similarly, Ferrari et al. (24) studied a cohort of prepubertal girls (37% beginning Tanner stage 2 at trial termination) for 1 to 2 years and reported a 3.8% increase after 1 year in LS aBMD, and a

4.7% increase after 2 years. Southard et al. (155) investigated 218 children (134 girls) aged 1-19 yrs. of mixed racial origin (28 black females and 28 black males) and found that higher LS aBMD was significantly associated with increased body mass, chronological age and pubertal status. There were no significant differences in LS aBMD between blacks or whites or between genders for any age group. The largest gains in LS aBMD occurred between 1-4 and 12-17 yrs.; these results were suggested to correspond with the early childhood and pubertal growth spurts, respectively. A significant increase in LS aBMD occurred with each successive pubertal stage; however, after controlling for body mass, there was no difference between subjects at Tanner stages I and II. In multiple regression analyses, chronological age and body mass separately accounted for r^2=0.77 and Tanner stage alone r^2=0.76. Body mass and Tanner stage together provided the best prediction of LS aBMD (r^2=0.85) (155). Moreira et al. (179) conducted a study with 121 children (52 girls, 69 boys) aged 3-18 yrs. There was a significant effect of puberty with an increase of 40% in the LS from the time of T1 through T4. Inspection of the data suggested that the increases in LS aBMD ceased earlier in girls than in boys. Similar to other investigations, Tanner stage and body mass were the best predictors of LS aBMD (179).

Slemenda et al. (55) completed a three-year prospective trial of calcium supplementation in 90 children (32 female and 13 male pairs of monozygotic twins) divided into postpubertal, peripubertal and postpubertal groups. The more sexually mature children had greater LS aBMD at baseline, suggesting a positive affect of sex steroids on bone mass. During the three years all three of the groups had significant gains in aBMD at all sites investigated; however the peripubertal children had the largest gains in aBMD over the three-year period. When aBMD gains in the peripubertal group were compared to the prepubertal group there were significant gains at the LS, but not at the radius. Increases in height and mass were positively associated with aBMD at all sites and changes in height were more closely associated with radial aBMD and changes in mass more closely associated with LS aBMD. The authors postulated that the increase in trabecular bone density was linked to estrogen exposure in the females.

Ferrari et al. (24) longitudinally studied a cohort of prepubertal girls (37% beginning Tanner stage 2 at trial termination) for 1 to 2 years and reported a 2.6% increase after 1 year in FN aBMD,

and a 4.7% increase after 2 years, clearly demonstrating the increases bone mineral accrual that occurs with the onset of puberty. Further, Ferrari et al. (24) reported a 6.0% increase after 1 year in femoral shaft (FS) aBMD, and a 7.0% increase after 2 years.

Rubin et al. (178) assessed 299 children (163 girls and 136 boys) between 6 and 18 yrs. for changes in distal radial aBMD. After puberty, there was an accelerated increase of aBMD in both sexes. Simple regressions showed radial aBMD to be significantly correlated with age, height, mass and Tanner stage. Multiple linear regression showed that peripheral aBMD had significant associations with height, mass, pubertal stage and age (76%).

The effect of estrogen on bone mass is well described through its association with menarche. Boot et al. (177) reported that girls who experienced menarche had higher density than those who were premenarcheal, regardless of age, and earlier age of menarche was significantly associated with higher aBMD at all sites. In another investigation during the initial 2 yrs. following menarche there was a substantial increase in FN and FS aBMD (133). There was a small increase between the second and fourth year postmenarche, followed by a decrease in aBMD after the fourth year up to the eighth (133). In yet another investigation a significant gain in aBMD was observed in the two years postmenarche in aBMD at the TB, FN and LS (150).

In summary, the largest gains in aBMD occur during the pubertal growth spurt, after which gains slow into adulthood. In females, there are significant gains in aBMD following the onset of menarche which are likely a result of an increase in circulating sex hormone (estrogen).

2.5.3.1.3 *Gender differences in aBMD*

Prepubertally, there is little difference in aBMD between genders; the differences observed following the prepubertal period are the result of earlier initiation of puberty in females, and the longer and greater magnitude of adolescent bone growth typically observed in males (59).

Nelson et al. (165) found no significant gender differences in TB aBMD in a cohort (n=773) of third grade children (mean age 8.9 yrs.) and later when they were in fourth grade (mean age = 9.9 yrs.). Log body mass was found to be the major correlate for TB aBMD. Similarly, there were no significant differences in TB aBMD between genders in a group of 234 individuals between 8 and 16 yrs. (152) and in another large cross-sectional investigation of males and females aged 4

though 20 yrs. there was no difference between genders for TB aBMD at any age (177). In contrast, Faulkner et al. (61) found that males exhibited significantly greater TB aBMD than the females after age 16 yrs.

Miller et al. (170) investigated aBMD in girls and boys (average age = 9.47 yrs. with 27 male and 43 female pairs of identical twins). In this sample 67% of the girls and 90% boys were Tanner stage 1. There were no gender differences at the radius, but the girls possessed greater LS aBMD at all ages (170). This investigation may have been biased in that the females would have been more sexually mature than the males at the end of the trial. However, in a number of investigations females have significantly greater LS aBMD when compared to males of the same age or pubertal stage (61;170;177). Different from the above investigations, in a cohort of 9-18 yrs. males and females (n=207), between the ages of 12 and 15 yrs. the females had slightly higher LS aBMD compared to the males, but not significantly so. When males and females where compared at equal pubertal stages there was still no significant differences in LS aBMD (133).

In contrast to the LS, males typically possess higher densities than females in other, more cortical, sites such as the TB, FN and FS. In a Canadian study females had significantly lower FN aBMD at all ages, which was suggested to be a factor of greater physical activity (61) In a similar investigation, at 17-18 yrs. males tended to have higher aBMD at the FN and FS, but not the LS and for any given pubertal stage the males tended to have greater aBMD than the females at the FS and FN, but not the LS (133).

Gunnes et al. (58) investigated changes in SPA-derived BMD accrual with growth at two regions of the ulna and radius – one being of primarily trabecular constitution and the other cortical. They recruited 494 children (247 of either sex) between the ages of 8-17 yrs. and determined distal (65% cortical) and ultra distal BMD measures (65% trabecular). In the prepubertal children (those <11 yrs. mean = 9.6yrs.), mean BMDd (distal radial BMD) was 6.8% ($p<0.0001$) higher in boys compared to girls, while BMDud (ultra-distal radial BMD) was similar between the genders. BMDd prior to puberty did not correlate significantly with anthropometric variables in either sex, while BMDud was positively correlated with both height and body mass in both sexes. Surprisingly, age was not a significant predictor in either measure prepubertally.

When both mass and height were entered into multiple linear regression analyses it was concluded that height was significantly associated with BMDud in the females as was mass in the males. In adolescents (mean age=13.4 yrs.) mean BMDud was 8.2% ($p<0.0001$) higher in boys than girls while BMDd was similar in both sexes. Age, height and mass were all significantly correlated with both measures in both sexes. In multiple linear regressions, female BMDd correlated positively with age and mass but negatively with height, and BMDud was positively correlated with age and weight. In the boys, both densities correlated well with age and mass. BMDd increased dramatically from age 11 in the females and 13 in the males until age 17 yrs. (study end). During this period, the average increase rate was 10% higher in the females. The only difference in the adolescent group was in the BMDd where females had greater values in the 15-16 yrs. age group. BMDud increased from age 10 in boys and 11 yrs. in girls. After 11 yrs. of age, all boys groups had greater BMDud (not significant in 15-16 yrs. group). In the females, both the increases in BMDd and BMDud were highest in the two years following menarche. BMDd continued to increase after the third year following menarche, but BMDud did not. By the age of 17 yrs. girls had adult values in BMD. Trabecular aBMD was at a mature state in the girls by 14 yrs. The authors concluded that female cortical and trabecular aBMD are regulated by different factors; from 11 through 16 yrs. of age, 33% and 22% of the cortical and trabecular density, respectively, were accumulated.

In summary, it appears that prepubertally there is little difference between genders for aBMD and that following puberty males tend to have greater aBMD at more cortical sites (FN, TB, FS) and equal or slightly less aBMD at the more trabecular-dominated LS when compared to females. However, since males typically possess greater bone size as compared to females it is impossible to determine whether the differences described between the genders are a true or whether the differences are primarily a result of DXA not adequately accounting for size differences.

2.5.4 Changes in volumetric bone mineral density

2.5.4.1 Volumetric BMD and chronological age

Several investigations that have examined the true volumetric density of growing bones have concluded that the observed increase in aBMD with age is unfounded (103;180). Trotter (181) compared ash density in the radius, humerus, tibia and femur in 143 cadavers, who at death ranged from birth to adulthood (22 yrs.). All density calculations were done by ash density where the volume of the bone was established by displacement and the mass by ash weighing. She observed an early and rapid increase in density from birth through 5 yrs. after which there was a gradual leveling off of increases in bone density, which seemed to be continued into the early twenties, at which time losses began. Most importantly, there was no discernable increase in bone density accumulation during puberty as suggested by aBMD investigations (181;182).

Schonau et al. (103) determined distal radial BMD by pQCT in eight healthy children (4.8 – 8.3 yrs.) and concluded that density remained stable throughout growth and that the density measurements were similar to those found in adults. The authors surmised that bone likely adapted with changes in bone biomechanics and structural characteristics rather than density during growth. Unfortunately, this investigation was intended to be a pilot study and there is an extremely small sample size (103). Similarly, Zamberlan et al. (183) x-rayed the non-dominant wrist in 167 females and 158 males and estimated that the volumetric BMD of the radius remained stable from 3 through 21 yrs. They concluded that skeletal growth is mainly related to bone enlargement and relative diminution of the medullary cavity in the appendicular skeleton, whereas volumetric bone density is identical in males and females and increases after puberty as a result of diminished bone turnover (183). In another cohort of girls aged 4-15 yrs. of age radial cortical BMD as assessed by QCT was reported to remain unchanged over the entire growth period (184).

2.5.4.2 Volumetric BMD and pubertal stage

In a QCT study by Gilsanz et al. (175) pubertal children had significantly greater trabecular and cortical LS BMD compared to prepubertal children. There was a gradual maintenance of LS BMD during childhood with a rapid gain during puberty (~20-25%) after which it plateaued.

However, the methodology in this investigation was questionable as there were not many postpubertal subjects, so a true plateau cannot be supported (160). In another QCT investigation, adolescent girls (mean = 15.9 yrs.) had significantly higher trabecular BMD as assessed by QCT when compared to young, adult women (mean age = 30.3 yr; 196.2 vs. 180.3 mg/cc K2HPO4). Further, multiple regression analyses failed to find a significant relationship between trabecular LS BMD and height, weight, surface area or BMI (r = 0.09-0.30). The authors concluded that trabecular vertebral density reached its peak in females around the time of cessation of longitudinal growth and epiphyseal closure, not the end of the third decade as suggested by others (175).

Mora et al. (180) cross-sectionally conducted a QCT investigation with 96 Caucasian girls (4-20 yrs.) to differentiate LS BMD in both the trabecular and cortical bone compartments. Both compartments of bone generally increased with age. Cortical bone increased gradually with each successive stage of puberty; however, cancellous bone was generally stable through Tanner stages 1-3 but increased from 3 to 4 and then plateaued. Interestingly, small decreases of cancellous bone in early puberty occurred, resulting in the values for girls in early puberty being slightly less than prepuberty. Significant correlations occurred with age, height, weight, BMI, BSA and cortical BMD, but there were no significant correlations between these variables and cancellous bone in the entire age group (180).

A separate analysis of only the prepubertal girls (n=38; 4-12 yrs.) showed a trend of increasing cortical bone with age, but decreasing cancellous BMD. Additionally, as the volume and the height of the vertebrae increased there was increased cortical density but decreased cancellous density. The cortical bone was positively associated with anthropometric variables whereas the cancellous bone was negatively associated in this prepubertal subsample. The results of this study suggest that there are differing patterns of cortical and cancellous vertebral density throughout growth (180). The authors concluded that DXA investigations, which show a gradual increase of aBMD with age, are influenced by the amount of cortical bone and/or the size of the vertebrae and that cortical bone is most responsive to loading. Cancellous bone may be less impacted by loading during puberty, but is more dependent upon endocrine status in the first

two decades (180). However, due to the high degree of error associated with the measurement of cortical bone at the LS with QCT (a result of the extremely thin cortical shell and errors in the pixilation of it), caution must be used in interpreting these results.

2.5.4.3 Gender Differences in Volumetric BMD

In an investigation by Gilsanz et al. (160), true volumetric bone density, as assessed by QCT, did not differ between boys and girls (n=101; 2-18 yr. of age) as long as maturity was controlled for. One of the flaws of this investigation, however, was the classification of pubertal status based on age. Basically, the authors categorized children as prepubertal, pubertal or postpubertal entirely based on chronological age. Multiple regression analyses failed to find a significant relationship between trabecular LS BMD and height, weight, surface area or BMI (r = 0.1-0.29), similar to Mora et al. (180), differing from almost all investigations that have assessed LS aBMD with DXA. Further, Gilsanz et al. (108) concluded in an investigation of primarily prepubertal children that males and females have identical LS trabecular and cortical BMD and that the apparent gender differences in bone mass in children are simply a reflection of bone size.

2.5.5 Summary

As individuals grow and mature, their bones grow in both size and increase in BMC and aBMD as assessed by DXA. These increases are typically rapid during early childhood (0-2 yrs.), slowly increasing through childhood, and once again accelerate for the period surrounding the pubertal growth spurt. The earlier punctuation in bone mass and density in females during puberty reflects the fact that females typically experience puberty two years earlier than their male counterparts. Following the pubertal growth spurt, bone mass gains in females slow or cease, whereas males tend to continue a slow increase into their third decade. Unfortunately, most of the information gathered regarding bone mass and density and growth has been garnered from cross-sectional investigations. Dependence on cross-sectional investigations can lead to errors in the interpretation of growth-related data as it often tends to distort the normal growth curves that would be observed longitudinally. To date, only one longitudinal cohort has been of sufficient sample size to adequately describe the changes in bone mass during adolescence (59). Compounding

the degree of error in pediatric bone research is the fact that the DXA calculates an areal bone mineral density as compared to a true volumetric density. This areal density is highly dependent on bone size and any fluctuation in this parameter can lead to gross errors in the assessment of bone change over time. The paucity of investigation that has been carried out with QCT has suggested that the dramatic increases observed in aBMD over puberty are most likely erroneous. Lastly, very few investigations have attempted to control for pubertal status, almost certainly introducing error in to the BMD patterns described.

2.6 *Changes in geometrically corrected areal bone mineral density*

Numerous investigators have attempted to correct aBMD for bone size in adults. Reid et al. (185) reported that after adjusting for height the differences observed between TB aBMD men and women were eliminated. They further postulated that any linear measure of body size would provide an adequate size correction, as the skeleton tends to scale proportionately. In contrast, Faulkner et al. (186) observed that after adjusting for height there was still a difference in aBMD between elderly men and women. The elderly males were heavier and taller than the elderly females and possessed significantly greater aBMD values than the females at the LS, FN and TB. However, after adjusting for bone projected area (based on the Kroger equation) there were no significant differences in estimated volumetric BMD between the genders (186).

2.6.1.1 Geometric corrected aBMD and chronological age

Kroger et al. (68) compared aBMD and statically corrected volumetric BMD (volBMD) at the LS and FN in 84 Finnish children and adolescents (44 females and 40 males) aged 6-19 yrs. There was a significant increase in LS aBMD with age and BMC and aBMD were significantly correlated with mass, height and BMI at the LS. When LS aBMD was adjusted for bone size, density only increased minimally, far less than what had been presented based on the evidence from aBMD investigations (68). Kroger et al. (56) performed another investigation with 65 Finnish children (37 females and 28 males) aged 7-20 yrs. to determine changes in BMD with growth and maturation. Male LS aBMD significantly increased with age (r=0.89) and there was significant

linear trend between age and LS volBMD (r=0.70). In the females there were similar correlations between age and LS aBMD and volBMD (r=0.77 and 0.54, respectively). In females the most marked increase in LS aBMD occurred between 11 and 13 yrs. (13% per year.). Long bone growth in females decreased markedly following menarche. LS BMC gain before menarche was primarily dependent upon expansion of the vertebral envelope, whereas after it was ascribed to an increase in volBMD. In males, the maximum increase in LS aBMD was between 13 and 17yrs. (11% per year). The maximum increases in LS volBMD occurred at the same time, but with muted gains (5-6% per year). In a similar study, Boot et al. (177) reported that LS BMAD increased with age and after adjusting for age, height had no significant association with BMAD in either sex.

When volumetric corrections were made to FN aBMD, correlations between volBMD and size measures were no longer significant (68). Surprisingly, in this cohort there was no significant correlation of age to FN volBMD suggesting that the observed increases in FN aBMD with aging were an artifact of bone size. Unfortunately, this investigation was confounded by lack of control for pubertal stage (68). Similar to other investigations, Kroger et al. (56), found a significant increase in FN aBMD with increased age. The males in their study had a significant increase in FN aBMD with age (r=0.68), but following adjustment for apparent volume, no significant relationship was found between age and FN volBMD, as was found in their previous investigation. The boys had significantly higher FN aBMD in the 6-7 and 18-19 yrs. age groups as compared to the girls, whereas the FN volBMD was significantly higher in the males for the 18-19 yrs. group only (56). For the adjusted values the FN volBMD remained constant over childhood and adolescence for both sexes. The authors suggested that before puberty the increase in bone size is a more important constituent of bone mass accumulation than increases in material density. Bone mass accumulation was more rapid and longer lasting in the males with the onset of puberty, especially at the FN and expansion of the bone envelope was especially pronounced in the males. In the females, accumulation of BMC, bone expansion and height gain slowed soon after the menarche (56).

In a mixed-longitudinal study, when FN aBMD was adjusted as with Kroger et al. there was no apparent change with age or a sex difference among males and females aged 6-18y. for FN

volBMD (61) . Despite the dramatic change in aBMD observed in the Ferrari et al. (24) longitudinal trial there were little, if any, changes in BMAD at the LS, FN or FS during puberty/prepuberty.

Finally, Lu et al. (187) investigated changes in LS, FN (Kroger technique) and FS volBMD in 209 subjects (109 males) aged 5-27y. The mid-femur was estimated by

Formula 14: pi x length of femur section x (diameter/2)^2 (diameter = area/height).

The femoral neck was assumed to be cylindrical as well, therefore

Formula 15: pi x height x (diameter/2)^2 .

Lu et al. observed a significant age-dependent increase in LS aBMD in both genders with increases that plateaued at 17.4 yrs. of age for males and 15.7 yrs. of age for females. LS aBMD was height and mass dependent even after controlling for age. LS volBMD was significantly associated with size variables but after controlling for age, height lost its significant interaction with LS volBMD. FN aBMD was significantly associated with age, height and weight (age-controlled as well), whereas FN volBMD had no association with age or weight in either sex, similar to other investigations. FN volBMD was related to height in females but not males and there was no significant sex difference. Femoral shaft (FS) aBMD increased with age in both sexes until 17.2 yrs. in the boys and 13.5 yrs. in the girls. Again, height and weight were significantly correlated with aBMD even after controlling for age. Conversely, FS volBMD measures had no significant association with height or age as volBMD stayed consistent across the age ranges. When corrected for volume, males had significantly higher FS volBMD than the females (187). The authors concluded that the volBMD of the FN and shaft were independent of age through childhood and early adulthood (187), similar to what is seen with QCT studies (103;148).

It is possible that the true density of the LS increases with age because of an increase in the number of trabeculae during the growth period. Anatomically, the lumbar spine vertebral body is neither a cylinder nor a cube, which invariably adds error to the estimation of true density in the static or geometric-based equations. To further add error, the posterior aspects of the transverse processes are inevitably included in the quantification of BMC, but not in the bone projected area. A combination of posterior-anterior and lateral DXA scans may provide a more realistic assessment of the bone dimensions of the LS, however the technology is currently limited by the

large error in the lateral scan assessment of bone projected area. It appears that in the more cortical regions (FN and FS) that after correction for volume there is minimal association between age and volBMD, as supported by QCT and cadaveric investigations.

2.6.1.2 Geometrically corrected aBMD and pubertal stage

In a mixed pubertal sample (9-21 yrs) with few subjects in each pubertal stage (3 prepubertal, 8 in early puberty, 15 in midpuberty, and 19 in late puberety), acceleration in BMC and aBMD was most rapid in the early teens and tapered off at approximately 16 yrs. of age (69). Contrasting to this, BMAD increased with age at the spine and midradius, but there were no significant changes associated with age at the whole body or femoral neck. When BMC, aBMD and BMAD were correlated with measures of weight, height and Tanner score, the highest correlations were seen with BMC, followed by weaker correlations with aBMD and the weakest with BMAD. All of the predictors of bone mass were found to be highly intercorrelated ($r = 0.78$-0.89). The results demonstrated that the increase in height and weight associated with pubertal growth in this group of girls was largely completed by the age of 16 yrs. and that the greatest increases in bone mineral occurred in the younger girls and from those who had recently begun to menstruate. The authors suggested that 50% of the change in lumbar spine BMC is accounted for by expansion in vertebral dimensions alone. Similarly, they suggested that 96% of the changes in femoral neck mineral, 55% of the midradial shaft, and 99% of the adolescent increase in TB BMC was due to bone expansion, rather than increased mineralization, implying that the expansion of cortical area accounts for the BMC changes. The two most important determinants of bone mass acquisition were found to be pubertal stage and body size (69).

Plotkin et al. (188) measured LS bone density in 433 Argentinean girls between the ages of 2 and 20 yrs. They corrected LS aBMD for body height according to the formula:

Formula 16: LS volBMD = BMC/(BA x height).

aBMD was also measured by lateral DXA, and a volumetric estimate was made. Between Tanner stages 1, 2 and 3 there were significant differences in LS aBMD for both PA and lateral scans, but no significant differences between Tanner stages 3 to 5. The most rapid growth in LS aBMD occurred from 11-15 yrs. of age. LS volBMD values showed significant differences between

Tanner stages 1-3 and no differences between 3-5. Interestingly, there were no differences observed for lateral LS volBMD for any stage. The authors concluded that the rapid gain in aBMD observed from 11-15 yrs. was not realized when corrected for volume (188).

Uusi-Rasi et al. (163) investigated 176 eight to 20 yr. old females to determine growth patterns of the LS. LS BMAD was assumed to describe the bone composition and trabecular architecture as a lumped, size-independent parameter of average porosity. Mass and height were significantly associated (0.62-0.90) with LS BMC and LS aBMD with the strongest correlations with LS BMC, as expected. Only body mass was significantly correlated with spinal BMAD of the pre and post-pubertal subjects, with no significant correlations in the peripubertal group. BMC, aBMD and BMAD at the spine increased with age at all measurement sites and both age and Tanner stage had high correlations with the bone variables (0.41-0.86). Radial BMAD had no relations with age or Tanner stage. In regression analyses Tanner stage was a better predictor than age in both radial and LS bone measures, demonstrating the importance of sex steroids during puberty.

2.6.1.3 Gender differences in geometrically corrected aBMD

In the investigation by Boot et al., girls had higher LS BMAD than males at all ages (177). Similarly, in Kroger et al., before the age of 12 yrs. there were no significant differences in LS volBMD between genders, but in the 12-13 yrs., group and the 16-17 yrs. group, the females had significantly higher LS volBMD (68).

When FN aBMD was adjusted as with Kroger et al. there was no significant change with age or a sex difference in a large Canadian cohort (40). In contrast, the mean adjusted FN volBMD was significantly higher in the males as compared to the females in a Finnish cohort (68).

2.7 *Changes in statistically corrected bone mineral density*

2.7.1.1 Statistically corrected aBMD and chronological age

Warner et al. (128) utilized the dynamic correction methods of Prentice et al. to generate predictive formulae for BMC based on PA, age, height, weight, pubertal stage and gender. A mean regression line of BMC was created that could be used to monitor healthy children or to examine those with a deficiency of normal bone metabolism. All aBMD measures were significantly

($P<0.001$) related to PA, elucidating the size dependence of aBMD. They concluded that a more appropriate model for aBMD would be BMC/BA^{lambda}, where lambda was the power coefficient from the regression equation of BMC on BA. The power coefficients after regressing BMC on PA were: whole body (1.39), LS (1.66), total hip (1.44), femoral neck (1.63), trochanteric region (1.26) and intertrochanteric region (1.42); all of the regression coefficients were significantly different from unity ($p<0.01$). After sBMD was calculated using this method the correlation of PA to BMC became insignificant at all sites ($r=0.02$-0.03). Lastly, after correction for pubertal stage and body size there were no differences between genders. Unfortunately, the very small sample size utilized was likely insufficient to be setting guidelines for norms (58 subjects over 5 pubertal stages and two genders). Further, their research was cross-sectional in nature and susceptible to the bias associated with the establishment of norms based on that form of data analysis (128).

It is evident that there is a need for scaling or some type of correction to be performed in order for a clear picture to emerge of what is actually happening during growth and development in terms of true volumetric density. Until the time when bone size can be controlled for it will be impossible to accurately longitudinally assess the changes that are occurring in bone mass during adolescence and childhood.

2.8 *Increased incidence of fracture during the adolescent growth spurt*

Increased incidences of distal forearm fracture during adolescence, particularly around the period of the growth spurt, are a frequent observation in an abundance of epidemiological investigations (189-195). In fact, the peak fracture incidence in both sexes corresponded approximately with the time of PHV (194). After this period of apparent skeletal fragility, fracture rates decrease into adulthood and remain low in men but again increase substantially in women after the age of 50, coincident with the occurrence of menopause (1).

2.8.1 Increased physical activity or increased body mass as a cause for greater fracture rate

Frequently, the increase in fracture incidence associated with the growth spurt has been attributed to increased levels of physical activity, and thus a higher probability of incurring a traumatic accident of some sort (44). While this theory does seem to have merit, on closer inspection of physical activity patterns in children and adolescents, this period is actually when there is decreased physical activity as compared to years previous (194). It is also unrealistic to assume that there is an increase in physical activity for the years during the growth spurt, and for significantly lower levels just before and after this period. Distal forearm fracture incidence seems to be climbing when activity is falling, leading to the conclusion that they are not causally related.

During the growth spurt, a concomitant significant increase in body mass is witnessed in both males and females (65). It is a fundamental dictum in bone biology that bone mass and architecture are intimately related to load bearing. However, in a time of rapid linear growth, it may be the case that all exogenous calcium (and almost certainly some endogenous from trabecular sources) is shunted to those areas of rapid growth of the long bones, as consolidation can and does occur afterward (44;146). During the growth spurt, trabecular mass may be relatively unchanged from prepubescence, but now must support the mass of an individual 15-20 kg heavier than when that structure and mass of trabecular bone was appropriate to withstand dynamic loading of this magnitude. At this time should a traumatic stress related to body mass be encountered at the distal forearm, it would have significantly more inertia than the trabeculae were

designed to resist and theoretically be more prone to fracture. Other factors may be at play as well to worsen the situation. Since the bone is sensitive to the loads placed upon it (34;123), it is probable that at some time the trabeculae of the distal forearm would be structurally inadequate for the stresses placed upon them. Bone adapts to increased stress by either altering its material properties or changing its architecture to better suit its loading environment. During the growth spurt it has been postulated that the calcium is shunted to the areas of longitudinal bone growth, therefore the building blocks for accruing more mass are not available (44). In light of the inability to accrue mass at this time, the trabeculae may attempt to remodel to some extent to better be suited to the loads commonly encountered. However, the inherent weak link in the chain is the chronology of the remodelling cycle in bone. While it only takes a few days to excavate the bone for remodelling, it can take up to as long as 4 months for replacement and full mineralization to occur afterwards (109). Thus, the remodelling space increases in the trabecular bone, causing both less mineral mass and decreased biomechanical competence, both of which decrease the strength of the structure and make it susceptible to fracture under lower loads.

A further theory is that the epiphyseal cartilage of the growth plate may act as an "attenuator" of force before it is mineralized for the distal forearm. Since a large proportion of the plate is of cartilage, and cartilage in terms of a material is relatively malleable, it can be seen as a "crash pad" for the distal forearm in that any stresses coming through the distal forearm will be passed directly to the cartilage. As the cartilage becomes more completely mineralized near the end of the growth spurt, it will attenuate less of the forces placing the trabecular portion of the distal forearm in a position where it many be crushed by the stress of the load on one side and by the greater mechanically stiff cortical bone on the other. This would put the site in the greatest mechanical disadvantage experienced to that time. Later, consolidation would allow more proper mineralization of the trabeculae and corticies, allowing for this type of fracture to occur less easily. However, there is a time of fragility for the distal forearm when it has lost the structure to maintain its strength, has lost some mass, and has now lost its "shock absorber".

Therefore, the question remains as to why there is an increased fracture incidence in the distal forearm during the growth spurt. While there has been exceedingly little direct research

performed to address this question, there have been a number of investigations that have addressed it indirectly.

2.8.2 Bone turnover during adolescence and menopause

During bone turnover osteoclastic resorption forms pits on and in the bone that subsequently are filled with osteoid from osteoblasts. These pits alone can lead to biomechanically weaker bone. After the osteoid is laid down from the osteoblasts it typically takes about two weeks before any mineralization occurs and it slowly becomes further mineralized over the next six months. Since it takes nearly half a year for bone to become fully mineralized, recently deposited matrix has a lower material density than does that of relatively older bone matrix (84). Therefore, the material density of a given bone is inversely related to the rate of bone turnover as with higher turnovers there will be relatively more undermineralized bone than in situations with lower rates of turnover (84;196).

Bone turnover and remodeling are dramatically increased during growth, as evidenced by bone resorption markers; for example, urinary excretion of hydroxylysyl and lysyl-pyridinoline peak during adolescence and decline with the cessation of growth and skeletal maturity (197). Bone turnover markers peak in boys and girls at approximately 12 and 14 yrs. of age, respectively, approximately the same time as PHV , and peak fracture incidence (53;197). Unfortunately, remodeling carries with it the burden of a small amount of bone lost per BMU, but more importantly it always leaves a remodeling space as a result of the inability of the osteoblasts to immediately fill in the areas excavated by the osteoclasts (198). The remodeling space normally represents about 2-5% of the total volume of a given bone, but is increased as the turnover of a bone increases. Therefore, an increase in the porosity of the bone is witnessed in high turnover conditions (38). This increased porosity would lead to decreased mechanical strength.

At times of high turnover, cancellous bone, such as that seen in the vertebrae after menopause in women, can become biomechanically weakened and fail under smaller loads (38;198). In the femur, cortical bone during times of elevated turnover become mechanically impaired due to increased porosity (29), which is alarming since the cortical shell at the distal radius is thinner and thus a given resorption cavity would take up a greater proportion of its

thickness, further compromising its strength (109). Further, Cadogan et al. (143) concluded that peak bone turnover rates occurred 1-2 years in advance of peak bone mineral accretion. Therefore, remodeling is occurring at its most rapid rate, thereby increasing the remodeling space and decreasing biomechanical integrity, well before peak mineral accretion.

The only other time in the lifespan when there is a dramatic increase in the fracture rate is postmenopausally in women. One of the characteristic responses to the loss of estrogen is an increased turnover of bone during the years immediately following menopause. There are many negative effects of increasing turnover; since there is a slight loss of bone in each BMU, an increased turn-over would mean that there would be increased losses of bone (there is evidence that there is even more bone lost per BMU in postmenopausally) and a dramatic increase in the remodeling space which can represent up to 10% of the skeletal volume. With high levels of bone turnover, increased rates for fracture especially in those areas with high components of trabecular bone such as the radius, the vertebrae, and the femoral neck invariably result.

2.8.3 Calcium requirement and adolescence: inadequate supply?

The adolescent growth spurt is punctuated with dramatic increases in total body bone mass and is one of the only periods during life that there is concomitant deposition of bone on both the periosteal and endosteal surfaces (131). During this time of peak growth in bone the requirements for calcium are excessive, even to the extent that optimum intake and absorption may be insufficient to meet the demands for calcium (145;146;199). In order to meet the demands of rapid growth in the long bones, it is essential for adequate calcium to be available. Rarely do children meet the RNI for calcium in a given day, further compounding the insufficiency of exogenous calcium (146). If exogenous sources are unable to supply the needed calcium for growth, then it must come from endogenous sources of sites not undergoing rapid growth. The most readily available supply of endogenous calcium is from trabecular bone owing to its high metabolic activity (5-10 times higher than cortical bone) (109). Calcium for longitudinal bone growth has also been suggested to originate from the cortical shell (40). Therefore, bone must be taken from regions where there is little growth occurring to be used for foundational building at

areas that are undergoing extensive collagenous matrix formation, and thus need the calcium in order to mineralize this tissue, if even incompletely.

Interestingly, Bonjour et al. (200) concluded that calcium supplementation in pubertal aged girls showed a preferential effect on the appendicular skeleton, demonstrating perhaps a persistent deficiency in this area during growth, furthering the hypothesis that this region may be somewhat calcium deficient as a whole during rapid growth.

2.8.4 Mechanical changes in bone with adolescent growth

The ultra-distal portions of both the radius and the ulna are comprised of predominantly trabecular bone, encapsulated by an exceedingly thin (~200 μm thick) shell of cortical bone (38). The trabeculae at this site provide the majority of strength needed for support during dynamic loading (201). A greater proportion of rigid cortical bone is found moving proximally into the diaphysis of each bone. Since the majority of functional strength is recognized as originating from the trabecular bone at the distal radius, it is interesting that no studies have yet addressed the affects of this compartment of bone at the distal forearm as related to fracture risk during adolescence.

Bone strength is most highly correlated with bone density at all times during the lifespan, accounting for between 70-80% of the material strength of the structure (38). However, architecture also plays a large role in strength, and it has been observed that with rapid growth there is an increase in the porosity of the cortical shell (44). Unfortunately, there have not been any investigations that have questioned the effects of rapid growth on the trabecular bone in this region, but one would suspect that the losses here would be more acute that those witnessed at the cortical region, since trabecular bone is more sensitive to hormonal fluxes than is cortical bone (109). Cortical bone porosity may be a conservative marker for what may be occurring underneath the thin cortical shell. It is well established that trabecular structure, in terms of connectivity and the actual width of the trabecular struts, account for much of the mechanical strength (38). Further evidence by Turner et al. has demonstrated that trabecular architecture mimics the external and internal forces engendered upon it to better withstand typical loading

environments (81). Accordingly, any loss of trabeculae mass in the distal forearm could lead to diminished biomechanical strength in the region.

Differences in the matrix and mineral, the modulus of elasticity, bending strength and tensile strength of cortical bone are all lower in children as compared to adults (202), but are no lower during adolescence than at any other time during childhood, so probably do not independently account for the increase in distal fracture rate observed. However, during rapid growth imperfect alignment of collagen fibers with the principle directions of loading has been reported (203), which leads to a decrease in the mechanical properties of the tissue, most specifically tensile strength, which would lead to increased fragility. The modulus of elasticity and the breaking strength of bone are directly proportional and attributed to the orientation and quantity of collagen fibers, as the direction and organization of fibers will have a large influence on the patterns of mineral deposition (203) There has not been a study completed that has utilized technology that would be appropriate to detect the microarchitectural changes that could be occurring, or the variations in density of the trabecular bone during this time of seeming fragility.

Hagino et al. (190) measured metaphyseal and diaphyseal BMD at the distal radius in children at all stages of growth. They observed that BMD of the metaphysis increased slowly before puberty, whereas the diaphysis increased linearly throughout the period of skeletal growth. Accordingly, the metaphyseal/diaphyseal ratio was lowest at the age when the peak incidence of distal radial fractures occurred. It has been previously described by QCT that cortical bone increases linearly through childhood and adolescence, whereas trabecular bone stays relatively steady throughout adolescence, but gains rapidly after linear growth had arrested in late adolescence (180). In other words, there is an arrest of accretion to the trabecular bone at this time (maintenance of status quo), with all the efforts of the system being expended into growth of the metaphyses.

As a testament to the rapidity of trabecular bone lost in the distal part of the radius as compared to the diaphyses, an investigation of children who fractured their arm had their BMD assessed before and after casting, to study the amount and patterns of bone loss. An immobilization period of between three to six weeks resulted in a reduction of the bone density of

up to 44% (mean 16%) in the distal part of the radius, whereas no significant change could be seen in the diaphyseal part of the same bone (204); the most rapid site of loss being that of the highest proportion of trabeculae.

A crude semi-quantitative measure of radial cortical porosity, the striation index, indicates high porosity in childhood that peaks at adolescence, but then falls dramatically into adulthood (205). This increased porosity has been suggested to be one of the causes of the increased fracture rates (198). After the cessation of linear growth, there is a slowing of intracortical remodelling and a decrease in cortical porosity, which leads to bone mass gain and is termed consolidation. Bailey et al. (206) also suggested that there is a temporary increase in the porosity of bones during the most rapid period of linear growth. Thus a pattern of both extensive trabecular and cortical loss appears during the adolescent growth spurt. Loss of any amount of bone in an area such as the distal radius, which is particularly susceptible to traumatic fracture owing to its anatomical location, could theoretically be the cause of the increased fracture incidence witnessed.

Skaggs et al. (184) recently reported that in a group of young girls (4-15 yrs. of age) the primary cause of radial fracture was a decreased relative cross-sectional (8% smaller) area as compared to controls and an increased body mass. Interestingly, BMD, as assessed by QCT, was not different between the fracture and nonfracture groups.

Parfitt (44) postulates that one of the causes of a weaker bone during the adolescent growth spurt may be due to the inability of newly laid down periosteal bone to achieve full mineralization before it is subsequently absorbed on the endocortical surface of the bone.

2.8.5 Dissociation between bone expansion and bone mineralization

Bone mineral accrual continues after the cessation of linear growth, as the bone becomes consolidated (142;207). Peak bone mineral content velocity occur approximately 1.2-1.6 yrs. after PHV in boys and girls, respectively (146), elucidating a dissociation between volumetric growth of bone and its subsequent mineralization. Interestingly, in a large cohort of girls followed longitudinally it was concluded that the age of onset, peak growth velocity, and completion of growth of a regions external dimensions preceded the mineral accrued within the periosteal envelope (137). Further, they demonstrated that the deceleration in bone mineral

accrual occurred 1 year later than the deceleration in bone length at both the vertebral body and the femoral diaphysis, demonstrating dissociation between bone mineral accrual and bone expansion.

Clearly there is physiological rationale behind the increased prevalence of fracture surrounding PHV and it is most likely attributable to dissociation between expansion of the bone envelope and its subsequent mineralization.

2.9 *Statement of problem*

Numerous investigations have sought to describe the changes that occur with bone mineral density during growth and maturation and the majority have concluded that BMD generally increases throughout childhood, accelerates through puberty, and slows and finally plateaus in early adulthood. This generally supported conclusion has its foundations in a large number of investigations that described the changes in DXA-derived aBMD over the pediatric years, with the preponderance of these studies cross-sectional in nature and with chronologically-based groupings.

Cross-sectional investigations provide inaccurate and often misleading data for the development of growth standards. Further, since the timing of puberty is variable and because it has such a large impact on both bone size and bone accrual, it is imperative to have some form of control for maturation in order to control for this variability and accurately describe growth; very few investigations have attempted to categorize children with respect to their pubertal status and of those that have, few have been methodologically sound. Unfortunately, aBMD is confounded by bone size in its representation of true density. A paucity of cross-sectional investigations have utilized techniques to partially control for the effect of bone size during growth and maturation on the measurement of aBMD by DXA, with none controlling for maturity.

Therefore, the true volumetric BMD patterns during adolescence are largely unreported and/or inaccurate due to a strong dependence on cross-sectional studies, a lack of proper grouping of individuals based on maturational development, and the reporting of only size-dependent aBMD.

This investigation is unique because:

1. No previous investigation has had the serial data available to **longitudinally** compare different measures of bone density over the growth period or to describe the patterning and tempo of bone density changes precisely through velocity analyses; velocity analyses are a unique strength of longitudinal data as they allow for the rate of accrual to be determined. Further, longitudinal analyses allow for the retention of

individual growth curve characteristics, which is essential in the production of normal curves.

2. No previous investigation has investigated these bone density variables grouped by an objective, definitive **maturational** benchmark such as PHV.
3. No other investigation has compared aBMD, BMAD and sBMD in an adolescent population to try and accurately describe the **size-independent bone density patterns** that occur with growth and maturation, which were, up to this point, largely unknown.

The purpose of this investigation was to longitudinally describe changes in BMD patterns across childhood and adolescence in a cohort that was maturationally aligned to minimize variability associated with differential timing of puberty. Further, patterns in aBMD over the investigated growth period were compared with two measures of BMD that attempted to correct for the size-mediated errors in DXA assessment of aBMD.

2.10 *Hypotheses*

This investigation tested the following hypotheses:

H1: aBMD is significantly influenced by body height, body mass and the bone projected area of the respective site.

$H1_1$: Body height, body mass and bone projected area will be significantly positively correlated with areal BMD at all sites.

$H1_2$: All regression coefficients of PA regressed upon BMC will be significantly different than unity at all measurement sites.

H2: aBMD corrected for bone size will not be significantly influenced by body height, body mass and the bone projected area of the respective site.

$H2_1$: Body height, body mass and bone projected area will not be significantly correlated with bone mineral apparent density (BMAD) at any site.

$H2_2$: Body height, body mass and bone projected area will not be significantly correlated with statistically corrected BMD at any site.

H3: The age-related increase in aBMD in both genders is eliminated when corrected for bone size.

$H3_1$: aBMD will increase significantly throughout the growth period at all sites in both genders.

$H3_2$: BMAD will not change significantly throughout the growth period at all sites in both genders.

$H3_3$: sBMD will not change significantly throughout the growth period at all sites in both genders.

H4: The gender difference observed in aBMD will be eliminated when corrected for bone size and maturation.

$H4_1$: Males will possess significantly greater aBMD at all sites as compared to females, when corrected for maturation.

$H4_2$: No statistical difference will exist between genders for BMAD at all sites when corrected for maturation.

H5: The age-related acceleration in aBMD velocity during the pubertal years is eliminated when corrected for bone size.

$H5_1$: aBMD velocity will remain positive throughout the growth period at all sites in both genders.

$H5_2$: BMAD velocity will be negative or zero through the growth period at all sites in both genders.

$H5_3$: sBMD velocity will be negative or zero through the growth period at all sites in both genders.

H6: There will be a significant lag period between the age at peak PA velocity and age at peak BMC velocity.

Throughout the thesis sections pertaining to a specific hypothesis will be preceded by a designation of the hypothesis number and subhypothesis denotation, when appropriate (ie. for hypothesis 4 sub 2 = $H4_2$).

2.11 *Limitations*

As the distance curves generated in this investigation are of a mixed-longitudinal design and not a true longitudinal design, the conclusions that come from the distance curves may be subject to bias and should be viewed as such. Growth curves generated from cross-sectional data tend to be smoothed and can mask the wide range of individual variation inherent in any age group of children (62).

Although the non-parametric smoothing spline (in this investigation, the cubic spline) technique preserves the major features of the velocity curves of growth it has some drawbacks; most notably, the spline may significantly underestimate the value of peak velocity of growth during the adolescent spurt. This phenomenon is the result of serial velocity measures put together in a piecewise fashion, which may not adequately reflect changes in velocity, and are often truncated (208).

Since DXA does not provide separate cortical and trabecular measures, it is only possible to provide an integrated estimate of aBMD.

The conclusions made here should be limited to primarily Caucasian populations, as approximately 98% of the cohort was of that race.

In the mixed-longitudinal data (distance data) a general linear multilevel ANOVA was utilized to determine significant age and gender effects. Since the data was not independent in each age group (there was a degree of serial correlation as there was longitudinal and cross-sectional data combined) the assumption of independence for ANOVA analyses was broken. However, this was the most appropriate method of analysis based on the study design.

3 Methods and Procedures

3.1 *Subjects*

Participants were males and females from the Saskatchewan Pediatric Bone Mineral Accrual Study (SPBMAS). The SPBMAS is a mixed-longitudinal investigation of seven years in duration (1991-1997) that's primary objective was to describe the effects of growth and maturation on bone mass through adolescence. At study initiation, 375 children (8-14 yrs.) from two Saskatoon (Saskatchewan, Canada) elementary schools were invited to participate, of which 228 (115 females and 113 males) accepted and obtained parental written consent. Over 98% of the sample was Caucasian, with Aboriginal, Asian, and African individuals accounting for the remaining 2%. During the seven years of study, numerous individuals were added to the sample, while some individuals chose to discontinue or were lost to follow-up. Consequently, the sample size fluctuated from year to year. Mixed longitudinal (or multiple longitudinal) designs have the advantage of being able to isolate main effects, such as age effect, from interfering effects such as time of measurement and cohort (64). Additionally, measures of dietary intake and physical activity habits were collected throughout the investigation to assess the impact of these variables on bone mineral accrual; these variables were not addressed in the current investigation. The SPBMAS was given approval by the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation (Appendix A) and all subjects, and their legal guardians, gave signed, informed consent prior to participation.

After seven years of data collection, a sub-sample of the SPBMAS (longitudinal database) was established that consisted of 70 males and 67 females that possessed enough growth data to allow for the accurate determination of their peak height velocity (PHV) through cubic spline linear regression. This longitudinal dataset was used for all of the analyses in this investigation as it allowed for the quantification of pubertal status based on PHV to correct for differences in maturational status of study participants.

Age at PHV was used as a maturational benchmark in this investigation. Maturational differences in the cohort were controlled by grouping individuals based on their respective age of PHV; all subjects were aligned on their respective age of PHV and were assigned maturational age values in relation to PHV. For example, a child at 2 years before his/her calculated PHV would be termed –2 PHV whereas a child 1 year after would be labeled 1 PHV. This method to control for differences in maturity is accepted in the pediatric literature as the gold standard (62), but has not been previously used to compare individuals for density measures since this information can only come from rare longitudinal investigations with serial density measures.

While bone measures at both chronological and PHV-based ages were determined in this investigation, only the PHV-based age data is presented and discussed in the formal body of the document as it is one of the unique strengths of this investigation; all chronological-based measures can be found in the Appendices for comparison with previous trials.

For the generation of the distance curves, all of the subjects in the longitudinal database were used. In this dataset, each individual was not necessarily represented in each data cell, therefore creating a mixed-longitudinal dataset. In other words, while the investigation reports values from 11 through 18 yrs. of age (-4 to 4 yrs. PHV), a particular individual may only have measurements for a smaller age span (ie. 12-16 yrs. or -3 to 2 yrs. PHV). Numerous individuals with different age spans are combined to allow for the estimation of bone measures over the entire investigated age span.

For the generation of the velocity curves, only a subset of the longitudinal data set was used; only those subjects that possessed data spanning a predetermined age span (ie. –2 to 2 years PHV or from 11 to 15 years of age). In this dataset, each individual was represented in each data cell, therefore creating a pure longitudinal dataset.

3.2 *Anthropometric and maturational assessment*

Anthropometric assessments were completed every six months for all individuals in the investigation (fall and spring measurements). At each visit, anthropometric measurements were completed three times, with the average of the two closest values taken as the criterion measure. Body mass was established by a digital scale (Toledo Scale, Canada; Model 2038), accurate to

0.1 kg, with the subject dressed only in light shorts and shirt. Stature was established without footwear by a fixed stadiometer accurate to 1 mm. During the assessment of stature, participants were instructed to fully inhale and hold their inspiration while light traction was applied to the occipital lobes of the cranium; since the intervertebral discs become compressed throughout the ambulatory day as a result of weight bearing, traction was performed in an attempt to decompress the vertebral discs to attain a true stature measurement. Skinfold and girth measurements were also collected on these occasions, but are not utilized in this investigation. Decimal age was assessed at each measurement point (years + (days/365.25)).

3.3 *Determination of peak height velocity*

Peak height velocity (PHV) is defined as the maximum rate (cm/y) of statural gain during growth. The age of PHV is defined as the age at which the maximum rate of statural growth occurs. Age at PHV has been found to be a useful landmark against which the tempo and timing of other tissues development can be anchored (62). In females the attainment of PHV is highly correlated with Tanner breast stage 2 and menarche, and in males moderately correlated with genital and pubic hair Tanner stage 2, although there is some degree of variation in the timing of the indicators of somatic and sexual maturation (62). Further, PHV is the most sensitive indicator of skeletal growth as it essentially based on skeletal length. Therefore, by aligning growth on PHV, individuals can be accurately compared maturationally. All individuals in this investigation were assessed for stature twice yearly, as described above. To allow for the assessment of velocity, a minimum of two time points are required to calculate the rate of change over that period. Height velocity was calculated by calculating the statural difference between two time points (Stature time 2 – Stature time 1, in cm) and dividing this by the time between the points (Time 2 – Time 1, in y). The product of this calculation leaves a velocity measure (in cm/y) and the calculated velocity value is ascribed to the midpoint between the two time points.

It is well established that statural growth, as well as linear bone measures, display a seasonal growth pattern of rapid growth during the summer months and slower growth during the winter months (209). To smooth seasonal growth differences, a rolling average technique was utilized which paired adjacent fall-fall measures and spring-spring measures and established their

velocities at the midpoint, thus avoiding the deleterious effects of seasonal growth on the velocity curves produced.

For each subject growth velocities and corresponding ages were plotted and analyzed by cubic spline regression with the use of a software analysis package (GraphPad Prism, San Diego, CA, USA). The cubic spline analysis provides a smoothed curve that allows the maximum velocity to be determined over the growth period. The cubic spline technique has been commonly used with growth data as it accurately models normal growth processes (138;164;210-215). Each peak velocity, as well as the corresponding age at which it occurred, was recorded as the PHV and age of PHV, respectively.

3.4 *Bone mass assessment*

Bone mass measurements were completed each fall during the investigation (October through November) by dual-energy x-ray absorptiometry (DXA), using a Hologic quantitative digital radiographic (QDR) 2000 bone densitometer (Hologic, Waltham, MA, USA) in the Department of Nuclear Medicine at the Royal University Hospital, Saskatoon, SK.

Bone mineral content (BMC) and bone projected area (BA) were measured yearly for the total body (TB), AP lumbar spine (LS; L1-L4), and the femoral neck (FN), with the exception of the initial year when no LS measurements were completed. All scans were made in the array mode utilizing enhanced global software version 7.10. TB scans were analyzed using software version 5.68A and the LS and FN scans utilized software version 4.42:1. Following analyses, all bone densitometry results were forwarded to a Nuclear Medicine physician for clinical assessment.

DXA assumes the bone mass of a particular region to be proportional to the absorption of ionizing radiation by the hard and soft tissues of the body. The QDR-2000 alternately pulses two energies of x-rays (70 kVp and 140 kVp) from an x-ray generator that is mounted below the subject during the assessment. These two voltages maximize differentiation between hard and soft tissues. The DXA collects the x-ray photons not absorbed in the body via a collimator above the anterior surface of the subject. The collimator compares the attenuation of the x-ray photons to a reference standard and is able to assess the composition of that particular region or pixel based on that reference. The QDR-2000 employs high-resolution images made possible by the fine (1.5

mm) collimation aperture, allowing for better bone edge detection than with previous generations of DXA. Additionally, shorter scanning times and lower doses of ionizing radiation are characteristic of the QDR 2000.

Subjects wore shorts and a light T-shirt during DXA scans and were free of all metal objects. All participants had their body mass and stature measured prior to DXA bone mass assessment and females were screened for pregnancy.

TB scans were made with the subject lying supine in the longitudinal midpoint of the scan, with the body composition phantom approximately 5 cm. lateral to the right foot. The body composition phantom is necessary for body composition estimation (lean body mass and fat mass). The subject's head was positioned so that the first scan line was air and not more than six scan lines below the lateral demarcation on the superior aspect of the scanning mat. The chin was raised and the shoulders depressed to prevent overlap of mandible and scapulae on the densitometer image. For tall individuals, the feet were dorsi-flexed or the knees bent, depending on the amount of tissue off the scan table. The arms were placed equidistant from the body, while both remaining on scan mat and not touching the body, with the hands pronated. The feet were rotated internally and masking tape was applied to join the great toes to immobilize the lower body and to aid in repeat positioning. The TB scan required approximately 5 minutes and 20 seconds to complete. In addition to BMC (g) and aBMD (g/cm^2), the DXA TB scan also provided an estimate of fat mass (g) and lean body mass (g) (LBM).

Anterior-posterior LS measurements were completed with the subject supine with the lower legs raised on a padded box (38 cm by 32 cm by 45 cm) with the femora as vertical with respect to the scan bed as was possible in order to decrease lumbar lordosis. The subject was positioned on the midline of the scan bed with the lumbar region between the lateral upper and lower spine limit lines. The hands were placed beneath the head maximally raising the rib cage out of the region of interest. The laser guidance point was placed on the midline of the subject approximately 5 cm below the level of the lateral aspect of the iliac crest. The scan ideally included L5 through T12 and took approximately 1.5 minutes, depending on the size of the subject.

The left proximal femur scans were completed with the subject supine and the left leg inwardly rotated (30 degrees) and fixed to a standardized positioning aid (lucite positioning wedge, Hologic) to both expose the head of the femur more completely and to standardize measurement. The femoral head, lesser trochanter and greater trochanter are all included in the region of interest analysis. To allow for proper analysis the lateral aspect of the greater trochanter was at least 5 lines (7.5 mm) from the outer edge of the ROI and the lesser trochanter was at least 10 lines (15 mm) away from the inferior aspect of the ROI. The proximal femur scan takes approximately 3 minutes.

All scan analyses were completed as outlined in the HOLOGIC QDR Operator's Manual and User's Guide, Hologic Inc., Waltham, MA., 1991. The compare function was used in all sequential scans at all sites to allow for increased consistency in ROI placement between analyses.

All scans in the first six years were analyzed by the same individual, and scans in the last year were analyzed by another. As an assessment of consistency, the last year's scans were reviewed by the initial analyst for consistency of region of interest placement and landmarking techniques.

Quality control of the DXA was performed by a Nuclear Medicine Department physicist and a senior technologist with the use of the supplied quality control (QC) phantom and software installed on the QDR 2000. QC lumbar spine phantom scans were performed daily and QC plots were recorded daily for any evidence of systemic drift in the DXA.

3.5 *Calculation of bone mineral density*

Areal bone mineral density (g/cm^2) was calculated by dividing DXA-determined BMC (g) by the respective PA (cm^2) for all sites.

Bone mineral apparent density (BMAD), an estimate of volumetric density, was established for all sites by the formulae of Katzman et al. (69):

1.) TB BMAD = $BMC/(BA^2/height)$

2.) LS BMAD = $BMC/BA^{1.5}$

3.) FN BMAD = $BMC/(BA^2/1.5)$

Since Katzman et al. (69) used a Hologic QDR-1000, which set the ROI at the FN to be 1cm, and the QDR-2000 used in this investigation set the ROI at 1.5cm, the FN equation was modified to reflect this (corrected formula as shown above).

Statistically-adjusted BMD (sBMD) was calculated according to Prentice et al. (67). All sites were calculated in the same manner. BMC and PA from the complete longitudinal sample were first converted to their natural logarithms to obtain a more linear relationship between the variables, following which univariate linear regression analyses of BMC on PA was completed. The resulting regression coefficient (lambda) between the two variables is used as an adjustment of the aBMD equation in the following manner:

4.) $sBMD = BMC/BA^{lambda}$

According to Prentice et al. (67) this equation is sensitive to the site of investigation, the population group investigated, and can account for all of the variance between the two variables. For normal aBMD the allometric equation is $aBMD = k \times BA$. For the statistically-corrected BMD the allometric equation is $sBMD = k \times BA^{lambda}$. Gender-specific values for lambda for each site, as generated from the SPBMAS data, are reported in Table 4.11. in the Results section.

3.6 *Development of mixed-longitudinal distance curves*

All variables (PA, BMC, BMD, BMAD, sBMD) for each individual, along with their respective age (both chronological and PHV-aligned age) were entered into a program that performed a cubic spline regression (GraphPad Prism 2.0, San Diego, CA, USA). The cubic spline program provided exact decimal year values of each variable for the particular individual. All values for each decimal year were averaged for each variable and distance growth curves were created for time points –4 through 4 yrs. PHV (10 through 18 yrs. of age). Since this was a mixed-longitudinal design, not all individuals were represented in each data cell.

3.7 *Development of longitudinal velocity curves*

A set of longitudinally developed normal curves was established for each of the following variables for both chronological and PHV-aligned age: BMC, PA, aBMD. BMAD, and sBMD. Each individual had their respective age and each of the bone variables entered into a program

that performed a cubic spline regression (GraphPad Prism 2.0, San Diego, CA, USA). The cubic spline program was programmed to provide exact decimal year values of each variable for the particular individual. Each of these curves was developed from only those individuals who had all bone mineral values from –2 to 2 yrs. (11 to 14 yrs. in the females; 12 to 15 yrs. in the males), with the exception of the LS where limited data only allowed for curves from –2 to 1 yrs. PHV in the males and –1 to 2 yrs. PHV in the females. These time periods were selected as they represented the points at which enough data was available for analyses.

3.7.1 Using normal velocity curves as predictive curves for estimating missing velocity data

As this investigation involved mixed-longitudinal data, full growth information was not available for all subjects; as a consequence of this some subjects did not have their full spectrum of growth represented in the dataset (individuals that did not have a value for –2 years PHV to 2 years PHV or for ages 11 or 15 yrs.; the longitudinal velocity analyses required subjects with at least that span of growth data). For those subjects that were short a year of growth to satisfy the analyses requirements, a normal growth curve, based on the results of the subjects with complete growth data, was used to estimate their growth velocity for the missing data point. Those subjects with greater than one year of missing data were excluded from further velocity analyses.

Predictions were calculated by simple algebraic solution in the following manner (BMC as an example):

$$\frac{\text{BMC (-1 PHV)}}{\text{BMC (-1 PHV normal curve)}} = \frac{\text{X BMC (-2 PHV)}}{\text{BMC (-2 PHV normal curve)}}$$

$$\text{solving for X} = \frac{\text{BMC (-1 PHV)} * \text{BMC (-2 PHV normal curve)}}{\text{BMC (-1 PHV normal curve)}}$$

This technique assumes that the missing value generated from the normal growth curve is not significantly different from that which would have occurred should observation permitted; it assumes that the missing value is typical of that observed in the cohort.

3.8 *Establishing size dependence of density measures*

Two methods were utilized to determine the dependency of aBMD on size measures. First, since aBMD has been found to be highly dependent on PA, the correlation between these two variables is typically high, exemplifying the dependence of aBMD on bone size. As size-independence is obtained, the resulting correlation between the density measure and the PA should be insignificant. Correlation analyses was used to discern the relatedness of the aBMD measures with PA, body mass and body height and the relationship between the corrected measures and PA, body mass and body height.

A further measure of the appropriateness of the aBMD measure can be established from the sBMD procedure. The theoretical underpinnings of aBMD assume that BMC and PA remain in direct proportion to one another throughout the size spectrum. If the resulting lamda from the regression of BMC on PA was significantly different from unity, aBMD assumed an inaccurate measure of true density at the particular site as BMC and PA change in different proportions from one another, negating the assumption of direct proportionality. However, if lambda was not significantly different from one, then aBMD was considered a valid measure of density at the particular site.

3.9 *Male and female comparisons of bone mineral*

PA, BMC, aBMD, BMAD distance and velocity values were compared between males and females at all sites for PHV-aligned age. Since sBMD was developed from different equations for both the male and females, no gender comparisons were completed.

3.10 *Statistical Procedures*

For all statistical procedures alpha was set at $p<0.05$. Analyses were completed utilizing SPSS for Windows Version 10.05. The statistical procedures used to answer each of the research questions (where statistical analyses were appropriate) are delineated below.

Size dependence of aBMD, BMAD and sBMD was established at each site by performing bivariate correlations (Pearson's Product Moment correlations – two-tailed) between each of the

density variables and PA, stature and body mass. Higher correlations indicated a higher dependence of the density measure on PA, stature or body mass. Statistical independence of density from PA, stature or body mass was achieved when bivariate correlations were insignificant.

The appropriateness of aBMD as a measure of density was determined by the regression of BMC on PA at each site. If the resulting regression coefficient was significantly different from one, as established by the Wald's test, aBMD was considered an invalid predictor of true bone density at that particular site.

A general linear multivariate model (gender x age) ANOVA was completed for all distance variables. Differences between males and females for any measure were established by the presence of a significant main effect for gender and associations with age determined by a significant main effect for age. If a significant interaction (gender x age) was found, post hoc comparisons were performed between males and females for each age group to delineate where significant differences occurred.

Observations as to whether the velocity was negative or positive were performed without any formal statistical procedures. In addition, a repeated-measures ANOVA (gender x age) was performed for each variable at each site. A significant effect of age was determined through a main effect for age, and a significant effect for gender was determined through a main effect for gender. If a significant interaction (gender x age) was found, post hoc comparisons were performed between males and females for each age group to delineate where significant differences occurred.

No statistical analyses were made between genders for sBMD as different equations were used for the estimate of sBMD.

4 Results

Only PHV-aligned data are presented and discussed in the formal body of this investigation. Chronological based data can be found in Appendix B (Tables 9.1-9.21 and Figures 9.1-9.9).

4.1 *Subject Characteristics*

4.1.1 Mixed-longitudinal sample: distance curves

Data from 70 males and 67 females were used to construct a series of mixed-longitudinal growth curves, from which all distance measures were obtained. These subjects were selected from the greater PBMAS study pool solely based on adequate statural data to determine PHV. Due to the nature of mixed-longitudinal data all subjects were not represented at each age; rather, subjects possessed datapoints that spanned a limited portion of the entire age ranges. Chronological-based data was available from 10 through 18 years of age and PHV-based data was available from –3 through 5 years relative to PHV. Table 4.1. provides the sample size for each time point for the TB and FN (both chronological and phv-based).

Table 4.1. Mixed-Longitudinal/Distance Curve Sample Sizes at Each Investigated Age: Total Body and Femoral Neck (Male and Female).

Chronological Age	*Male n*	*Female n*	*PHV-aligned Age*	*Male n*	*Female n*
10	19	33	-3	25	15
11	32	51	-2	36	29
12	45	56	-1	48	42
13	58	61	0	65	52
14	66	54	1	63	65
15	58	41	2	54	58
16	46	27	3	41	47
17	34	15	4	29	31
18	16	8	5	15	17

Since LS data was not collected in the initial year of the SPBMAS there are consequently fewer measurements available at the LS and therefore less data, as reflected in the sample size (Table 4.2.).

Table 4.2. Mixed-Longitudinal/Distance Curve Sample Sizes at Each Investigated Age: Lumbar Spine (Male and Female).

Chronological Age	*Male n*	*Female n*	*PHV-aligned Age*	*Male n*	*Female n*
10	9	22	-3	14	12
11	19	31	-2	26	18
12	32	51	-1	34	30
13	45	54	0	46	42
14	55	51	1	59	54
15	56	41	2	54	58
16	44	27	3	42	47
17	34	15	4	29	31
18	16	8	5	15	18

4.1.2 Longitudinal sample: velocity curves

In the longitudinal dataset, each individual was represented at each investigated age. Table 4.3. presents the sample size at each age (both chronological and phv-based), for the TB and FN.

Table 4.3. Longitudinal/Velocity Curve Sample Sizes at Each Investigated Age: Total Body and Femoral Neck (Male and Female).

Chronological Age	*Male n*	*Female n*	*PHV-aligned Age*	*Male n*	*Female n*
11		44	-2	31	29
12	43	44	-1	31	29
13	43	44	0	31	29
14	43	44	1	31	29
15	43		2	31	29

Since LS data was not collected in the initial year of the SPBMAS there are consequently fewer measurements available at the LS and therefore less data, as reflected in the sample size (Table 4.4.).

Table 4.4. Longitudinal/Velocity Curve Sample Sizes at Each Investigated Age: Lumbar Spine (Male and Female).

Chronological Age	*Male n*	*Female n*	*PHV-aligned Age*	*Male n*	*Female n*
11		35	-2	28	
12	29	35	-1	28	27
13	29	35	0	28	27
14	29	35	1	28	27
15	29		2		27

4.2 *Dependence of aBMD on body height, body mass, and PA (H1)*

4.2.1 aBMD correlations with body height, body mass and bone projected area ($H1_1$).

PHV-aligned bivariate Pearson product moment correlations between aBMD and body height, body mass, and PA for males and females for all sites can be found in Tables 4.5-4.10.

Table 4.5. PHV-Aligned Correlations of Total Body BMD, BMAD and sBMD with Age, Bone Projected Area, Body Mass, and Stature (Males).

	Age	*BA*	*WT*	*HT*
aBMD (g/cm^2)	0.823c	0.888c	0.808c	0.780c
BMAD (g/cm^3)	-0.493c	-0.600c	-0.640c	-0.543c
sBMD (g/cm^3)	.116a	0.061	-0.001	-0.060

a = p<0.05; b = p<0.01; c = p<0.001.

Table 4.6. PHV-Aligned Correlations of Total Body BMD, BMAD and sBMD with Age, Bone Projected Area, Body Mass, and Stature (Females).

	Age	*BA*	*WT*	*HT*
aBMD (g/cm^2)	0.805c	0.855c	0.711c	0.765c
BMAD (g/cm^3)	-0.372c	-0.649c	-0.705c	-0.570c
sBMD (g/cm^3)	0.257c	0.040	-0.089	-0.041

a = p<0.05; b = p<0.01; c = p<0.001.

Table 4.7. PHV-Aligned Correlations of Femoral Neck BMD, BMAD and sBMD with Age, Bone Projected Area, Body Mass, and Stature (Males).

	Age	*BA*	*WT*	*HT*
aBMD (g/cm^2)	0.678c	0.465c	0.691c	0.608c
BMAD (g/cm^3)	0.083	-0.356c	0.062	-0.099a
sBMD (g/cm^3)	0.369c	-0.009	0.361c	0.221c

a = p<0.05; b = p<0.01; c = p<0.001.

Table 4.8. PHV-Aligned Correlations of Femoral Neck BMD, BMAD and sBMD with Age, Bone Projected Area, Body Mass, and Stature (Females).

	Age	*BA*	*WT*	*HT*
aBMD (g/cm^2)	0.710c	0.516c	0.691c	0.728c
BMAD (g/cm^3)	0.455c	-0.103a	0.313c	0.295c
sBMD (g/cm^3)	0.509c	-0.009	0.381c	0.372c

a = p<0.05; b = p<0.01; c = p<0.001.

Table 4.9. PHV-Aligned Correlations of Lumbar Spine BMD, BMAD and sBMD with Age, Bone Projected Area, Body Mass, and Stature (Males).

	Age	*BA*	*WT*	*HT*
aBMD (g/cm^2)	0.809c	0.814c	0.767c	0.737c
BMAD (g/cm^2)	0.593c	0.489c	0.609c	0.460c
sBMD (g/cm^3)	0.200c	0.002	0.279c	0.025

a = p<0.05; b = p<0.01; c = p<0.001.

Table 4.10. PHV-Aligned Correlations of Lumbar Spine BMD, BMAD and sBMD with Age, Bone Projected Area, Body Mass, and Stature (Females).

	Age	*BA*	*WT*	*HT*
aBMD (g/cm^2)	0.798c	0.895c	0.757c	0.808c
BMAD (g/cm^2)	0.728c	0.692c	0.678c	0.637c
sBMD (g/cm^3)	0.271c	-0.003	0.234c	0.015

a = p<0.05; b = p<0.01; c = p<0.001.

At all sites aBMD was significantly (p<0.001) associated with bone projected area, body mass, and body height (Tables 4.5-4.10.). Further, PHV-aligned age was significantly (p<0.001) associated with aBMD at all three sites.

4.2.2 Regression coefficients of PA regressed on BMC ($H1_2$)

Table 4.11. presents the linear regression coefficients from the regression of lnBA on lnBMC. All of the regression coefficients were significantly different from unity.

Table 4.11. PHV-Aligned Linear Regression Coefficients (Lambda values) after the Regression of lnBA on lnBMC at the TB, FN and LS (Male and Female).

	Male	*Female*
Site	*PHV-aligned*	*PHV-aligned*
TB	1.446	1.432
FN	1.591	1.866
LS	1.807	1.953

4.3 *Independence of corrected bone mineral density measures (BMAD and sBMD) from body height, body mass or bone projected area (H2)*

4.3.1 BMAD correlations with body height, body mass and bone projected area ($H2_1$)

PHV-aligned bivariate Pearson product moment correlations and significance levels between BMAD and body height, body mass, and PA for males and females for all sites can be found in Tables 4.5-4.10.

At the TB and LS there were significant negative ($p<0.001$) correlations between BMAD and age, PA, body height and body mass for both genders. At the FN the males had significant correlations with BMAD in only PA and height, while the females had significant associations between BMAD and all four measures.

4.3.2 sBMD correlations with body height, body mass and bone projected area ($H2_2$)

PHV-aligned bivariate Pearson product moment correlations and significance levels between SBMD and body height, body mass, and PA for males and females for all sites can be found in Tables 4.5-4.10.

At the TB only age in females was significantly ($p<0.001$) correlated with sBMD. At the FN sBMD was significantly ($p<0.001$) correlated with all measures except PA in both genders. Lastly, at the LS, sBMD was only significantly ($p<0.001$) correlated with age and body weight in both genders.

4.4 *Bone mineral density through adolescence (mixed-longitudinal growth curves – distance) (H3 and H4)*

PHV-based age group averages ± SEM for all PA, BMC, aBMD, BMAD and sBMD distance curves are reported in Appendix C in Tables 10.1-10.6.

4.4.1 PHV-aligned PA through adolescence

Growth curves for PA at the TB, FN and LS are found in Figure 4.1. As shown in Figure 4.1a., male TB PA increased with age, with acceleration in the year preceding and the two years following PHV. PA for females increased linearly from –3 through 2 years PHV, with a slowing thereafter. There was a significant ($p<0.001$) main effect for maturational age. While the PHV-aligned pattern of PA change was similar between genders, males had significantly greater PA at all ages as supported by the significant ($p<0.001$) main effect for gender.

As shown in Figure 4.1b., FN PA increased in males from –3 through –1 years PHV, with rapid expansion between –1 and 1 years PHV, and dramatic slowing of expansion from 1 through 4 years PHV. In females, PA increased from –4 through 2 years PHV, after which PA decreased from 2 through 4 years PHV. There was a significant ($p<0.001$) main effect for maturational age. At -3 yrs. PHV male and female PA was equal, however after this point males had greater rates of expansion leading to significantly greater PA after -2 yrs. PHV, as evidenced by the significant ($p<0.001$) main effect for gender and interaction between gender and maturational age.

As seen in Figure 4.1c., males' LS PA followed a sigmoidal pattern with increases throughout the age range and acceleration between –1 and 2 years PHV. In females, PA increases were relatively linear from –3 through 3 years PHV, after which PA stabilized. There was a significant ($p<0.001$) main effect for maturational age. LS PA was similar between the genders, however males possessed a significantly higher PA at most ages investigated, with a significant ($p<0.001$) main effect for gender.

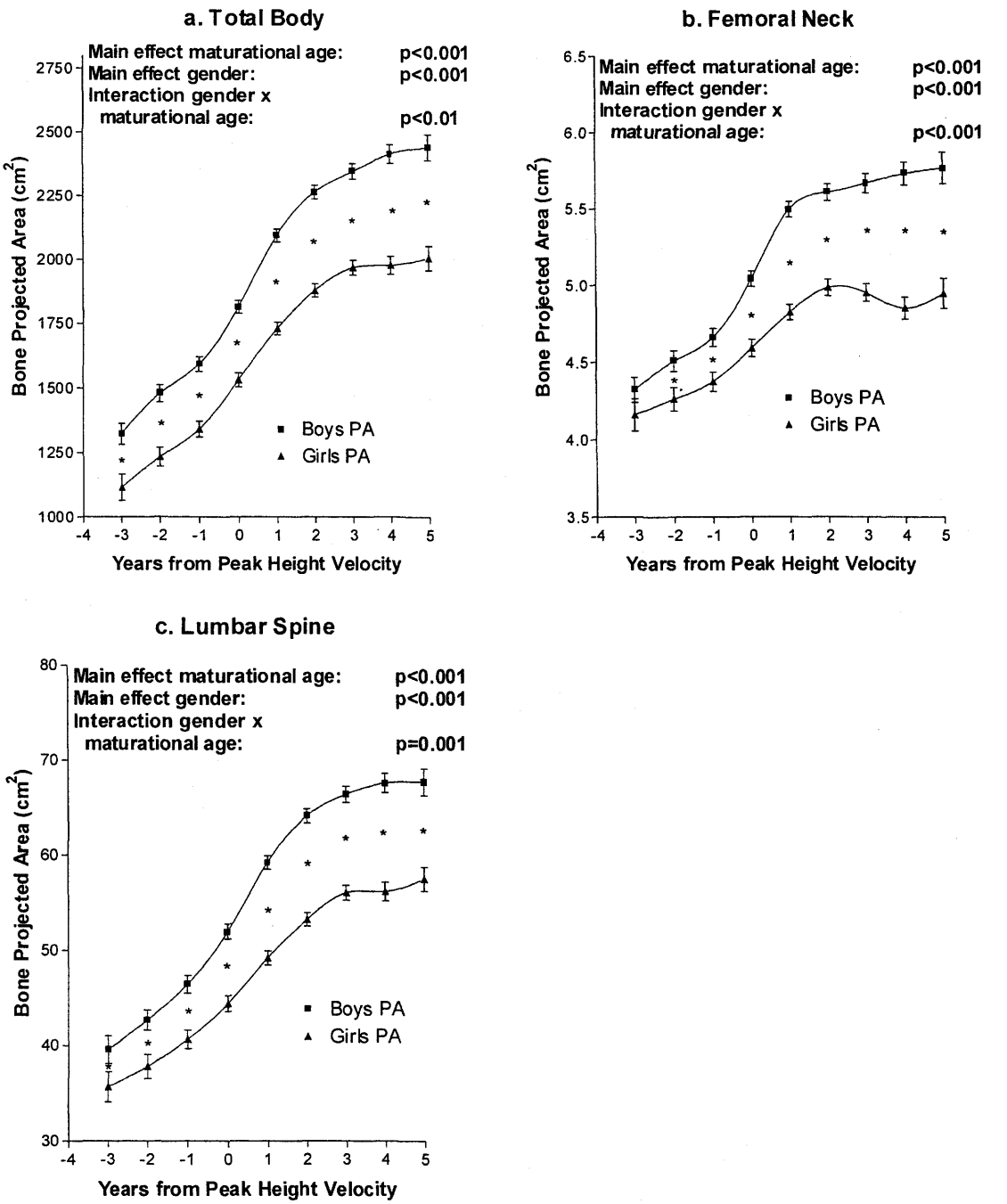


Figure 4.1. Male and female PHV-aligned growth curves for bone projected area (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

* denotes significant differences between genders at a specific maturational age in the instance where a significant age x gender interaction is present.

4.4.2 PHV-aligned BMC through adolescence

Results for BMC at the TB, FN and LS are found in Figure 4.2. As shown in Figure 4.2a. TB BMC accrual in males mirrored the changes in BA. Female TB BMC accrual increased throughout the age range, with acceleration between –1 and 2 years PHV. There was a significant ($p<0.001$) main effect for age. BMC accrual was similar between genders, however males had significantly greater BMC at all ages investigated; there was a significant ($p<0.001$) main effect for gender.

FN BMC in males followed a sigmoidal pattern, as shown in Figure 4.2b., with increases throughout the growth period and a period of acceleration between –1 and 1 year PHV. BMC females increased from –3 through –1 years PHV, accelerating through –1 to 3 years PHV, then stabilizing. There was a significant ($p<0.001$) main effect for maturational age. Bone mineral was higher in males at all time points, as is evidenced by the significant ($p<0.001$) main effect for gender.

As seen in Figure 4.2c., LS BMC accrual in males mirrored PA with increases throughout the age range with acceleration between –1 and 2 years PHV. BMC increased in females throughout the age range until 3 years PHV, with acceleration between 0 and 3 years PHV, after which BMC plateaued. There was a significant ($p<0.001$) main effect for maturational age. The pattern of accrual was similar between the genders, however, males possessed significantly greater BMC at all ages, as evidenced from a significant ($p<0.001$) main effect for gender and an insignificant interaction between gender and maturational age.

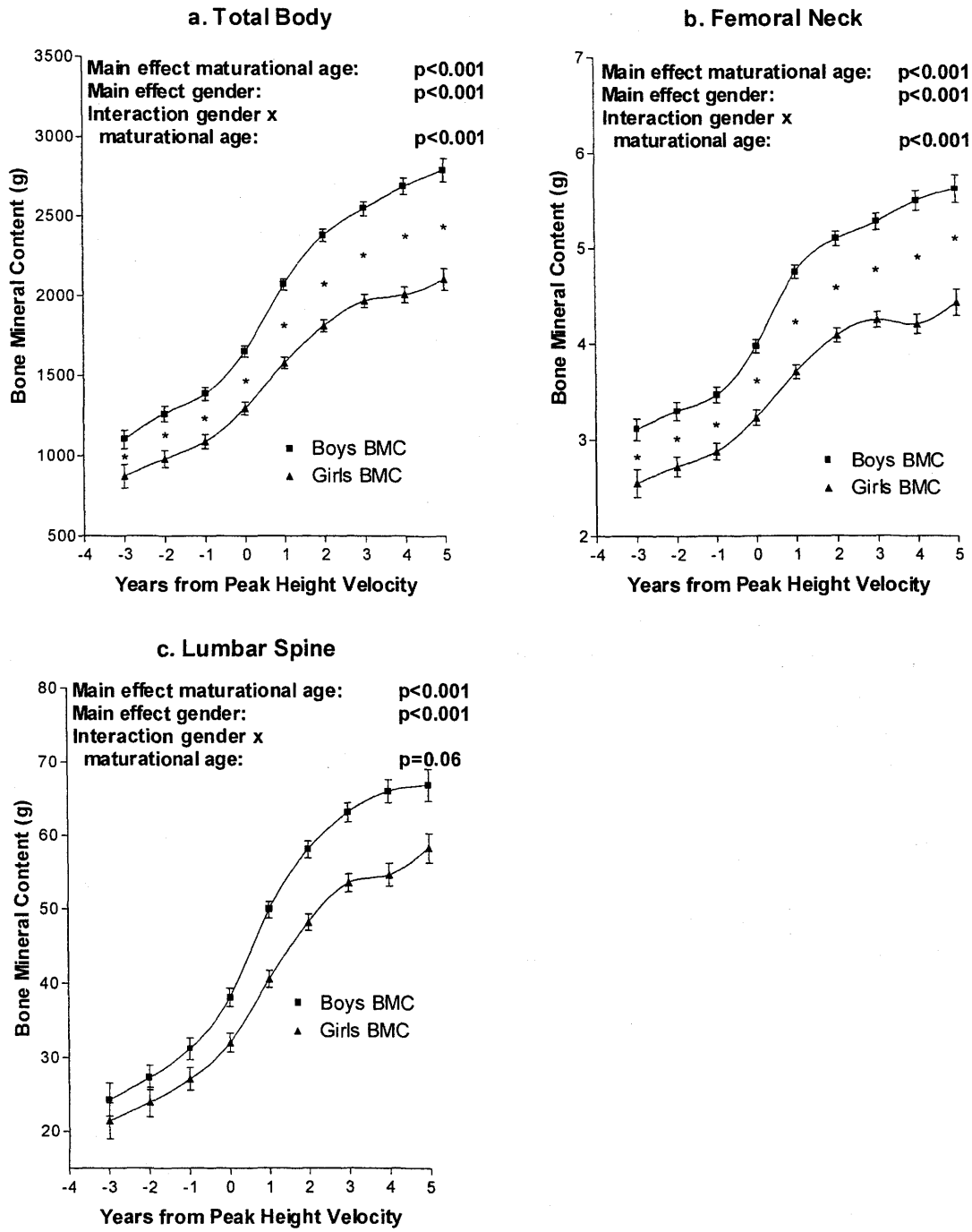


Figure 4.2. Male and female PHV-aligned growth curves for bone mineral content (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

* denotes significant differences between genders at a specific maturational age in the instance where a significant age x gender interaction is present.

4.4.3 PHV-aligned aBMD through adolescence ($H3_1$ and $H4_1$)

Results for aBMD at TB, FN and LS are found in Figure 4.3. As shown in Figure 4.3a., male and female TB aBMD increased with age, with a period of rapid density increases from –1 through 2 years PHV. There was a significant ($p<0.001$) main effect for age. Males had significantly greater aBMD values at all ages; a significant ($p<0.001$) main effect was found for gender, with no significant interaction between gender and age.

In males and females, FN aBMD mirrored the gains made in BMC, as shown in Figure 4.3b. A significant ($p<0.001$) main effect for maturational age was observed. Males possessed significantly higher aBMD at all ages, as evidenced by the significant ($p<0.001$) main effect for gender and no significant interaction between gender and maturational age.

As seen in Figure 4.3c. LS aBMD in males increased in a sigmoid fashion similar to both PA and BMC. Female aBMD increased linearly from –3 through 0 years PHV, after which there was an increase in aBMD between 0 and 2 years PHV, with slowing thereafter. There was a significant ($p<0.001$) main effect for maturational age. LS aBMD was identical between genders when aligned on PHV, as evidenced by a lack of significant main effect for gender or interaction between gender and maturational age.

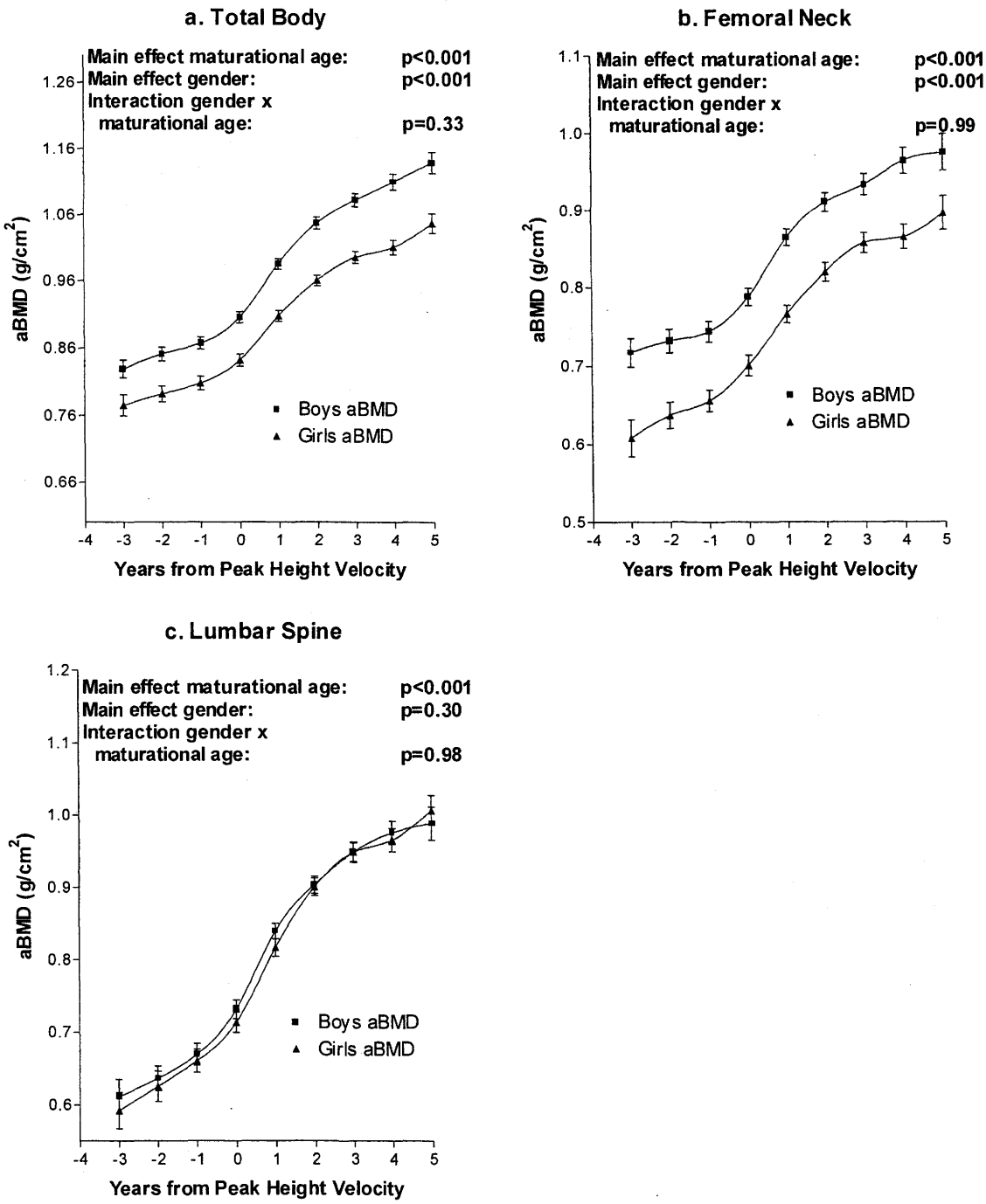


Figure 4.3. Male and female PHV-aligned growth curves for areal bone mineral density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

4.4.4 PHV-aligned BMAD through adolescence ($H3_2$ and $H4_2$)

Results for BMAD at TB, FN and LS are found in Figure 4.4. As shown in Figure 4.4a., TB BMAD in both males and females decreased from –3 through 0 years PHV, with a plateau thereafter. There was a significant ($p<0.001$) main effect for maturational age. Females had significantly greater TB BMAD at all ages as evidenced by a significant ($p<0.001$) main effect for gender with no interaction between gender and maturational age.

As shown in Figure 4.4b., FN BMAD in males decreased until PHV and then rebounded with increases until the end of the investigated age period. In females, FN BMAD was relatively stable to PHV, after which there was a consistent increase. There was a significant ($p<0.001$) main effect for maturational age. Male FN BMAD was significantly greater than female BMAD from –3 years PHV until –1 years PHV after which it was equal; there was no significant main effect for gender, but there was a significant ($p<0.01$) interaction between maturational age and gender.

In males, LS BMAD was stable from –3 through –1 years PHV, after which it increased until 3 years PHV, and then slowed until the end of the investigated age range, as seen in Figure 4.4c. BMAD in females constantly increased with rapid acceleration from 0 to 2 years PHV. There was a significant ($p<0.001$) main effect for maturational age. Female LS BMAD was greater at all ages as evidenced by a significant ($p<0.001$) main effect for gender and no significant interaction effect.

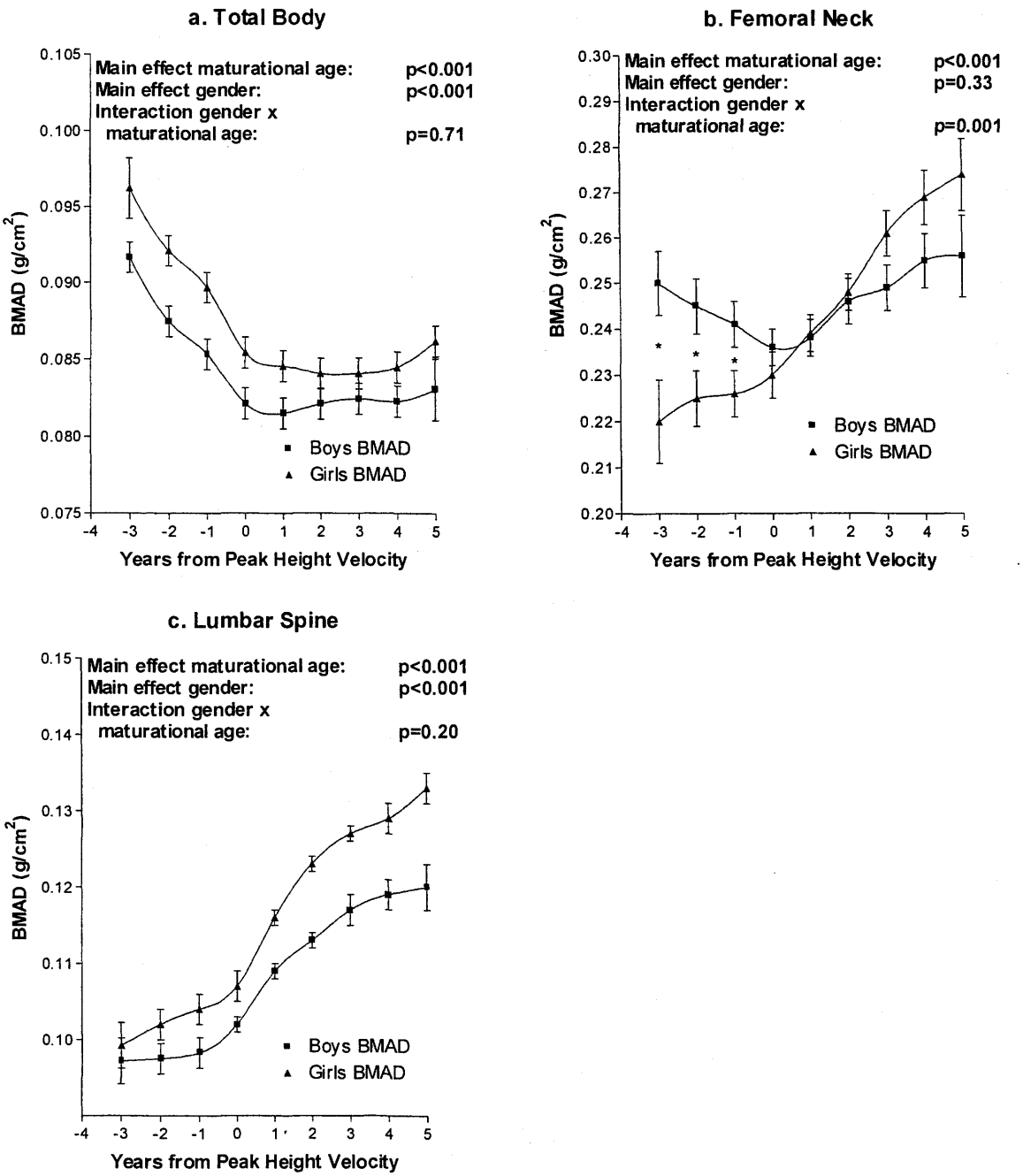


Figure 4.4. Male and female PHV-aligned growth curves for bone mineral apparent density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

* denotes significant differences between genders at a specific maturational age in the instance where a significant age x gender interaction is present.

4.4.5 PHV-aligned sBMD through adolescence ($H3_3$ and $H4_2$)

Results for sBMD at TB, FN and LS can be found in Figure 4.5. Male and female TB sBMD decreased from –3 through 0 years PHV, after which there was a deflection and an increase thereafter, as seen in Figures 4.5a. and 4.5b., respectively. When aligned on PHV males and females displayed identical patterns of sBMD over the age span. There was a definitive pattern of diminishing bone until PHV, with a subsequent increase in sBMD.

As shown in Figure 4.5c. FN sBMD in males remained stable from –3 through 0 years PHV, after which there was a linear increase in density until the end of the investigated age range. Similarly, in females, sBMD increased minimally from –3 through –1 years, with a linear increase in density thereafter, as shown in Figure 4.5d.

As shown in Figure 4.5e. LS sBMD in males decreased until PHV, increasing linearly thereafter. In females, sBMD decreased slightly from –3 though 0 years PHV, and then rebounded with rapid increases in sBMD thereafter, as seen in Figure 4.5f.

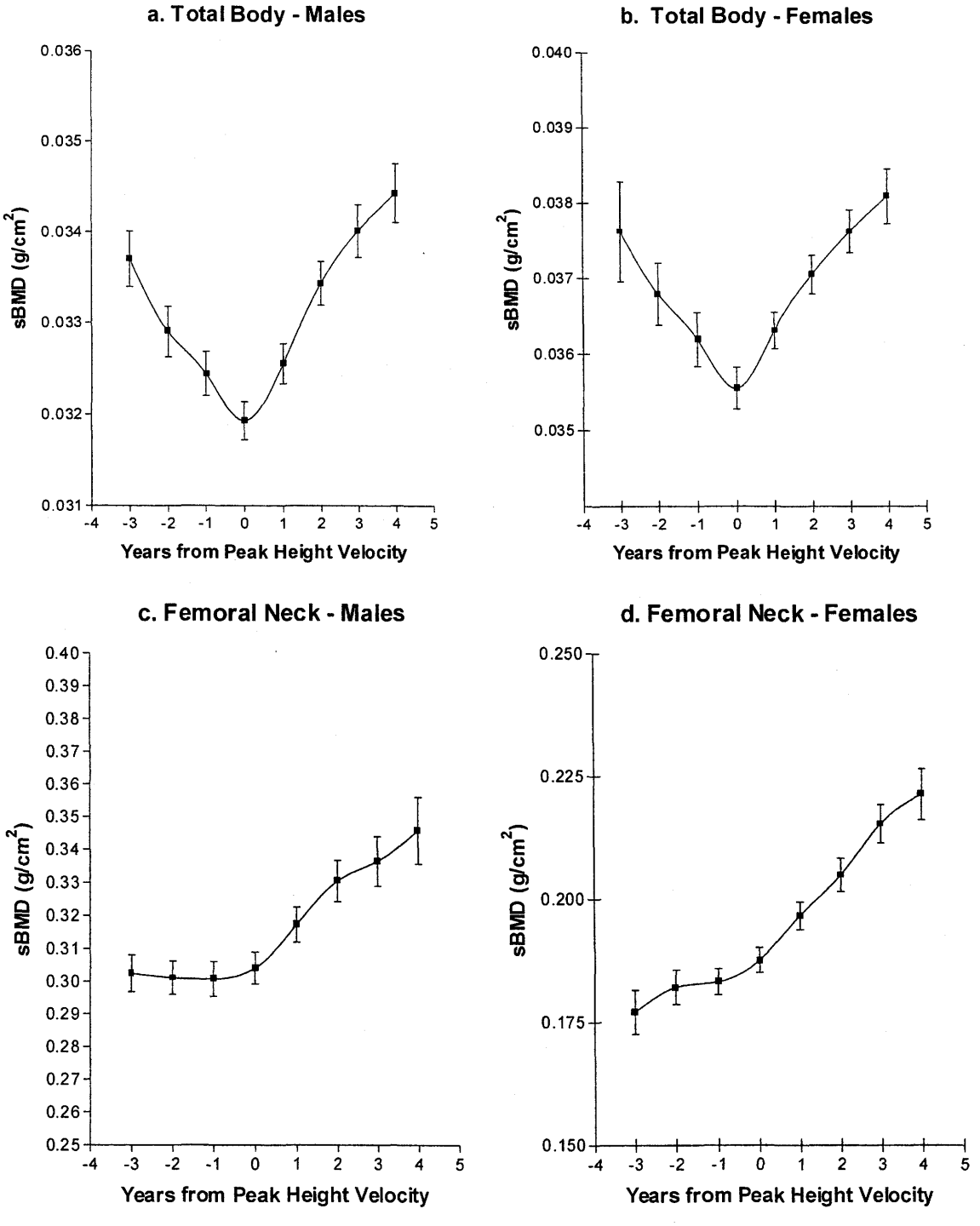


Figure 4.5. PHV-aligned growth curves for statistically-corrected bone mineral density (mean±SEM) of the a. Male total body; b. Female total body; c. Male femoral neck; and d. Female femoral neck. Figure 4.5. continued on following page.

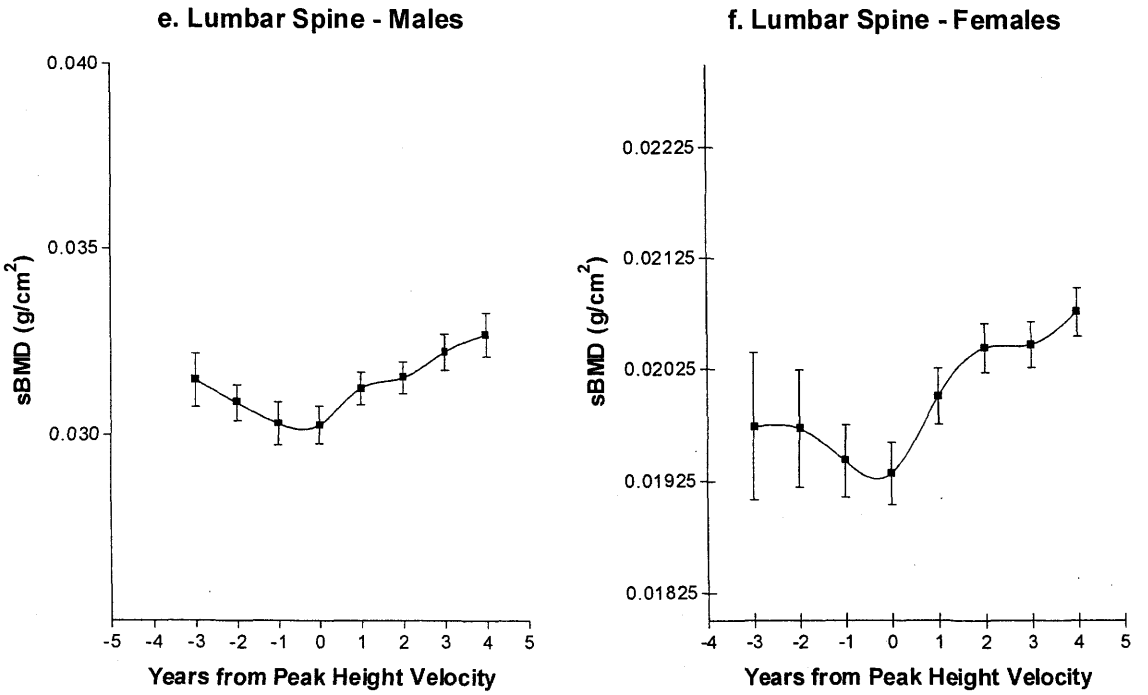


Figure 4.5. continued. PHV-aligned growth curves for statistically-corrected bone mineral density (mean±SEM) of the e. Male lumbar spine; and f. Female lumbar spine regions.

4.5 *Velocity of bone mineral density through adolescence (longitudinal growth curves - velocity)(H5)*

PHV-based age group averages ± SEM for all PA, BMC, aBMD, BMAD and sBMD velocity curves are reported in Appendix C in Tables 10.7-10.15.

4.5.1 PHV-aligned PA velocity through adolescence

All PA velocity measures for the TB, FN, and LS can be found in Figure 4.6. As shown in Figure 4.6a., TB PA in males and females accelerated from –2 to 0 years PHV, followed by a deceleration to 2 years PHV. There was a significant ($p<0.001$) main effect for maturational age. When aligned on PHV, males and females had similar PA velocity curves, at nearly identical developmental ages, with the only difference being that the males had a significantly greater magnitude velocity of expansion compared to the females at all ages. There was a significant ($p<0.01$) main effect gender, with no significant interaction between gender and maturational age (Figure 4.6a.).

As shown in Figure 4.6b, FN PA in males and females accelerated to PHV after which it decelerated for the remainder of the time span. There was a significant ($p<0.001$) main effect maturational age. When aligned on PHV, PA velocity was significantly greater in males at all ages with the exception of 2 years PHV. There was a significant ($p<0.001$) main effect for gender, as was a significant ($p<0.001$) interaction between gender and maturational age.

In males, LS PA accelerated to PHV, after which there was a deceleration, as seen in Figure 10c. PA in females accelerated from –1 to 0 PHV after which there was a deceleration to 2 years PHV. There was a significant ($p<0.001$) main effect for maturational age. Males possessed a significantly greater PA velocity only at PHV. There was significant ($p<0.001$) main effect for gender, and a significant ($p<0.05$) interaction between age and maturational age.

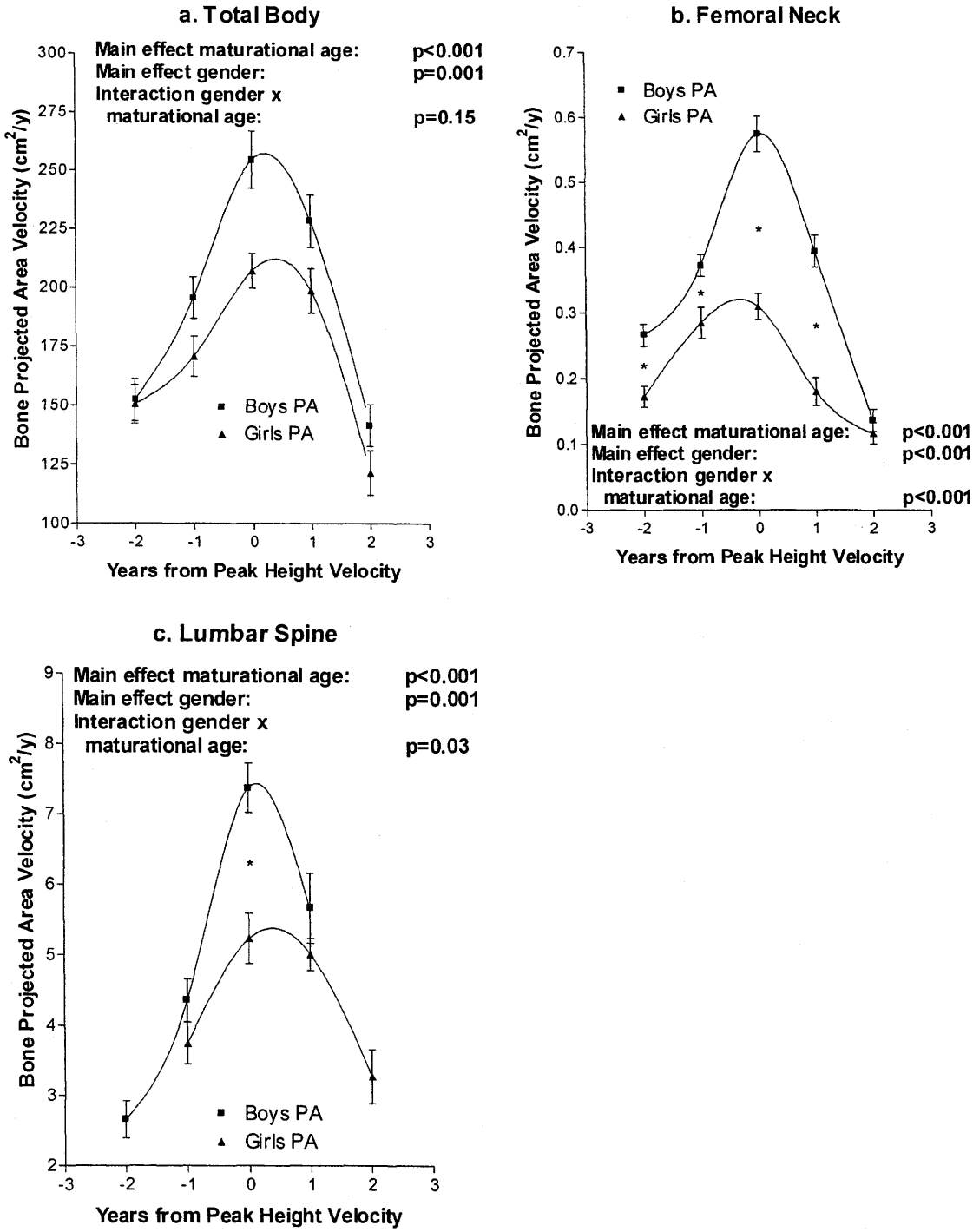


Figure 4.6. Male and female PHV-aligned velocity curves for bone projected area (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

* denotes significant differences between genders at a specific maturational age in the instance where a significant age x gender interaction is present.

4.5.2 PHV-aligned BMC velocity through adolescence

All BMC velocity measures for the TB, FN, and LS are found in Figure 4.7. As shown in Figure 4.7a., TB BMC in males and females accelerated from –2 to 1 year PHV, with a slowing thereafter. There was a significant ($p<0.001$) main effect for maturational age. Males had significantly greater BMC accrual velocities over the entire age range. There was a significant ($p<0.001$) main effect for gender.

As shown in Figure 4.7b., in males and female FN BMC gains accelerated to PHV and decelerated to 2 years PHV. There was a significant ($p<0.001$) main effect for maturational age. The curves for BMC accrual at the FN were the same in shape for males and females, however males possessed significantly greater BMC accrual velocities at all ages except for 2 years PHV. There was a significant ($p<0.001$) main effect for gender, with a significant ($p<0.001$) interaction between gender and maturational age.

As shown in Figure 4.7c., LS BMC in males accelerated from –2 to 0 years PHV and began to slow thereafter. In females BMC accelerated from –1 to 1, after which it decelerated. There was a significant ($p<0.001$) main effect for maturational age. BMC accrual velocity was significantly greater at PHV and 1 year after PHV in the males. There was a significant ($p<0.001$) main effect for gender, and a significant ($p<0.01$) interaction between gender and maturational age.

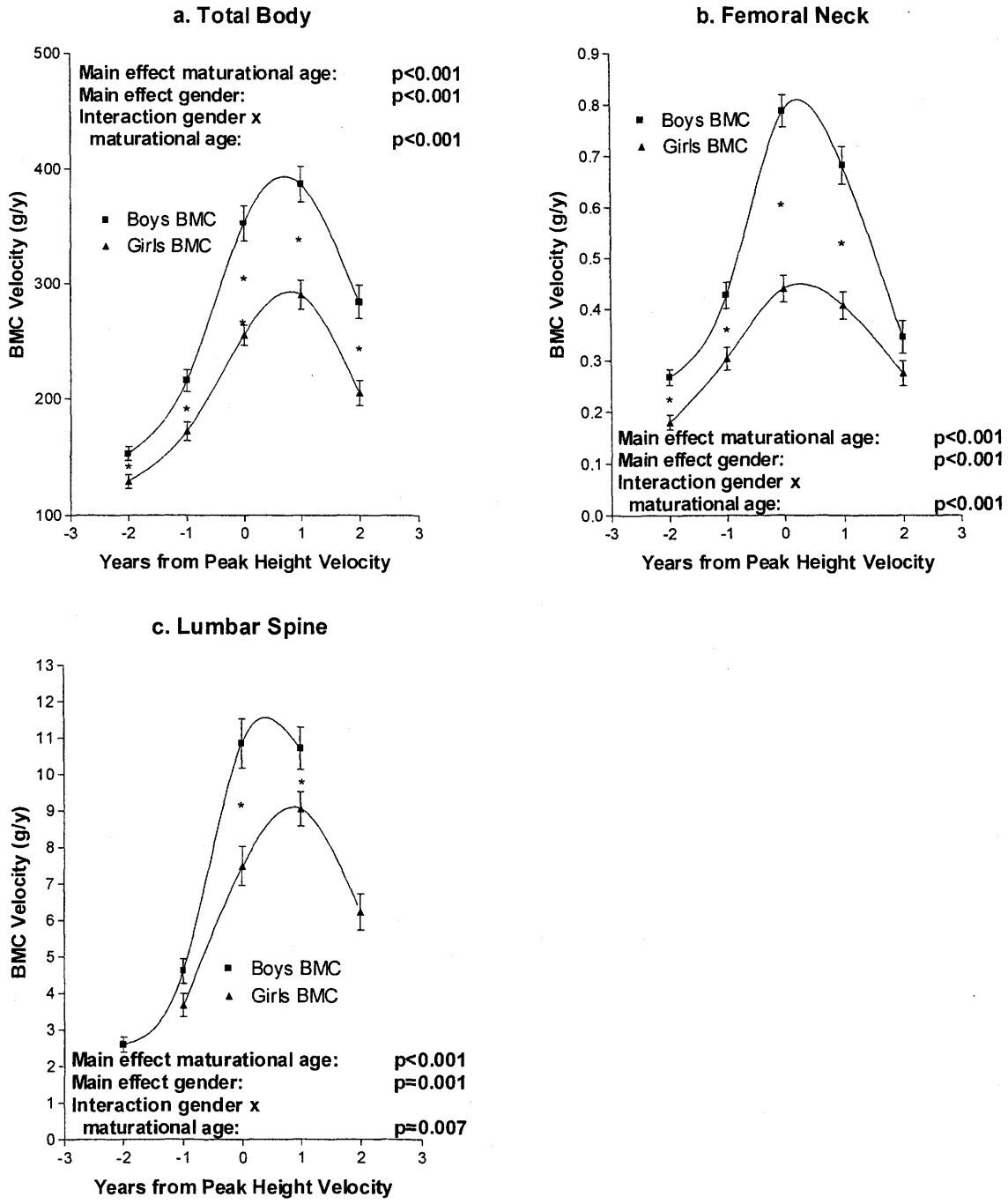


Figure 4.7. Male and female PHV-aligned velocity curves for bone mineral content (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

* denotes significant differences between genders at a specific maturational age in the instance where a significant age x gender interaction is present.

4.5.3 PHV-aligned aBMD velocity through adolescence ($H5_1$)

All aBMD velocity measures for the TB, FN, and LS can be found in Figure 4.8. As shown in Figure 4.8a., gains in male TB aBMD accelerated to 1 year PHV, with a deceleration thereafter. For females, aBMD increased in the same manner as BMC. There was a significant ($p<0.001$) main effect for maturational age. aBMD velocity curves were identical shape between males and females, with the peaks occurring at identical developmental ages and with males having significantly greater magnitude velocity at all ages; there was a significant ($p<0.001$) main effect for gender, with no significant interaction between gender and maturational age.

As shown in Figure 4.8b., FN aBMD in males mirrored the gains made in BMC. Areal BMD acceleration in females was steady between –2 and –1 year PHV, after which a rapid acceleration occurred until 1 years PHV, and a deceleration thereafter. There was a significant ($p<0.001$) main effect for maturational age. Areal BMD curves between the males and females were of a similar shape and magnitude, however, the females possessed significantly greater aBMD velocity at -2 years PHV, and the males significantly greater at PHV. There was no significant main effect for gender, but a significant ($p<0.01$) interaction term for gender and maturational age.

In males, LS aBMD increased from –1 to 0 years PHV, and a continued high rate of aBMD increases thereafter, as shown in Figure 4.8c. LS aBMD velocity in females remained relatively stable from –1 to 0 years PHV, with a dramatic acceleration to 1 year PHV, and a deceleration to 2 years PHV. There was a significant ($p<0.001$) main effect for maturational age. LS aBMD velocity was practically identical at all time points for males and females, with males reaching their peak slightly earlier than the females; there was no significant main effect for gender, nor was there one for interaction between maturational age and gender.

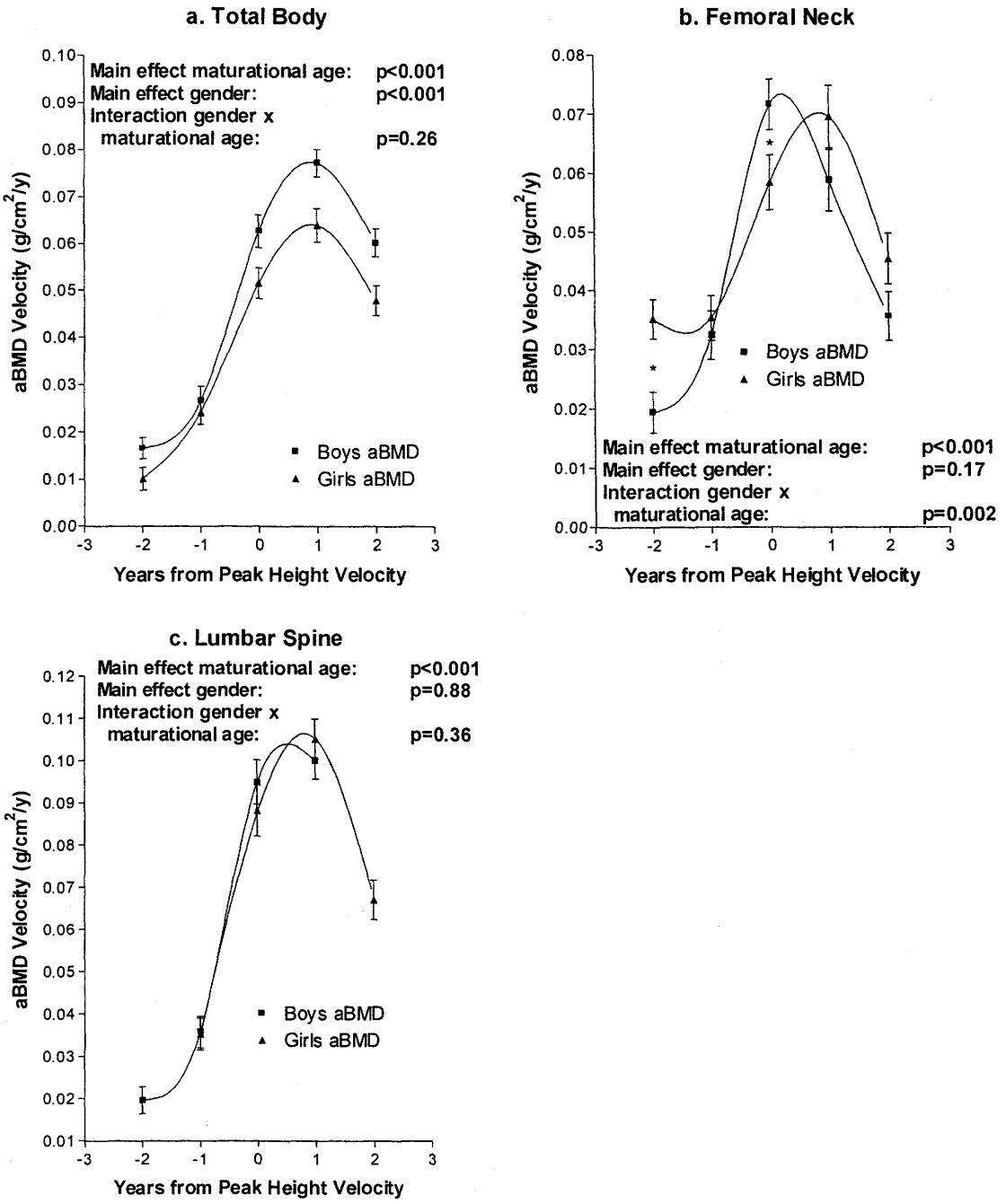


Figure 4.8. Male and female PHV-aligned velocity curves for areal bone mineral density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

* denotes significant differences between genders at a specific maturational age in the instance where a significant age x gender interaction is present.

4.5.4 PHV-aligned BMAD velocity through adolescence ($H5_2$)

All BMAD velocity measures for the TB, FN, and LS can be found in Figure 4.9. As shown in Figure 4.9a., TB BMAD in males was mostly negative, but accelerating from -2 years PHV to a positive velocity at 1 year PHV, decelerating thereafter. TB BMAD velocity in females was negative through the whole age range with the exception of the last year; however, a consistent acceleration was observed throughout the age range. There was a significant main effect ($p<0.001$) for maturational age. The males has significantly greater TB BMAD velocity as compared to the females at all ages. There was a significant ($p<0.01$) main effect for gender, but no significant interaction between gender and maturational age.

As shown in Figure 4.9b., FN BMAD in males constantly accelerated throughout the age span, with positive velocity values being reached by 0 years PHV and thereafter. In females, BMAD accelerated rapidly to 0 years PHV after which there was a stabilization of velocity until the end of the investigated age span. There was a significant ($p<0.05$) main effect for maturational age. Initially, BMAD velocity for females was negative, but by –1 year PHV became a positive velocity. There was a insignificant trend for BMAD velocity to be greater in the females from –1 PHV years onward. No significant main effect was observed for gender or interactions between maturational age and gender.

As shown in Figure 4.9c., LS BMAD velocity in males was stable from –2 to –1, but rapidly accelerated in the period from –1 to 0 years PHV, with a stabilization of the velocity thereafter. In females BMAD accelerated linearly from –1 to 1 year PHV, after which there was deceleration. There was a significant ($p<0.001$) main effect for maturational age. BMAD velocity was identical for males and females at all time points. There was no significant main effect for gender, nor was there a significant interaction between gender and maturational age.

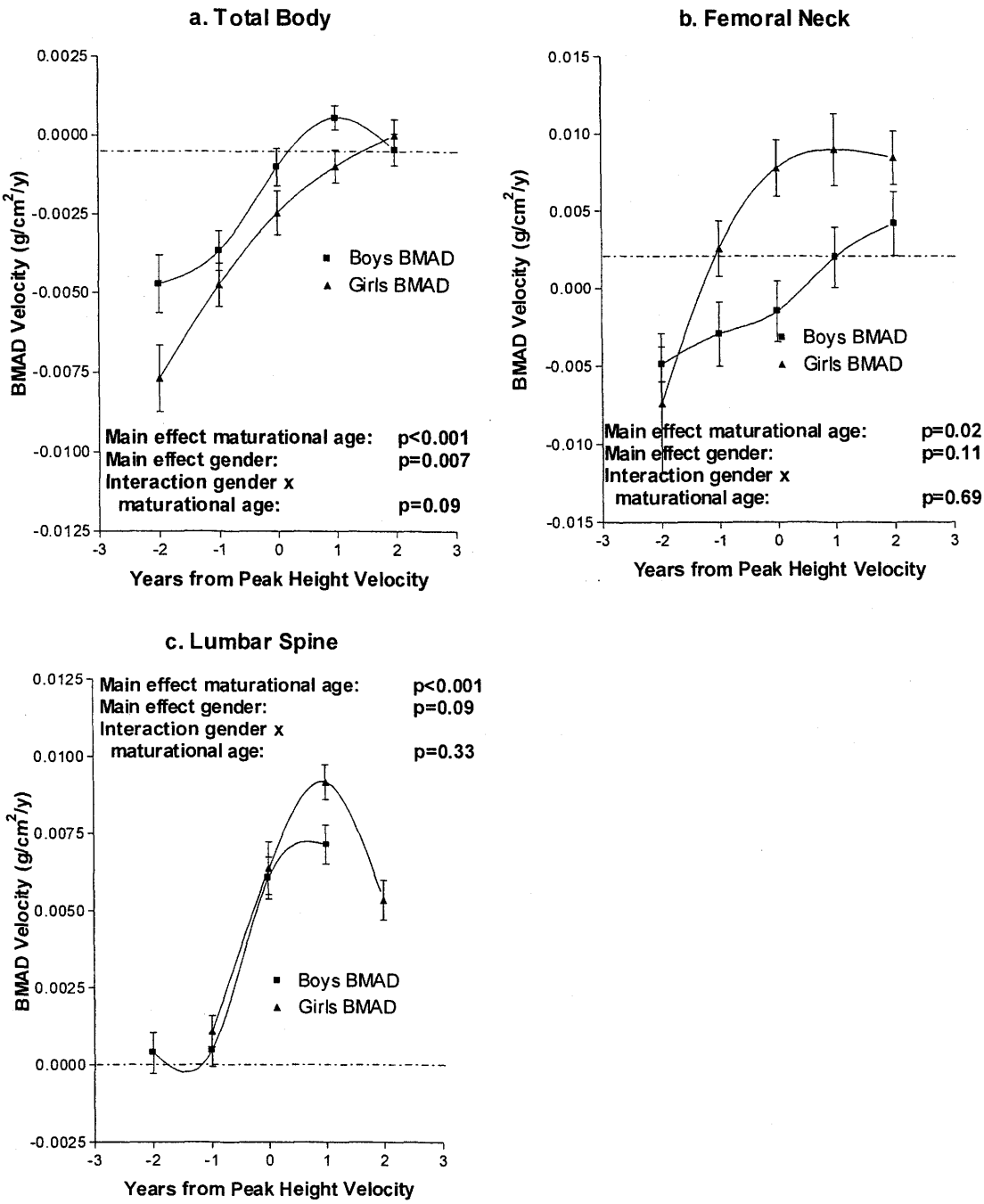


Figure 4.9. Male and female PHV-aligned velocity curves for bone mineral apparent density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

* denotes significant differences between genders at a specific maturational age in the instance where a significant age x gender interaction is present.

4.5.5 PHV-aligned sBMD velocity through adolescence ($H5_3$)

All sBMD velocity measures for the TB, FN, and LS can be found in Figure 4.10. As shown in Figure 4.10a., male sBMD velocity was negative until PHV, with a consistent acceleration throughout the age span. Females had a linear acceleration in TB sBMD from -2 to 1 year PHV, with a stabilization thereafter, as shown in Figure 4.10b.

As shown in Figure 4.10c., FN sBMD in males was stable through –2 to –1 years PHV, with a rapid acceleration to 0 years PH and minimal deceleration thereafter. In females, sBMD decelerated from –2 to –1 years PHV, rebounded to PHV and remained stable until the end of age range, as seen in Figure 4.10d.

As shown in Figure 4.10e., LS sBMD velocity for males was relatively stable until –1 year PHV after which rapid acceleration continued until 1 year PHV, the end of the measurement period. In females sBMD was stable from –1 to 0 years PHV, with acceleration and deceleration observed in the following two years, respectively, as seen in Figure 4.10f.

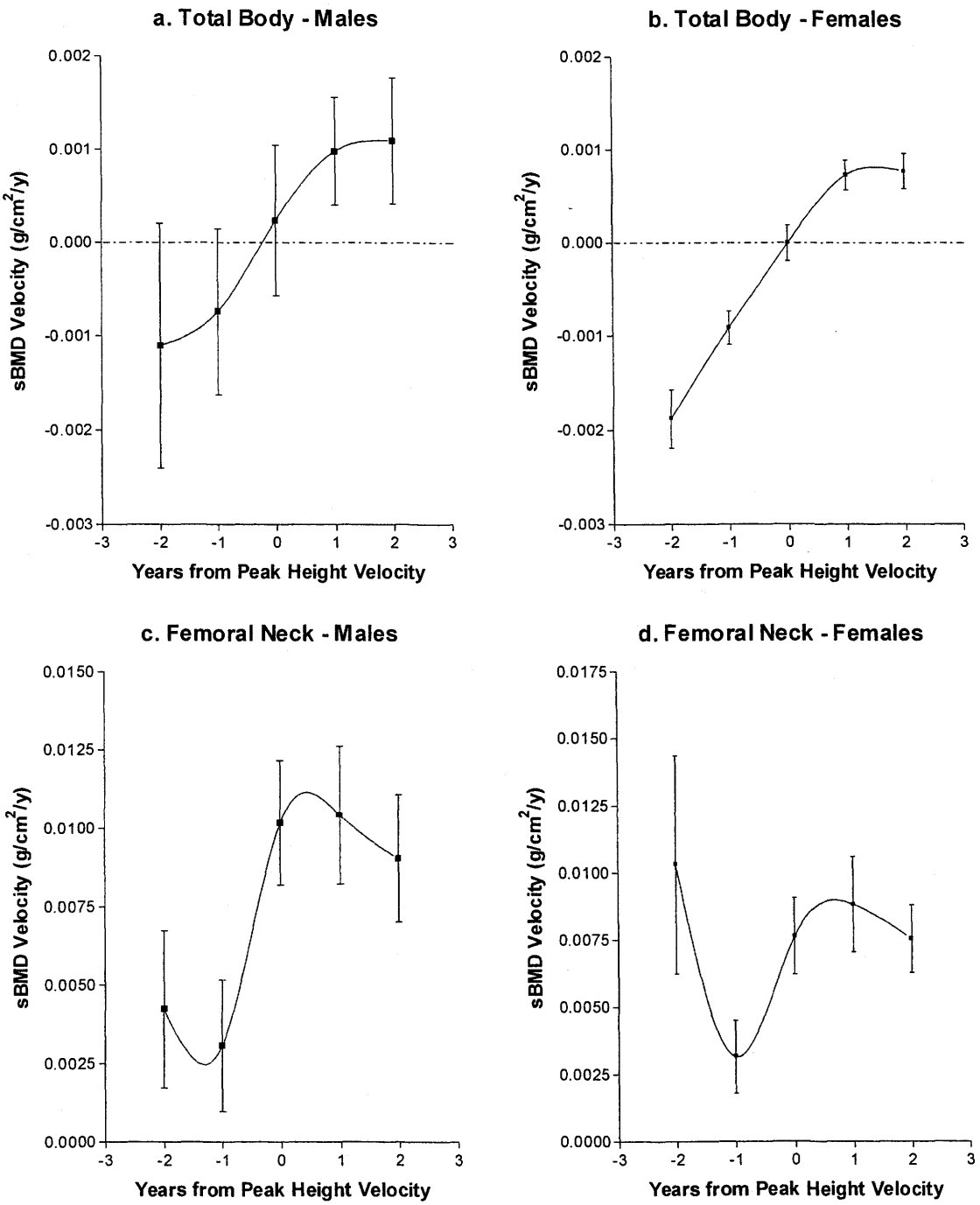


Figure 4.10. PHV-aligned velocity curves for statistically-corrected bone mineral density (mean±SEM) of the a. Male total body; b. Female total body; c. Male femoral neck; and d. Female femoral neck regions. Figure 4.10. continued on following page.

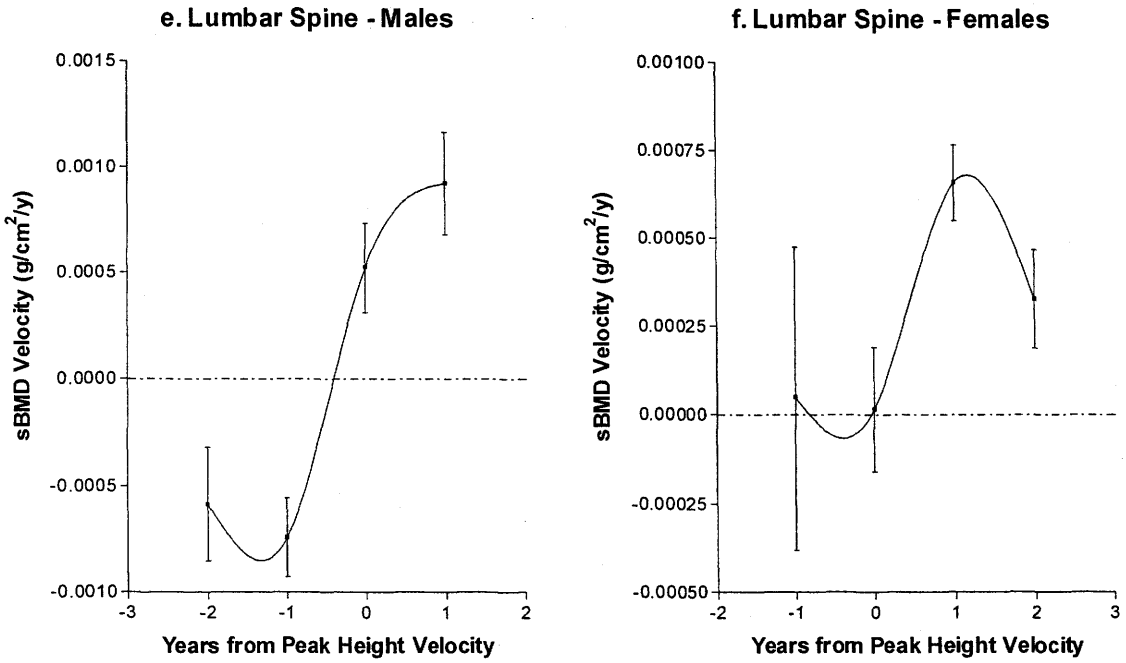


Figure 4.10. continued. PHV-aligned velocity curves for statistically-corrected bone mineral density (mean±SEM) of the e. Male lumbar spine; and f. Female lumbar spine regions.

4.6 *The dissociation between PA and BMC velocity curves (H6)*

As shown in Figure 4.11a., when males are aligned on PHV, TB PA velocity peaked at 0.22 years PHV with the peak BMC velocity occurring 0.49 years later at 0.71 years PHV. Peak PA velocity occurred at 0.40 years PHV in the females, as shown in Figure 4.11b., with the peak BMC at 0.81 years, resulting in a dissociation of 0.41 years.

As shown in Figure 4.11c., peak FN PA velocity occurred at 0.048 years in males while the peak BMC velocity occurred at 0.258 years, a dissociation of 0.21 years. As shown in Figure 4.11d., the peak FN PA velocity in the females occurred at –0.32 years and the peak BMC velocity at 0.29, a dissociation of 0.61 years.

In males, LS PA velocity peaked at 0.13 years PHV, as shown in Figure 4.11e., and BMC peaked at 0.40 years PHV, a dissociation of 0.27 years. As shown in Figure 4.11f., the peak LS PA velocity in the females occurred at 0.37 years PHV while the peak BMC velocity age was 0.86 years PHV, a dissociation of 0.49 years.

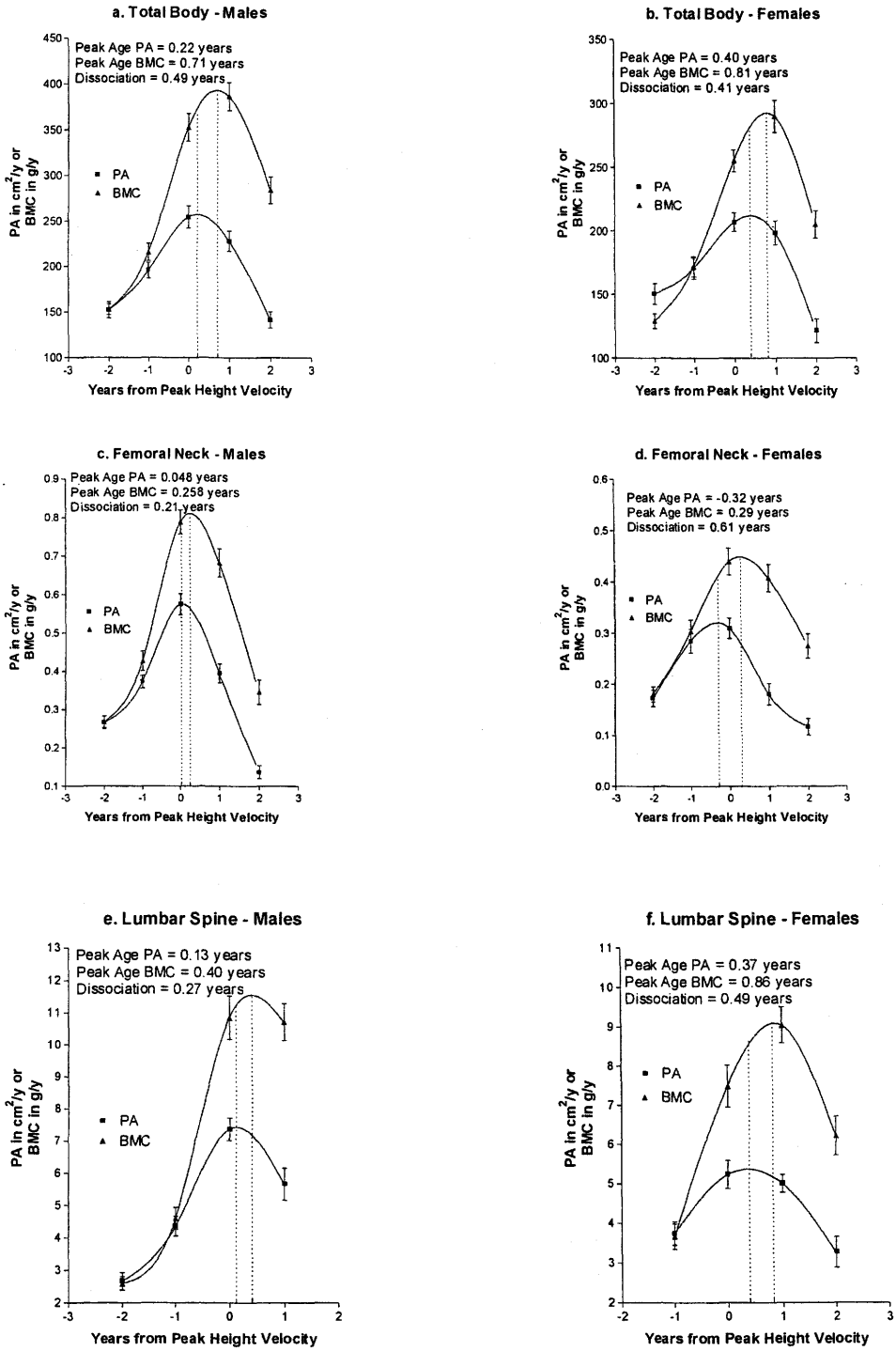


Figure 4.11. PHV-aligned velocity curves displaying the dissociation between bone projected area and bone mineral content (mean±SEM) for a. Male total body; b. Female total body; c. Male femoral neck; d. Female femoral neck; e. Male lumbar spine; and f. Female lumbar spine.

5 Discussion

The discussion is organized around the hypotheses. For each hypothesis, each respective sub-hypothesis is first addressed as it compares to the findings of prior investigations, if applicable. After each subhypothesis has been addressed, discussion of the overall hypothesis with emphasis on underlying physiological mechanisms is offered, where appropriate. At the conclusion of the hypotheses discussion, a brief summary of the findings is presented.

5.1 *General*

During childhood and adolescence three irrefutable phenomena occur in regards to the growth of bone in healthy children: 1.) There is an increase in the cortical thickness of the bones through both periosteal expansion and endosteal apposition (131), 2.) There is an increase in the length of the bone through growth at the epiphyseal growth plates until epiphyseal closure (62), and 3.) As a consequence of the increased cortical thickness and length of the bones, there is a corresponding increase in bone mineral (BMC) (59;61;152). While the BMC in a given region of bone and for the entire skeleton can be accurately estimated by DXA (96), it is infrequently reported in the literature owing to its dependence on bone size (67;97;101). BMC or bone size alone cannot adequately describe the qualitative changes in bone that occur with growth. PA and BMC are both measures of bone quantity. The most commonly practiced method of describing the qualitative characteristics of bone is bone mineral density, and typically an areal density. Bone density measures attempt to use data from both bone mass and bone size to estimate bone quality. The actual material density within bone, between bones within an individual, and between individuals, varies at any one time and over time (84). Areal density is only an estimate of the average density of a defined region, and includes both osseus and nonosseus materials.

Little is known regarding the true pattern of bone density changes during growth. This void in knowledge is due to a lack of longitudinal investigations that have corrected for differences in maturity status, and partially to DXA-based misrepresentation of true volumetric BMD changes. In the pediatric bone literature, aBMD is most frequently reported since it is hypothesized to at least partially correct for volume differences. Although there is a partial correction for size, the adequacy of size-correction with aBMD is hard to discern, and largely inadequate (67-69;101). Thus, it is difficult to establish true BMD patterns during growth with DXA-based aBMD measures as concomitant changes in bone size are observed throughout growth clouding the changes in true BMD.

As demonstrated in the literature review, several studies showed aBMD to be flawed in that it is consistently significantly dependent on bone size (67-69), and the high correlation of aBMD with PA, body height and body mass was further confirmed by the results in this investigation. Based on these findings the use of DXA-based aBMD should be discontinued in the pediatric population as it likely does not accurately reflect the true change in BMD occurring within the growing child.

When corrected for bone size the rapid increases in aBMD typically observed in children were either lost or at least attenuated, suggesting that the skeleton may not undergo a steady increase in density during growth as has been suggested by a preponderance of investigations that have utilized the size-dependent aBMD measures (55;61;133;150;152;155;178;216-218).

Conversely, in this investigation BMAD and sBMD results demonstrated that BMD does not increase throughout growth, but rather that there are generally both periods of decreasing density, as well as periods of increasing density, during growth. A frequent observation was that of diminishing or stable BMAD or sBMD during rapid skeletal expansion, followed by a subsequent increase in density of the bone as volume expansion slowed or arrested while bone accrual persisted. The point at which there was a nadir (most frequently almost precisely at PHV) in BMD could hypothetically represent the period of greatest skeletal fragility. This relative fragility hypothesis is supported by epidemiologic investigations which report the highest fracture rates during the period of PHV (189-195).

5.2 *aBMD is significantly influenced by the height of the subject, the mass of the subject and the bone projected area of the respective site (H1)*

5.2.1 Height, weight and bone projected area will be significantly positively correlated with areal BMD at all sites ($H1_1$)

True measures of density are independent from the physical space in which the material is found; much as the density of water at a constant temperature does not change whether it is in a thimble or a 45-gallon drum. Ideally, in the measurement of BMD the measure should be relatively independent from measures of BA; that is, when there is a significant association between measures of density and size then a confounding relationship exists and the estimate of density is flawed. Only when a size-independent measure of volumetric bone density can be performed *in vivo* can accurate representations of the actual bone accrual patterns over the lifespan be determined.

In this investigation aBMD measures at all sites were significantly ($p<0.001$) positively associated with measures of PA, stature and body mass, confirming the subhypothesis that height, weight and bone projected area are significantly positively correlated with aBMD at all sites. This consistent significant association confirms the dependence of the DXA-derived aBMD measures to size measures, particularly PA, which lends support to those techniques such as BMAD and sBMD that attempt to minimize the relationship between aBMD and BA.

Numerous other investigations have similarly found significant associations between aBMD and PA (67;127;128). In a mixed-gender sample of young children (mean = 12.9 yrs), Warner et al. (128) found that aBMD was a poor expression of density as it failed to remove the significant ($p<0.001$) association with PA ($r=0.81$ between LS aBMD and PA and $r=0.91$ at the TB). Prentice et al. (67) also found significant ($p<0.02$ – $p<0.001$) correlations between aBMD and PA in a cohort of healthy British men and women (TB, men $r=0.56$ and women $r=0.22$; LS, men $r=0.65$ and women $r=0.28$).

The results of this significant association with size are that larger bones will appear to have greater densities and smaller bones less, often regardless of their true densities. This finding is

particularly problematic when applied to the pediatric population where rapid growth and dramatic size differences between similarly aged children is common.

5.2.2 All regression coefficients of PA regressed upon BMC will be significantly different than unity at all measurement sites ($H1_2$)

DXA-derived aBMD assumes that both PA (a surrogate of volume) and BMC increase proportionally. If this relationship is valid then the regression of the natural logarithms of PA on BMC should result in a regression coefficient not significantly different from unity. If, however, the relationship is significantly different from unity then a better equation for the estimation of density should be introduced with the PA adjusted to reflect the proportionality delineated by the regression equation.

As at all sites regression coefficients were significantly different from unity; thus, the assumption that PA and BMC increase proportionally and the subhypothesis that all regression coefficients of PA regressed upon BMC would be significantly different than unity at all measurement sites, was supported.

The only other non-clinical investigations to perform similar regressions found analogous effects. In a study by Warner et al (128) power coefficients were similarly generated for a group of children and adolescents from the age of 6 to 17 years and all were found to be significantly different from 1 ($p<0.01$). The power coefficients were found to be 1.39 TB, 1.63 FN and 1.66 LS. These regression coefficients were similar to the coefficients found in my investigation: male/female (TB 1.446/1.432, FN 1.591/1.866, and LS 1.807/1.953). Molgaard et al. (126) regressed TB PA on BMC and found a regression coefficient of 1.43 for the females and 1.37 for the males in a cohort aged 5 to 19 yrs. These coefficients also were similar to the regression coefficients found in this investigation and further strengthen the argument to abandon the use of aBMD in the pediatric population, where changes in PA over growth and between individuals of similar chronological age are virtually inevitable.

5.2.3 Hypothesis 1 Conclusion

In this investigation both of the subhypotheses that aBMD is significantly affected by size measures were supported. These findings suggest the dependency of DXA-derived aBMD on size measures, particularly PA, and implore that the use of DXA-derived aBMD be limited in pediatrics.

Based on these findings the preponderance of pediatric literature describing changes in aBMD is most likely inaccurate due to aBMDs high dependency on bone size. Only when individuals are matched for PA could aBMD be used as a comparator. However, matching individuals prospectively by PA is impractical and exceedingly difficult.

The assumption that bone mineral density increases steadily with age through childhood and adolescence is most likely an error of DXA measurement related to its dependency on bone size. While there is a consistent increase in aBMD with growth, as observed in this investigation, there is a concomitant increase in PA as well, making it impossible to determine whether the increase in aBMD is due to a true increase in density, a size-related measurement artifact, or a combination of both. The size-corrected measures give a better representation of the actual density changes with growth.

5.3 *aBMD corrected for bone size will not be significantly influenced by the height of the subject, the mass of the subject and the bone projected area of the respective site (H2)*

5.3.1 Height, weight and bone projected area will not be significantly correlated with BMAD at any site ($H2_1$)

When aBMD was estimated for bone volume by the use of static geometric equations (BMAD), there was a significant decline in the variance accounted for by PA, body height and body mass; however the associations did remain significant between the variables and BMAD. BMAD removed some of the dependence of aBMD on bone size at the TB and LS (as compared with the respective correlations of aBMD with size measures at a given site), however all the size measures were still all significantly ($p<0.001$) correlated with BMAD. With the FN, the use

of BMAD again decreased the dependence of aBMD on bone size; however BMAD was still significantly correlated with PA and height in males, and mass and height in the females. At both the TB and FN PA was, in most instances, significantly negatively associated with BMAD, suggesting that as bone projected area increased, BMAD decreased. This may be interpreted as the inability of the body to adequately supply adequate calcium for the rapid skeletal expansion during adolescent growth (146). Perhaps as the bones increase in size the available mineral is used simply for the minimum architectural needs with full mineralization occurring sometime afterward when expansion has ceased and the body is able to supply the demands for this increased density (44).

Interestingly, weight was significantly negatively correlated with TB BMAD in both genders. This is the opposite to what would be expected based on the laws of mechanical loading and bone mineralization in that greater loads lead to localized greater mineralization (110;219-221). Possible explanations include that the TB site is 80% cortical in nature and not as responsive to loading as the LS site, or that generally too much of the skeleton is unloaded for vast proportions of the modern day to see a positive response of the whole skeleton to brief, localized loading. Similarly, the association between weight and BMAD at the FN in males was not significant. The FN is another more highly cortical region as compared to the LS. In the investigation by Katzman et al. (69) similar findings were reported for the TB and FN sites with correction by BMAD resulting in the loss of significant association between the BMAD and mass, while the significant association between mass and BMAD was retained at the LS. These observations suggest that this adjustment may not be appropriate for the TB in either gender or the FN in males. However, there may also be an alternative hypothesis where the largely cortical nature of the TB and FN sites are simply less responsive to loading from the total body mass as compared to the more metabolically active, primarily trabecular, LS.

The associations between BMAD and bone size were negative at the TB and FN, implying that smaller bones were denser. The epidemiological trials that hypothesize a period of decreased density during rapid bone expansion support these findings in that the peak rates of bone expansion and accrual differed by half a year on average. A smaller bone at the start of

rapid expansion may be denser than a bone afterwards, as a lot of the mineral that was found in the cortical shell may have been redistributed to allow for rapid expansion. It has been hypothesized that there is a "borrowing" of calcium from the cortical shell to supply the rapid growth regions with adequate calcium supplies (44). It has been postulated by Parfitt (44) that the cortical shell exhibits increased porosity during the period of rapid growth which would both decrease density as measured by DXA and lead to a decreased material strength within the cortical shell. Since the FN and TB sites are primarily cortical and the LS primarily trabecular, it is especially interesting to note that the only significant negative associations were observed in the TB and the FN, not the LS, which supports the theory that there is a pattern of decreasing cortical bone during growth with a relative maintenance of trabecular bone.

The results from this investigation were similar to the findings of Katzman et al. (69) in that the strongest associations with PA, body mass and body height were with BMC, followed by aBMD, and then BMAD. Similar to Kroger et al. (68) the results showed that when LS aBMD was corrected for bone size, the strength of the association with size measures decreased, but remained significant. Similarly, in the investigation by Lu et al. (187) after normalization for PA the association between density and height and weight decreased, but remained significant at the LS, but significant associations were lost at the FN with the exception for height in females.

Based on the mixed findings the subhypothesis that height, weight and bone projected area would not be significantly correlated with BMAD at any site was not supported. The correlations, however, were weaker than with aBMD in all instances suggesting that there was at least a partial independence gained from size or mass measures.

5.3.2 Height, weight and bone projected area will not be significantly correlated with statistically corrected BMD (sBMD) at any site ($H2_2$)

After correction of the data by the Prentice et al. procedure (67) the correlations between PA and sBMD were non-significant at all sites (r=-0.008-0.061). These results support the hypothesis that this equation was adequately accounting for PA in the assessment of density. At the TB, the association between weight and sBMD was eliminated, while at the FN and LS it was preserved ($p<0.001$). Numerous investigations have concluded that weight-bearing physical

activity places mechanical stimuli on the loaded bones to increase their density and to organize their trabecular infrastructure to accordingly resist the lines of strain to maintain mechanical integrity (110;123;222;223). The total body may be too large an area to be sensitive to specific mechanical loading. However, in the act of locomotion both the LS and the FN receive significant osteogenic stimuli to regulate bone density, most likely independently of bone size. One of the most consistent predictors of bone density is body mass, with higher masses leading to higher stresses. Therefore, it may be logical to see the significant relationship remain intact between weight and sBMD, even after the correction for bone size.

In the only other comparable investigation, Warner et al. (128) found that after statistical correction sBMD was no longer significantly correlated with PA (r=0.02-0.03). Based on these findings the subhypothesis that height, weight and bone projected area would not be significantly correlated with sBMD at any site was supported in all instances except for body mass where LS and FN sBMD were still significantly associated with mass. Theoretically, these corrected measures of density can be hypothesized to be the most accurate estimate of the true patterns of bone accrual during growth.

5.3.3 Hypothesis 2 Conclusion

In this investigation there was partial support for both subhypotheses. The use of BMAD partially corrected for the effect of size; however, the relationships between BMAD and size and mass measures remained significant in almost all instances, limiting its use in the pediatric population. Further, the trend for negative relations between body mass and BMAD is puzzling in light of the fundamental laws of mechanical adaptation in bone, apart from geometric or architectural changes (Wolff's Law; Frost's Mechanostat). The use of sBMD consistently led to a density measure that was independent from BA. This finding suggests that its use in correcting for density in growing children may be very useful. There was a trend for the highest correlations to be found with aBMD, then weaker with BMAD, and insignificant relations with sBMD. Similar results were found with Katzman et al. (69) in their investigation, but with BMC, aBMD, and BMAD.

These findings further suggest that the previous research in growth and development that has focused on aBMD as assessed by DXA is likely flawed in its dependence on bone size.

5.4 *The age-related increase in aBMD in both genders is eliminated when corrected for bone size (H3)*

5.4.1 aBMD will increase significantly throughout the growth period at all sites in both genders ($H3_1$)

This subhypothesis was supported in all of the sites investigated, in both males and females, with aBMD displaying significant gains over age as evidenced by both a consistent significant ($p<0.001$) main effect for maturational based age.

This investigation is unique in that maturation (through PHV) was controlled in the entire cohort. The few previous investigations in bone growth that did report pubertal status attempted to control for maturation by Tanner staging, which is more subjective and more imprecise than maturation correction by PHV as used in this investigation. Tanner staging, while relatively valid, is biased in both self- and investigator-based assessment and each one requires a degree of subjectivity. Tanner staging is a qualitative assessment of a continuous process. The use of self-assessment in Tanner staging is less accurate than investigator-assessed staging, however it is less stressful to the child (224). Conversely, the use of Tanner staging by the investigator is more accurate but is often stressful for adolescents at a time when their body image is particularly sensitive. Further, a frequent finding in Tanner staging is that of differing Tanner stages in a given individual, since generally an assessment of pubic hair development is performed in both sexes, along with breast development in females and genital development in males. Certainly, a child cannot be in two different stages of development during a precise moment in time. While the use of Tanner staging is easier than determining PHV, it doesn't easily allow for a comparison between genders and it only allows for very general categorization of maturity status when compared to the precise estimates that can be deemed from the estimation of PHV. Further, when assessing the maturity of the skeleton PHV would seem like a logical choice to determine the tempo of growth in the skeleton since height is directly a product

of skeletal growth. Tanner staging is a more appropriate measure of the endocrine status, which does have an impact on skeletal growth, but probably is not as sensitive an indicator of skeletal growth as height velocity (62). The use of PHV as a control for maturity has scarcely been used as a consequence of the time needed to identify it; while Tanner staging can be done during a single assessment, the determination of PHV can take years of diligent assessment during the proper age span to ascertain, necessitating tremendous time and personnel commitments. Tanner staging is most appropriate for cross-sectional investigations where the participant is only observed once, and in longitudinal investigations Tanner staging can also be used, but PHV would most likely be the preferred method as it is more precise and less subjective. A less used method is that of radiographic bone age, but due to the necessity of x-ray exposure and the labourious process of grading the many bones of the wrist region, it is infrequently used.

As is consistent from almost all investigations in the literature, aBMD increased with maturation. Surprisingly, the patterns of mineral accrual between the genders were strikingly similar when aligned on PHV, supporting the use of PHV-alignment for comparisons between the genders when possible. The positive relationship between maturational level and bone mineral accrual is logical since during puberty there is an increase in both growth hormone and sex steroids, which have potent effects on bone mineral accrual in both males and females (the specific affects of these hormones on bone discussed later (section 5.5.3).

The importance of sex steroids during puberty was demonstrated in an investigation by Slemenda et al. (55) where they reported that prepubertal, peripubertal and postpubertal children increased aBMD significantly at both the LS and FN over a three year period. They concluded that children who were peripubertal exhibited the greatest gains in aBMD, and at the LS the 3-year rate of increase was nearly threefold greater during puberty than before puberty ($p<0.001$). Similar differences were seen at the femoral neck in terms of before and during pubertal bone accrual, but to a lesser extent; this should be expected as the FN is more cortical in constitution and thus less sensitive to endogenous estrogen. At the end of the investigation the elder children (17 years of age) had attained peak bone mass values in the FN, but not the spine. Further, Southard et al. (155) concluded that there was a significant effect of puberty on

bone aBMD, with those more advanced possessing greater aBMD values in both genders. Moreira-Andres et al. (179) reported that in 88 children aged 10 though 18 years of age aBMD increased significantly with advancing states of puberty and Southard et al. (155) concluded that there was a significant effect of puberty on aBMD, with those more advanced possessing greater aBMD values. None of these previous investigations utilized the PHV-based maturity assessment, but rather subjective Tanner staging and all were cross-sectional.

5.4.1.1 Total Body

When controlled for maturation, TB aBMD in the males increased throughout the age span, with the most rapid mineral accrual occurring after PHV. The correlation between PHV-age and aBMD at the TB was significant ($r=0.823;p<0.001$), displaying the dependence of aBMD on pubertal age. Similarly, Boot et al. (177) reported significant increases in aBMD from Tanner stages 2-5, compared with the previous stage. Rico et al. (154) similarly found a significant increase in TB aBMD with higher Tanner stages in their cohort of adolescents.

After the females were aligned on PHV increases above that of the previous year were observed yearly throughout the age span. The PHV-age was significantly correlated with TB aBMD ($r=0.805;p<0.001$). Similar to the males, Boot et al. (177) reported that significant increases in female TB aBMD were observed from Tanner stages 2 and 4 and 5, compared with the previous stage.

5.4.1.2 Femoral Neck

Male FN aBMD was stable until PHV, then increased dramatically. The fact that PA had the same growth pattern suggests the increase may simply be an artefact of the measurement. PHV-age was significantly correlated with aBMD ($r=0.678;p<0.001$). Concurring with the findings of this investigation, Ruiz et al. (225) observed significant gains in aBMD at the FN in males in late puberty.

Similar to the males, girls had an increase in FN aBMD only after –1 year PHV. The correlation between the PHV-age and aBMD at the FN was also significant ($r=0.710;p<0.001$). The rapid increase in aBMD with a concomitant decrease in the years after puberty is supported

by investigations by Bonjour et al. (133) and Theintz et al. (150). They observed rapid increases in aBMD during puberty, and especially the years just after puberty. However, after 2 year following menarche, aBMD decreased substantially to the point where no more mineral was being laid down, suggesting that PBM had been attained at approximately 16 or 17 yrs. of age. Ruiz et al. (225) similarly reported a steady increase in aBMD at the FN in girls, with accelerated gains in mid and later pubertal stages.

5.4.1.3 Lumbar Spine

A dramatic increase in LS aBMD was found in the males after –2 years PHV, and the PHV-age was significantly correlated with aBMD ($r=0.809;p<0.001$). The findings in this investigation concur with previous studies. Boot et al. (177) reported that significant increases in LS aBMD from Tanner stages 2-5, compared with the previous stage. In another investigation, Ruiz et al. (225) reported that aBMD increased with pubertal maturation with the increases being insignificant between prepuberty (Tanner 1) and early puberty (Tanner 2 and 3), but vertebral density increased dramatically thereafter and measures from later puberty were significantly ($p<0.001$) higher than those in prepubertal children. Ruiz et al. (225) reported a significant and dramatic increase in LS aBMD in the final two stages of puberty (T4 and T5) in males, with relative stability or minimal gains in the first three stages. Grimston et al. (153) reported that there was little change in aBMD in Canadian children over the first three Tanner stages in both sexes, with a dramatic increase in aBMD of the FN and LS between stages 3 and 4 (16% increase) and stages 4 and 5 (11% increase). De Schepper et al. (162) found similar trends in the analysis of their Belgian database. Further, Rubin et al. (178) reported a consistent, linear increases in aBMD with pubertal stage and a number of other investigations have found significant increases in LS aBMD with higher Tanner stages (53;141;169).

Female LS aBMD increased dramatically from –1 years PHV onward until –3 PHV, with a significant correlation between PHV-age and LS aBMD ($r=0.798;p<0.001$). The most rapid periods of increasing aBMD were those observed around the pubertal years. Concurring with the positive effect of estrogen on bone status during puberty, Boot et al. (177) reported that significant increases in female LS aBMD were observed from Tanner stages 2-5, compared with

Tanner stage 1. Ruiz et al. (225) reported that LS aBMD was not significantly different between prepuberty (Tanner 1) and early puberty (Tanner 2 and 3), but vertebral density increased dramatically thereafter and measures from later puberty were significantly ($p<0.001$) higher than those in prepubertal females, similar as was seen in the males. Additionally, Rubin et al. (178) reported consistent, linear increases in aBMD with pubertal stage. In contrast, Plotkin et al. (188) reported significant increases in aBMD from Tanner stage 1 through 3, with insignificant changes from stages 3 to 5. With the exception of the Plotkin et al. (188), all of the above investigations support the findings of this study.

While it is not surprising that there would be an increase in BMC at all sites with increasing maturational states, as was observed in both genders, and is expected with increasing levels of growth hormone and sex steroids, it is questionable as to whether the increases in aBMD were real or an artefact of the rapidly expanding cortical shell.

5.4.2 BMAD will not change significantly throughout the growth period at all sites in both genders ($H3_2$)

The subhypothesis that BMAD would not change significantly through the growth period was not supported as there was a main effect for age observed at all sites. While there was some evidence of stabilization in the TB, this was not seen in either the LS or FN. Since there are very few previous investigations of this type to compare it to, the discussion will be primarily limited to the theoretical physiological rationale of the trends witnessed.

An increase in true volumetric LS BMD may be associated with both age and growth, with the increases theoretically attributed to increases in the quantity and thickness of individual trabeculae, artefacts in the mathematical extrapolation of bone volume and the volume ratio between the intravertebral disc and the vertebra (which influences the height of the ROI), or methodological limitations such as the inclusion of highly cortical posterior and transverse processes in the quantitation of BMC (but not of bone projected area) when the x-ray beam comes from a posterior-anterior direction (226).

As with the aBMD curves, aligning data on PHVs generally results in similar shaped curves between the genders, supporting its use for the comparison of genders.

5.4.2.1 Total Body

Both males and females displayed a similar pattern for PHV-aligned TB BMAD curves. TB BMAD decreased yearly from –4 to 0 years PHV and then remained steady for the duration of the study period. The correlations between BMAD and PHV-age were both significantly negative (males r= -0.493;p<0.001, females r= -0.372;p<0.001), suggesting a decrease in bone BMAD with increasing age. Certainly, this decrease is observed from –4 to 0 years PHV, but not for the period thereafter. This relative decrease and then stabilization suggests than as children pass in to puberty there is actually a lower bone density in peripubertal children than in prepubertal. There is evidence from investigations in adults that suggest that as the body loses bone mineral it will compensate for the loss in material strength by increasing its architectural strength through increasing its cross sectional area (104). Perhaps with an increased cross-sectional area, less density is needed to resist the strains engendered upon it and then with the stabilization of weight and PA expansion the body attains a state of homeostasis where the strength matches the loading environment. As osteons mature their material density increase, which may not occur to any great extent during growth as bone turnover is so rapid; only after the relative arrest of skeletal expansion would bone turnover slow enough to allow for enhanced mineralization of the osteons. Different from the findings in this investigation, Katzman et al. (69) reported that BMAD did not change with age in a cohort of adolescent females (r=0.004;p NS). The difference between this trials may be ascribed to the difference in sample size as Katzman et al. (69) only had 45 subjects in her subject pool and they were measured cross-sectionally using chronologically-based age.

It is interesting to note that many of the epidemiological trials where high fracture rates are witnessed occur at the point of bone mineral stabilization in BMAD (189-195). Perhaps the observed decrease in bone mineral density is a consequence of rapid bone envelope expansion, with too little exogenous calcium being supplied, forcing the body to redistribute a relatively equal bone mass over a greater volume, thus decreasing density, as was suggested by Parfitt (44). Certainly the data from this study regarding the dissociation between TB PA and BMC curves support this hypothesis. It is important to note that this observation is over a highly

cortical area. Parfitt (44) hypothesized that much of the calcium for rapid expansion is supplied from the cortical shell, as evidenced by increased cortical porosity. This hypothesis is supported in this investigation where during rapid skeletal expansion of the TB, a highly cortical region, there was little, if any, increase in BMAD. Only following the slowing of skeletal expansion was there stabilization or minimal increases in BMAD observed, hypothetically a result of a refilling of the excavation sites burrowed by osteoclastic resorption during rapid growth and high bone turnover.

5.4.2.2 Femoral Neck

In the males, PHV-aligned FN BMAD decrease each year from –4 through 0 years PHV after which it increases each year to 4 years PHV. The correlation between age and BMAD was not significant (r=0.083), suggesting that FN BMAD is independent of age. This rebound of bone density at the FN may be explained by the dissociation of the PA and BMC curves, with BMC accrual lagging behind PA expansion until BMC peaks while expansion decreases, allowing for rapid consolidation to occur. This makes particular sense when PA literally arrests shortly after PHV, while BMC continues to increase through the entire age range. Similar to this investigation, Kroger et al. (56) reported no significant association between age and volBMD (r=-0.08; NS) and Lu et al. (187) reported that after conversion to volBMD there was no longer any significant association between density and age or weight. In another investigation, Kroger et al. (68) concluded that there was no significant correlation of volBMD at the FN with age, suggesting that the observed increases in aBMD were due simply to increases in the bone envelope size.

In females BMAD was unchanged until year 1 PHV, after which it increased to the end of the measurement period. There was a significant correlation between BMAD and PHV-age ($r=0.455; p<0.001$), suggesting that BMAD is age-dependent at the FN in females.

Ferrari et al. (24) longitudinally studied a cohort of prepubertal girls (37% beginning Tanner stage 2 at trial termination) for 1 to 2 years and reported a 2.2% increase after 1 year in FN BMAD, and a 0.07% increase after 2 years. A smaller "rebound" can be seen in the female data, though not as apparent as with the males. It is important to note that this observation is in a moderately

cortical area. The phenomenon of a small rebound in early puberty is consistent with this investigation. In contrast, Kroger et al. (56) reported no significant association between age and volBMD (r=0.18; NS), Lu et al. (187) reported that after conversion to volBMD there was no longer any significant association between density and age or weight and Katzman et al . (69) reported that BMAD did not change with age. All of these other investigations were cross-sectional with limited sample sizes, and did not control for maturity, which may explain the differences in the findings.

5.4.2.3 Lumbar Spine

In males, when aligned on PHV, there was a maintenance of BMAD from –4 through –1 years PHV after which yearly gains were recorded; however a significant relationship was observed between PHV-age and BMAD ($r=0.593; p<0.001$). Similar to these findings, Boot et al. (177) described a curvilinear increase in LS BMAD across an age span of 5-18 y., Kroger et al. (56) reported a linear association between age and volBMD ($r=0.70$; $p<0.001$) and Lu et al. (187) reported that LS volBMD was significantly associated with age, height and weight. Both of the investigations above utilized chronological age, so are likely biased.

A rapid rate of BMAD increeases was observed between -1 and 1 yr PHV. These findings are similar to those of Boot et al. (177) who reported that significant increases in BMAD were only observed from Tanner stages 4 and 5, compared with the previous stages. In earlier stages of maturation, BMAD remained stable, suggesting that perhaps the dissociation between PA and BMC in the LS is less than in other sites and after PA expansion slows, the greater magnitude BMC gains account for augmented BMAD. Since the LS is primarily trabecular bone in composition, it may be that during rapid skeletal growth less bone is removed from the trabecular structure for growth, as it has been hypothesized that a large amount of endogenous calcium is supplied from the cortical bone of the body (44). Further, trabecular bone, owing to its high relative metabolic activity, can accrue bone at a much more rapid rate than cortical bone (109). This enhanced accrual ability may be utilized when the bone is loaded and senses a need for enhanced mineralization to withstand the stresses applied to it.

Similar to the boys, the girls demonstrated only minimal gains in BMAD until PHV, after which there was a dramatic increase in density from 0 to 2 yr PHV with slowing thereafter. The relationship between PHV-age and BMAD was strong and significant at the LS for the females ($r=0.728;p<0.001$), suggesting that BMAD is age dependent. Boot et al. (177) reported that significant increases in BMAD were only observed from Tanner stages 4 and 5, compared with the previous stage; in earlier stages BMAD remained stable. Ferrari et al. (24) longitudinally studied a cohort of prepubertal girls (37% beginning Tanner stage 2 at trial termination) for 1 to 2 years and reported a 1.1% increase after 1 year in LS BMAD, and a 1.4% increase after 2 years. Similarly, changes in FS BMAD were 26% after 1 year and 2.9% after 2 years. These increases were smaller in magnitude than the observed changes in aBMD at each site in this investigation as they were at least partially controlled for bone size.

For chronologically-based age, similar to the findings in the females here, Kroger et al. (56) reported a linear association between age and volBMD ($r=0.54$; $p<0.001$), Lu et al. (187) reported that LS volBMD was significantly associated with age, height and weight and Katzman et al. (69) reported that BMAD increased significantly with age. All the above investigations concur with the findings presented here for LS BMAD. Kroger et al. (68) reported that only minimal increases in LS volBMD were observed, far smaller than the increases suggested by aBMD data.

Interestingly, the rebound effect was not too evident in this trabecular-dominated area of the skeleton. However, there was a relative stability in bone mass until after PHV had been attained and expansion had slowed so that bone accrual could subsequently increase BMAD, with exceedingly rapid accrual for the 2 to 3 years immediately following PHV. These observations support the hypothesis that skeletal expansion is occurring at a pace that is too rapid for proper mineralization, whether it be a consequence of inadequate exogenous calcium supplies, exogenous protein intake, or endogenous calcium supplies. Interestingly, in the Boot et al. (177) investigation with adolescent females there was a trend for values to "rebound" for LS BMAD.

Looking over the three sites there was a trend for the largest rebound effect to be observed in the most cortical region (TB), the intermediate region had an intermediate rebound (FN), and the smallest rebound in the most trabecular region (LS). These findings support the hypothesis of Parfitt (109) who suggested that the endogenous calcium supplied for rapid skeletal growth is primarily "borrowed" from the cortical shell as evidenced by increased cortical porosity.

5.4.3 sBMD will not change significantly throughout the growth period at all sites in both genders ($H3_3$)

The hypothesis that sBMD would not change significantly with age was not supported in this investigation. Because very limited studies are available for comparison most of the discussion is theoretical.

5.4.3.1 Total Body

When aligned on PHV males and females had the same pattern of sBMD change, with the "rebound" effect prominent: TB sBMD decreased yearly from –4 to 0 years PHV after which increases in sBMD were observed until the end of the age span. The associations between sBMD and PHV-age were significant (males $r=0.116;p<0.05$, females $r=0.257;p<0.001$). The prominent rebound effect observed in this region lends further support to the cortical borrowing hypothesis of Parfitt (227). This rebounding of bone mineral may be a result of the dissociation and different timing and tempo of PA and BMC over the growth period. It is possible that the growth in envelope expansion may have been too rapid early in puberty for bone accrual to keep pace with, but following PHV the lag was made up and consolidation began to become more complete, leading to higher sBMD values with the slowing of PA expansion and the continuation of BMC accrual.

5.4.3.2 Femoral Neck

Both males and female FN sBMD remained stable until PHV, then increased. Significant associations were observed between sBMD and PHV-age (males $r=0.369;p<0.001$, females $r=0.509;p<0.001$). This dramatic increase in density in both of the genders is likely a

result of elevated growth hormone and sex steroid levels (affect of sex steroid and growth hormone on bone discussed in section 5.5.3).

5.4.3.3 Lumbar Spine

Both males and females exhibited a "rebound" effect for LS sBMD and exhibited significant relationships between age and sBMD (males $r=0.200;p<0.001$, females $r=0.271;p<0.001$), implying a close relationship between age of maturation and sBMD.

5.4.4 Hypothesis 3 Conclusion

The hypothesis that, when corrected for bone size, there would be no increase in BMD with age was not supported. However, interesting patterns did emerge from the data than are different from what is currently presented in the literature. The results show that there is a period of increasing BMD in later puberty, even early adulthood, but there is also a trend for decreasing density during early puberty when the skeleton is undergoing rapid expansion. The subhypothesis that BMAD and sBMD would not change significantly throughout the growth period at all sites in both genders was not supported; however, an extremely interesting physiological phenomenon was consistently found in the data. In almost every site, in both genders there was a strong trend for the BMD values for the most size-independent measure, sBMD, to decrease until approximately PHV, after which BMD was seen to increase until the end of the investigated end period.

The rebound effect makes physiological sense when combined with the epidemiological data of fracture rate during adolescence (189-195). Theoretically, the trough in the rebound curve represents a point of increased skeletal fragility at a time of increasing body mass.

Numerous other investigations have shown evidence of a rebound effect in their data, although it has rarely been highlighted. Gilsanz et al (160) performed a LS QCT investigation of boys and girls aged 3-18 years of age. In this investigation maturational grouping was done arbitrarily with prepubertal, indeterminate and pubertal subjects being assigned to groups purely on their age and not a measured maturity level. Certainly, the prepubertal group was valid (girls and boys under 9 and 9.5 years, respectively), however the indeterminate (girls 9-13.4 and boys

9.5-13.7 years, respectively), and the postpubertal (girls 13.4-18 and boys 13.7-18 years, respectively) would have been inappropriate as the indeterminate would have certainly included pubertal children and the pubertal would have certainly included postpubertal children. Regardless of the methodological problems with this investigation, it did present interesting volumetric data that lends strength to the findings of this study. For LS trabecular bone boys had slightly higher BMD prepubertally than in the indeterminate stage, which was then followed by dramatically higher BMD in the pubertal grouping. Similarly in the females trabecular bone was steady through prepubertal and indeterminate stages, after which a dramatic increase was observed in the pubertal grouping. This trend was similar to the LS findings of this investigation. For cortical bone, in both males and females, a trend of slightly lower volumetric density for the indeterminate subjects as compared to the prepubertal subjects followed by a dramatically higher pubertal density supports the estimated volumetric data found at the TB and FN in this investigation. In this QCT data, there was a similar trend of slight losses, or a minimal maintenance of BMD, with a "rebound" in BMD after puberty.

Similarly, Mora et al (180) performed a QCT investigation in a cohort of girls categorized by Tanner staging and found evidence of a "rebound" effect in trabecular bone mass in the LS; cortical bone mass was observed to increase progressively throughout childhood and adolescence whereas trabecular vertebral BMD changed little with age in the prepubertal girls, decreased slightly in early puberty, and then increased in later puberty, after which it reached its maximum value at sexual maturity. The authors concluded that hormonal or metabolic factors played a more important role than mechanical factors in the regulation of trabecular bone mass during the pediatric years.

In the BMAD data of Katzman et al. (69) there is some evidence of a rebound in the FN and TB, but none in the LS, similar to some of the findings in this investigation. The extremely small sample size of the is cohort, however, makes analysis far too subjective.

In a longitudinal study estimating volumetric BMD at the proximal radius Magarey et al. (228) reported a trend for females to have stable bone mass from 11 to 13 years of age, after which there was a dramatic increase in density. However, in the males, there was a decrease in

estimated volumetric bone density from 11 to 13 years of age, after which there was a rebound to higher values thereafter. When aligned on pubertal status, both males and females displayed the "rebound" with a decrease in BMD from Tanner stage 1-3 and a subsequent increase. The authors concluded that the decrease in BMD during midpuberty was a consequence of a lag between bone mineralization and periosteal expansion.

Milinarsky et al. (229) assessed 571 (4-24 years of age) children and adolescents in Chile with SXA at the distal (primarily cortical bone) and ultradistal (primarily trabecular bone) forearm. D-BMD and U-BMD increased very slowly from 4 to 8 years of age in girls, and from 4 to 12 years of age in the boys. Dramatic increases from 8 and 19 years of age in the females and 12 and 20 years in the males in both areas were observed. In terms of maturity, the boys and girls both had significant increases in D-BMD between Tanner stages 3 and 4 and 4 and 5, and in mean UD-BMD between Tanner stages 3 and 4. Significant increases in BMD after menarche were observed in the females for 2 years for D-BMD, and for 1 year in UD-BMD. In both of the genders height increases preceded increments in bone accrual. Interestingly, in this investigation two "rebounds" were observed for both the cortical and trabecular site – one at approximately the midgrowth spurt and one at the pubertal growth spurt.

The BMD volumetric data are consistent in showing a rebound effect during growth, with the age of minimum density theoretically the period of maximum fragility.

As bone grows, cortical BMD increases as a result of the replacement of relatively low density primary osteons with higher density secondary or tertiary osteons, along with secondary calcification (230;231). The prevalence of osteoid seams may give some insight as to why older bone may be denser; Frost et al (232) found 10% osteoid seams at age 5, 3% at age 15, 0.8% at age 30, and 0.8% at age 70. Theoretically, a greater prevalence of uncalcified osteoid seams would decrease the overall density of the cortical bone.

However, even after size-related controls have been made to correct for the inadequacies of BMD assessment by DXA, the measurement itself rarely reflects the underlying biological phenomenon. Changes in bone density with growth are commonly ascribed to changes in mineralization, regardless of whether the changes are due to increases in the

physical amount of bone within the envelope (as with increased trabecular thickness or a greater cortical thickness) or due to true increases in mineralization in the form of an incorporation of additional mineral into existing bone matrix (84).

5.5 *The gender difference observed in aBMD will be eliminated when corrected for bone size and maturation (H4)*

5.5.1 Males will possess significantly greater aBMD at all sites as compared to females, when corrected for maturation ($H4_1$)

The subhypothesis that males will possess significantly greater aBMD at all sites as compared to females, when corrected for maturation was supported at the TB and FN, but not the LS.

5.5.1.1 Total Body

The pattern of aBMD accrual was identical between the sexes, with males displaying significantly higher values at all ages. Further, aBMD velocity curves were identical between males and females, with the peaks occurring at identical developmental ages and with the males only having a slightly larger magnitude velocity at peak (males = 0.078 and females = 0.064 g/cm2/y.). By aligning velocity curves on PHV and effective normalization for males and females occurs, allowing for their direct comparison. The augmented BMD increase in the males may be a consequence of DXA-mediated error in the measure of aBMD of subjects of differing sizes as the males were physically larger than the females.

In chronological-based measures, Boot et al. (177) reported no significant differences between the genders for TB aBMD at any age, whereas Maynard et al. (218) reported that TB aBMD did not differ between the genders until age 15 after which aBMD was significantly greater in the males, similar to what was found in this investigation.

5.5.1.2 Femoral Neck

In the males aBMD was consistently higher for all time points when compared to female values. Similarly, Bonjour et al. (133) reported bone aBMD based on pubertal stage and

concluded that for all pubertal stages the males possessed greater aBMD (significantly so at T1 and T5) as compared to the females. Grimston et al. (153) found similar results in that the males in their investigation, at each Tanner stage, had greater FN aBMD as compared to their female counterparts. Differently from these results, Ruiz et al. (225) reported that only at Tanner stage 1 did the males possess significantly higher FN aBMD as compared to the girls; interestingly there was a trend for the females to have greater values at the other pubertal stages.

BMD curves between the males and females were of a similar shape and magnitude (males = 0.073, females 0.070 g/cm2/y) with the only difference being that of the timing of the peak with the males attaining their peak approximately half a year before the females, just after PHV.

By aligning velocity curves on PHV an effective normalization for males and females occurs, allowing for their direct comparison and it was found that they were extremely similar in regards to their patterns of BMD change, despite the size differences.

5.5.1.3 Lumbar Spine

aBMD was identical between the genders at the LS when aligned on PHV. Similarly, Bonjour et al. (133) concluded that when aligned on pubertal status there was no difference between males and females at the LS. Also, Rubin et al. (178) reported consistent, linear increases in aBMD with pubertal stage, however at midpuberty the females briefly possessed a greater LS aBMD. When adjusted for differences in body mass between the genders, the females had significantly higher LS aBMD at all pubertal stages as compared to the males. Ruiz et al. (225) reported that at no Tanner stage was there a significant difference between genders for LS aBMD, although there were strong trends towards the females having greater densities, particularly at the later stages of pubertal development. Southard et al. (155) reported no difference between the genders for LS aBMD art any pubertal stage, but did report a collapsed-group significant increase in LS aBMD with each succeeding pubertal stage.

5.5.2 No statistical difference will exist between genders for BMAD at the TB, LS and FN, when corrected for maturation ($H4_2$)

As there were no other trials in the literature to compare the data to no comparisons can be made with previous investigations. The greater increases in BMAD rates with the females might be attributed to the greater osteogenic potential of estrogen as compared to the androgens (233). During puberty GH as well as sex steroid increase, which both have a positive influence on bone accrual (234;235). The influence of puberty was more marked in females than in males in a study by Boot et al. (177), as in a multiple regression analysis the Tanner stage did not correlate significantly with aBMD in males whereas with the females it was a major determinant. Animal investigations have shown the dominant effect of estrogen as compared to androgens on the skeleton (233). Further, those girls that had an earlier menarche had higher BMD, suggesting the importance of estrogen on mineralization (177).

5.5.2.1 Total body

BMAD between males and females showed the same pattern of initial loss concluding with a plateau in density values from PHV onward; females had significantly higher TB BMAD than the males at all time points as reflected by the significant main effect for gender and no significant interaction effect for gender and age. When corrected for bone size it appears that females contain slightly higher bone density than do males. These findings support the contention that the differences observed between males and females in the aBMD literature are skewed due to the larger bone size of the males.

5.5.2.2 Femoral neck

BMAD of the FN was higher in males from –4 years PHV until –1 years PHV after which BMAD values were equal until 4 years PHV where females possessed greater BMAD as compared to the males; however there was no significant main effect for gender, but a significant interaction between age and gender.

These findings are similar to what have been reported in the chronological-based literature. Lu et al. (187) reported no significant difference between the genders for volBMD at

the FN. When aBMD was corrected for size, there was only a significant difference ion the 18-19 y. group with the males having a greater volBMD (68)

There was a difference in the shape of the BMAD curves between the males and females; the males exhibited a prominent rebound with the trough at PHV, but the females had a rebound with a trough at -3 or -2 years PHV.

5.5.2.3 Lumbar Spine

The females possessed higher BMAD at all ages past –2 years PHV when compared to males as reflected by the significant main effect for gender and no significant interaction term. In support of this trial, Boot et al (177) reported significantly greater LS BMAD at all ages (5-18 y) in females as compared to males and Kroger et al. (68) reported that after the age of 16-17 girls had significantly greater volBMD than the males.

Perhaps the effect of increasing estrogen elicits mineralization earlier or to a greater degree in this highly trabecular region than is seen in the boys, resulting in higher true density in trabecular-dominated regions.

5.5.3 Hypothesis 4 Conclusion

When aligned by maturation, only the FN displayed no difference between the genders, so the hypothesis was only supported at the FN. Perhaps there is a real difference in mineralization patterns between the genders at the TB and LS. For example, Zamberlan et al. (183), after measuring cortical thickness and bone density of growing males and females at the distal radius, concluded that most of the purported differences between males and females and the changes that are associated with age are primarily an artifact of skeletal dimension rather than changes in actual density. In fact, the estimated volumetric BMD of the radius remained stable from 3-21 years in the investigation. The distal radius can be most compared with the TB measure in this investigation as it is a highly cortical site.

In essence, differences between the genders in patterns of bone mineralization have to be at least partially attributed to differences in central, and perhaps local, hormonal action. Trabecular bone has been suggested to be more responsive to estrogen and cortical more to

testosterone; the preferential activity of these two gonadal hormones may provide some insight as to the patterns observed in this trial.

The products of the GH-IGF-I system induce proliferation without marked maturation of the epiphyseal growth plate and thus induce linear skeletal growth that lasts until sex-hormone mediated epiphyseal closure (236). The sex hormones, estrogen and testosterone are present in very low quantities during infancy and childhood and are produced in abundance during puberty and afterward. These hormones promote both proliferation and maturation of the epiphyseal growth plate. The direct actions of these hormones on the growth plate are difficult to ascertain owing to the indirect actions it has through increasing the activity of the GH-IGF-I axis and of insulin during puberty, and through the aromatization of testosterone to estrogens (236).

Childhood growth is largely GH-dependent, with pubertal growth being dependent on a combination of sex steroids and GH (237). During puberty there is a distinct rise in the release of GH (238-240). Further, sex steroids have a direct anabolic action and a modulating effect on GH secretion (237). During puberty the increase in sex steroids cause an increase in endogenous pulsalite secretion of GH in boys and girls (241).

An adequate pubertal growth spurt cannot occur without sufficient quantities of GH (242;243). However, the combined growth promoting effects of the gonadal axis and increased GH secretion are required for growth at puberty (244). In an investigation by Rose et al. (245) GH levels increased during puberty in both sexes, with the highest levels at midpuberty, with the pulse amplitude, rather than the temporality, responsible for the increase. Children with GH deficiency have dramatically lower bone mass as compared with their normal counterparts (246-248). In adults with childhood onset GH deficiency, the lack of serial change in bone mass in adulthood suggests that the reduced BMD observed may primarily be a consequence of reduced bone mineral accretion as compared to advanced rates of bone loss (249).

It is well established that estrogen has potent osteogenic effects on bone. The importance of estrogen in growing adolescent females is perhaps best observed in cases where estrogen is absent – amenorrhea. In a recent investigation 21 of the 27 amenorrheic women

investigated (mean age 27 yrs) showed pathological demineralization of the lumbar spine (250). To illustrate the importance of estrogen on growing bone, the degree of undermineralization in the investigation was higher in those subjects with primary as compared to secondary amenorrhea; the authors concluded that the lower bone mass in the primary amenorrheics was attributable to reduced bone formation in adolescence (250). In another investigation it was estimated that amenorrehic women had a 15% reduction of BMD at the LS as compared to normally menstruating women (251). Similarly, a QCT investigation concluded that LS trabecular bone mass was 20-30% decreased in patients with amenorrhea as compared to eumenorrheic women (252). Obviously, there is unequivocal evidence that a lack of estrogen is extremely deleterious to growing bone, particularly trabecular bone. However, estrogen is also important for cortical bone accrual as shown in an investigation by Dhuper et al (207) where the effect of estrogen on growing bone was demonstrated in that the girls with the lowest exposure to estrogen during puberty had the lowest cortical and overall bone mass.

The presence of estrogen is also important for normal mineralization in males; observations from males who lacked aromatase (253) or functional estrogen receptors (254) displayed delayed epiphyseal fusion, suggesting that even in male subjects estrogen plays a key role in the maturation and eventual fusion of the epiphyseal plate.

Theoretically, estrogen could be a limiting factor to the adolescent growth spurt (236). Blumsohn et al. (156) postulate that the effect of estrogen on growing bone in females is biphasic in that during early puberty when circulating estrogen levels are low, estrogen stimulates growth, while during later puberty, when estrogen levels are higher, it has a potent inhibitory effect on longitudinal bone growth and accelerates growth plate closure.

Testosterone has an important influence on bone development as evidenced by the concomitant increase in osteocalcin, a marker of bone formation, and serum testosterone during puberty (255). In an investigation of adolescent male hypogonadism it was found that cortical bone density was decreased in adolescent male patients with hypogonadism as comparison with controls; gonadal sex steroid testosterone deficiency was attributed to this lower BMD (256). Clearly, there was an effect of testosterone on cortical density.

Testosterone may promote mineralization in bone through a number of pathways: activation of the PTH-vitamin D axis, enhancement of GH secretion, which results in augmented IGF-I action on bone, and most likely a direct effect on bone. The action of testosterone on bone is thought to be direct even in the absence of systemic growth hormone and IGF-I (256), however, the effect of testosterone on bone has been shown to be retarded without the presence of GH (257).

It has been suggested that testosterone has a preferential effect on the axial skeleton (primarily cortical bone). However, evidence from investigations with children afflicted with primary hypopituitarism suggest that the growth of the lower body is primarily under the control of GH, whereas the growth of the upper body segment is more under the control of testosterone (258;259).

In a recent study of central precocious puberty and congenital adrenal hyperplasia it was concluded that adrenal androgens appeared to have a smaller role in bone maturation than estrogen despite the fact that both estrogen and adrenal androgen are involved in increasing BMD (260). Adrenal androgens may act indirectly on bone cells by conversion to testosterone and dihydrotestosterone (261). Androgens can also be aromatised into estradiol or estrone (262).

Sex steroids influence circulating levels of GH and IGF-I, suggesting that an interaction between these hormones may be important to the acquisition and maintenance of bone mass (263). It is possible that the influence of GH deficiency on endogenous sex steroids may explain the presence of reduced bone mass in GH deficient children (263). IGF-I has chrondrocyte mitogenic effects, but is also anabolic to bone tissue through the stimulation of osteoblast proliferation and differentiation, and matrix formation (264).

Based on these findings it seems as though estrogen has a preferential effect on more trabecular regions, whereas the androgens a preferential effect on the more cortical regions, although there seems to be no clear answer in the literature as yet.

This preferential effect of estrogen on trabecular bone mass may partially explain the higher BMAD found in the females in this investigation, and the higher aBMD found in a number

of investigations, despite their smaller PA as compared to males of a similar age or developmental stage.

5.6 *The age-related acceleration in aBMD velocity during the pubertal years is eliminated when corrected for bone size (H5)*

5.6.1 aBMD velocity will remain positive throughout the growth period at all sites in both genders ($H5_1$)

aBMD velocity remained positive in all sites for all genders, supporting the subhypothesis. There are very few comparable studies with which to contrast the findings of this investigation.

As with the distance data, when the velocity values were aligned on pubertal status based on PHV the variance observed decreased and the velocity curves of the genders became similar. If anything, this investigation gives credence to the methods used here; the conclusions drawn from the maturity-aligned data have greater validity compared to the chronological data. With the chronological data subjects were grouped according to a set chronological age, but invariably are at differing points of maturation, since puberty can vary widely in terms of age. In essence, by grouping the children based on chronological ages, there is a grouping of differing maturity levels and thus differing growth tempos. The result of this erroneous grouping would be to artificially attenuate those periods of rapid growth and enhance the lower levels – creating a curve that is less reflective of individual growth. When aligned on PHV these problems are eliminated and the true shape of the growth patterns can be determined and from that mineralization patterns ascertained. The use of maturity based data is essential when comparing or combining velocity curves.

5.6.1.1 Total Body

In the males all TB aBMD velocity measures remained positive, with the age at peak aBMD velocity occurring at 0.89 years PHV. TB aBMD velocity remained positive over the entire measurement period for the females, and the peak aBMD velocity was observed at 0.91 years

PHV. There are no trials with which to compare this data. It is interesting to note that for both the males and females the peak in BMC followed PHV by almost a year, giving support to the theory that first the bone expands, and then becomes consolidated. It is possible that at the time of rapid bone expansion the body simply cannot provide enough calcium to fully mineralize the newly laid down osteoid, as supported by longitudinal research by Martin et al. (146).

5.6.1.2 Femoral Neck

When aligned on PHV the male peak aBMD velocity occurred at .021 years PHV and all values were positive throughout the measurement period. All measures of FN aBMD velocity were positive in the females throughout the measurement period with the peak occurring at 0.83 years PHV. The peak for the FN occurred earlier in the males than it did for the TB, showing some form of temporal spacing. In the females there was a relative equality between the age at peak in both the TB and FN.

5.6.1.3 Lumbar Spine

When aligned on PHV, male LS aBMD velocity remained positive at all time points, with the peak velocity occurring at 0.52 years PHV. In females, all aBMD velocity values were positive and the peak occurred at 0.81 years PHV. Again, the peak for the females was exactly in the range of the other two sites, but the peak for the males was intermediate between the sites.

5.6.2 BMAD velocity will be negative or zero through the growth period at all sites in both genders ($H5_2$)

This subhypothesis was not supported in totality; however, there were notable portions of the velocity curves that exhibited a negative velocity. The negative velocities observed are in contrast to the findings of aBMD investigations which show a constant increase in density with growth (55;61;133;150;152;155;178;216-218). From this data it seems safe to claim that there are periods of decreasing density during early puberty and periods of increasing density with later puberty.

5.6.2.1 Total Body

When aligned on PHV, male BMAD velocity peaked at 1.04 years PHV. Only at the peak did BMAD velocity attain a positive velocity. At no time did the female BMAD reach a positive velocity with the peak occurring at 2 years PHV. The negative velocities described in both the males and females in this investigation may be a result of dissociation between the volume and the mineral content of the bone. As the curves are observed, they are accelerating towards zero or positive values. This may be the result of bone volume slowing and BMC accelerating, causing bone which at first was expanding more quickly than it could be mineralized, to become more and more mineralized as the lag between the two processes come to a union before BMC accrual dominates. This highly cortical sample largely supports the subhypothesis in both genders.

5.6.2.2 Femoral Neck

When the males were aligned on PHV, BMAD velocity was negative until 1 year PHV, with the peak occurring at the end of the measurement period. In the females, the –2 year PHV had a negative velocity associated with it, but a positive velocity was witnessed from –1 years onward with the peak occurring at 1 year PHV. The male curve suggests that increases in BMD are beginning to rapidly occur at the end of the measurement period – this may be the point at which BMC gain overtakes bone envelope expansion. In the female curve, a stabilization and the beginning of a downward deflection approximately 2 years after PHV support the findings of Theintz et al. (150) and Bonjour et al. (133), who both concluded that the most rapid time of aBMD gain is in the two years directly after menarche. Once again, the intermediate cortical site displays a pattern intermediate between the highly cortical and highly trabecular site.

5.6.2.3 Lumbar Spine

BMAD velocity in the males was consistently positive with a dramatic acceleration occurring from –1 to 0 years PHV. The male peak BMAD velocity occurred at 0.68 years PHV. BMAD velocity was not negative at any time point in the females and the peak occurred at 0.93 years PHV. In both the males and females, extremely rapid gains were made in bone density;

the steepness of the velocity curves were much greater than that of the TB or FN, perhaps reflecting the fact that trabecular bone is much more metabolically active as compared to cortical bone (109) and that the sex hormones present during puberty have a rapid effect on sites such as this with large proportion of trabecular bone. It is interesting to note that the boys' peak value was 0.0072 g/cm3/y and the females' 0.009 g/cm3/y., one of the rare instances where the females had a greater peak, perhaps to the greater metabolic activity of estrogen in bone as compared to the androgens (233).

Note that the most trabecular region was the only region with little or no negative velocity values.

5.6.3 sBMD velocity will be negative or zero through the growth period at all sites in both genders ($H5_3$)

5.6.3.1 Total Body

Male TB sBMD velocity remained negative until PHV after which positive velocities were observed and peak occurred at 1.75 years PHV. Female TB sBMD velocity remained negative until PHV after which positive velocities were observed, with the peak observed at 1.49 years. These observations support the rebound hypothesis. Density is thought to decrease during periods of rapid skeletal expansion and rebounds with increasing density after growth in skeletal size has all but arrested allowing for increased mineralization and the filling of cortical porosity (109).

5.6.3.2 Femoral Neck

Male FN sBMD data was positive at all time points with the peak occurring at 14.26 years. In the females, FN sBMD was positive at all time points with the peak velocity occurring at the start of the measurement period. Although a positive velocity was observed at all time points, there was a dramatic decrease in velocity in both the boys and girls that fit in with the rebound hypothesis.

5.6.3.3 Lumbar Spine

With PHV-aligned data, the males had a negative sBMD velocity at –2 and –1 years PHV, after which they attained a positive velocity. The peak velocity was attained at the end of the measurement period. The peak velocity in sBMD occurred in the females at 1.16 years at the LS, with only some midpoint values between –1 and 0 PHV being negative velocities. A definite trend for a rebound effect is observed in the data once again. Interestingly, the males had a greater period of negative velocity as compared to the females, perhaps due to the greater osteogenic response of trabecular bone to estrogen as compared to testosterone.

5.6.4 Hypothesis 5 Conclusion

In many of the circumstances, BMAD velocity was negative or close to zero. For BMAD females only had negative values for TB, whereas the other two sites were primarily positive. For the males, negative values were obtained for most of the TB measures, and from -2 to 0 years PHV at the LS. When aligned on PHV the hypothesis is supported at the TB, a highly cortical site, partially supported at the FN, and intermediate site, and not supported at the LS, a primarily trabecular site. Overall, however, these findings display a far different pattern of BMD change as suggested by the linear increases of aBMD data at all sites.

There appears to be a preferential mineralization pattern during growth with trabecular bone acutely more responsive to some factor for optimization of mineralization during puberty, as compared to cortical bone. Certainly the higher metabolic activity of trabecular bone can be one factor associated with this rapid growth as the LS would be the most responsive to central hormone activity, such as the sex steroids. Further, the potent effect of endogenous estrogen at this time in life in females could partially explain the significantly higher bone mass at the LS during this period of life.

A consistent finding in this investigation is that after controlling for size there is evidence of a period of decreased density, or a slowing of BMD acceleration, just prior to the PHV being attained. This consistency suggests that bone is less dense at PHV that any other point during growth and thus could be more susceptible to fracture. Interestingly, the most cortical regions

seem to be the most effected, perhaps a consequence of the increased cortical porosity espoused by Parfitt (44), or possible due to a difference in sex steroid or GH action on bone.

5.7 *There will be a lag period between the age at peak PA velocity and age at peak BMC velocity (H6)*

At every site, for both genders there was a dissociation observed between the PA velocity curve and the BMC velocity curve, with the PA curve always attaining peak values before that of the BMC curve.

When aligned on PHV the data truly exhibits the dissociation that is occurring between skeletal expansion and subsequent bone accrual. In both the males and females there is a significant dissociation between TB PA and BMC velocity curves with males having a dissociation of 0.71 years and females one of 0.41 years. In both the males and females there is a significant dissociation between FN PA and BMC velocity curves with males having a dissociation of 0.21 years and females one of 0.61 years. In both the males and females there is a significant dissociation between LS PA and BMC velocity curves with males having a dissociation of 0.27 years and females one of 0.49 years.

These dissociations may be responsible for the transient osteopenia alluded to in numerous investigations regarding higher fracture rates in adolescents during the period surrounding PHV (189-195).

5.7.1 Hypothesis 6 Conclusion

A definitive dissociation between bone mineral size and bone mineral accrual occurred in this population. In the females the FN was the first to expand and subsequently achieve its peak bone mineral accrual, with the LS and TB following relatively equal to one another temporally in terms of their peak PA expansion and BMC accrual. In the males, the FN similarly achieves its peak first, followed by the LS and then the TB.

After investigating the relationship between the asynchrony of height gain and bone mass accrual during puberty, Fournier et al. (216) concluded that during pubertal maturation there is a time lag between bone mineral acquisition and statural growth, and that the lag was

different for different sites depending on their constitution. They concluded that overall the dissociation was generally larger in the males as compared to the females, particularly at the FN and FS. The delay was found to be maximal at PHV. The findings of this investigation concur with those of Fournier et al. (216).

These investigations, as well as this one, give support to the hypothesis that during adolescence initially bone rapidly increases in size and then later undergoes consolidation. After the period of maximal bone envelope expansion and before the period of rapid mineralization, there hypothetically should be a period of heightened skeletal fragility.

6 Summary and Conclusions

The use of a longitudinal database and the control of maturity by PHV is the unique asset of this investigation. The design made the observations robust and meaningful as they eliminated a tremendous amount of variability that would be present with cross-sectional data or if comparisons were simply based on chronological age.

DXA-based aBMD is the most frequently reported measure of bone in paediatric growth literature. Based on abundant aBMD evidence the pattern of BMD changes during growth has been described to consistently increase with an accelerated rate of BMD increases during the pubertal growth spurt, with BMD increases then slowing into adulthood coincident with the attainment of PBM. These assumptions were supported in the data presented here. However, the increase of aBMD with growth is at odds with epidemiological evidence (189-195) that demonstrates a period of skeletal fragility precisely during the time when the most rapid skeletal expansion is occurring.

DXA-derived aBMD was found in this investigation to be significantly dependent on bone size, through both correlational and linear regression analyses. The result of the size dependence of aBMD is that larger bones will have artificially inflated density values and smaller bones artificially lower. During growth bone size changes dramatically and when a child is measured serially over the growth period it is impossible to determine whether the changes observed are due to a true increase in bone density or whether it is an artefact of the measurement technology. Further, a comparison between individuals of different sizes cross-sectionally with aBMD is flawed for the same reasons. Therefore, the pattern of BMD changes with growth as described by aBMD is most likely not accurate as it is confounded by changes in bone size.

When aBMD was corrected for bone size through either geometric (BMAD) or statistically-based techniques (sBMD) the association between bone size and density was either

diminished (BMAD) or eliminated altogether (sBMD) at all sites, as determined by correlational analyses. Interestingly, BMD patterns with growth were determined based on the corrected equations there was a definitive trend of decreasing BMD until PHV, with a subsequent "rebound". This pattern is different from that typically described by aBMD investigations. This phenomenon of decreasing BMD until PHV is supported by the epidemiologic investigations (189-195) which suggest a maximum fragility of bone approximately the time of PHV. The corrected measures of BMD more closely explain the epidemiological phenomena of maximal fracture incidence during rapid growth than do the uncorrected values that suggest fractures are occurring during periods of relatively maximal density. Interestingly, the region with the greatest proportion of cortical bone (TB) displayed the most consistent and pronounced rebound phenomenon, followed by the intermediately cortical FN, and least frequently and pronounced with the highly trabecular LS. The different mineralization patterns with cortical and trabecular bone may be a consequence of preferential resorption of cortical bone during rapid growth in order to supply periosteal expansion.

BMAD may not be the most appropriate size-correction as it was shown to consistently be significantly associated with PA and led to some patterns of BMD change that were difficult to rationalize physiologically. The strict geometric assumptions used in the determination of BMAD are probably not appropriate in this population. sBMD did attain independence from PA and led to patterns of bone density that was physiologically plausible. The downfall of using sBMD is that it must be developed for each specific population before it can be applied; however, it does have promise for norm development if developed from a large sample.

When the growth curves of males and females were aligned on PHV-based maturation a similarity in BMD patterns was consistently observed. While there were generally few differences, there was a slight trend for females to have higher density measures at the more trabecular LS site. It is likely that cortical and trabecular bone development are regulated by different factors, whether they be environmental or endogenous, as supported by the work of Gunnes et al. (265) in a large cohort of children. Slemenda et al. (55) concluded that the different rates of change observed in sites with differing bone composition may be a

consequence of preferential action of hormones on trabecular bone. However, after correction for pubertal status and correction for bone size, density measures between the genders were strikingly similar suggesting that a large part of the gender difference observed may simply be a consequence of bone size.

When the velocity of BMD was determined, PA-dependent aBMD displayed consistent positive acceleration values at all time points and at all sites, suggesting that increases in aBMD continues throughout growth with a period of particularly rapid aBMD increase during the pubertal years. However, when density was corrected for bone size there was a pattern of negative velocity in the years prior to PHV, with positive velocity after. This trend was most evident and strongest in the TB, intermediate in the FN, and least obvious in the LS; the more cortical sites displaying the greatest decreases in BMD prior to PHV. Parfitt (227) suggested that during periods of rapid skeletal expansion the calcium requirements that cannot be met completely by exogenous supplies are augmented by endogenous calcium removed from cortical bone resulting in increased porosity of cortical bone during growth. Consequently, those regions with the highest percentage of cortical bone (TB) should preferentially lose bone mass before that of those regions with primarily trabecular bone (LS).

Lastly, when comparing velocity curves for PA and BMC a definitive and consistent lag period was seen with the peak in bone projected area preceding the increases in bone mass accrual anywhere from 0.21 years to 0.61 years. This dissociation between bone projected area and bone mass suggests that bone first expands and then becomes further mineralized. Theoretically, as supported by epidemiologic fracture investigations (189-195), the point of maximal bone expansion, as seen in this investigation as the age at peak velocity of PA is the point at which there is the greatest fragility as complete mineralization has not yet occurred. In terms of the temporality of bone turnover this lag may make sense. In the basic multicellular unit a packet of bone is removed relatively quickly (7-10 days). After bone has been removed by osteoclasts there is a period of reversal where osteoblasts lay down osteoid and begin preliminary ossification. However, after the osteoid has been laid down, it can take anywhere from 3-6 months to see complete mineralization of the osteoid. During that period of increasing

mineralization there would certainly be mechanically inferior bone and if enough regions were undergoing this mineralization simultaneously, such as is seen with growth as evidenced by high levels of biochemical markers for bone turnover, the bone as a whole would be more susceptible to failure. Another theoretical rationale for the lag between expansion and mineralization may be simply due to an outpacing of increases in size with the physical ability of the body to mineralize that much osteoid; there simply is not enough calcium present from both endogenous and exogenous sources to supply both the rapid expansion and mineralization needed to keep these two parameters in balance.

The findings of this investigation suggest that aBMD as determined by DXA is not appropriate for assessing changes in BMD in children during growth. Further investigations need be completed to precisely determine volumetric BMD patterns during growth as it probably has not been accurately described up until this point. The evidence from the corrected BMD in this trial consistently suggests that there is a period of skeletal fragility during PHV that is a consequence of an early pubertal decrease in density until PHV, with increasing thereafter.

While patterns of BMD change do explain some of the changes that occur in terms of the mechanical integrity of the bone, a great deal of variability can most likely also be attributed to changes in bone geometry and architecture over the growth period. In future investigations there is a need to measure these mechanical parameters as well as measures of BMD to completely understand the changes occurring with growth as there is most certainly an interplay between these factors.

6.1 *Hypotheses summary*

H1: aBMD is significantly influenced by body height, body mass and the bone projected area of the respective site.

SUPPORTED.

$H1_1$: Height, weight and bone projected area will be significantly positively correlated with areal BMD at all sites.

SUPPORTED.

$H1_2$: All regression coefficients of PA regressed upon BMC will be significantly different than unity at all measurement sites.

SUPPORTED.

H2: aBMD corrected for bone size will not be significantly influenced by height, body mass and the bone projected area of the respective site.

$H2_1$: Height, weight and bone projected area will not be significantly correlated with BMAD at any site.

NOT SUPPORTED.

$H2_2$: Height, weight and bone projected area will not be significantly correlated with statistically corrected BMD (sBMD) at any site, demonstrating its independence from size measures in the estimate of bone density.

SUPPORTED.

H3: The age-related increase in aBMD in both genders is eliminated when corrected for bone size.

NOT SUPPORTED.

$H3_1$: aBMD will increase significantly throughout the growth period at all sites in both genders.

SUPPORTED.

$H3_2$: BMAD will not change significantly throughout the growth period at all sites in both genders.

NOT SUPPORTED.

$H3_3$: sBMD will not change significantly throughout the growth period at all sites in both genders.

NOT SUPPORTED.

H4: The gender difference observed in aBMD will be eliminated when corrected for bone size and maturation.

NOT SUPPORTED.

$H4_1$: Males will possess significantly greater aBMD at all sites as compared to females, when corrected for maturation.

SUPPORTED AT TB AND FN, not LS.

$H4_2$: No statistical difference will exist between genders for BMAD at the TB, LS and FN, when corrected for maturation.

NOT SUPPORTED.

H5: The age-related acceleration in aBMD velocity during the pubertal years is eliminated when corrected for bone size.

$H5_1$: aBMD velocity will remain positive throughout the growth period at all sites in both genders.

SUPPORTED.

$H5_2$: BMAD velocity will be negative or zero through the growth period at all sites in both genders.

NOT SUPPORTED.

$H5_3$: sBMD velocity will be negative or zero through the growth period at all sites in both genders.

NOT SUPPORTED.

H6: There will be a significant lag period between the age at peak PA velocity and age at peak BMC velocity.

SUPPORTED.

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8 Appendix A: Ethics Approval

Appendix B

UNIVERSITY ADVISORY COMMITTEE ON ETHICS IN HUMAN EXPERIMENTATION

(Medical Ethics)

NAME AND EC #: Dr. D.A. Bailey
Physical Education
88-102
NHRDP (#6608-1261-OS)

DATE: February 15, 1994

The revised consent form for the study entitled "A mixed Longitudinal Study of Bone Density Change During the Adolescent Years in Boys and Girls with Special Reference to Physical Activity Patterns and Nutritional Factors" has been reviewed by the University Advisory Committee on Ethics in Human Experimentation (Medical Sciences) and approval has been provided for renewal of the study.

Therefore you are free to proceed with the project subject to the following conditions:

APPROVED.

Please submit the revisions requested in 1(a) to the Director of Research Services, Room 210 Kirk Hall.

2. Any significant changes to your protocol should be reported to the Director of Research Services for Committee consideration in advance of its implementation.

3. Please submit to the Committee all adverse events reports received from the study sponsor.

4. Upon discontinuation or closure of the research study, please notify the Director of Research Services in writing.

for E. A. McKenna
Chair
University Advisory Committee on Ethics in Human Experimentation

cc: Royal University Hospital

9 Appendix B: Chronological-based Data

9.1 *Chronological-based tabular data*

Table 9.1. Chronological Correlations of TB BMD, BMAD and sBMD with Age and Size Measures (Males).

	Age	BA	WT	HT
BMD (g/cm^2)	0.814c	0.888c	0.805c	0.779c
BMAD (g/cm^3)	-0.414c	-0.589c	-0.639c	-0.524c
sBMD (g/cm^3)	0.151b	0.055	-0.013	-0.063

a = p<0.05; b = p<0.01; c = p<0.001.

Table 9.2. Chronological Correlations of TB BMD, BMAD and sBMD with Age and Size Measures (Females).

	Age	BA	WT	HT
BMD (g/cm^2)	0.773c	0.851c	0.719c	0.770c
BMAD (g/cm^3)	-0.325c	-0.657c	-0.714c	-0.588c
sBMD (g/cm^3)	0.291c	0.043	-0.071	-0.029

a = p<0.05; b = p<0.01; c = p<0.001.

Table 9.3. Chronological Correlations of FN BMD, BMAD and sBMD with Age and Size Measures (Males).

	Age	BA	WT	HT
BMD (g/cm^2)	0.678c	0.466c	0.682c	0.604c
BMAD (g/cm^3)	0.095	-0.370c	0.037	-0.118a
sBMD (g/cm^3)	0.384c	-0.008	0.350c	0.216c

a = p<0.05; b = p<0.01; c = p<0.001.

Table 9.4. Chronological Correlations of FN BMD, BMAD and sBMD with Age and Size Measures (Females).

	Age	BA	WT	HT
BMD (g/cm^2)	0.645c	0.520c	0.696c	0.728c
BMAD (g/cm^3)	0.382c	-0.096	0.323c	0.297c
sBMD (g/cm^3)	0.432c	-0.005	0.389c	0.371c

a = p<0.05; b = p<0.01; c = p<0.001.

Table 9.5. Chronological Correlations of LS BMD, BMAD and sBMD with Age and Size Measures (Males).

	Age	BA	WT	HT
BMD (g/cm^2)	0.736c	0.811c	0.762c	0.732c
BMAD (g/cm^3)	0.527c	0.488c	0.614c	0.458c
sBMD (g/cm^3)	0.158a	0.001	0.295c	0.028

a = p<0.05; b = p<0.01; c = p<0.001.

Table 9.6. Chronological Correlations of LS BMD, BMAD and sBMD with Age and Size Measures (Females).

	Age	BA	WT	HT
BMD (g/cm^2)	0.747c	0.894c	0.762c	0.811c
BMAD (g/cm^3)	0.678c	0.688c	0.687c	0.639c
sBMD (g/cm^3)	0.250c	-0.005	0.249c	0.021

a = p<0.05; b = p<0.01; c = p<0.001.

Table 9.7. Male Total Body Chronological Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
10	19	1260.9	27.52	1031.7	26.71	0.818	0.0089	0.0934	0.00162	0.0323	0.00042
11	32	1427.1	24.59	1200.4	27.09	0.839	0.0073	0.0884	0.00105	0.0313	0.00027
12	45	1571.4	27.57	1361.9	30.23	0.864	0.0069	0.0858	0.00102	0.0309	0.00026
13	58	1767.6	29.40	1594.3	35.61	0.898	0.0075	0.0828	0.00086	0.0305	0.00023
14	66	1962.1	30.66	1887.5	43.68	0.955	0.0089	0.0826	0.00062	0.0309	0.00019
15	58	2160.3	31.63	2211.7	51.09	1.017	0.0106	0.0823	0.00059	0.0314	0.00021
16	46	2300.2	31.53	2453.0	54.54	1.062	0.0118	0.0819	0.00069	0.0319	0.00027
17	34	2364.0	36.78	2596.7	63.46	1.095	0.0131	0.0828	0.00080	0.0325	0.00032
18	16	2420.5	61.73	2730.3	111.23	1.122	0.0198	0.0827	0.00089	0.0329	0.00037

Table 9.8. Female Total Body Chronological Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
10	33	1267.7	44.37	1020.9	45.60	0.799	0.0090	0.0921	0.00176	0.0387	0.00046
11	51	1396.7	35.62	1159.5	41.01	0.823	0.0084	0.0890	0.00118	0.0381	0.00031
12	56	1597.7	35.23	1397.7	45.53	0.866	0.0098	0.0854	0.00090	0.0378	0.00029
13	61	1772.2	32.47	1643.3	45.10	0.920	0.0098	0.0841	0.00082	0.0384	0.00029
14	54	1870.3	29.36	1804.0	41.97	0.960	0.0088	0.0841	0.00071	0.0391	0.00026
15	41	1916.7	25.18	1905.1	38.72	0.991	0.0091	0.0850	0.00070	0.0399	0.00029
16	27	1967.1	31.21	1999.4	53.51	1.013	0.0131	0.0850	0.00091	0.0404	0.00042
17	15	2013.6	48.07	2089.7	79.66	1.034	0.0172	0.0850	0.00132	0.0408	0.00055
18	8	2046.2	94.63	2174.8	142.35	1.059	0.0270	0.0862	0.00272	0.0416	0.00101

Table 9.9. Male Femoral Neck Chronological Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
10	18	4.225	0.072	2.968	0.084	0.703	0.0162	0.2506	0.00712	0.3057	0.00765
11	32	4.505	0.057	3.259	0.065	0.723	0.0105	0.2418	0.00432	0.3030	0.00466
12	45	4.685	0.059	3.451	0.065	0.736	0.0096	0.2370	0.00410	0.3017	0.00436
13	58	4.953	0.060	3.891	0.069	0.786	0.0103	0.2400	0.00455	0.3123	0.00480
14	66	5.238	0.062	4.394	0.082	0.838	0.0107	0.2418	0.00417	0.3223	0.00456
15	57	5.545	0.068	4.938	0.098	0.891	0.0142	0.2431	0.00505	0.3317	0.00590
16	46	5.658	0.069	5.203	0.094	0.923	0.0173	0.2474	0.00659	0.3403	0.00774
17	34	5.691	0.076	5.402	0.118	0.952	0.0212	0.2535	0.00784	0.3497	0.00925
18	16	5.696	0.123	5.644	0.213	0.992	0.0331	0.2635	0.01152	0.3637	0.01373

Table 9.10. Female Femoral Neck Chronological Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
10	33	4.262	0.067	2.763	0.088	0.645	0.0881	0.2277	0.00407	0.1829	0.00317
11	51	4.446	0.056	3.023	0.084	0.676	0.0841	0.2286	0.00357	0.1846	0.00284
12	56	4.652	0.050	3.402	0.087	0.728	0.0871	0.2352	0.00386	0.1911	0.00311
13	61	4.858	0.048	3.805	0.090	0.781	0.0899	0.2416	0.00407	0.1974	0.00328
14	54	4.925	0.053	4.039	0.080	0.819	0.0803	0.2508	0.00440	0.2052	0.00348
15	41	4.909	0.054	4.159	0.072	0.848	0.0722	0.2605	0.00506	0.2130	0.00396
16	27	4.904	0.075	4.217	0.098	0.861	0.0983	0.2653	0.00721	0.2168	0.00563
17	15	5.027	0.105	4.333	0.130	0.863	0.1303	0.2594	0.00902	0.2127	0.00697
18	8	5.090	0.177	4.496	0.235	0.882	0.2352	0.2616	0.01025	0.2149	0.00775

Table 9.11. Male Lumbar Spine Chronological Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
10	9	39.237	1.708	23.433	1.644	0.592	0.0229	0.0947	0.00282	0.0302	0.00088
11	19	41.883	1.139	26.170	0.994	0.623	0.0131	0.0965	0.00194	0.0302	0.00068
12	32	46.477	1.025	30.978	1.035	0.664	0.0128	0.0976	0.00180	0.0296	0.00060
13	45	50.909	1.035	37.050	1.239	0.722	0.0135	0.1014	0.00162	0.0299	0.00050
14	55	56.785	0.964	46.153	1.401	0.805	0.0144	0.1068	0.00151	0.0304	0.00042
15	56	61.497	0.955	54.284	1.560	0.874	0.0149	0.1114	0.00146	0.0309	0.00038
16	44	64.750	0.920	60.156	1.665	0.924	0.0164	0.1148	0.00172	0.0313	0.00046
17	34	66.737	1.058	64.019	1.843	0.956	0.0182	0.1172	0.00206	0.0317	0.00058
18	16	67.118	1.204	65.284	2.470	0.970	0.0272	0.1185	0.00313	0.0319	0.00086

Table 9.12. Female Lumbar Spine Chronological Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
10	22	38.637	1.047	24.857	1.388	0.635	0.0185	0.1021	0.00206	0.0193	0.00036
11	31	42.248	1.249	29.621	1.709	0.687	0.0190	0.1058	0.00189	0.0193	0.00035
12	51	45.757	0.951	34.825	1.451	0.748	0.0157	0.1105	0.00155	0.0194	0.00026
13	54	50.482	0.929	42.983	1.591	0.839	0.0166	0.1181	0.00167	0.0198	0.00027
14	51	53.231	0.824	48.486	1.405	0.903	0.0137	0.1238	0.00130	0.0203	0.00022
15	41	54.997	0.901	51.693	1.431	0.934	0.0122	0.1261	0.00117	0.0204	0.00024
16	27	56.255	0.896	54.712	1.798	0.967	0.0174	0.1289	0.00169	0.0206	0.00025
17	15	57.409	1.329	57.804	2.787	1.000	0.0244	0.1320	0.00210	0.0208	0.00029
18	8	58.221	2.348	60.181	5.368	1.022	0.0472	0.1337	0.00384	0.0210	0.00043

Table 9.13. Male Total Body Chronological Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
12	43	190.6	11.70	212.4	13.94	0.028	0.0036	-0.0043	0.00082	-0.0011	0.00035
13	43	222.7	10.33	286.3	16.00	0.044	0.0043	-0.0024	0.00052	-0.0002	0.00015
14	43	209.0	11.40	345.1	16.28	0.071	0.0040	0.0007	0.00050	0.0009	0.00014
15	43	183.8	11.68	313.2	17.69	0.059	0.0038	-0.0006	0.00036	0.0006	0.00012

Table 9.14. Female Total Body Chronological Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
11	44	203.4	9.19	204.8	11.81	0.027	0.0037	-0.0100	0.00189	-0.0002	0.00040
12	44	203.9	8.18	259.6	11.57	0.052	0.0036	-0.0025	0.00055	0.0001	0.00017
13	44	169.3	8.88	251.5	12.09	0.055	0.0032	-0.0008	0.00043	0.0007	0.00014
14	44	113.9	8.46	181.0	10.17	0.041	0.0024	-0.0007	0.00038	0.0006	0.00011

Table 9.15. Total Body Chronological Peak Data.

Measure	Age at Peak Velocity		Peak Velocity	
	Males	Females	Males	Females
BA (cm)	13.15	11.59	223.3604	206.7081
BMC (g)	14.11	12.39	345.9016	265.3701
BMD (g/cm^2)	14.16	12.65	0.0718	0.0568
BMAD (g/cm^3)	14.17	14.00	0.0008	-0.00074
sBMD (g/cm^3)	14.24	13.30	0.0009	0.000745

Table 9.16. Male Femoral Neck Chronological Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
12	43	0.199	0.0301	0.294	0.0380	0.030	0.0045	-0.0013	0.00192	0.0048	0.00190
13	43	0.330	0.0399	0.516	0.0491	0.052	0.0051	0.0002	0.00196	0.0083	0.00198
14	43	0.303	0.0245	0.582	0.0392	0.061	0.0050	0.0033	0.00166	0.0120	0.00186
15	43	0.245	0.0358	0.511	0.0477	0.056	0.0055	0.0074	0.00558	0.0112	0.00224

Table 9.17. Female Femoral Neck Chronological Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
11	44	0.223	0.0308	0.361	0.0338	0.048	0.0047	0.0040	0.00167	0.0044	0.00131
12	44	0.236	0.0301	0.417	0.0279	0.055	0.0046	0.0065	0.00193	0.0065	0.00149
13	44	0.181	0.0374	0.428	0.0386	0.060	0.0044	0.0097	0.00221	0.0089	0.00165
14	44	0.029	0.0202	0.250	0.0230	0.048	0.0036	0.0122	0.00182	0.0102	0.00135

Table 9.18. Femoral Neck Chronological Peak Data.

Measure	Age at Peak Velocity		Peak Velocity	
	Males	Females	Males	Females
BA (cm)	13.24	11.83	0.3359	0.2371
BMC (g)	13.88	12.65	0.5833	0.4419
BMD (g/cm^2)	14.05	12.89	0.0612	0.0603
BMAD (g/cm^3)	15.00	13.99	0.0074	0.0122
sBMD (g/cm^3)	14.26	14.00	0.0122	0.0102

Table 9.19. Male Lumbar Spine Chronological Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
12	29	4.581	0.4556	4.819	0.5477	0.033	0.0052	-0.0001	0.00058	-0.0002	0.00454
13	29	5.842	0.4532	8.218	0.7024	0.072	0.0074	0.0041	0.00082	0.0001	0.00024
14	29	5.909	0.4101	10.221	0.7156	0.092	0.0075	0.0065	0.00091	0.0009	0.00027
15	29	4.833	0.4330	9.571	0.7461	0.088	0.0067	0.0065	0.00068	0.0010	0.00019

Table 9.20. Female Lumbar Spine Chronological Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
11	35	4.158	0.2675	5.111	0.4772	0.052	0.0051	0.0028	0.00051	-0.0001	0.00133
12	35	5.137	0.2835	8.177	0.6343	0.090	0.0072	0.0069	0.00081	0.0002	0.00073
13	35	4.290	0.2565	7.797	0.4539	0.084	0.0049	0.0069	0.00059	0.0004	0.00063
14	35	2.585	0.2259	5.230	0.3085	0.060	0.0039	0.0050	0.00050	0.0003	0.00057

Table 9.21. Lumbar Spine Chronological Peak Data.

Measure	*Age at Peak Velocity*		*Peak Velocity*	
	Males	**Females**	**Males**	**Females**
BA (cm)	13.54	12.05	6.0527	5.1399
BMC (g)	14.17	12.36	10.2690	8.4428
BMD (g/cm^2)	14.25	12.32	0.0924	0.0923
BMAD (g/cm^3)	14.39	12.45	0.0067	0.0074
sBMD (g/cm^3)	14.56	13.19	0.0010	0.0004

9.2 *Chronological-based figures*

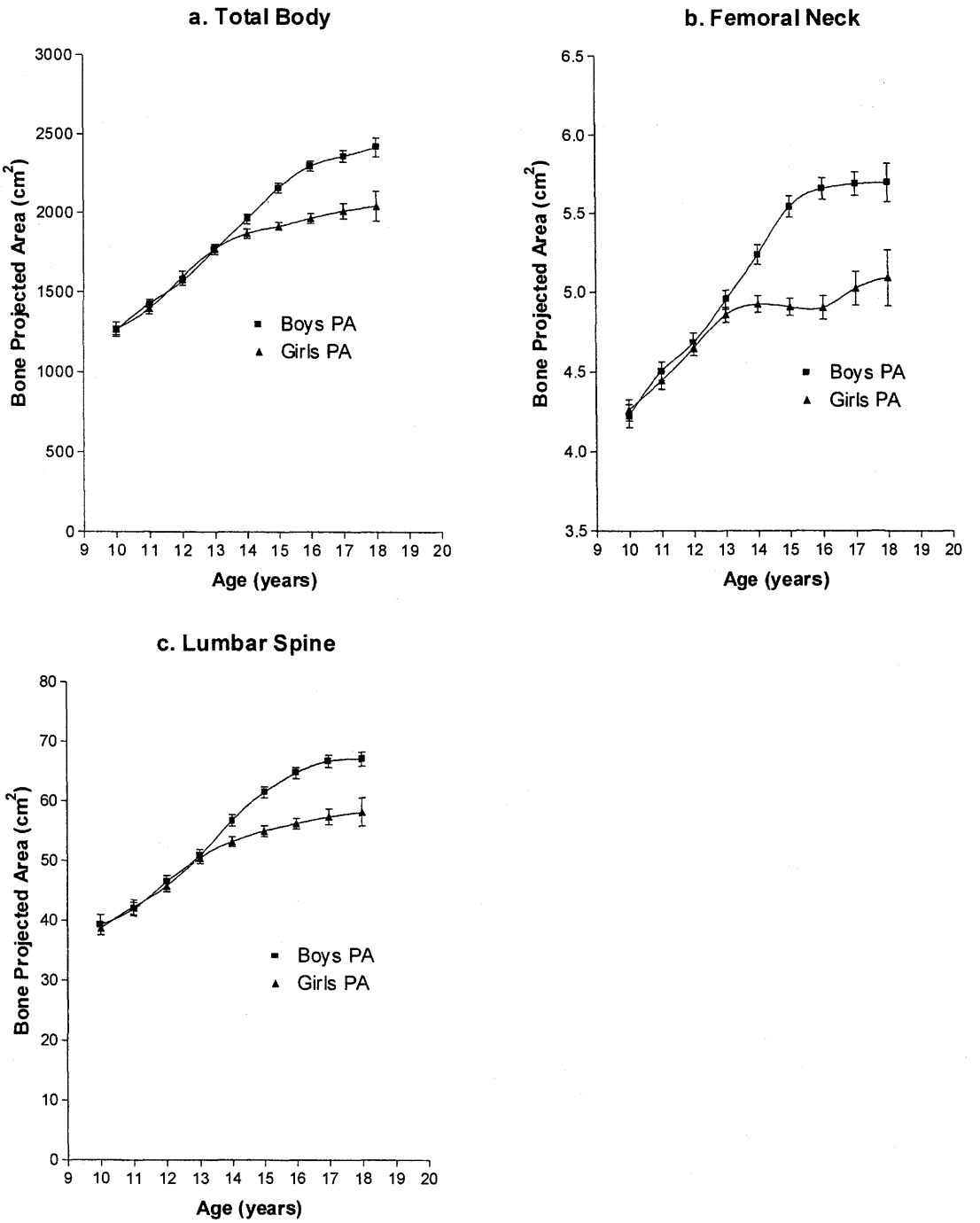

Figure 9.1. Male and female chronological-based growth curves for bone projected area (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

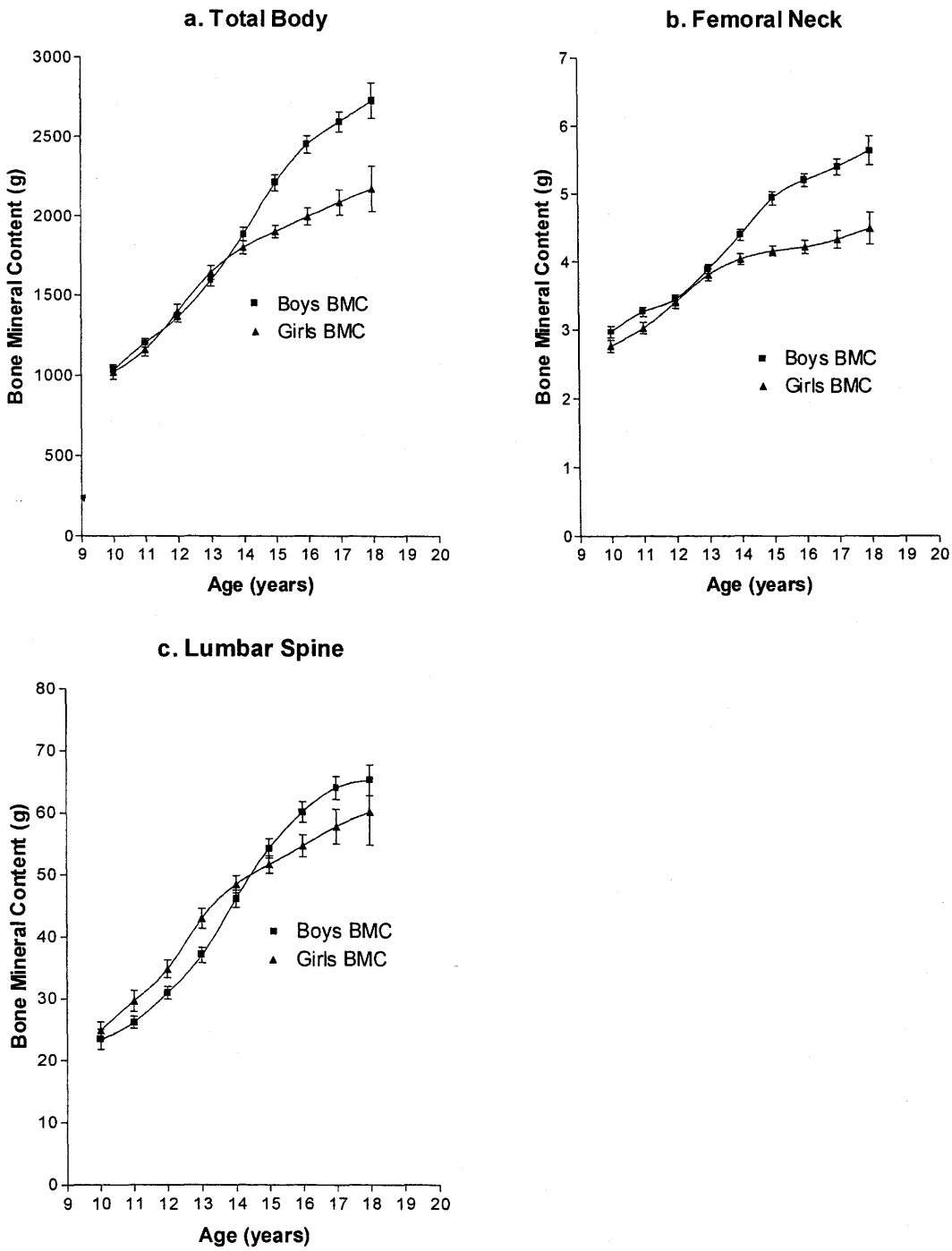

Figure 9.2. Male and female chronological-based growth curves for bone mineral content (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

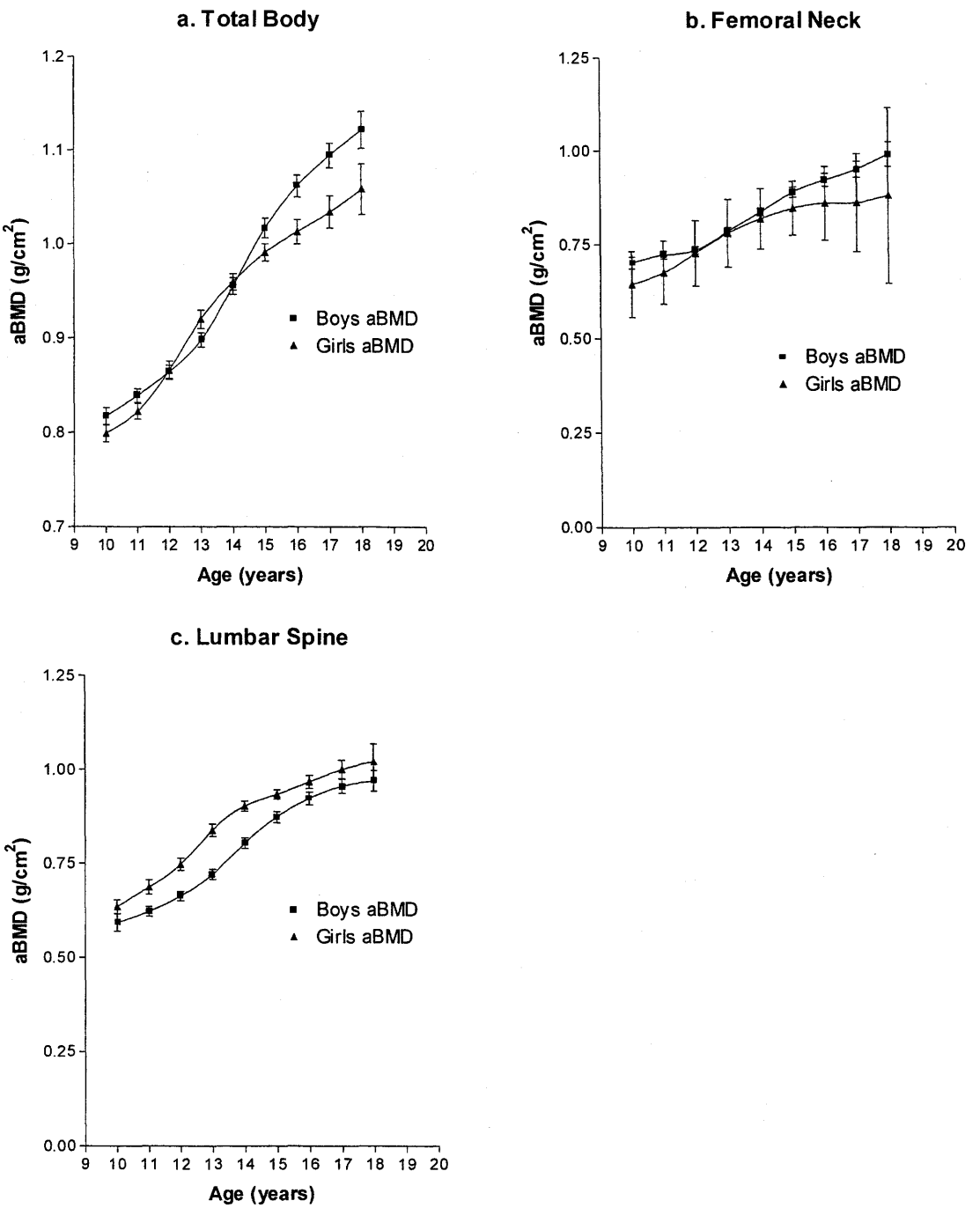

Figure 9.3. Male and female chronological-based growth curves for bone mineral density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

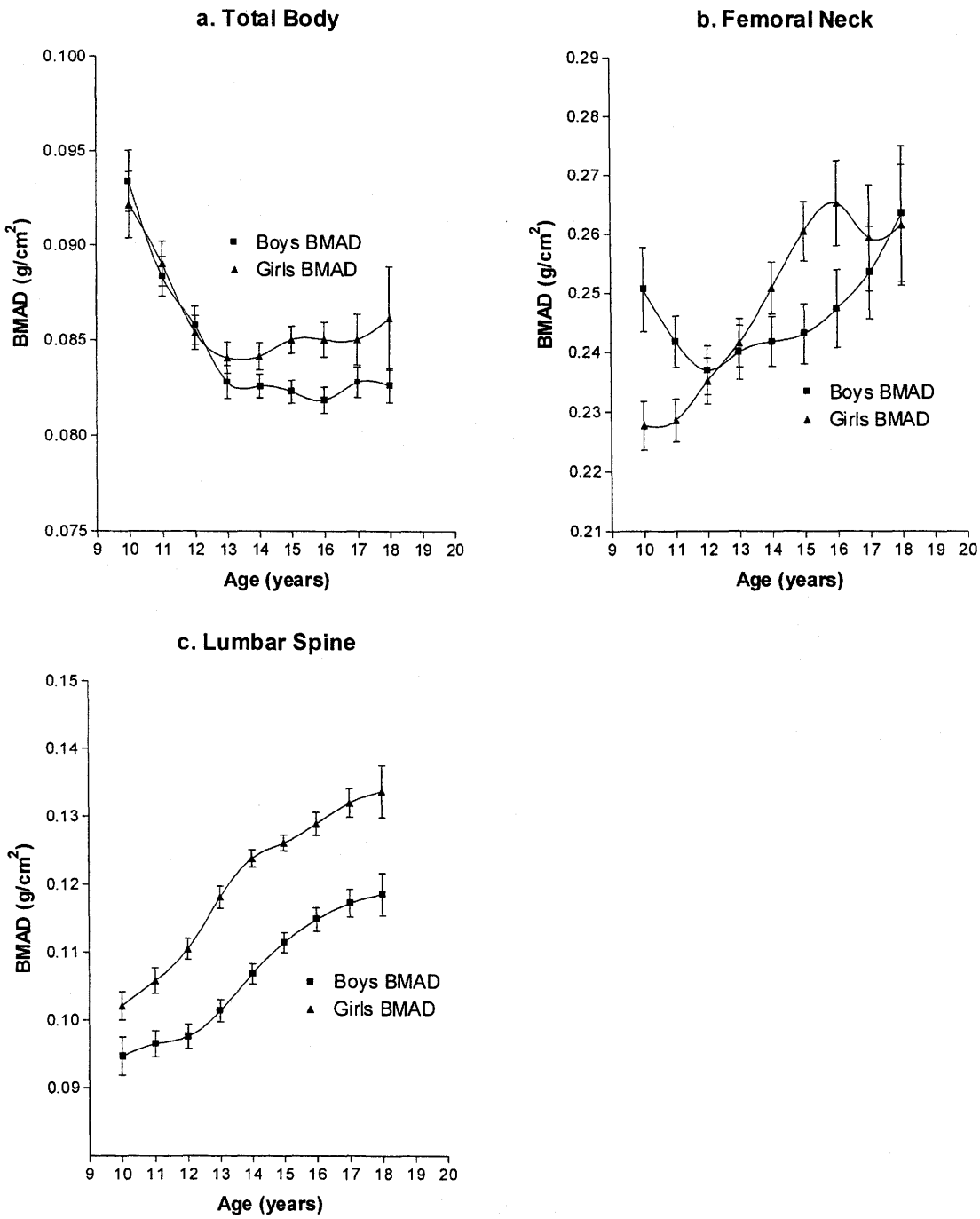

Figure 9.4. Male and female chronological-based growth curves for bone mineral apparent density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

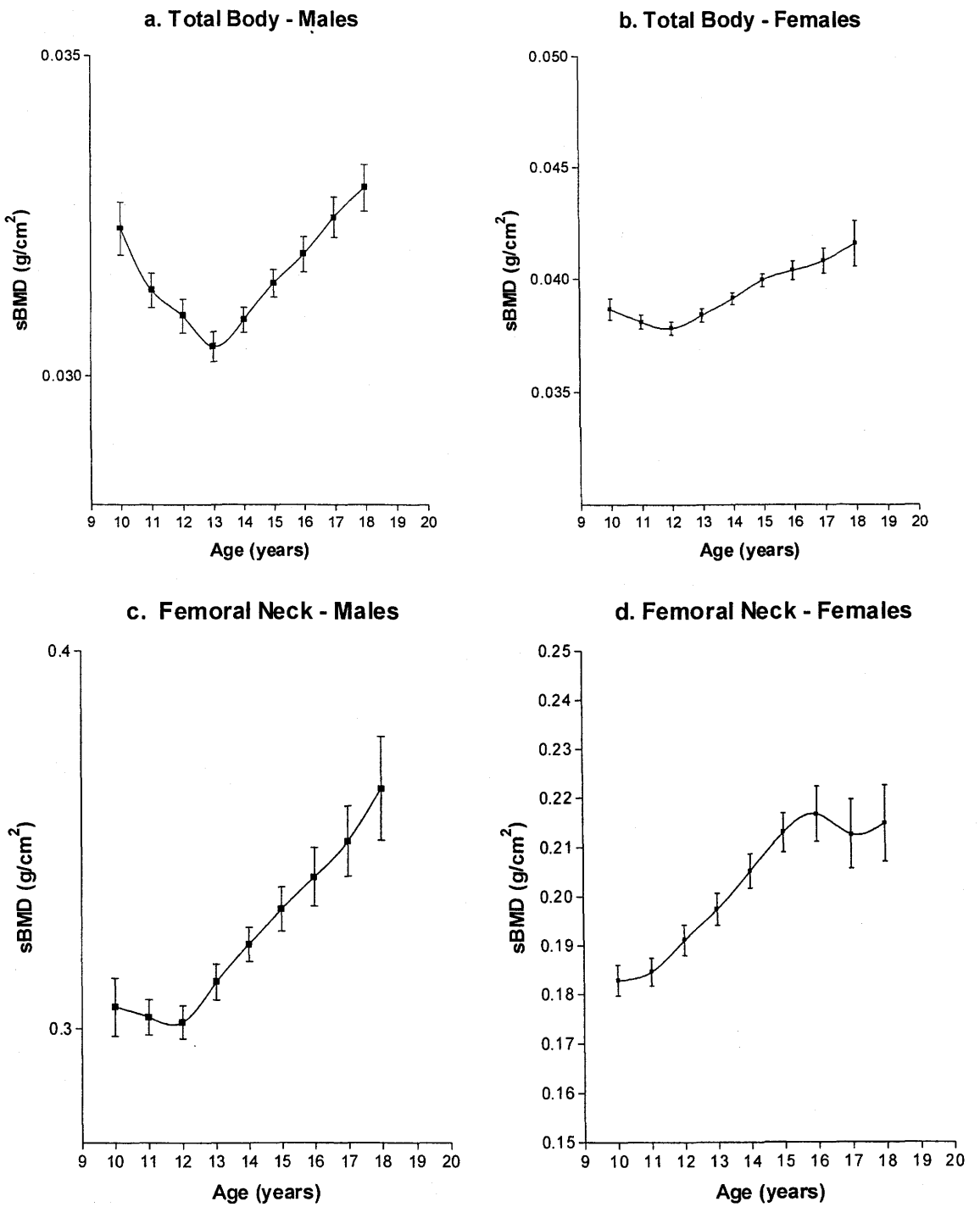

Figure 9.5. Chronological-based growth curves for statistically-corrected bone mineral density (mean±SEM) of the a. Male total body; b. Female total body; c. Male femoral neck; and d. Female femoral neck. Figure 9.5. continued on following page.

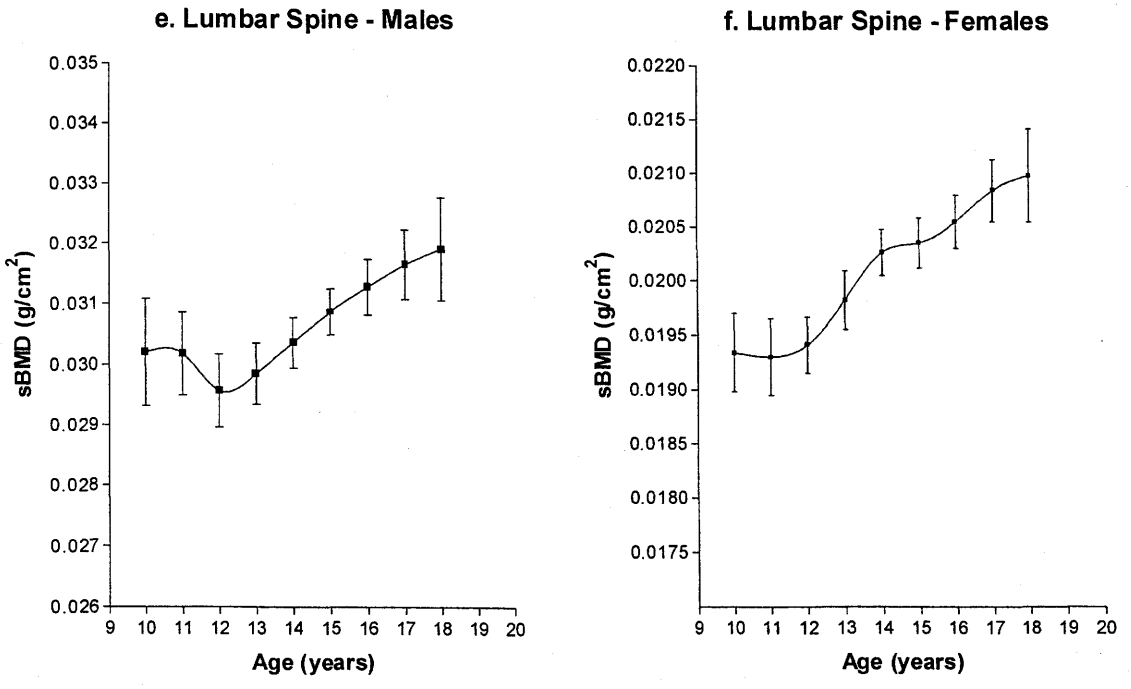

Figure 9.5. continued. PHV-aligned growth curves for statistically-corrected bone mineral density (mean±SEM) of the e. Male lumbar spine; and f. Female lumbar spine regions.

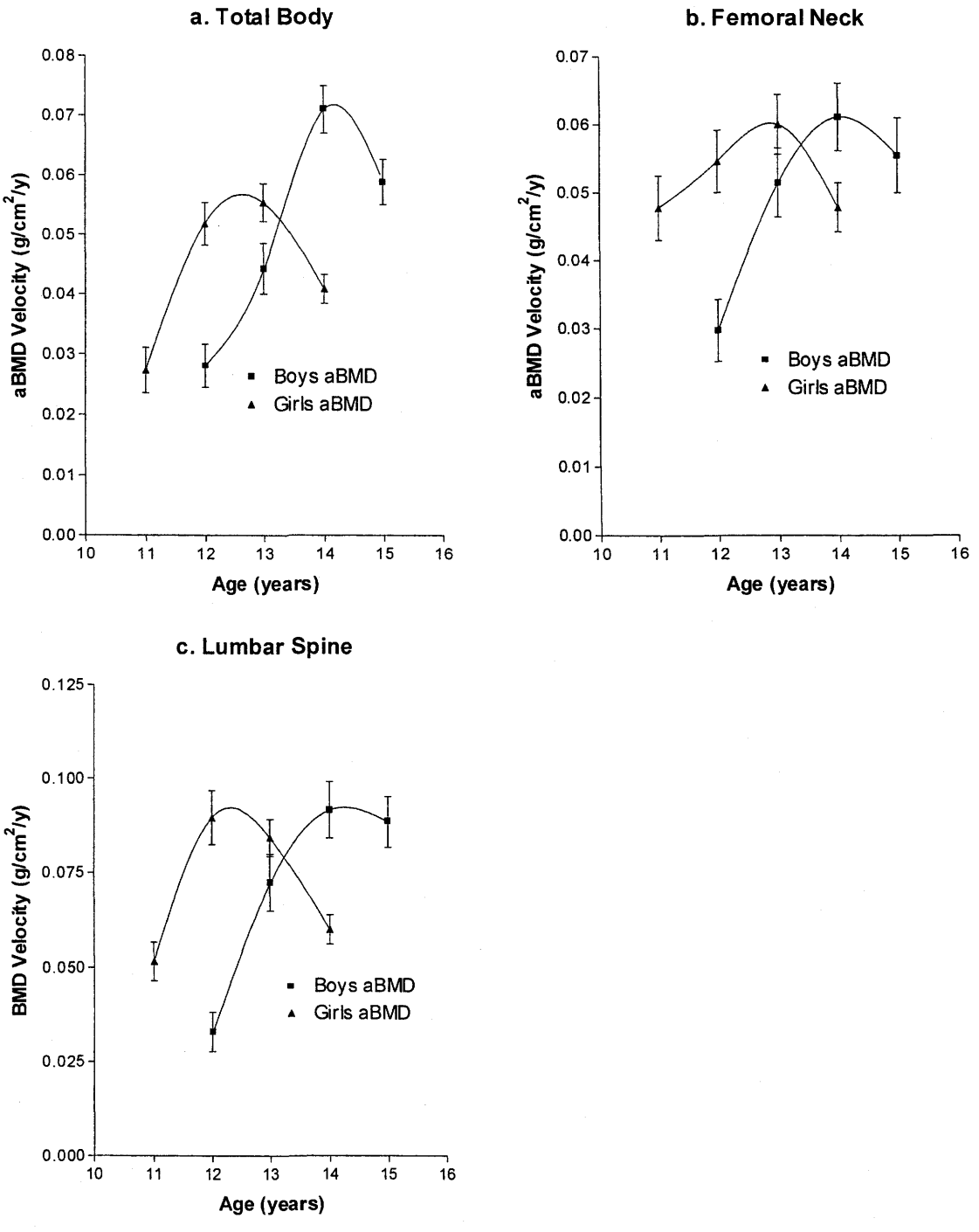

Figure 9.6. Male and female chronological-based velocity curves for areal bone mineral density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

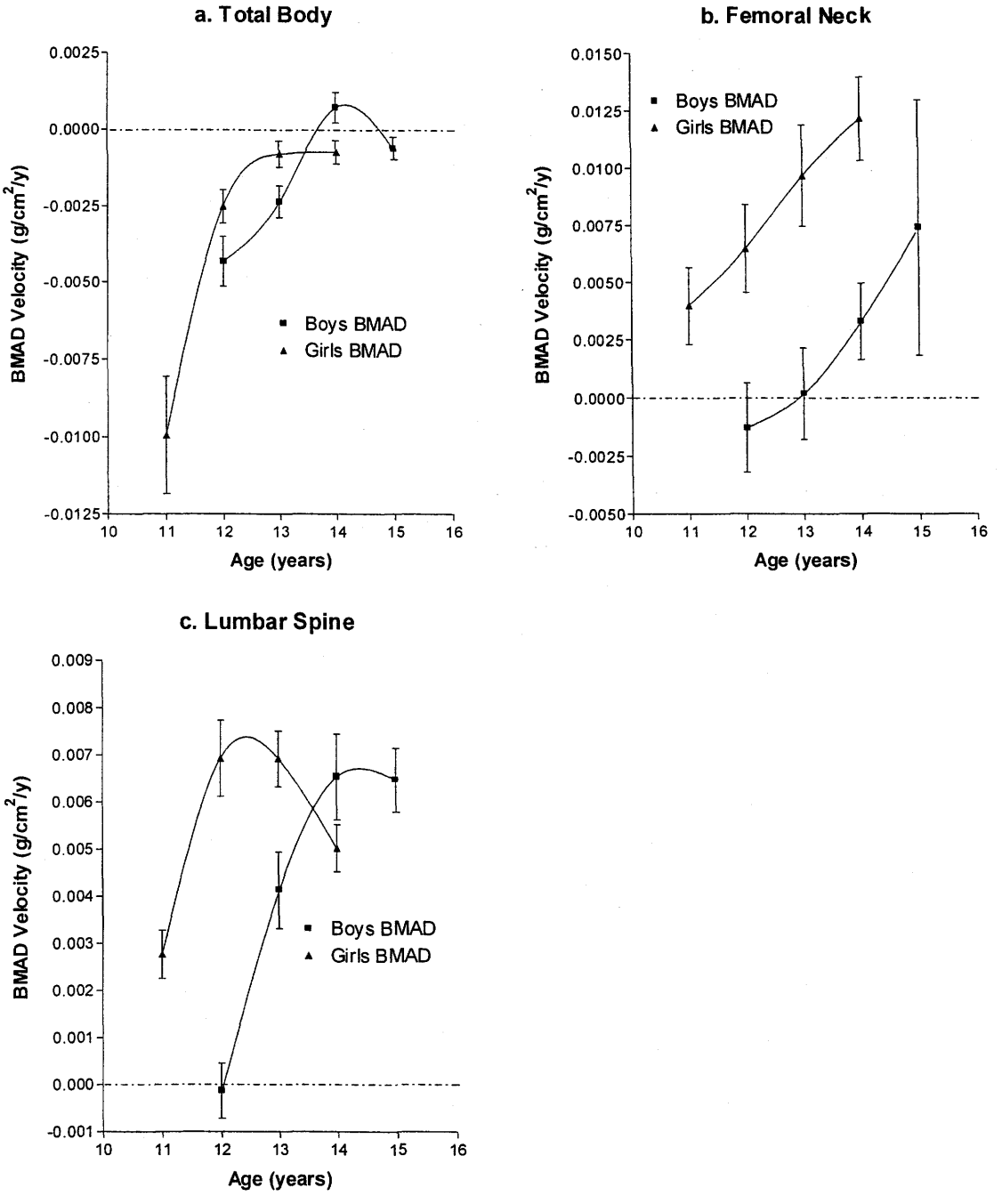

Figure 9.7. Male and female chronological-based velocity curves for bone mineral apparent density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

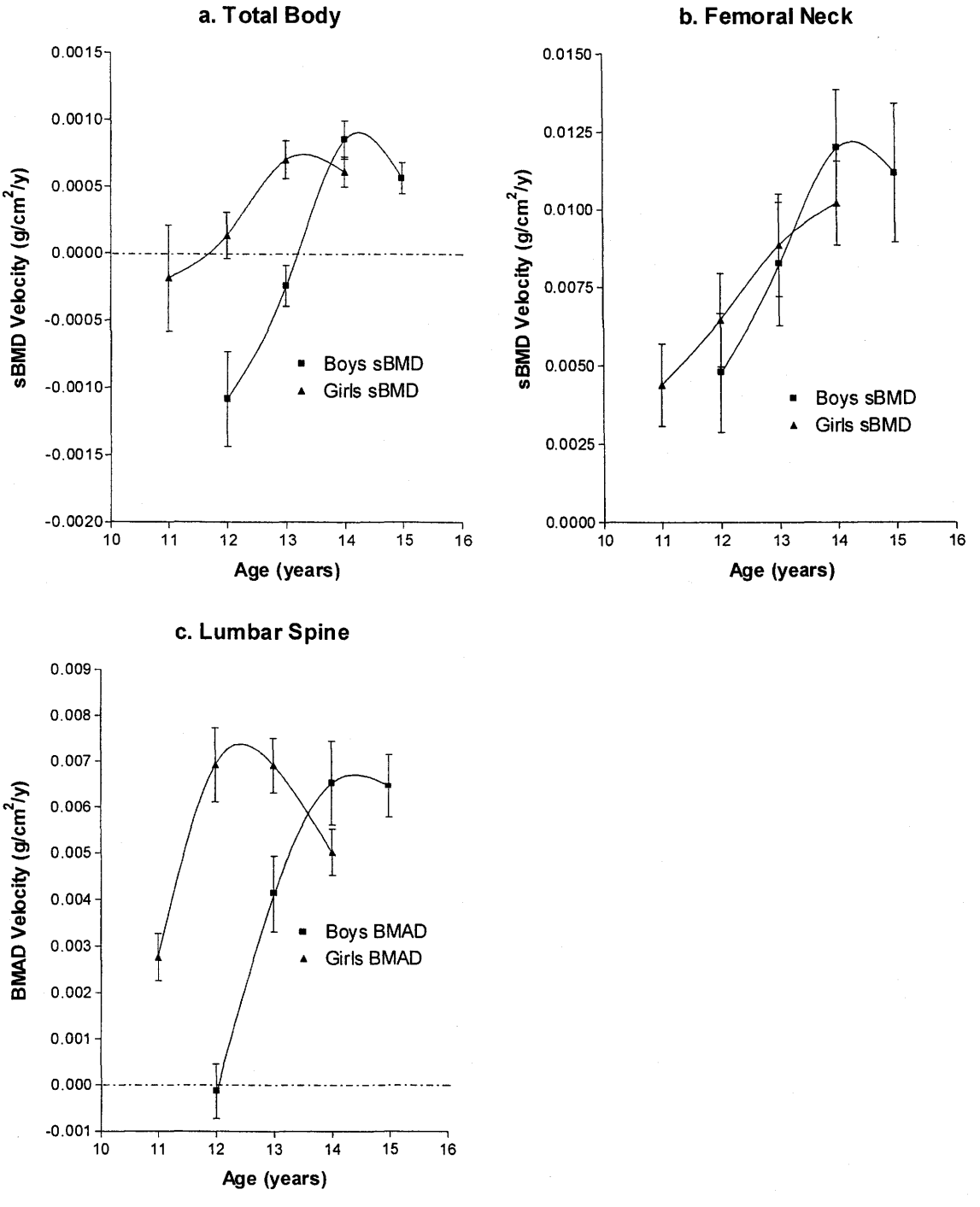

Figure 9.8. Male and female chronological-based velocity curves for statistically-corrected bone mineral density (mean±SEM) of the a. Total body; b. Femoral neck; and c. Lumbar spine regions.

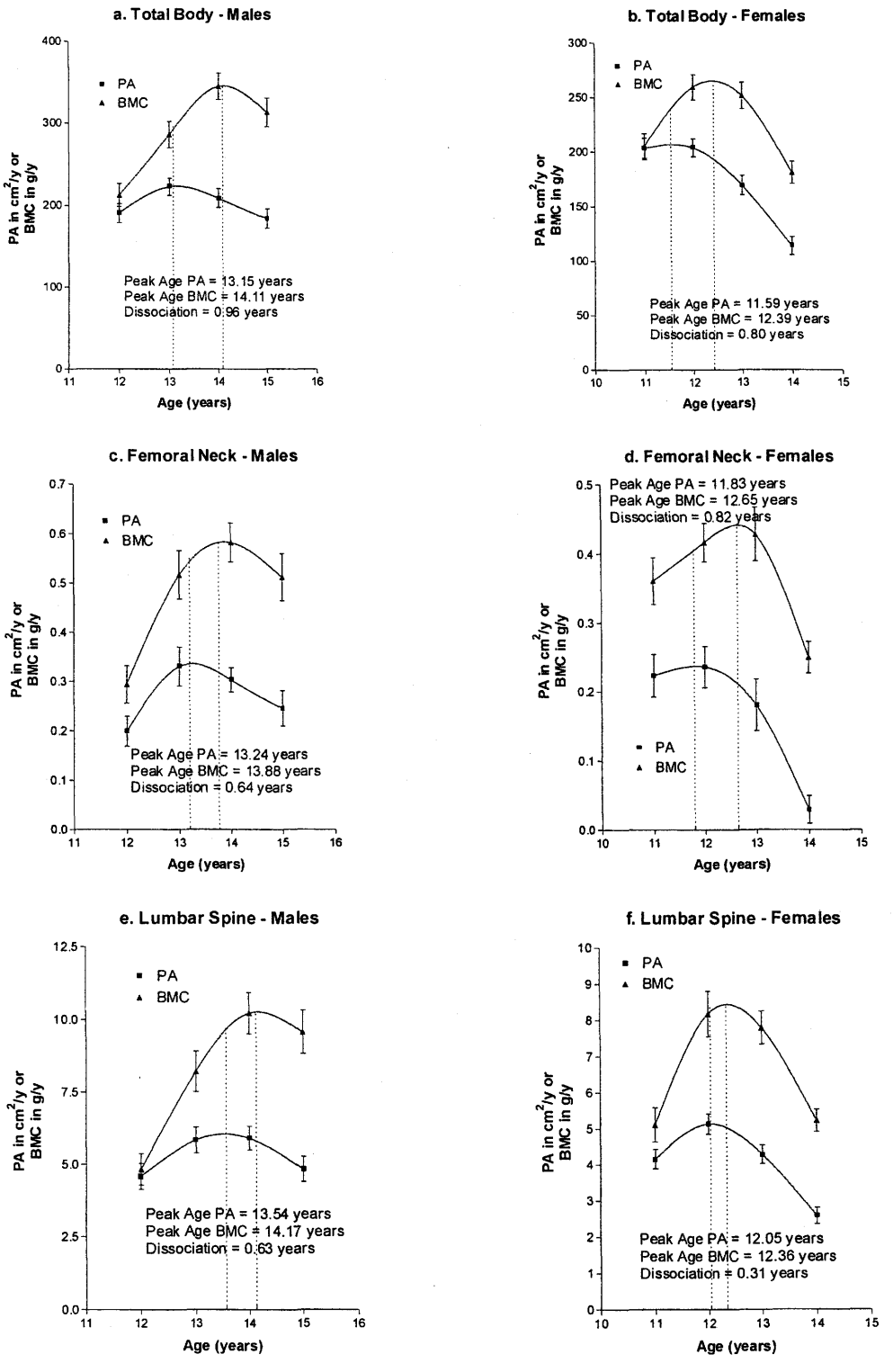

Figure 9.9. Chronological-based velocity curves displaying the dissociation between bone projected area and bone mineral content (mean±SEM) for a. Male TB; b. Female TB; c. Male FN; d. Female FN; e. Male LS; and f. Female LS.

10 Appendix C: Supplementary Peak Height Velocity-Aligned Tabular Data

10.1 *PHV-aligned tabular data*

Table 10.1. Male Total Body PHV-Aligned Data.

Age	n	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-4	13	1176.9	29.21	971.0	33.91	0.823	0.00483	0.0983	0.00213	0.0352	0.00055
-3	25	1321.4	31.40	1100.5	36.20	0.829	0.00480	0.0917	0.00129	0.0337	0.00031
-2	36	1478.0	25.59	1262.3	30.91	0.851	0.00506	0.0875	0.00103	0.0329	0.00028
-1	48	1592.4	25.40	1385.6	30.12	0.867	0.00494	0.0853	0.00090	0.0324	0.00024
0	65	1814.3	24.46	1650.8	33.69	0.905	0.00637	0.0822	0.00065	0.0319	0.00021
1	63	2093.7	24.54	2070.2	38.85	0.985	0.00690	0.0815	0.00058	0.0326	0.00022
2	54	2263.5	26.53	2379.1	45.07	1.047	0.00715	0.0821	0.00058	0.0334	0.00024
3	41	2345.2	31.62	2546.4	54.08	1.082	0.00728	0.0825	0.00069	0.0340	0.00029
4	29	2414.0	38.00	2689.2	68.55	1.110	0.00724	0.0823	0.00082	0.0344	0.00033

Table 10.2. Female Total Body PHV-Aligned Data.

Age	n	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-4	4	908.9	11.816	677.2	50.58	0.744	0.0174	0.1059	0.00334	0.0394	0.00122
-3	15	1114.6	20.647	869.9	56.65	0.775	0.0137	0.0962	0.00218	0.0376	0.00066
-2	29	1232.9	19.199	980.6	36.80	0.792	0.0078	0.0921	0.00146	0.0368	0.00041
-1	42	1340.8	21.891	1088.5	35.46	0.808	0.0074	0.0897	0.00142	0.0362	0.00035
0	52	1530.5	22.066	1295.3	35.60	0.842	0.0075	0.0854	0.00100	0.0356	0.00027
1	65	1729.8	22.705	1578.7	37.27	0.907	0.0078	0.0846	0.00076	0.0363	0.00024
2	58	1878.1	19.165	1810.2	35.54	0.960	0.0082	0.0841	0.00070	0.0371	0.00026
3	47	1967.8	19.168	1966.9	43.57	0.995	0.0099	0.0841	0.00072	0.0376	0.00029
4	31	1977.4	18.035	2007.6	56.42	1.011	0.0133	0.0845	0.00078	0.0381	0.00037

Table 10.3. Male Femoral Neck PHV-Aligned Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-4	13	4.243	0.068	2.996	0.079	0.708	0.0192	0.2513	0.00897	0.3019	0.00953
-3	25	4.323	0.073	3.108	0.089	0.717	0.0138	0.2499	0.00518	0.3024	0.00562
-2	36	4.507	0.058	3.300	0.073	0.732	0.0120	0.2446	0.00465	0.3011	0.00510
-1	48	4.661	0.052	3.468	0.063	0.744	0.0115	0.2412	0.00497	0.3007	0.00531
0	65	5.043	0.051	3.973	0.065	0.789	0.0109	0.2363	0.00433	0.3041	0.00480
1	63	5.497	0.057	4.751	0.080	0.865	0.0126	0.2380	0.00455	0.3172	0.00526
2	54	5.609	0.066	5.101	0.089	0.911	0.0144	0.2460	0.00547	0.3304	0.00623
3	41	5.670	0.068	5.282	0.103	0.934	0.0178	0.2493	0.00650	0.3364	0.00757
4	29	5.733	0.083	5.506	0.115	0.965	0.0225	0.2554	0.00894	0.3458	0.01019

Table 10.4. Female Femoral Neck PHV-Aligned Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-4	4	3.953	0.133	2.405	0.133	0.609	0.0399	0.2320	0.01846	0.1858	0.01424
-3	15	4.162	0.100	2.546	0.100	0.608	0.0183	0.2196	0.00575	0.1771	0.00450
-2	29	4.262	0.072	2.722	0.072	0.637	0.0114	0.2253	0.00462	0.1822	0.00353
-1	42	4.375	0.063	2.880	0.063	0.656	0.0096	0.2259	0.00347	0.1833	0.00266
0	52	4.591	0.053	3.228	0.053	0.701	0.0100	0.2297	0.00319	0.1877	0.00252
1	65	4.824	0.045	3.703	0.045	0.766	0.0106	0.2392	0.00351	0.1967	0.00280
2	58	4.986	0.051	4.088	0.051	0.820	0.0117	0.2480	0.00425	0.2049	0.00337
3	47	4.953	0.056	4.252	0.056	0.858	0.0146	0.2609	0.00489	0.2153	0.00391
4	31	4.853	0.063	4.208	0.063	0.866	0.0193	0.2688	0.00653	0.2213	0.00523

Table 10.5. Male Lumbar Spine PHV-Aligned Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-4	5	38.015	1.296	22.846	1.400	0.601	0.0289	0.0976	0.00484	0.0320	0.00172
-3	14	39.553	0.835	24.283	1.014	0.611	0.0167	0.0972	0.00224	0.0315	0.00071
-2	26	42.675	0.773	27.267	0.839	0.636	0.0112	0.0975	0.00145	0.0309	0.00048
-1	34	46.436	0.739	31.199	0.964	0.669	0.0138	0.0983	0.00183	0.0303	0.00057
0	46	51.974	0.764	38.122	0.985	0.731	0.0123	0.1015	0.00159	0.0303	0.00050
1	59	59.215	0.726	49.852	1.122	0.839	0.0124	0.1091	0.00146	0.0312	0.00043
2	54	64.116	0.749	58.065	1.209	0.903	0.0126	0.1129	0.00145	0.0315	0.00042
3	42	66.387	0.932	63.133	1.519	0.948	0.0146	0.1165	0.00166	0.0322	0.00049
4	29	67.590	1.109	66.030	1.632	0.975	0.0154	0.1189	0.00190	0.0327	0.00059

Table 10.6. Female Lumbar Spine PHV-Aligned Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-3	12	35.653	1.4805	21.407	1.848	0.592	0.0262	0.0992	0.00326	0.0197	0.00066
-2	18	37.801	1.2009	23.919	1.554	0.625	0.0206	0.1018	0.00256	0.0197	0.00052
-1	30	40.640	0.9739	27.093	1.248	0.660	0.0137	0.1036	0.00152	0.0194	0.00032
0	42	44.412	0.9372	32.017	1.278	0.713	0.0126	0.1072	0.00131	0.0193	0.00028
1	54	49.206	0.8475	40.585	1.341	0.816	0.0131	0.1164	0.00134	0.0200	0.00025
2	58	53.248	0.7031	48.194	1.114	0.900	0.0109	0.1234	0.00116	0.0204	0.00022
3	47	56.078	0.7953	53.530	1.437	0.948	0.0136	0.1266	0.00130	0.0205	0.00020
4	31	56.214	0.9396	54.647	1.886	0.965	0.0176	0.1286	0.00159	0.0208	0.00022

Table 10.7. Male Total Body PHV-Aligned Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-2	31	152.20	8.878	152.75	6.051	0.017	0.0022	-0.0047	0.00092	-0.0011	0.00131
-1	31	195.64	8.834	215.63	9.438	0.027	0.0031	-0.0037	0.00063	-0.0007	0.00089
0	31	254.53	12.272	352.92	15.272	0.063	0.0035	-0.0010	0.00060	0.0002	0.00081
1	31	228.26	11.220	386.86	15.337	0.077	0.0029	0.0006	0.00039	0.0010	0.00058
2	31	141.33	8.834	284.16	14.602	0.060	0.0030	-0.0004	0.00051	0.0011	0.00068

Table 10.8. Female Total Body PHV-Aligned Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-2	29	150.37	8.159	150.37	8.159	0.010	0.0024	-0.0077	0.00104	-0.0019	0.00031
-1	29	170.62	8.469	170.62	8.469	0.024	0.0026	-0.0047	0.00069	-0.0009	0.00018
0	29	207.05	7.299	207.05	7.299	0.052	0.0033	-0.0025	0.00070	0.0000	0.00019
1	29	198.55	9.389	198.55	9.389	0.064	0.0036	-0.0010	0.00052	0.0007	0.00016
2	29	121.38	9.415	121.38	9.415	0.048	0.0032	-0.0077	0.00051	0.0008	0.00019

Table 10.9. Total Body PHV-Aligned Peak Data.

Measure	PHV-Age at Peak Velocity		Peak Velocity	
	Males	Females	Males	Females
BA (cm)	0.2178	0.3980	257.2641	212.1414
BMC (g)	0.7134	0.8135	393.5000	292.9398
BMD (g/cm^2)	0.8936	0.9086	0.0775	0.0642
BMAD (g/cm^3)	1.0438	1.9950	0.0006	0.000002
sBMD (g/cm^3)	1.7497	1.4944	0.0011	0.0008

Table 10.10. Male Femoral Neck PHV-Aligned Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-2	31	0.266	0.01695	0.2678	0.01528	0.019	0.0034	-0.0049	0.01116	0.0042	0.00251
-1	31	0.373	0.01683	0.4274	0.02585	0.032	0.0041	-0.0030	0.00206	0.0031	0.00209
0	31	0.575	0.02753	0.7885	0.03144	0.072	0.0043	-0.0015	0.00196	0.0102	0.00198
1	31	0.395	0.02486	0.6825	0.03667	0.059	0.0052	0.0020	0.00196	0.0104	0.00219
2	31	0.137	0.01684	0.3460	0.03184	0.036	0.0041	0.0042	0.00207	0.0090	0.00203

Table 10.11. Female Femoral Neck PHV-Aligned Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-2	29	0.1723	0.01607	0.1801	0.01469	0.035	0.0033	-0.0074	0.00448	0.0103	0.00406
-1	29	0.2851	0.02359	0.3039	0.02234	0.035	0.0038	0.0026	0.00179	0.0031	0.00135
0	29	0.3099	0.01972	0.4402	0.02590	0.058	0.0046	0.0078	0.00184	0.0076	0.00142
1	29	0.1803	0.02107	0.4078	0.02656	0.070	0.0053	0.0090	0.00234	0.0088	0.00178
2	29	0.1167	0.01627	0.2751	0.02413	0.045	0.0043	0.0085	0.00173	0.0075	0.00126

Table 10.12. Femoral Neck PHV-Aligned Peak Data.

Measure	PHV-Age at Peak Velocity		Peak Velocity	
	Males	Females	Males	Females
BA (cm)	0.0476	-0.3179	5.7619	3.2075
BMC (g)	0.2578	0.2879	8.1095	4.4945
BMD (g/cm^2)	0.2078	0.8335	0.0734	0.0703
BMAD (g/cm^3)	1.9950	1.0038	0.0042	0.0090
sBMD (g/cm^3)	0.4431	-2	0.0111	0.0103

Table 10.13. Male Lumbar Spine PHV-Aligned Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-2	28	2.662	0.2650	2.591	0.2081	0.020	0.0032	0.0004	0.00066	-0.0006	0.00027
-1	28	4.354	0.3043	4.601	0.3382	0.036	0.0037	0.0005	0.00054	-0.0007	0.00019
0	28	7.369	0.3516	10.849	0.6780	0.095	0.0053	0.0061	0.00068	0.0005	0.00021
1	28	5.662	0.4957	10.712	0.5790	0.100	0.0043	0.0071	0.00063	0.0009	0.00024

Table 10.14. Female Lumbar Spine PHV-Aligned Velocity Data.

Age	N	BA (cm^2)		BMC (g)		BMD (g/cm^2)		BMAD (g/cm^3)		sBMD (g/cm^3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
-1	27	3.744	0.2973	3.671	0.3171	0.035	0.0037	0.0011	0.00051	0.00005	0.00043
0	27	5.234	0.3564	7.490	0.5386	0.088	0.0060	0.0064	0.00085	0.00001	0.00018
1	27	5.007	0.2293	9.046	0.4636	0.105	0.0047	0.0092	0.00057	0.00066	0.00011
2	27	3.269	0.3847	6.223	0.4970	0.067	0.0047	0.0053	0.00064	0.00033	0.00014

Table 10.15. Lumbar Spine PHV-Aligned Peak Data.

Measure	*PHV-Age at Peak Velocity*		*Peak Velocity*	
	Males	**Females**	**Males**	**Females**
BA (cm)	0.1289	0.3742	7.4319	5.37765
BMC (g)	0.4030	0.8623	11.5569	9.09822
BMD (g/cm^2)	0.5156	0.8060	0.1040	0.10648
BMAD (g/cm^3)	0.6809	0.9337	0.0072	0.00919
sBMD (g/cm^3)	0.9962	1.1665	0.0009	0.00068