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Influences of Muscle, Fat and Hormones on Bone Development in Women

A Cross-Sectional and Longitudinal Study Spanning Three Generations

JYVÄSKYLÄN YLIOPISTO

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"As a well spent day brings happy sleep, so a life well used brings happy death."

— Leonardo da Vinci

勤劳一日，可得一夜安眠；勤劳一生，可得幸福长眠。

— 达 • 芬奇

To my family and mentors for their love and support

ABSTRACT

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Finnish Summary
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Understanding how bone growth and loss are regulated by mechanical and hormonal factors is important for optimizing the attainment of peak bone mass and prevention of fragility fracture in late life. The aim of this study was to investigate the influences of muscle, fat and hormonal factors on bone development in peripubertal girls, and pre- and postmenopausal women. The study subjects were 396 girls aged 10 to 13 years at baseline, 257 mothers and 154 grandmothers. Body composition was assessed using DXA and bone properties by pQCT. Hormones were determined by fluoroimmunoassays or ELISA. We *first* compared the peak growth velocity times (PVTs) of musculoskeletal variables in the lower leg. We found that the growth of muscle lagged behind the bone growth in size (length and width), but preceded bone mass accrual in pubertal girls. This finding does not support the view that muscle drives bone growth in size, but accords with the mechanostat postulate that muscle drives bone mass accrual. We *then* analyzed the associations between growth/sex hormones and bone traits using hierarchical models. Circulating IGF-1 promoted peripubertal bone growth largely in a muscle-dependent fashion. The effects of estradiol and testosterone on peripubertal bone growth were time-dependent. They stimulated bone growth before menarche, but the stimulatory effects waned or became inhibitory after menarche. We *further* evaluated how well bone adapted its strength to the applied load from body weight in girls, mothers and grandmothers. The results showed that bone did not strengthen adequately to maintain equilibrium with the load from greater body weight, leading to an age-dependent relative bone strength deficit. This was largely attributable to fat mass accumulation, because the beneficial effects of increased fat mass on bone did not compensate for the mechanical burden that it imposed. We *finally* assessed bone mass distribution at the shafts of weight-bearing tibia and non-weight bearing radius in girl-mother-grandmother trios. The girl-mother and mother-grandmother differences were used to represent the patterns (amount and direction) of bone mass accrual and loss, respectively. We found that both bone accrual and loss were direction-specific in tibia, but relatively uniform in radius, suggesting a load-driven bone mass distribution. However, pronounced bone loss during ageing did not occur exactly at the sites of preferential bone deposition during early life. This suggested that bone loss, from a directional perspective, is not a complete reversal of the bone accrual. In conclusion, this study provides new insights into the complex relationships of bone development with muscle, fat and hormonal regulation, and corroborate the importance of early intervention and optimal body composition for bone health improvement and consolidation.

Key words: Puberty, girls, premenopause, postmenopause, body composition, bone, muscle, fat mass, lean mass, insulin-like growth factor-1, estradiol, testosterone

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Jyväskylä, November 2011 Leiting Xu
徐 雷 艇

LIST OF ORIGINAL ARTICLES

This dissertation is based on the following papers, referred to as I-IV in the text.

I Xu L, Nicholson P, Wang Q, Alén M, Cheng S. Bone and muscle development during puberty in girls: a seven-year longitudinal study. J Bone Miner Res. 2009;24:1693-8.

II Xu L, Wang Q, Wang Q, Lyytikäinen A, Mikkola T, Völgyi E, Cheng S, Wiklund P, Munukka E, Nicholson P, Alén M, Cheng S. Concerted actions of insulin-like growth factor-1, testosterone and estradiol on peripubertal bone growth - A 7-year longitudinal study. J Bone Miner Res. 2011;26:2204-11.

III Xu L, Nicholson P, Wang QJ, Wang Q, Alén M, Cheng S. Fat mass accumulation compromises bone adaptation to load in Finnish women: a cross-sectional study spanning three generations. J Bone Miner Res. 2010;25: 2341-9.

IV Wang Q, Xu L, Wang Q, Chen D, Tian H, Lu C, Cheng S, Völgyi E, Wiklund P, Munukka E, Nicholson P, Alén M, Cheng S. Is bone loss the reversal of bone accrual? - Evidence from a cross-sectional study in daughter-mother-grandmother trios. J Bone Miner Res. 2011;26:934-40.

ABBREVIATIONS

ANOVA	Analysis of variance
AR	Androgen receptor
BM	Bone mass
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMSI	Bone muscle strength index
BSI	Bone strength index
cBMD	Cortical volumetric bone mineral density
cCSA	Cortical cross-sectional area
CSA	Cross-sectional area
CSMI	Cross-sectional moment of inertia
DXA	Dual-energy X-ray absorptiometry
E2	Estradiol
ECM	Extracellular matrix
ER	Estrogen receptor
FGF-2	Fibroblast growth factor-2
FM	Fat mass
GH	Growth hormone
GHR	Growth hormone receptor
IGF-1	Insulin-like growth factor-1
LM	Lean mass
LSD	Least significant difference
LTPA	Leisure time physical activity
mCSA	Muscle cross-sectional area
PC	Periosteal circumference
pQCT	Peripheral quantitative computerized tomography
PVT	Peak velocity time
RBSI	Relative bone strength index
T	Testosterone
tBMC	Total bone mineral content
tCSA	Total cross-sectional area
TL	Tibial length
TRM	Time relative to menarche

CONTENTS

1 INTRODUCTION

Osteoporosis is a progressive, systematic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue that increases bone fragility and hence susceptibility to fractures(1). This disease is highly prevalent worldwide and poses a great socio-economic burden on society (2, 3). In Finland, among 57-65 year old Finns, 40% of men and 56% of women have osteopenia and 2% of men and 14% of women have osteoporosis (4). Thus, an emphasis should be placed on developing cost-effective preventive measures to combat or offset this rise in osteoporosis and related fracture. Numerous studies have endeavored to decipher the pathogenesis of this disease. The pathogenesis of osteoporosis is attributable to a number of factors, including genetic predisposition, previous fracture history, inadequate vitamin D and calcium intake and physical inactivity, etc (5). In women, estrogen deficiency after menopause is the main cause of rapid bone loss and regarded as the mechanism underlying the vulnerability of women to osteoporosis in old age (6).

However, accumulating evidence has shown that osteoporosis has its origins in ear ly life. Bone traits in adulthood track from the position established early in life (7, 8). The bone mass of an individual in later life depends upon the peak bone mass attained during growth and the subsequent rate of bone loss (9). Theoretically, people who acquire maximal bone mass in their early years should be at a reduced risk of skeletal fragility and fracture in later life (10). The period from the onset of puberty to young adulthood is most critical for the bone development (11), since during this period bone grows fast and bone mass approximately doubles (12). The growing bone at peripuberty adapts vigorously to mechanical loading (13, 14), and is under strong regulation of hormonal factors (7). Knowledge of the mechanical and hormonal determinants accounting for the physiologic variations in peripubertal bone growth will provide the best means toward optimization of peak bone mass attainment (10), as well as the early prevention, diagnosis and treatment of osteoporosis (15).

The mechanical loads exerted on bone mainly comprise of dynamic loads from muscle contraction and passive loads from gravitational forces associated with impact which scale to body weight (16). Body weight is mainly composed

of fat mass and lean mass (mainly muscle mass). Bone supports muscle, and muscle contraction produces the dominant physiological loads applied on bone (17-19). Therefore, the development of bone and muscle are inseparably associated and connected, forming a functional "bone-muscle unit" (19). However, how the developing bone and muscle relate to each other during growth remains unclear. Fat mass accumulation causes larger load on bone, stimulating bone formation. Adipose tissue also secretes bone active hormones such as estrogens and leptin which can have both stimulatory and inhibitory effects on bone formation (20, 21). The effects of adipose tissue on bone growth and development have been extensively studied (22-27). Unfortunately, the results are rather controversial, reflecting the complexity of the fat-bone relationship.

Bone adapts its shape and mass distribution to the prevailing loads which decrease during ageing to a greater extent at those skeletal sites where loads increase most from the young age to adulthood (28, 29). Accordingly, the loss of bone during ageing may occur preferentially at sites where more bone is deposited in earlier life. In other words, the bone loss during ageing, from a directional perspective, is a reversal of bone accrual during early life. This hypothesis that "bone loss is the reversal of accrual" is seemingly attractive and in accordance with the principles of mechanostat theory. However, it lacks supportive evidence due to the infeasibility of longitudinal cohort studies from young to old age.

Bone development is also under the strong influence of the hormonal milieu (7). The growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis augments longitudinal growth by stimulating chondrogenesis in growth plate (30, 31) and promotes radial bone growth via enhancing periosteal apposition (32, 33). Estrogen and androgen both have profound, but different, effects on bone metabolism. Estrogens induce a linear growth spurt (34) but inhibit periosteal apposition (35), whereas androgens stimulate periosteal apposition (36) and probably directly act on growth plate (37). Our knowledge of the skeletal effects of growth/sex hormones is largely derived from animal models or from patients with a deficiency or excess of these hormones, and direct evidence from longitudinal studies conducted in normal population is lacking. In addition, these hormones also play a role in muscle hypertrophy (38, 39). It remains unclear whether the effects of these hormones on bone development are mediated through the changes in muscle.

The aim of this study is to investigate the influences of muscle, fat and hormonal factors on bone development in women, with an emphasis on growing bone at peripuberty. In addition, the relationship between bone loss and accrual was also evaluated. The results of this study will deepen our understanding of how bone development is regulated by these factors, which is essential for promotion of bone health in early life and prevention of osteoporosis in later life.

2 REVIEW OF THE LITERATURE

2.1 Basic bone biology

Bone is a rigid but dynamic connective tissue. Each bone serves one or more specific functions including mechanical support for body movement, protection for internal organs, maintenance of mineral homeostasis, and provision of the environment for hematopoiesis (40). At the macroscopic level, two types of bone are distinguishable: cortical (compact or dense) and trabecular (spongy or cancellous) bone. The former is relatively dense and has a slower turnover, whereas the latter has a much larger surface area per unit volume and a greater rate of metabolic activity (41). Histologically, bone is composed of cells and an extracellular matrix. The bone matrix has organic and inorganic components. The organic matrix, which provides the ductility and ability to absorb energy, is mainly composed of type I collagen. The inorganic matrix, which confers the rigidity to a bone, primarily consists of calcium and phosphorus in the form of hydroxyapatite (42). There are three types of mature bone cells found in bone tissue: osteoblasts, osteoclasts and osteocytes. Osteoblasts develop from mesenchymal stem cells and are responsible for the bone formation. When osteoblasts are trapped in the bone matrix secreted by themselves, they become osteocytes that are isolated in lacunae (42) . Although the functions of osteocytes are still unclear, they are believed to be the major mechanosensory cells in bone (43). Osteoclasts are specialized macrophage polykaryons derived from hematopoietic stem cells and responsible for bone resorption (44). They also play a role in the regulation of immunity (45) and hematopoiesis (46) .

Bone constantly undergoes modeling and remodeling in order to achieve and maintain its functions (47, 48). Bone modeling occurs mainly during growth and brings in changes in both the size and architecture of the bone in response to physiological or mechanical factors (47). During modeling, bone formation and resorption are not tightly coupled (49). Both bone formation without prior bone resorption on periosteal surface and bone resorption without subsequent bone formation on endosteal surface are referred to as bone modeling (50, 51). Bone remodeling is the process by which bone is renewed to maintain bone

strength and mineral homeostasis (52). Bone remodeling occurs predominantly on the bone's inner surface and much less on its periosteal surface. Therefore, bone remodeling does not change the size or shape of the bone (53). Unlike modeling, osteoclasts and osteoblasts closely collaborate in the remodeling process in what is called a basic multicellular unit (BMU) (54), in which bone resorption by osteoclast is followed by the formation of new bone of comparable amount by osteoblast (55, 56). A greater volume of bone resorbed than formed by each BMU produces a net negative balance, which is the basis of bone loss (57).

2.2 Bone growth

2.2.1 Longitudinal bone growth

The longitudinal growth of long bones occurs in the growth plates where chondrocytes synthesize cartilage that is subsequently ossified (58, 59). The growth plate is located between epiphyseal and metaphyseal bone at the ends of the long bones. It can be divided into horizontal zones containing chondrocytes at different stages of differentiation, from a reserve zone at the epiphyseal end, through a proliferative zone to prehypertrophic and hypertrophic zones at the metaphyseal end (**Figure 1**)(59).

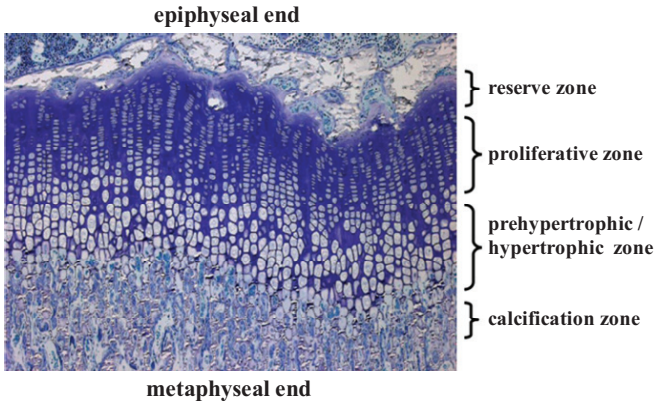

FIGURE 1 Micrograph of a 2 µm thick section of a rat proximal tibial growth plate showing the reserve zone, as well as the proliferative and hypertrophic zones where chondrocytes firstly proliferate and subsequently enlarge (undergo hypertrophy). Reproduced from Villemure (59) with permission.

Although the whole process of longitudinal bone growth is complicated and the exact mechanisms remain unclear, it is known that the combination of chondrocyte proliferation, the enlargement of maturing chondrocytes in the hypertrophic zone, and the production of extracellular matrix (ECM) proteins in the growth plate are the major contributors to longitudinal bone growth (60).

Briefly, the relatively inactive resting chondrocytes, also referred to as stem cells, migrate into the proliferating zone, and then divide in a longitudinal direction, organize in a typical columnwise orientation and synthesize ECM proteins (60-62). The proliferating chondrocytes finally lose their capacity to divide and start to differentiate through prehypertrophic into hypertrophic chondrocytes with an increase in size (63, 64). The hypertrophic chondrocytes start the mineralization of the surrounding matrix by secreting calcium-phosphates, hydroxyapatite, and matrix metalloproteinases (65-67). These processes of chondrocyte proliferation, hypertrophy, and cartilage matrix secretion result in chondrogenesis. The mineralized chondrocytes undergo apoptosis, leaving a scaffold for osteoblasts from invaded blood vessels to lay down new metaphyseal trabecular bone. Thus, the synchronized processes of chondrogenesis and cartilage ossification pushes the older chondrocytes towards the diaphysis and "squeezes" new material in between the growth plate's reserve zone and the zone of provisional calcification (62). This entire process continues as an orderly progression of activity that extends toward both ends of the developing bone. As a result, the bone gains length.

The postnatal longitudinal bone growth and hence the increase in height continues throughout childhood (68, 69). The growth velocity in humans is greatest in late fetal life (70). After age 1, the postnatal growth in length slows considerably until the age 2 years. Thereafter the growth usually continues at a fairly steady rate until adolescence when the adolescent growth spurt, indicated by a rapidly increases in height, occurs (**Figure 2**) (68, 69). In addition to the two growth spurts in first year of life and at puberty, some evidence suggests that there is a mid-growth spurt around age 6-7 (71), due to a transient increased growth rate of the bones, particularly the long bones (72), but this is not a consistent finding.

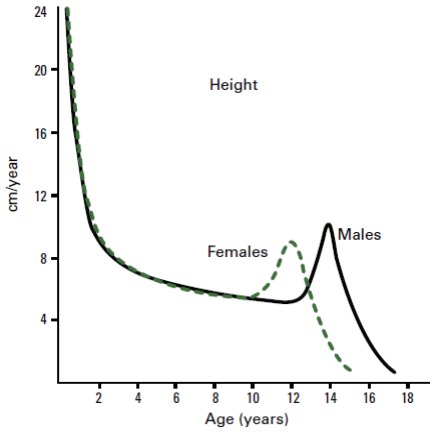

FIGURE 2 Growth velocity of body height over childhood and adolescence (male and female). Reproduced from Simm (69) with permission.

Puberty is associated with sexual development and is a time of significant increase in height and hence in bone length. Longitudinal bone growth rapidly increases during pubertal growth spurt, which contributes more than 15% to the final height of the individual (73). The onset of puberty corresponds to a skeletal (biological) age of around 11 years in girls and 13 years in boys (74, 75). On average, thus girls have 2 less years of prepubertal growth because of a earlier onset of puberty, and their puberty lasts for 3 years rather than the 4 years in boys (76). The shorter duration of prepubertal and pubertal growth in girls results in the difference in adult height hence bone length as well as other bone traits between men and women (12). Cartilage growth slows down from the late puberty and virtually ceases in the early twenties (12).

The decline in growth rate after the pubertal growth spurt is caused primarily by a decrease in the rate of chondrocyte proliferation (77), accompanied by structural senescent changes, such as a gradual decline in the overall growth plate height, proliferative zone height, hypertrophic zone height, size of hypertrophic chondrocytes, and column density (78). The mechanisms underlying this so-called "growth plate senescence" remain elusive (77). In growth plate-transplantation experiments, the growth rate of the transplanted growth plate depends on the age of the donor animal, not that of the recipient (79). This finding indicates that the decline in growth rate is caused by a mechanism intrinsic to the growth plate itself, not to hormonal or systemic mechanisms that would be a property of the recipient (78), perhaps because stem-like cells in the resting zone have a finite proliferative capacity. Gradual proliferative exhaustion is followed by epiphyseal fusion during which the growth plate completely ossifies so that only a thin epiphyseal line remains and the bones can no longer grow in length (68, 78).

2.2.2 Radial bone growth

Bones get wider through a process called periosteal apposition. Periosteal bone acquisition occurs in periosteum, where osteoblasts add mineralized tissue on the outer bone surface. The periosteum is a layer of dense connective tissue covered the external surfaces of most bones, and serves as a transitional region between cortical bone and the overlying soft tissue or musculature (80). Long bones exhibit a continuous periosteal surface, except at the articular surfaces and tendon insertions (80). Blood vessels and different types of nerve fibers permeate the periosteum, providing nourishment for bone via the blood supply (81) and making it very sensitive to manipulation (82, 83).

Histologically, periosteum is composed of two distinct layers of connective tissues (**Figure 3**) (80). The outer layer is mainly composed of fibrous tissue, providing elasticity and flexibility (80, 84). The inner layer, called the cambium layer, is in direct contact with the bone surface. It contains progenitor cells which develop into osteoblasts that are responsible for generating new bone during growth, allowing for bone growth in width (80, 84). Radial growth of the diaphysis is caused by direct apposition of cortical bone by osteoblasts from the inner cambial layer of the periosteum.

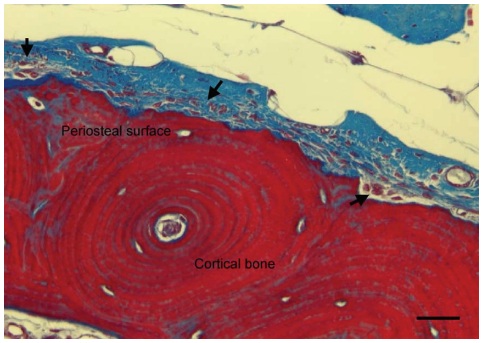

FIGURE 3 Periosteal covering of the human femoral midshaft. The cambium layer (arrowheads) near the periosteal surface is abundant in cells. Reproduced from Allen (80) with permission.

The cellular and molecular mechanisms that accelerate the periosteal expansion during growth or decelerate it with age remain poorly understood. Anatomical and histological differences have been observed between the periosteum of growing and adult bones (84-86). In children, the periosteum is thick and loosely attached to the cortex, allowing for rapid production of new bone. In adult bone, the periosteum is thinner and more adherent to the cortex, producing new bone less readily. The thinning of the periosteum and the attenuated capacity of the periosteal apposition with age may be attributed to the changes in morphology and reduction in number of periosteal fibroblasts and osteoblasts. In addition, the decline of the vessel density throughout the periosteum with age may also contribute (84-86).

The bone growth in width, as measured at the mid-shaft of long bones, is rapid during early life, but then continuously slows down until reaching a nadir during early school age (62, 87). The growth rate then peaks during puberty, and decreases dramatically thereafter (62, 87) (**Figure 4**). The pattern of change in radial bone growth rate resembles that of height velocity, indicating that the processes of longitudinal and radial bone growth are probably well-coordinated (88). This may confer a biomechanical advantage to the growing bones, because bone lengthening tends to undermine bone mechanical competence, while widening augments it (62, 89). A slower rate of bone widening relative to growth in bone length may produce a slender bone with compromised ability to tolerate loads. Conversely, if the bone widening surpasses the lengthening, it would produce a bulky bone which hampers agile movement. However, little is known about the co-regulation of bone lengthening and widening during growth.

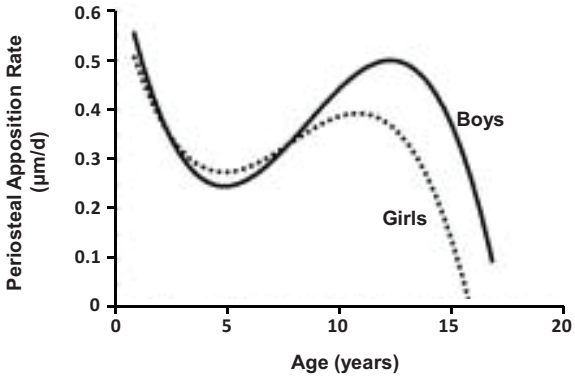

FIGURE 4 Second metacarpal periosteal apposition rates in Caucasian children. Reproduced from Rauch (62) with permission.

It has been tacitly assumed that after skeletal maturity bones do not get wider, since with increasing age bone cells respond less vigorously to mechanical and hormonal stimuli (90). But studies of age-related changes in bone mass, volume and cross-sectional area have provided conclusive evidence that human periosteal bone apposition can continue after skeletal maturity (85, 90, 91). Recent longitudinal studies have also confirmed, although the growth in length ceases in early adulthood (92), long bones continue to increase in size via expansion in cross-sectional area throughout life (93)(**Figure 5**). Even after menopause, minute but observable periosteal apposition occurs with advancing age (94, 95). Through continual periosteal apposition, bones adapt efficiently to the changing mechanical loads, since the body weight continues to increase after the cessation of longitudinal growth, and perhaps partly compensate for age-related loss in bone mass (95).

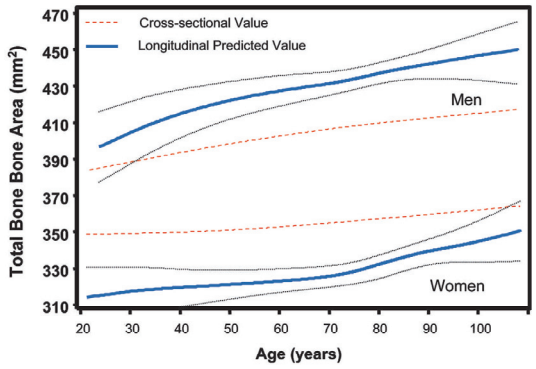

FIGURE 5 Age-related trajectories of the bone cross-sectional area in men and women during life span, estimated from the cross-sectional (red line) and longitudinal data (blue line) and their 95% CIs (black lines). Adapted from Lauretani (93) with permission.

2.2.3 Bone mass accrual

Bone mass can be measured as bone mineral content (BMC) and bone mineral density (BMD). BMC is defined as the mass of mineral in a given bone or part of the bone. Areal BMD is the BMC per unit projected bone area of the bone in the coronal plane (96). It is not an accurate measurement of true BMD, because it is confounded by differences in bone size due to the missing depth value in the calculation (97). This limitation is overcome by the measurement of volumetric BMD which is the BMC per unit volume of bone (97).

Bone mass increases through changes in outer dimensions (length and width) and through the increased degree of tissue mineralization (98-100). The bone mineral deposition begins *in utero* (99). During the period of a normal human pregnancy, the fetus accumulates approximately 30 g of calcium; the majority of this is accrued during the last trimester (9, 101). The BMC reaches around 2,000 g by 15-20 years of age (102). As described previously, the amount of bone mineral contained within the periosteal envelope increases as bone size expands (103). Meanwhile, minerals including calcium, phosphorus, and others are progressively incorporated into matrix at sites of newly formed organic bone matrix (osteoid) (104). Continuous matrix mineralization adds more minerals within a given bone volume, increasing the degree of tissue mineralization and leading to a higher volumetric BMD on a material level (98). Young bone tissue has higher water content, allowing for the active interaction between growing apatite crystals and the water from the bone matrix, leading to a higher tissue mineralization rate compared to the mature bones in which the water content is low and these exchanges are considerably lower (100). In fact, mineralization is rarely complete and stops at about 90-95% of the expected maximum level (100).

More than half of the adult bone mass is accrued during the pubertal years of rapid bone growth and 85-90% of adult peak bone mass (PBM) is achieved by the end of puberty (105). PBM is regarded to be the bone bank for the remainder of life (15). It is a major determinant of osteoporosis and fractures in the elderly (15, 106), because the amount of bone in the skeleton in later life is the result of the amount of bone gained during growth to skeletal maturity and the loss of bone that occurs with aging (15). Despite its importance, the timing of PBM attainment is of considerable controversy. It varies significantly from 17 to 18 years of age to as late as 35 years of age (15), depending on the different bone sites, genders, ethnicities, as well as the different techniques used to determine bone mass. But most studies indicate that bone mass does not significantly increase after the third decade (107).

During fast pubertal growth, total body BMC increases significantly, while the volumetric BMD increases only moderately (108), even decreases during certain period (109, 110), indicating that the increase in bone mass during puberty is largely due to the increase in bone size (108) and the tissue mineralization lags behind the rapid expansion in size (109). This inference is in accordance with the observation that the increase in height precedes the increase in BMC (111). The dissociation between bone growth in size and mass,

leads to a "temporary skeletal debt" or the "pubertal mineral debt" (111). This confers a biomechanical disadvantage to the growing bone, resulting in a time of relative bone fragility, which may explain the increased fracture incidence observed during the adolescent growth spurt (109-112).

2.2.4 Bone mass distribution

Bone must be strong for bearing load, yet light for facilitating mobility (113, 114). The strength of a bone is determined by both its material and structural properties. During growth, optimal bone strength is achieved by modifying mass distribution rather than increasing mass alone (114). The diversity of bone mass distribution is attributable to the different degrees of focal modeling around the periosteal perimeter (periosteal apposition) and remodeling at the corresponding point on the endocortical surface (endocortical resorption) (115, 116). Bone modeling strategically deposits bone mineral where it is needed, to modify bone size and shape; remodeling removes it from where it is not, to avoid bulk (117).

Bone adapts its shape and mass distribution to the prevailing loads applied on it. Therefore, the pattern of bone mass accumulation resembles the change of strain distribution during growth (113, 118). For example, periosteal apposition adds twice the amount of bone anteriorly and posteriorly than medially and laterally in pubertal girls during a 2-year follow-up (**Figure 6**) (119). Consequently, estimates of bending strength increased more in the anterioposterior than mediolateral direction (119), since the mechanical strain applied on bone in daily activity is more pronounced in the anterioposterior direction than the mediolateral direction. This ability of bone to increase its strength in response to loading by adapting its structural design rather than increasing its mass is convincingly attested to by the structural difference in the playing and non-playing arm of young tennis players: the strength of the humerus of the playing arm is optimized via modifying the bone size, shape, and mass distribution without changing its mass and density (120).

2.3 Bone loss

Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk (1). The World Health Organization has defined osteoporosis as a BMD 2.5 standard deviations or more below the mean value for young adults (T score < -2.5) (121). The development of osteoporosis is largely a result of age-related bone loss if left untreated (6), due to the negative balance during the remodeling process: a greater volume of bone is removed than is replaced within the sites of remodeling on the inner surface of bone (94).

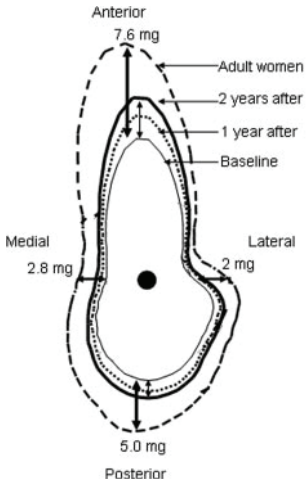

FIGURE 6 Bone mass distribution around the bone mass center at tibial midshaft in different age groups (the black dot in the center of bone). Adapted from Wang (119) with permission.

Both women and men lose bone with normal aging until the end of life (122), but the overall pattern of age-related bone loss differs in men and women, and varies in different bone sites (123). Bone loss starts at relatively slow rates from its peak from the fourth decade of life. Women lose bone more rapidly from the onset of menopause (6, 124), whereas the bone loss in men is rather constant and does not show the rapid phase, because they do not have the equivalent of a menopause (123). In addition, although the age-related bone loss occurs in both cortical and trabecular bone, menopause in women is associated with a rapid loss of trabecular bone, while less dramatic loss occurs in the cortical compartment (123, 124) (**Figure 7**). Overall, women lose 35–50% of trabecular and 25–30% of cortical bone mass with advancing age, whereas men lose 15–45% of trabecular and 5–15% of cortical bone (123, 125).

The increase of bone loss following the menopause in women is mainly a result of a marked reduction in circulating estradiol concentrations (126). Estrogen deficiency increases the rate of bone remodeling with a negative balance between bone formation and resorption within each BMU (127). As indicated by biochemical markers, bone resorption increases by 90% at menopause, whereas bone formation markers increase by only 45% (128). Estrogen deficiency induces cancellous as well as cortical bone loss (129). Highly increased bone resorption in cancellous bone leads to general bone loss and destruction of local architecture. In cortical bone the estrogen withdrawal enhances endocortical resorption and increases intracortical porosity (130). These changes lead to decreased bone mass, disturbed architecture and reduced bone strength (130).

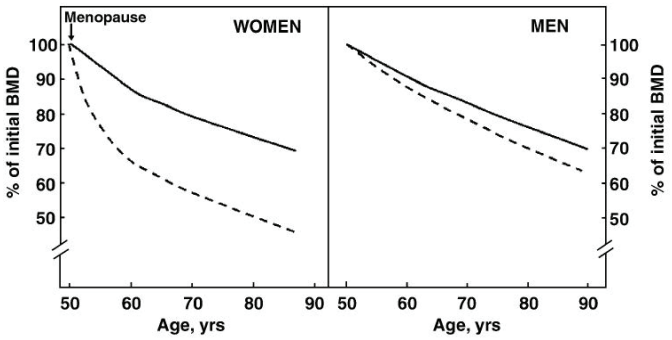

FIGURE 7 Patterns of age-related bone loss in women and men. Dashed lines represent trabecular bone and solid lines, cortical bone. Reproduced from Khosla et al (123) with permission.

Postmenopausal estrogen deficiency enhances endocortical remodeling and thus removes more bone from the inner surface, excavating the bone marrow cavity and decreasing the cortical thickness (124). On the other hand, estrogen deficiency removes a constraint on periosteal apposition which continues even after menopause (94). As a result, mineralized cortex is displaced outwards, further away from the neutral axis of bone. This increases the bending strength of bone and partially offset the decrease in bone strength resulting from enhanced endocortical bone loss (94), because at a given mass the bending strength of a unit area of bone is proportional to the fourth power of its distance from the neutral or long axis of the bone (131).

2.4 Factors related to bone development

2.4.1 Genetics

Genetic factors are a major determinant of bone growth and development. Chick limb buds removed and grown in vitro developed the shape of the proximal femur, implying that bone development is imprinted in the genetic code (132). Longitudinal human studies have found that familial resemblance in bone structural strength is established during the first year of life (133), and bone traits track from the position established early in life (7, 8). This suggests that the bone growth may, to a considerable extent, follow a programmed trajectory which is preprogrammed by a master gene or a set of genes (134). Furthermore, studies in twins have shown that between 50% and 85% of the variance in peak bone mass is genetically determined, depending on skeletal site and the age of the subjects studied (135-137). Family studies have also suggested a high heritability of bone mass. BMD is highly correlated between mothers and daughters (133, 138), and daughters of osteoporotic women have lower BMD compared to age-matched controls (139). As a result, a woman

whose mother has had a hip fracture has higher risk of fracture than women without such family history (140).

The data are conflicting with regard to the influence of genetic factors on age-related bone loss. This is probably due to the reality that bone loss, indicated by BMD change with age, is a noisier phenotype than BMD alone, and the interval of follow-up must be long enough to ensure that inter-individual variation exceeds the measurement error (141). However, accumulating evidence supports a heritable contribution to age-related bone loss, although it is weaker than for peak bone mass. Studies have shown that heritability of the BMD change ranges from 34% to 76% (135, 142-147). However, two twin studies did not find evidence for a significant genetic effect on bone loss in male wrist (135) and female hip (147). This suggests that the genetic influence on bone loss is probably gender- and site- and cohort-specific. In addition, genetic factors may also play a key role in regulating other phenotypes that predispose to osteoporotic fractures such as femoral neck geometry (148).

2.4.2 Mechanical loading and physical activity

According to mechanostat theory, bone adapts its size, shape, and mass to the mechanical loads applied on it (18, 149). The adaptation of bone to loads is achieved through the process of bone modeling and remodeling. When loading-induced strains stay below the lowest remodeling threshold, the remodeling removes bone from trabecular and subcortical surfaces, reducing the bone strength. Otherwise it conserves the current bone architecture and mass. When strains exceed the modeling threshold, modeling strengthens the bone (149).Through this neuron-equivalent mechanism, bone is able to maintain strength within a safety margin under habitual mechanical challenges.

In addition to the magnitude of the mechanical strain, bone adaption also depends on other characteristics of the strain, such as strain rate and frequency. At given loading frequency and peak strain magnitude, increasing strain rate was found to be a positive determinant of changes in bone mass (150). It was also demonstrated that only a few loading cycles of relatively high magnitude were enough to optimize the bone formation response; increasing the number of loading cycles by 10-fold had no additional effect (151). Turner (152) proposed three rules of bone adaptation to load: First, bone adaptation is more responsive to a dynamic than static loading. Secondly, a short duration of mechanical loading suffices to initiate an adaptive response, while longer duration does not further enhance the bone adaptation. Thirdly, bone adaptation is sensitive to the changes of the loading environment, but less responsive to the routine mechanical loading.

The influence of physical activity (PA) on bone development starts in prenatal life. Involuntary muscle contraction in utero, such as that occurring during the regular fetal kicks against the uterine wall, is believed to modulate cartilage growth, ossification and bone modeling and remodeling (104, 153, 154). After birth, the further bone growth is strongly influenced by the mechanical

strains associated with PA. Childhood and adolescence represent a critical time period for strengthening bones via PA. During this time, the body continues to accrue bone mass until reaching peak bone mass and the skeleton's adaptive response to loading is most pronounced before adulthood (155, 156). A plethora of cross-sectional and longitudinal studies have shown that physically active children generally have greater BMC and BMD than their inactive counterparts (157-163). More importantly, the beneficial effect of PA in childhood persists to adulthood (164, 165). The effects of PA on bone in adults are much less pronounced than in the young. Studies conducted in adult population have shown modest, even no effects on bone, depending on the gender, age and physical activity regimens (166-169). This is probably due to the fact that the adult bone is less responsive to load. Thus, during adulthood, the primary goal of physical activity should be to maintain, rather than increase, bone mass (170).

The beneficial effects of PA on skeleton depend on the type, intensity, frequency and duration of physical activity. For example, weight bearing exercise has been shown to be more osteogenic than non-weight bearing activity (159, 171). Therefore, runners have higher total body, femoral neck and leg BMD than swimmers and greater leg BMD than cyclists (172). The American College of Sports Medicine recommends high intensity (60% of 1 repetition maximum) impact activities with duration and frequency of 10–20 min/day and 3 days/week for improving bone health in children and adolescents; and moderate to high weight-bearing endurance activities with a duration of 30–60 min/day and a frequency of 3–5 times per week for preserve adult bone health (170).

2.4.3 GH/IGF-1 axis

Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are the most important hormones for normal postnatal bone growth. GH is synthesized and secreted by the anterior pituitary gland under the control of central and peripheral signals (173). IGF-1 is synthesized and secreted mainly by the liver under stimulation of GH, and partly by other tissues, such as muscle and bone, under control of other factors (174). Liver-derived IGF-1 acts as a circulating hormone, while the extrahepatically originated IGF-1 serves as a local growth factor (175).

GH exerts its effects by binding to growth hormone receptor (GHR) which is highly expressed in multiple tissues. GH recruits resting chondrocytes into a proliferative state (176), stimulates the proliferation of cultured chondrocytes (30), and induces the proliferation of cells of the osteoblastic lineage (177). Therefore, it plays a key role in the longitudinal growth of bone and in the attainment of peak bone mass. There is a good correlation between growth velocity and growth hormone secretion, expressed as the 24-h mean concentration (178). GH excess due to pituitary adenomas in childhood results in gigantism (179) and in adults leads to acromegaly (180), while GH deficiency or insensitivity due to GHR mutations or defects in GH signaling pathways markedly impairs postnatal growth (181). The *somatomedin hypothesis* postulates

that the effects of GH on longitudinal bone growth are realized indirectly by stimulating hepatic IGF-1 synthesis which in turn activates chondrocyte proliferation in the growth plate (182). However, local GH injection stimulates tibial bone growth significantly, but the contralateral tibia did not show this increase (183), suggesting an IGF-1-independent role of GH in longitudinal bone growth. Corroborated by other studies, the *dual effector theory* was formulated (184), stating that GH acts both directly on the growth plate and indirectly via stimulating systemic and local IGF-1 synthesis.

IGF-1 is essential for both pre- and postnatal bone growth. In one animal study, *igf1* null mice showed reduced bone length, total cross-sectional bone area, and cortical bone area compared with wild-type mice (185). IGF-1 mediates most of the effects of GH on growth, but it also has GH-independent effects during postnatal bone growth, as indicated by the finding that *igf1* null mice exhibit greater impairment in bone accretion than mice lacking GHR or GH (185, 186). IGF-1 regulates the physiology of growth plate via endocrine, paracrine or autocrine regulation (31, 187). IGF-1 is available to skeletal tissues through de novo synthesis by osteoblasts and osteoclasts and also by release of stored IGF-1 from bone matrix during osteoclastic bone resorption (188, 189). Liver-specific *igf1* gene-deleted mice exhibit similar growth rates compared with wild-type mice, despite the greatly reduced serum IGF-levels (190, 191), suggesting the IGF-1 locally produced in bone is probably more important than circulating IGF-1 in the regulation of growth plate physiology. However, a recent study reported that, in the absence of tissue *igf1* gene expression, maintaining a long-term elevation in serum IGF-1 is sufficient to establish normal body size, body composition, and both skeletal architecture and mechanical function (192). The controversy suggests a threshold concentration of circulating IGF-1 is necessary for normal bone growth (60, 193, 194). In human, serum IGF-1 is significantly correlated with height at puberty, and with bone age and growth velocity (111), which supports the idea of an essential role for circulating IGF-1 in longitudinal growth.

IGF-1 promotes radial bone growth and plays an important role in maintenance of adult bone mass (32, 33). Periosteum is abundant in receptors for IGF-1 (32), providing a stage for IGF-1 to exert its anabolic effects on the periosteal surface. Studies have shown that circulating IGF-1 stimulates periosteal bone growth along the cortex (195, 196), whereas reduced serum levels of IGF-1 in mice lacking liver-specific IGF-1 are associated with impaired periosteal apposition, leading to the development of slender bones during growth (195). Furthermore, the *igf1* knockout mice exhibit a significant deficit in bone mass accrual, mainly resulting from reduced cortical bone mass, while the trabecular bone of such mice is not significantly reduced (197). In one study, a reduction of trabecular bone formation was observed after administration of IGF-1 in normal mice, which is probably due to the mechanical compensation for increased periosteal bone formation (198).

IGF-1 synthesis and secretion decrease with age. The mean serum IGF-1 level is 40% lower in the 41- to 60-year-old age group compared to the 23- to 40-year-old age group (199). From 20 to 60 years of age, the concentration of IGF-1

in human femoral cortical bone declines by 60% (200). Several cross-sectional studies have demonstrated a strong correlation of serum IGF-1 with bone turnover markers (201, 202) and BMD (203, 204) in postmenopausal women. Longitudinal studies have also showed that low circulating levels of IGF-I in elderly women are associated with greater bone loss (205). It was reported that serum IGF-1 concentration was a sensitive parameter that had the capacity to early differentiate women with low bone mass/osteoporosis from normal women, suggesting that the measurement of serum IGF-1 in young women may help in the early identification of those at risk for developing low bone mass and osteoporosis (204).

2.4.4 Sex steroids

Sex steroids, including estrogens and androgens, are derived from cholesterol (206). Estrogens are produced mainly in the ovaries, and also produced in smaller amount in extragonadal tissues, such as adrenal glands, placenta, fat tissue, liver and breasts (207). There are three major endogenous estrogens in women: estrone (E1), 17β-estradiol (E2), and estriol (E3) (206). Among them, E2 has been intensively studied with regard to its role in bone metabolism. Androgens are secreted primarily from the testes and the adrenals (208). The most important and well-known androgen is testosterone. Estrogens and androgens play a very profound role in almost all aspects of bone development and metabolism, such as skeletal growth, the sexual dimorphism of the skeleton, attainment of peak bone mass, and the maintenance of bone mass and architecture in adults.

2.4.4.1 Estrogens

Estrogen exerts effects on bone via binding to estrogen receptors (ERs) which are found in osteoblasts, osteoclasts and chondrocytes (209, 210). The estrogenic regulation of bone growth starts from prenatal life. Alterations in the maternal estrogenic levels during pregnancy can influence early phases of fetal bone tissue development and subsequently result in permanent changes in the skeleton (211). The E2 level increases from the onset of puberty (212). The pubertal increase in growth velocity has traditionally been attributed to testicular androgen secretion in boys, and to estrogen or adrenal androgen secretion in girls. However, E2 concentration is significantly associated with growth velocity in health girls (213) as well as in boys at puberty (214). This indicates that estrogen may be the principal hormone stimulating the pubertal growth spurt in both girls and boys (73). Interestingly, the E2 concentration is positively correlated with growth velocity at early puberty, but negatively later (214), suggesting that the effect of estrogen on longitudinal bone growth is probably biphasic: at low level, estrogen stimulates growth, while higher levels of estrogen have potent inhibitory effects on longitudinal growth via accelerating epiphyseal closure (34, 73, 215). This view is supported by the findings that low-dose estrogen treatment can accelerate growth in both

prepubertal boys and girls (216), whereas patients with estrogen deficiency fail to undergo epiphyseal fusion during puberty, resulting in tall stature in adulthood (217).

The increase in E2 is associated with bone gain in pubertal girls, probably by suppressing bone resorption at the endosteal surface, rather than through enhancing periosteal apposition (34, 218). Conversely, animal studies suggest an inhibitory effect of estrogens on periosteal apposition and bone size expansion (219). Therefore, estrogen partly mediates the sexual dimorphism of bone size and mass, which is one of the factors that predispose women to a higher risk for fractures later in life. In adult bones, estrogens are essential for the maintenance of bone mass and architecture. Estrogen inhibits bone remodeling by concurrently suppressing osteoblastogenesis and osteoclastogenesis from marrow precursors (220). Estrogen deficiency, as happens in postmenopausal women, increases the bone remodeling rate with greater volume of bone resorbed than formed in each of numerous BMUs. This results in a net loss of bone and micro-architectural deterioration (125). In addition, estrogens and their receptors have an impact on the mechanical sensitivity of the skeleton by decreasing the threshold of the mechanostat and thereby increasing the sensitivity of bone to mechanical stimuli (221). The increased bone formation in response to mechanical loading is diminished when estrogen is deficient (222).

2.4.4.2 Androgens

Androgens affect bone metabolism via androgen receptors (ARs). Androgen-deficient animal models exhibit an increase in apoptosis and a decrease in the proliferation of chondrocytes in the growth plate (223), suggesting a stimulatory role of androgens in the longitudinal bone growth. The subcutaneous administration of androgen in normal mice accelerates epiphyseal maturation, compromising the bone length (224). Similarly in human, individuals with increased androgen levels, such as those with congenital adrenal hyperplasia, have an increased height velocity during the prepubertal years, premature epiphyseal maturation and compromised adult height (225). Therefore, like estrogens, androgens may also have a biphasic effect on longitudinal bone growth: at the start of puberty, androgens stimulate endochondral bone formation, whereas they induce epiphyseal closure at the end of puberty (226). Androgens can be converted into estrogens via the P450 aromatase enzyme complex (206), therefore at least part of the effect of androgens on bone can be explained by their aromatization into estrogens (226). In fact, controversy still remains whether androgenic effects on growth plate physiology are directly mediated via the AR or are secondary to aromatization of androgen to estrogen and hence ER mediated.

Androgens enhance bone formation by stimulating osteoblast proliferation, increasing the lifespan of both osteoblasts and osteoclasts by affecting apoptosis (126); and inhibit bone resorption though suppressing osteoclastogenesis (227). Animal data from male mice with disrupted AR or

orchidectomy provide convincing evidence that androgens stimulate male periosteal expansion (226, 228). Male subjects with inactive ARs have a female bone phenotype with a typical decline in bone cross-sectional area, mass and areal density, supporting the view that androgens stimulate bone size expansion and mass accrual (229). However, androgen deficiency plays a less important role in the pathogenesis of male osteoporosis than does estrogen in female osteoporosis, because the testes do not suddenly stop producing androgens as the ovary does for estrogens (226). In addition, older men treated with an aromatase inhibitor show significant decreases in spinal BMD despite the increases in testosterone level (230), demonstrating the importance of aromatization of testosterone to E2 for skeletal maintenance in older men.

2.4.5 Body composition

2.4.5.1 Lean mass and muscle

The largest customary load on bone arises from muscle contractions. Therefore, muscle strength is regarded as the major regulator of development of bone architecture and strength (18, 19, 231). It has been known for more than three decades that muscle mass and bone mass are closely associated (232). Total body lean mass (LM), a surrogate for muscle strength, is a consistent predictor of bone mass, regardless of the gender and age of the studied cohorts (17, 233-235). This relationship is especially close during growth and development (236-238). The change of LM is strongly correlated to the change of total body BMC in pubertal girls (239). As much as 76% of the variation in bone strength index in the distal radius of children and adolescents can be explained by grip strength alone (240), whereas in adults, the predictive power reduced to less than 50% (241). In addition, maximal increase in LM precedes peak BMC accrual at different sites (242). This result coincides with the view that bone development is driven by muscle as postulated by mechanostat theory (19), but the possibility cannot be ruled out that the growth of bone and muscle are independently genetically determined. In addition, muscle also secretes bone regulating growth factors, such as IGF-1 and FGF-2 (32). Hence, the integrated growth and development of bone and muscle is likely to be regulated in part by paracrine mechanisms at the muscle-bone interface involving growth factor signaling (32).

It has been suggested that the loss of muscle mass with aging is the principal cause of age-related bone loss (149). However, the view that "muscle loss causes bone loss" may be oversimplified. After menopause the exponential loss of bone is not accompanied by an accelerated loss of muscle strength and mass. The loss of muscle strength follows a more gradual course and is not affected significantly by a sudden decline in hormones, as is the case with bone loss (243). Indeed, there was not a close correspondence between changes in bone strength and changes in habitual load as assessed using lean body mass, total skeletal muscle mass, nor any consistent pattern (244). Moreover, inter-individual variation in the strength-to-load ratios was substantial (244).

Therefore, the decline in muscle strength or mass contributes to, but does not dominate, age-related bone loss (6).

2.4.5.2 Fat tissue

There are strong reasons to expect a pathophysiologic linkage between fat and bone tissue. Osteoblasts and adipocytes in bone marrow originate from the same mesenchymal stem cells (245). Normal aging is associated with both a high incidence of osteoporosis and bone marrow adiposity (246, 247), and both bone remodeling and adiposity are regulated by the hypothalamus and sympathetic nervous system (246).

Adipose tissue affects bone metabolism mainly through biomechanical and endocrine pathways. Biomechanically, higher fat mass exerts greater load on bone, which may stimulate bone formation. From an endocrine perspective, fat tissue secretes bone active hormones, such as estrogens and leptin, which can have both stimulatory and inhibitory effects on bone formation (20, 21, 248). The association between fat mass and bone properties found in population-based studies in fact reflects the integrated effects of both biomechanical and endocrine influence of fat tissue on bone. Such studies have been conducted extensively during the past three decades, but have yielded conflicting results. In several studies on children and adolescents, fat mass was positively and independently associated with BMC (22, 239, 249, 250). It was therefore suggested that adipose tissue acts to stimulate bone growth (22). Conversely, fat mass was not found to influence proximal femur geometry and strength independently of lean mass (251). In another study, fat mass was found to be a even better positive determinant of whole body BMD than lean mass in girls, whereas in boys, it was found to be a negative determinant of whole body BMD and lumbar spine BMD (23). For a given weight, obese children have been reported to have a lower bone mass in several previous studies (24, 26). In adults, the results are also conflicting, with positive, negative or no association between body fat and bone variables all having been reported (25, 27, 252). The controversy is partly attributable to the cohort effects or the different bone sites studied, and partly due to the different approaches used in the statistical analysis.

2.5 Summary of the literature review

The postnatal skeleton growth in length, width and mass as is under the control of a complex web of regulating factors including heredity, mechanical loading, growth/sex hormones, body composition and other factors. These factors regulate the establishment of the morphological features of bone during growth, the attainment of optimal peak bone mass, as well as the bone loss with age. GH/IGF-1 axis initiates longitudinal bone growth, while sex hormones accelerate bone growth at early puberty but decelerate it at late puberty. These hormones are also essential for bone mass attainment and maintenance in adults. The bone loss at old age is largely due to the deficiency of sex steroids.

Muscle or lean mass is consistently the strongest predictor for bone mass. Therefore, enhancing muscle strength via exercise is beneficial to bone health. However, the relationship between fat and bone is more complicated and still remains controversial.

3 PURPOSE OF THE STUDY

Understanding the regulations of the skeletal development into a mechanically competent structure is important for optimizing the attainment of peak bone mass and to prevent or postpone the occurrence of fragility fracture. Therefore, the main purpose of this mixed longitudinal and cross-sectional study spanning three generations was to explore the influences of muscle, fat and hormonal factors on bone development in women, with an emphasis on the growing bone at peripuberty. More specifically, the objectives were the following:

1) To depict the growth patterns of the bone-muscle unit and explore the relationship between bone and muscle growth from prepuberty to early adulthood using a longitudinal study design. The purpose of this analysis was to test whether the growth of muscle size precedes that of bone size and mass, and thereby to support or disprove the causality of the relationship between bone and muscle growth (I).

2) To assess the influence of serum IGF-1, E2 and T concentrations on bone lengthening, widening and mass accrual from prepuberty to early adulthood using a longitudinal study design. Using hierarchical models, we attempted to address whether the hormonal effects on bone growth differ before and after menarche, and whether the skeletal effects of these hormones are independent of muscle growth (II).

3) To investigate how fat mass accumulation affects bone mechanical competence at different developmental stages of women, i.e. at puberty, early adulthood, and pre- and postmenopause, respectively. This analysis used a cross-sectional design spanning three generations (III).

4) To test the hypothesis that bone loss in old age is possibly a directional reversal of bone accrual in early life. This analysis was based on cross-sectional data spanning three generations, the differences in bone mass distribution among girl-mother-grandmother trios were used to represent the bone accrual from the early adulthood to middle age, and the bone loss from middle to old age (IV).

4 MATERIALS AND METHODS

4.1 Subjects and study design

This observational study was a part of the Calex and Calex-family study. The recruitment of the study population is presented schematically in **Figure 8**. A detailed description of the participant recruitment is described elsewhere (253, 254). In brief, the girls were first contacted via class teachers teaching grades 4 to 6 (age 9-13 yrs old) in 61 schools in the city of Jyväskylä and its surroundings in Central Finland (96% of all the schools in these areas). Of 396 eligible girls, 258 (mean age at baseline 11.2 yrs) participated in DXA and pQCT measurements over a maximum period of 8 years (mean duration of total follow-up was 7.5 years). Among the 258 girls having baseline bone measurements, 200 girls were present at 1-year, 221 at 2-year, 87 at 3.5-year and 101 at 7-year follow-up. The main reasons for dropout were loss of interest and relocation. In addition, 134 girls from the original cohort who had no baseline measurement were re-invited to participate in the laboratory tests in years 2007 and 2008, thus resulting in 235 participants (mean age 18.3 years) at 7-year follow-up measurement.

In addition, biological mothers and grandmothers (maternal and paternal) of the girls were invited to participate in the bone measurements in the years 2003, 2004, 2007 and 2008. Of the mothers, 108 were measured in 2003 and 2004, and 214 in 2007 and 2008. 65 mothers had two bone measurements. This resulted in 257 mothers with at least one bone measurement. Of the grandmothers, 75 were measured in 2003 and 2004, and 120 in 2007 and 2008. Among them, 41 had two bone measurements. This resulted in 154 grandmothers with at least one bone measurement. Postmenopausal mothers, premenopausal grandmothers, and those who are currently taking medication or had diseases known to affect bone metabolism were excluded.

The study design for objective I & II was longitudinal. 258 girls who had baseline measurement and had at least two measurements during follow-ups were included. The study design for objective III and IV was cross-sectional,

with all 396 girls and their premenopausal mothers and postmenopausal grandparents being included.

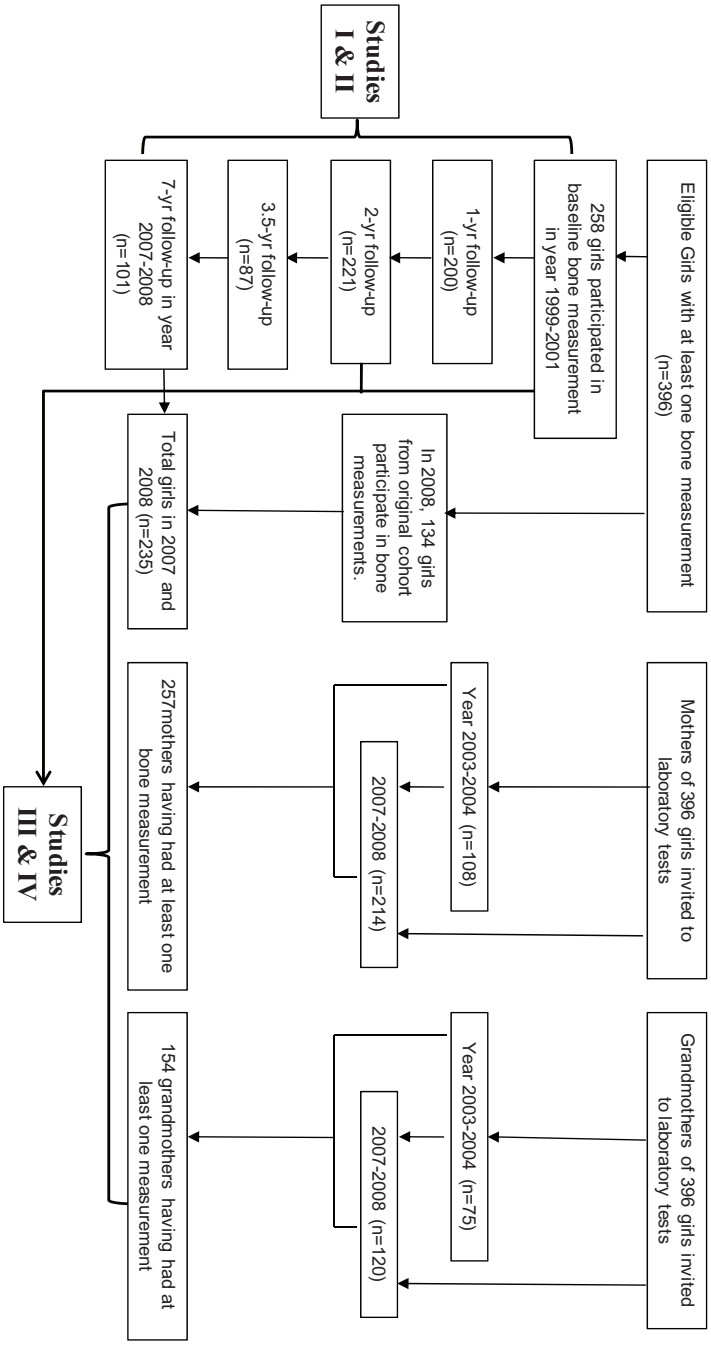

FIGURE 8 Flowchart of the CALEX and CALEX –family study

4.2 Measurements

4.2.1 Anthropometry

Body weight and height were measured using an electronic scale and stadiometer with subjects wearing light clothes and on bare feet. Body mass index (BMI) was calculated as weight (kg)/ height $(m)^2$.

4.2.2 Body composition

Body composition was assessed using dual-energy X-ray absorptiometry (DXA) (Prodigy; GE Lunar Corp., Madison, WI, USA). Lean tissue mass (LM) and fat mass (FM) were used in the current study. The coefficient of variation (CV) of two repeated measurements on the same day was on average 1.0% for LM and 2.2% for FM.

4.2.3 Bone measurements

The left tibial and radial shafts were scanned using peripheral quantitative computerized tomography (pQCT) (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany). The scan locations were at 60% of lower leg length up from the lateral malleolus and 30% of forearm length proximal to the wrist joint surface. Image processing and calculations of bone parameters were done by Stratec software. Total bone cross-sectional area (tCSA), cortical cross-sectional area (cCSA), total bone mineral content (tBMC), cortical volumetric bone mineral density (cBMD), periosteal circumference (PC), muscle cross-sectional area (mCSA) and polar cross-sectional moment of inertia (CSMI) were the outcome variables. The coefficient of variation (CV) of two repeated measurements on the same subject on the same day was on average 1% for tCSA, cCSA, mCSA and total BMC, and <1% for cBMD. The tibial length (TL) was measured from DXA scans. TL was defined as the distance between the proximal edge of tibia (middle point of the line from medial to lateral condyle) and distal border of tibia. The CV of three repeated measurements of TL in all participants across three generations was on average 2.7%.

4.2.4 Bone mass distribution analysis

Polar bone mass distribution analysis was performed using the Geanie 2.1 software (Bonalyse Oy, Jyväskylä, Finland) as has been described elsewhere (255). Briefly, as illustrated in **Figure 9**, an orthogonal coordinate system was established on the cross-section of the tibial and radial shaft. The y-axis was defined to coincide with the direction of the greatest width of the tibial or radial shaft, passing through the mass center of the cross-section. The x-axis was defined as perpendicular to the y-axis through the mass center. Then the total cross-section was divided into seventy-two sectors, each of an angle of 5°. The BMC within the area of each sector was given automatically by the software.

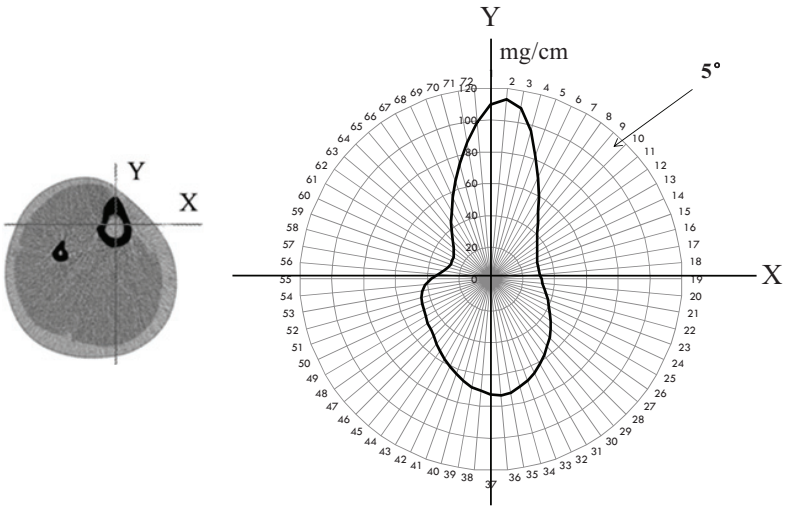

FIGURE 9 Illustration of polar distribution analysis bone mineral content (BMC) at tibial shaft.

4.2.5 Derived strength indices

Three strength indices were calculated based on the pQCT measurement at tibial shaft. 1) Bone strength index (BSI) was the product of CSMI and cBMD (256), which integrates both the material and structural properties of bone. This BSI has been validated by its close correlation with the actual, mechanically-determined bending breaking force of bones (256, 257). 2) Bone-muscle strength index (BMSI) was calculated as BSI/(mCSA×TL), where mCSA was used as a surrogate for muscle force and the product of mCSA and TL represented the bending moment exerted on the tibia by muscle on the lever length of the tibia (258). BMSI was used to indicate the equilibrium between bone strength and muscle force. 3) Relative bone strength index (RBSI), a new parameter, was introduced in study III, in order to reflect the relationship between tibial bone strength and the load applied on it. RBSI was computed as BSI/(TL × body weight). The product of body weight and tibial length, reflecting the bending moment of body weight acting on the lever length of the tibia, was used as a surrogate for load (259). Here, body weight, instead of muscle mass or size, was included in the calculation, because the impact forces generated from falls and other similar traumas scale with body weight (259). Raw RBSI was multiplied by 10^7 for presentational convenience.

4.2.6 Determination of hormones

Fasting blood samples were collected in the morning between 7:00 and 9:00 a.m. at each time point. If the girls had begun menstruation, the blood samples were

collected between 2 and 5 days after the onset of menstrual bleeding. Serum was stored at -80 °C until analyzed. Insulin-like growth like factor-1 (IGF-1) was assessed using time-resolved fluoroimmunoassays (IMMULITE; Siemens Healthcare Diagnostics, IL, USA). Estradiol (E2) and testosterone (T) were determined using ELISA (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany). Inter- and intra-assay coefficients of variation (CVs) were 6.1% and 3.1% for IGF-1, 3.2% and 5.4% for E2, and 3.9% and 6.2% for T, respectively.

4.2.7 Health questionnaire

Health history and lifestyle background information including the date of first menstruation of the girls, menopause status in mothers and grandmothers, disease and medication history and physical activity in all participants were obtained by self-administered questionnaire. Time relative to menarche (TRM) was calculated as the difference (in months) between the age at measurement and the age at menarche. Leisure time physical activity score (LTPA) was calculated as previously described (260).

4.3 Statistical analysis

All data were checked for normality before further analysis. If data were not normally distributed, their natural logarithms were used. The cross-sectional analyses were done in SPSS 15.0. Analysis of variance (ANOVA) with the least significant difference (LSD) post-hoc test was used to compare the differences among different groups. Pearson correlation coefficients or simple linear regression models were used to evaluate the associations between any two parameters of interest. In study III, a *t*-test with dummy variables was used to test whether the regression slopes (βs) in adult women were different from those in girls. In study IV, repeated measures analysis of variance was used to assess the difference in bone mass distribution in girl-mother-grandmother trios.

The longitudinal analyses (I & II) were performed using MLwiN 2.02 software. Hierarchical (multilevel) models with random effects were used to depict the growth patterns of musculoskeletal variables. In study I, the peak velocity time (PVT) of each musculoskeletal variable was determined from the maximum in the predicted velocity curve. In study II, the associations between bone parameters and hormones were assessed by regression coefficients obtained from multilevel models with or without adjustment of muscle CSA. By introducing a dummy variable to denote the respective period of before or after menarche, the associations between hormones and bone properties were assessed respectively before and after menarche. A $p < 0.05$ was considered statistically significant.

5 RESULTS

5.1 General characteristics

Table 1 presents the results of the variables used in this study of girls at baseline and 7-year follow-up, and of their premenopausal mothers and postmenopausal grandmothers. The mean menarche age of girls was 12.9 years. At 18 years of age, girls had similar body height but lower body weight in comparison with premenopausal adult women. The BMI and fat mass increased from early adult girls, through premenopausal adults, to postmenopausal women (all $p<0.01$). The LTPA score was highest at 18 years of age ($p<0.01$). For musculoskeletal variables, tibial length, tCSA, tBMC, cCSA, cBMC, cBMD, CSMI, muscle CSA at lower leg, and radial tCSA and tBMC increased in girls from baseline to 7-year follow-up (all $p<0.05$); at 18 years of age, girls already had similar TL as their premenopausal mothers, while other musculoskeletal variables at tibial and radial shaft were still significantly lower (all $p<0.01$). Similarly, almost all musculoskeletal parameters were lower in postmenopausal than premenopausal women (all $p<0.01$), except the tCSA which showed a trend to increase throughout the women's lifespan. The IGF-1 level was lower in adults than in the young girls (all $p<0.001$). The E2 level was significantly lower in 11 year old girls and postmenopausal women than 18 year old girls and premenopausal women (all $p<0.001$). The T level was the highest in 18 year old girls ($p<0.001$).

TABLE 1 Anthropometry, body composition, bone and muscle variables and hormones of girls at baseline and 7-year follow-up, their premenopausal mothers and postmenopausal grandmothers

Variables	Girls		Premenopausal Mothers	Postmenopausal Grandmothers
	Baseline	7-yr follow-up		
n	258	235	138	114
Age (years)	11.2 (0.8)	18.3 (1.1)	44.7 (4.1)	70.7 (6.3)
TRM (months)	-21.1 (12.9)	66.5 (18.6)		
Anthropometry				
Height (cm)	145.6 (8.0)	165.8 (5.7)	165.3 (5.7)	159.2 (5.2)
Weight (kg)	39.1 (8.5)	60.0 (10.0)	69.7 (13.4)	71.6 (11.3)
BMI (kg/m^2)	18.3 (2.9)	21.8 (3.2)	25.5 (4.5)	28.2 (4.2)
Physical Activity				
LTPA score	30.6 (9.3 - 100.5)	39.3 (10.5- 146.9)	29.1 (7.8 - 108.8)	27.7 (6.3 - 121.5)
Body composition				
Lean mass (kg)	27.3 (4.3)	38.1 (4.2)	42.0 (4.8)	40.0 (4.1)
Fat mass (kg)	10.5 (5.4)	19.4 (7.5)	25.0 (10.2)	29.3 (8.4)
pQCT				
Measurement				
Tibia shaft				
TL(cm)	33.3 (2.4)	37.2 (1.7)	36.9 (1.8)	35.9 (1.7)
tCSA (mm^2)	372.5 (54.7)	471.7 (56.0)	487.8 (59.1)	490.3 (49.8)
tBMC (mg/mm)	246.9 (36.0)	354.2 (42.0)	376.6 (48.0)	337.3 (40.8)
cCSA (mm^2)	198.5 (30.8)	271.1 (34.7)	288.8 (39.4)	260.2 (34.6)
cBMC (mg/mm)	207.0 (33.1)	310.9 (39.5)	334.8 (45.4)	287.9 (44.4)
cBMD (mg/cm^3)	1042.7 (28.1)	1134.6 (20.9)	1160.3 (21.6)	1103.6 (43.9)
CSMI (mm^4)	24750 (7421)	40268 (9408)	43363 (10840)	43481 (9252)
mCSA (mm^2)	4311 (833)	6326 (1060)	7012 (1057)	6656 (1048)
Radial Shaft				
tCSA (mm^2)	NA	101.8 (13.5)	108.0 (13.3)	113.6 (13.4)
tBMC (mg/mm)	NA	94.5 (10.2)	103.7 (11.6)	92.3 (13.7)
Hormones				
IGF-1 (nmol/L)	32.5 (12.7)	31.8 (7.8)	18.0 (4.8)	13.5 (5.1)
E2 (nmol/L)	0.09 (0.04 - 0.24)	0.14 (0.03 - 0.60)	0.14 (0.04 - 0.53)	0.06 (0.01 - 0.25)
T (nmol/L)	0.41 (0.05 - 1.38)	2.85 (0.85 - 9.51)	1.26 (0.41 - 3.83)	0.63 (0.19 - 2.12)

Data are presented as mean (SD), except for LTPA score, E2 and T, for which the median and its 5-95 percentile were given.
NA, not available, radial shaft was not scanned by pQCT at baseline.

5.2 Bone and muscle growth (Study I)

The timings of peak growth velocity of bone properties at tibial shaft and muscle cross-sectional area at left lower leg of peripubertal girls are shown in **Figure 10**. The peak velocity time (PVT) of the growth of tibial length and total CSA were located at around 20 months prior to menarche. The growth velocities of total BMC and muscle CSA peaked approximately 1 year later, at around 5 and 8 months prior to menarche, respectively. The PVT of cortical CSA was found to be 7 months prior to menarche, 2 months earlier than total BMC.

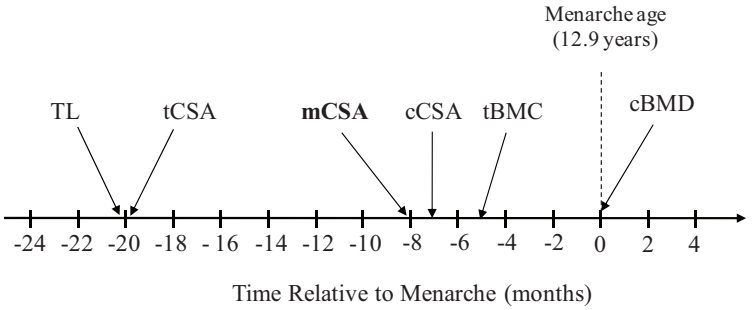

FIGURE 10 Peak velocity time (PVT) of the growth of bone and muscle variables at left tibial shaft. PVT of each variable was determined from the maximum in the growth velocity curve derived from the prediction equation of a hierarchical model.

No significant difference was found in bone muscle strength index (BMSI) from baseline to 3.5-year follow-up, but at 7-year-follow-up. When the girls were 18 years old, BMSI was higher than those at other measurement time points (**Figure 11**).

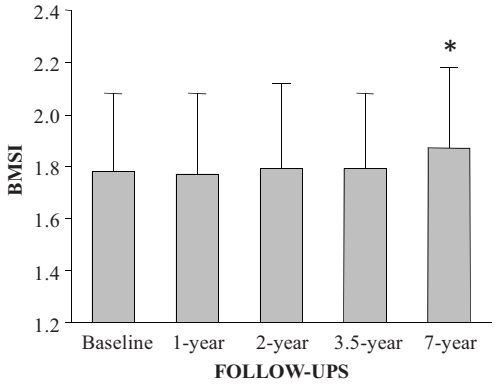

FIGURE 11 Comparison of bone muscle strength index (BMSI) at baseline (mean age 11.2 years), 1-, 2-, 3.5- and 7-year (mean age 18.3 years) follow-ups. Error bars represent SDs.
* $p<0.05$, compared to other time points by ANOVA

5.3 Effects of IGF-1 and sex steroids on bone growth (Study II)

IGF-1 was positively associated with tibial length, periosteal circumference (PC) and total BMC both before and after menarche, but not with cortical volumetric BMD. E2 level was associated with tibial length positively before menarche, but negatively after menarche. No associations were found between E2 and PC, total BMC or cortical volumetric BMD throughout growth after controlling for IGF-1 and T. Before menarche, T was positively associated with tibial length, PC, total BMC and cortical volumetric BMD, whereas after menarche T was only associated with PC (**Table 2**).

TABLE 2 Associations between hormones and bone variables at tibial shaft

Predictors	Outcome			
	TL	PC	tBMC	cBMD
Before menarche				
IGF-1	+	+	+	NS
E2	+	NS	NS	NS
T	+	+	+	+
After menarche				
IGF-1	+	+	+	NS
E2	−	NS	NS	NS
T	NS	+	NS	NS

+ and − indicate a significantly positive and negative association, respectively; NS represents no significant association was found. The associations were analyzed by a hierarchical model regressing bone variables (TL, PC, tBMC or cBMD) on TRM and its square and cube, IGF-1 and natural logarithm transformed E2 and T, with dummy variables indicating the respective period of before and after menarche.

After adjusted for muscle CSA, IGF-1 was only associated with tibial length before menarche, whereas the associations between E2 and bone properties did not change: positive before but negative after menarche. In addition, the positive associations between T and bone properties remained before menarche, but disappeared after menarche. Muscle CSA is positively associated with bone variables except for cBMD throughout growth (**Table 3**).

TABLE 3 Associations between hormones and bone variables at tibial shaft after adjustment for muscle cross-sectional area

Predictors	Outcome			
	TL	PC	tBMC	cBMD
Before menarche				
IGF-1	+	NS	NS	NS
E2	+	NS	NS	NS
T	+	+	+	+
mCSA	+	+	+	NS
After menarche				
IGF-1	NS	NS	NS	NS
E2	−	NS	NS	NS
T	NS	NS	NS	NS
mCSA	+	+	+	NS

+ and − indicate a significantly positive and negative association, respectively; NS represents no significant association was found. The associations were analyzed after introducing muscle CSA in addition to TRM and its square and cube, IGF-1 and natural logarithm transformed E2 and T.

5.4 Fat mass and bone mechanical competence (Study III)

Girls at age of 11 and 18 yrs and premenopausal women had similar relative bone strength index (RBSI) ($p>0.05$), whereas 13-year-old girls and postmenopausal women had lower RBSI than other age groups (all $p<0.05$) (**Figure 12**).

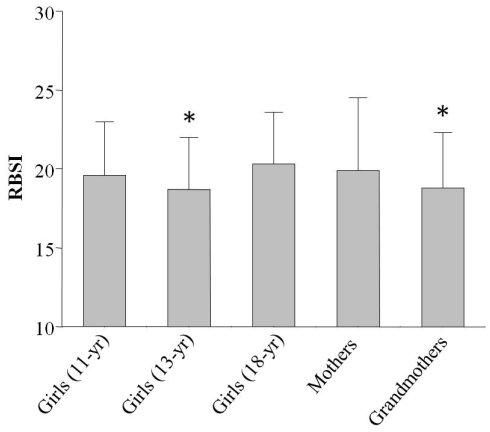

FIGURE 12 Comparison of relative bone strength index (RBSI) among different age groups. Error bars represent SDs.
* $p < 0.05$ vs. values of 11-, 18-yr-old girls or premenopausal women.

Table 4 shows the associations between RBSI and body weight, fat mass and lean mass. RBSI was negatively associated with body weight across age groups and generations (all $p<0.001$), with steeper regression slopes in pre- and postmenopausal women than in girls (all $p<0.05$). A similar pattern was found in the association between RBSI and fat mass. In contrast, no significant association between lean mass and RBSI was found in growing girls and premenopausal women (all $p>0.05$). Unexpectedly, in postmenopausal women, the association between lean mass and RBSI became negative ($p<0.001$).

TABLE 4 Associations of body weight, lean mass and fat mass with relative bone strength index (RBSI) in females in different age groups.

	RBSI				
	11-yr Old Girls	13-yr Old Girls	18-yr Old Girls	Premenopausal Mothers	Postmenopausal Grandmothers
Body weight	-0.065	-0.067	-0.077	-0.14*	-0.18*
ln (Fat mass)	-2.12	-2.05	-3.54	-6.26*	-7.08*
Lean mass	NS	NS	NS	NS	-0.26

Data are regression slopes (βs) obtained from linear regression models with bone parameters as dependent variables and weight, lean mass and natural logarithm transformed fat mass as independent variable, respectively.
ln, natural logarithm transformed.
*$p<0.05$, compared to girls; NS, not statistically significant

5.5 Relationship between bone accrual and bone loss (Study IV)

In the analysis of bone mass distribution, the measurements of the members of each girl-mother-grandmother trio were matched and treated as if they had been in one woman at three time points (i.e. young adulthood, middle and old age, respectively).

At the tibial shaft, more bone mass was located in the anterior and posterior regions than in the lateral and medial regions in all subjects, leading to an elliptical shape (**Figure 13A**). Compared with mothers, 18-yr-old girls had significantly less bone mass in the anterior and medial-posterior regions, whereas no difference was found in the lateral-posterior region (**Figure 13B**). The distribution of site-matched differences in BMC between mothers and grandmothers approximated the shape of butterfly, with the more bone mass reduction occurring in the anterior and posterior regions than in the lateral and medial regions (**Figure 13C**).

In contrast, the BMC distribution in radial shaft was more homogeneous in all directions, apart from a bulge in the ulnar direction (**Figure 14A**). Similarly, the site-matched difference of bone mass between the girls and the mothers was relatively homogenous except in the ulnar-lateral direction (**Figure 14B**). Compared with the mothers, the grandmothers' radial shaft had a similar mass in the ulnar region but uniformly less in other regions (**Figure 14C**).

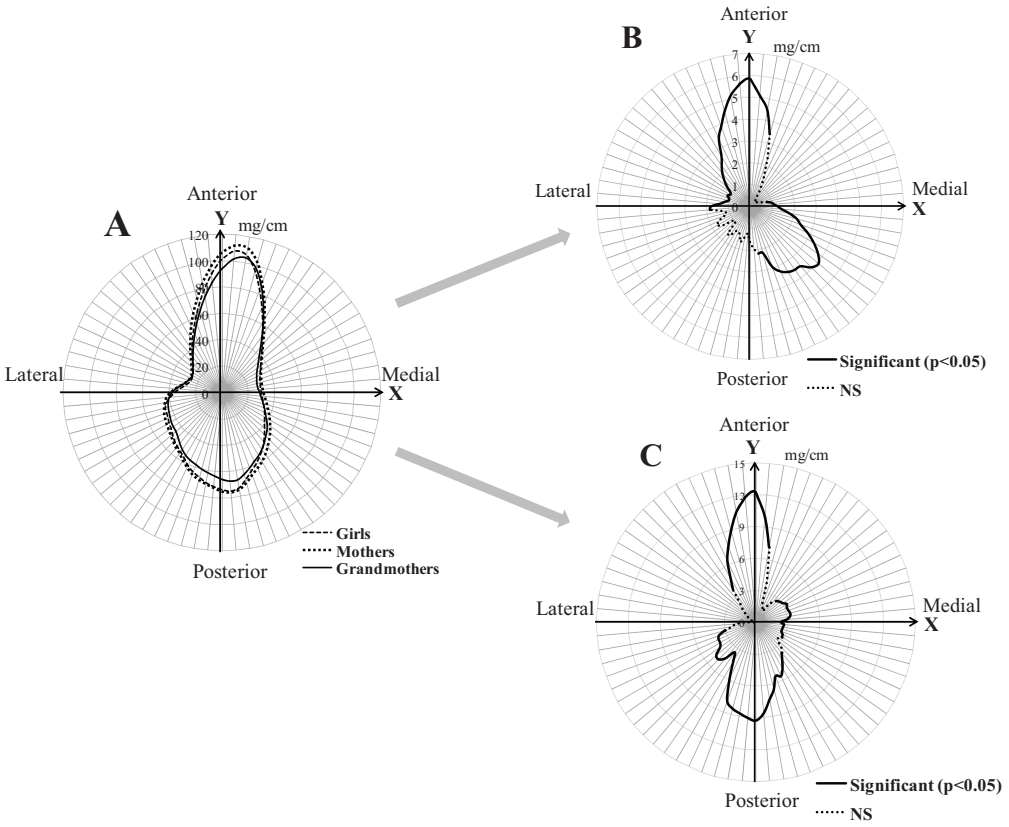

FIGURE 13 Polar distribution of BMC and site-matched BMC differences at tibial shaft. Panel **A**: polar distribution of BMC in girls, premenopausal mothers and postmenopausal grandmothers; Panel **B**: polar distribution of BMC differences between girls and mothers; Panel **C**: polar distribution of BMC differences between mothers and maternal grandmothers.

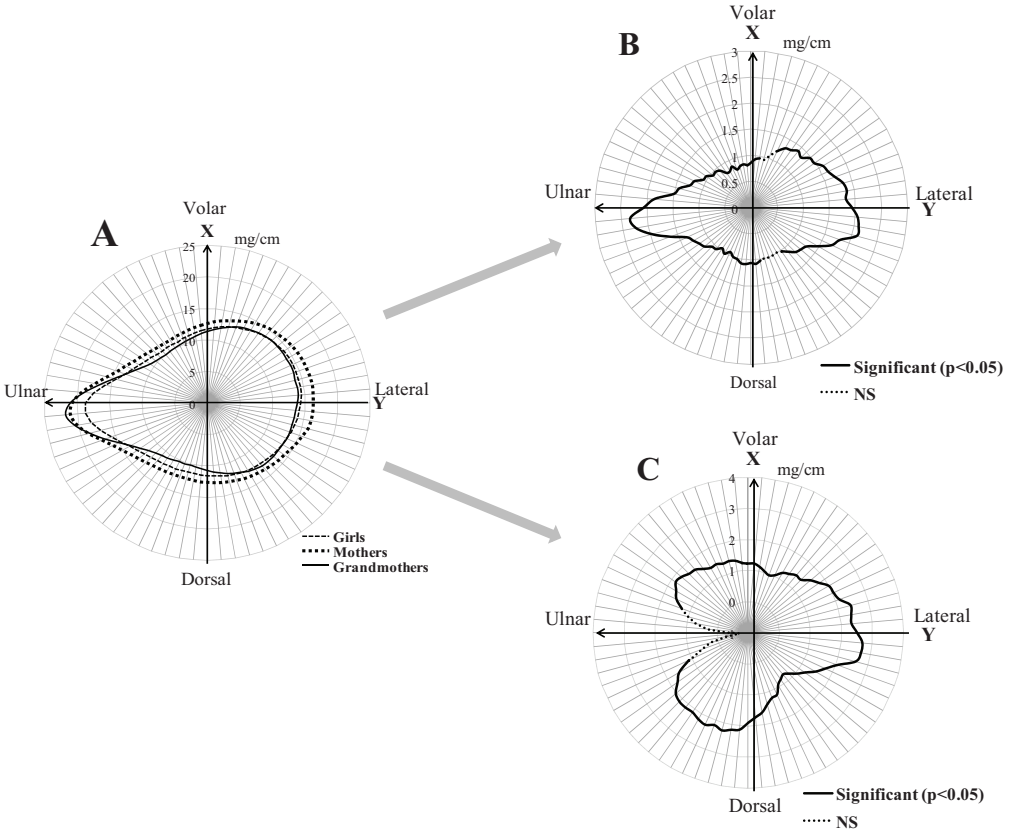

FIGURE 14 Polar distribution of BMC and site-matched BMC differences at radial shaft. Panel **A**: polar distribution of BMC in girls, premenopausal mothers and postmenopausal grandmothers; Panel **B**: polar distribution of BMC differences between girls and mothers; Panel **C**: polar distribution of BMC differences between mothers and maternal grandmothers

6 DISCUSSION

This study of the influences of muscle, fat and hormones on women's bone development had three components. *Firstly*, the growth pattern of bone-muscle unit and the hormonal effects were longitudinally explored. *Secondly*, the relationship between fat mass and bone mechanical competence was cross-sectionally examined in pubertal, early adult, pre- and postmenopausal adult women. *Finally*, the relationship between bone accrual and loss was assessed in girl-mother-grandmother trios.

6.1 Relationship between peripubertal bone and muscle growth

In our 7-year longitudinal study (I) from prepuberty to early adulthood, we found that the timings of peak growth velocities (PVTs) differ among tibial length, tCSA, tBMC, cCSA, cBMD and muscle CSA; whereas BMSI, an index indicating the equilibrium between bone strength and muscle force, remained relatively constant, suggesting an asynchronous but well-coordinated growth pattern of the bone-muscle unit during the rapid growth period.

The PVTs of bone length and total CSA preceded that of BMC and BMD, indicating that the mass accrual lags behind the size expansion of the bone. The rapid growth in bone dimensions without similar increase in bone mineral content and density may contribute to lower mineralization of bone tissue which is considered one of the reasons underlying the elevated risk of fracture during puberty (111, 261). In addition, both the PVTs of bone length and width (CSA) were located at around 20 months prior to menarche, indicating that bone lengthening and widening are synchronized. The synchrony between bone lengthening and widening may be significant in avoiding possible biomechanical disadvantages during rapid growth, because bone lengthening tends to undermine bone mechanical competence, while widening augments it (62, 89). However, the factors that coordinate growth in width relative to growth in length (i.e., slenderness) are unknown. It is possible that both bone lengthening and widening are controlled by the same set of genes controlling

body size, or controlled by different genes which became well-coordinated during evolution.

Muscle force is regarded as the primary driving force for bone growth, because bone constantly adapts to mechanical loads applied on it, which are considered primarily to arise from muscle contractions (19, 262). If this view is true, muscle growth must precede bone growth, since in a cause-and-effect relationship, the causal factor has to occur prior to its effects. In this study, we found that the growth velocity of muscle CSA peaked prior to that of bone mass increment. This result is consistent with previous report by Rauch et al. (242) and supports the view that muscle drives bone growth in mass. In contrast, the growth velocity of muscle CSA peaked one year later than tibial outer dimension (length and width), which does not accord with the postulated "muscle drives bone" paradigm and suggests that bone growth in size is relatively independent of muscle strength increment. Comparison of the timings of the peak growth rates cannot prove cause-and-effect relationships between variables, but it can *disprove* such relationships. Muscle force is probably not the primary driving force, but a powerful modifier, for pubertal bone size expansion.

6.2 Effects of IGF-1 and sex steroids on bone growth

The growth of the bone-muscle unit is under strong regulation by the growth and sex hormones (263). In study II, we found that circulating IGF-1 promotes peripubertal bone growth largely in a muscle-dependent fashion. The sex steroids strongly stimulate bone growth before menarche, but the stimulatory effects wane or become inhibitory after menarche. These results are largely but not totally in agreement with current views on the relationships between bone growth and related hormones (214, 219, 264).

There was a strong positive association between serum IGF-1 level and tibial length, and the association was independent of muscle CSA before menarche. This confirmed the essential role of circulating IGF-1 establishing and maintaining skeletal architecture during growth (192). In contrast, the positive association between serum IGF-1 and periosteal circumference disappeared after adjustment for muscle CSA, suggesting that circulating IGF-1 promotes periosteal apposition, to a large extent indirectly through stimulating muscle growth and hence increasing mechanical loading from growing muscle. This finding disagrees with previous reports that liver-derived IGF-1 stimulates periosteal apposition directly or via enhancing bone response to mechanical loading (33, 196), but accords with evidence from other studies against a direct effect of systemic IGF-1 on periosteal apposition (265). In addition, serum IGF-1 is associated with BMC, not with cortical volumetric BMD, suggesting that IGF-1 promotes bone mass accrual probably by adding more bone on the periosteal surface. But again, the effect is not independent of muscle growth.

In contrast to IGF-1, the effects of sex steroids on bone growth are largely independent of muscle growth. Estradiol level was associated with tibial length

positively before menarche, but negatively after menarche. This finding is consistent with the previously suggested biphasic effects of estrogen on longitudinal bone growth: at low level, estrogen stimulates growth, while higher levels of estrogen have potent inhibitory effects on longitudinal growth via accelerating epiphyseal closure (34, 73, 215). The positive association between testosterone level and tibial length before, but not after, menarche suggests that androgen promotes bone lengthening in women in early, but not late puberty. Therefore, the effect of androgen on longitudinal bone growth seems also "biphasic": it promotes endochondral bone growth before menarche, but this effect is arrested by estrogen-related growth plate closure after menarche.

It was assumed that estrogen and androgen have different endocrine effects on bone widening: androgen stimulates periosteal bone formation, while estrogen inhibits it (36, 103), which is regarded as being responsible for the development of a sexually dimorphic skeleton (36). Our results partially support this view, because estradiol levels were not associated with periosteal circumferences throughout growth, but testosterone were so associated before menarche. The smaller bone size in females is more likely due to their shorter prepubertal and pubertal growth periods (12) and earlier estrogen-related epiphyseal closure (73). In addition, In addition, testosterone, but not estradiol, was positively associated with BMC and volumetric BMD before menarche. This suggests that testosterone plays an important role in female bone consolidation during the growth spurt in females, whereas estrogen may play a permissive role in bone mass accrual by modifying the modeling and/or remodeling thresholds (266), and hence limiting or augmenting the anabolic effects of other hormonal or mechanical stimuli on bone.

6.3 Influence of fat mass on bone mechanical competence

It is best to evaluate the biomechanical stability of a bone in the context of the customary mechanical challenges which it has to withstand. For example, an elephant's bone, in absolute value, is much stronger than a mouse's. However, there is much less mechanical load applied on the mouse's bone. If adjusted for body weight, a mouse's bone may be as strong as, or even stronger than an elephant's. Similar phenomenon exists with regard to lean, normal weight and obese human. Therefore, in study III, the bone strength was evaluated by a newly-introduced parameter, relative bone strength index (RBSI), which reflects the bone strength relative to the applied load.

We found that RBSI was similar in 11- and 18-year-old girls and premenopausal women. This is consistent with the idea that bone adapts to load to maintain an adequate safety margin without extra bulk. Lower RBSI implies a reduced ability of bone to sustain the imposed load, and thus a greater vulnerability to fracture. Indeed, girls aged around 13 yrs and postmenopausal women had a significantly lower RBSI compared to other age groups. This is in

accordance with the known peaks of peripheral limb fracture incidence in peripubertal girls (267, 268) and in postmenopausal women (269, 270).

RBSI was inversely associated with body weight across age groups and generations, suggesting a relative bone strength deficit associated with greater load from body weight. The slopes of the regression lines were steeper in pre- and postmenopausal women than in girls, indicating that every unit increase in body weight was associated with a greater decrease in RBSI in adult women than in girls. This indicates that the bone strength deficit is age-dependent. RBSI was inversely associated with fat mass in girls and in adults, with steeper regression slopes in pre- and postmenopausal women than in girls, suggesting that bone does not adapt sufficiently to the mechanical loads from fat. This is similar to the pattern of the relationship between RBSI and body weight. By contrast, RBSI remained relatively constant with increasing lean mass in girls and premenopausal women, indicating that bone adapts its strength efficiently to the mechanical challenge from lean mass. Since body weight is mainly comprised of lean mass and fat mass, we can make an inference that the relative bone strength deficit to body weight gain is attributable to the fat mass accumulation, not lean mass increment. In addition, the adverse effect of fat mass on bone mechanical competence is age-dependent, which is probably due to different adaptive capacity of young, adult and senile bone to increasing load.

6.4 Relationship between bone accrual and bone loss

In the Study IV, the relationship between bone accrual and bone loss was addressed by comparing the bone mass distribution at the shafts of weight-bearing (tibia) and non-weight-bearing (radius) bones in girl-mother-maternal grandmother trios. The differences in bone mass between mothers and grandmothers, as expected, were mainly found in the anterior and posterior regions; whereas the most pronounced girl-mother differences, unexpectedly, existed in the anterior and medial-posterior regions. Similarly, the pattern of bone loss at radial shaft approximated to, but did not precisely mirror, that of bone accrual, although both of them were more uniform than at tibial shaft. These findings did not perfectly support the "bone loss is the reversal of accrual" hypothesis. The bone loss in old age is largely, but not completely, a reversal of the preferential deposition of bone in the most highly loaded regions during early life.

The partial discordance between the patterns of bone accrual and loss suggested that the change of the strains along the tibial and radial shaft was not homogeneous from site to site throughout life. For example, in the lateral-posterior region of the tibial shaft, the girls' BMC resembled their mothers' value, indicating that in this region the bone mass has already approximately reached its peak value at age 18 years. In contrast, the bone mass increased pronouncedly from early adulthood to middle age in the medial-posterior region of the tibial shaft and on the ulnar side of the radial shaft, whereas less or no significant bone loss occurred in these two regions from middle to old age.

This is probably due to the fact that the soleus muscle in the lower leg and the interosseous membranes connecting the radius and ulna frequently exert great mechanical load in these two regions, stimulating bone accrual and preventing bone loss. Despite the complex biomechanical surroundings, the possibility cannot be ruled out that the bone mass accrual and loss patterns are largely genetically determined.

6.5 Limitations

The studies have some limitations. The influences of other factors which may contribute to bone growth, such as exercise, vitamin D, calcium, protein and other nutrients intake, were not taken into consideration in studies I & II. Therefore, they are limited in their ability to provide a comprehensive view of peripubertal bone growth. In addition, in study II, the serum level of sex steroids showed a large variation, although the timing of menstruation had been strictly controlled for throughout the study, indicating that one snapshot measurement cannot reflect the real hormonal exposure.

In study III, RBSI may not have reflected all the factors which determine the bone stability, because the calculation of BSI only included the cortical volumetric BMD and cross-sectional moment of inertia, without taking into consideration the material and structural traits of the trabecular compartment and collagen characteristics which may also have substantial influences on bone strength. However, the agreement of the timing of the trough in RBSI in girls with the observed peak in childhood fractures and in postmenopausal women who had higher risk of fracture indicates that RBSI captures important aspects of bone biomechanical competence. Moreover, the consistency of RBSI among 11-, 18-year-old girls and premenopausal women is in accordance with the evolutionary view that bone is designed by nature to meet the conventional biomechanical needs of a species.

In study IV, the age of mothers (around 43 years old) in this study was older than that of peak bone mass (30-39 yrs). Thus the continued modification of bone mass distribution (either increase or decrease) may be underestimated.

The studies I, II, III and IV were all based on bone measurements from limb bones (tibia and radius) in women. Bone growth and loss do not take place at a uniform rate throughout the skeleton; bone structure and mechanical properties differ between genders and among different skeletal sites, and the relationships between bone, muscle, fat and hormones may also be gender- and site-specific. Therefore, care should be taken if seeking to generalize from our results to other skeletal sites or to males.

7 MAIN FINDINGS AND CONCLUSIONS

On the basis of our results, we conclude that:

1) The growth of muscle lags behind growth of bone size but precedes that of bone mass in pubertal girls. This finding does not support the hypothesis that muscle force drives the growth of bone size, whereas the possibility remains that muscle exerts an effect on bone mass accrual. In addition, BMSI remained relatively constant throughout puberty, suggesting an asynchronous but well-coordinated development of the bone-muscle unit.

2) Circulating IGF-1 promotes peripubertal bone growth largely in a muscle-dependent fashion. The effects of sex steroids on peripubertal bone growth are time-dependent. They strongly stimulate bone growth before menarche, but the stimulatory effects wane or become inhibitory after menarche. These findings imply that the timing of menarche is critical for peripubertal bone growth.

3) Bone does not strengthen adequately to maintain equilibrium with the load from greater body weight, leading to an age-dependent relative bone strength deficit in women from peripuberty to postmenopause. This is largely due to the fact that the weight gain in women from puberty onwards is, for the most part, attributable to fat mass accumulation, and the beneficial effects of increased fat mass on bone, if any, do not compensate for the mechanical burden that it imposes.

4) The distribution of bone mass undergoes modification from early adulthood through middle age into old age. Both bone accrual and loss are direction-specific in weight-bearing bone, but relatively uniform in non-weight-bearing bone. The bone loss in old age is largely, though not completely, a reversal of the preferential deposition of bone during early life.

TIIVISTELMÄ

Luukato eli osteoporoosi on merkittävä terveysongelma iäkkäillä. Osteoporoosin juuret voivat kuitenkin olla lapsuudessa ja nuoruudessa, jolloin saavutetaan merkittävä osa luunhuippumassasta. Tämän vuoksi luuston kasvua ja luukudoksen uudismuodostusta säätelevien mekaanisten ja hormonaalisten tekijöiden ymmärtäminen kasvuiässä on tärkeää luun optimaalisen huippumassan saavuttamiseksi ja osteoporoottisten murtumien ehkäisemiseksi. Tämän tutkimuksen tavoitteena oli selvittää lihas- ja rasvakudoksen sekä hormonaalisten tekijöiden vaikutuksia luuston kasvuun ja kehitykseen naisilla. Tutkittavina oli 396 alun perin 10-13–vuotiasta tyttöä, 257 äitiä ja 154 isoäitiä. Kehon koostumus määritettiin kaksienergiaisella röntgenabsorptiometriamenetelmällä (DXA) ja luun mineraalitiheys perifeerisen kvantitatiivisen tietokonetomografian (pQCT) avulla. Hormonimääritykset suoritettiin immunofluorimetrisesti tai entsyymivälitteisen immunosorbenttimäärityksen (ELISA) avulla. Tulokset osoittivat, että tytöillä luun pituus- ja leveyskasvu edeltää lihasmassan kasvua, mutta lihasmassan kasvu edeltää luumassan kasvua. Tämä tulos ei tue aikaisempaa käsitystä, jonka mukaan luun koko kasvaa lihasvälitteisesti, mutta vahvistaa käsitystä, jonka mukaan lihaksen kasvu edeltää luumassan kasvua. Hormonaaliset muutokset kasvuiässä tukevat tätä havaintoa, sillä insuliinin kaltaisen kasvutekijän (IGF-1) havaittiin edistävän luun kasvua puberteetti-iässä pääasiassa lihavälitteisesti. Estradiolin ja testosteronin vaikutukset luuston kasvuun puberteetti-iässä ovat puolestaan aikariippuvaisia. Ennen kuukautisten alkamista estradioli ja testosteroni kiihdyttävät luun kasvua, mutta kuukautisten alkamisen jälkeen niiden kasvua kiihdyttävät vaikutukset vaimenevat tai muuttuvat luun kasvua ehkäiseviksi. Tarkasteltaessa luun lujuuden mukautumista kehon painoon havaittiin, että naisilla luun lujuus suhteessa kasvavaan kehon painoon heikkenee iän myötä, johtuen kehon rasvamassan kasvusta. Ikääntyessä myös luumassan jakautumisessa havaittiin muutoksia. Kasvun vaikutuksia luustoon pyrittiin kuvaamaan vertaamalla tyttöjen kehonpainoa kantavan sääriluun ja painoa kantamattoman värttinäluun luumassan jakaumaa heidän äitiensä luumassan jakaumaan ja vastaavasti ikääntymisen vaikutuksia vertaamalla äitejä isoäiteihin. Äideillä oli tyttöjä enemmän luukudosta sääriluun anteriorisella ja mediaalis-posteriorisella alueella. Isoäideillä puolestaan oli vähemmän luukudosta anteriorisella ja posteriorisella alueella kuin äideillä. Luumassan eroavaisuudet värttinäluussa sen sijaan olivat samansuuntaisia tyttärien, äitien ja isoäitien välillä. Erot sääri- ja värttinäluun välillä viittaavat siihen, että mekaaninen kuormitus ohjaa luumassan jakautumista. Koska luumassa ei ikääntyessä vähene täysin samoilta alueilta kuin missä se kasvun aikana eniten lisääntyy, luumassan kato ei ole luumassan kasvun täydellinen peilikuva. Kaiken kaikkiaan tutkimuksen tulokset viittaavat siihen, että luun koon kasvu murrosiässä ei ole riippuvainen lihasmassan kasvusta. IGF-1, estradiolin ja testosteronin vaikutukset luun kasvuun ennen kuukautisten alkamista ovat ensisijaisen tärkeitä luuston optimaaliselle kasvulle. Kehon rasvamassan lisääntyminen heikentää luun mukautumiskykyä kehon painon kasvusta aiheutuvaan kuormitukseen. Luukato myöhemmällä iällä on suurelta osin käänteinen ilmiö luun kehitykselle kasvuiässä.

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ORIGINAL PAPERS

I

BONE AND MUSCLE DEVELOPMENT DURING PUBERTY IN GIRLS: A SEVEN-YEAR LONGITUDINAL STUDY

by

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Bone and Muscle Development During Puberty in Girls: A Seven-Year Longitudinal Study

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ABSTRACT: The growth of lean mass precedes that of bone mass, suggesting that muscle plays an important role in the growth of bone. However, to date, no study has directly followed the growth of bone and muscle size through puberty and into adulthood. This study aimed to test the hypothesis that the growth of muscle size precedes that of bone size (width and length) and mass during puberty. Bone and muscle properties were measured using pQCT and DXA in 258 healthy girls at baseline (mean age, 11.2 yr) and 1-, 2-, 3–4- and 7-yr follow-up. Growth trends as a function of time relative to menarche were determined from prepuberty to early adulthood for tibial length (TL), total cross-sectional area (tCSA), cortical CSA (cCSA), total BMC (tBMC), cortical volumetric BMD (cBMD), and muscle CSA (mCSA) in hierarchical models. The timings of the peak growth velocities for these variables were calculated. Seventy premenopausal adults, comprising a subset of the girl's mothers (mean age, 41.5 yr), were included for comparative purposes. In contrast to our hypothesis, the growth velocity of mCSA peaked 1 yr later than that of tibial outer dimensions (TL and tCSA) and slightly earlier than tBMC. Whereas TL ceased to increase 2 yr after menarche, tCSA, cCSA, tBMC, and mCSA continued to increase and were still significantly lower than adult values at the age of 18 yr (all $p < 0.01$). The results do not support the view that muscle force drives the growth of bone size during puberty.
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Key words: BMD, bone growth, girls, muscle cross-sectional area, peak growth velocity time, puberty

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INTRODUCTION

The growth of bone and muscle is inseparably associated. Bone adapts its structure and strength to withstand mechanical loads, which are considered primarily to arise from muscle contractions.[1–4] The balance between the strength of bone and the force applied on it is established and maintained during growth.

Both bone and muscle characteristics are largely genetically determined,[5] and both are under the strong influence of growth hormone IGF-I and sex steroids. However, how the developing bone and muscle relate to each other during growth remains unclear. It is important to understand this relationship, for example, for promoting bone growth without inducing injuries. Rauch et al.[6] reported that the growth of total body lean mass preceded that of BMC during puberty and interpreted this as supportive of the view that bone adapts its strength to the muscle contraction during growth. However, lean mass is not the same as muscle mass, and BMC is not the same as bone size or strength. Hence, further data are needed to probe this complex relationship of bone and muscle during growth.

In this study, we aimed to test the hypothesis that growth of muscle size precedes that of bone size and mass as measured in the middle of lower leg in pubertal girls in a 7-yr longitudinal study spanning from prepuberty to early adulthood.

The authors state that they have no conflicts of interest.

MATERIALS AND METHODS

Subjects

A total of 258 healthy girls 10–13 yr of age (mean age, 11.2 yr) were recruited from local schools in the city of Jyväskylä and its surroundings in central Finland. These girls were initially enrolled in a calcium and vitamin D intervention (CALEX study) during the first 2 yr.[7,8] After the conclusion of the intervention, these girls were further invited to participate in bone measurements at 3–4- and 7-yr follow-up. To be eligible for the study, the participants had to have no history of medical conditions or medications known to affect bone metabolism. Because no intervention effects[9] on bone structural and material properties and mCSA were found at follow-up, data were pooled for this study.

Body weight and height were measured, with subjects wearing light clothes and on bare feet. The age at menarche was defined as the first onset of menstrual bleeding and was determined by questionnaire or phone call during the follow-up. Among the 258 girls at baseline, 200 girls were

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present at 1-yr, 221 at 2-yr, 87 at 3–4-yr, and 102 at 7-yr follow-up. The main reasons for dropout were loss of interest, lack of time, and relocation. In addition, 255 mothers (age, 32–58.9 yr) were invited for participation. Among them, 70 were premenopausal (mean age, 41.5 yr; range, 32–45 yr) and were included in this study to provide adult values for comparative purposes. The study protocol was approved by the ethical committee of the University of Jyväskylä, the Central Hospital of Central Finland, and the Finnish National Agency of Medicines. Informed consent was given by all subjects and their parents before the assessments.

Bone measurement

The left lower leg was scanned using pQCT (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany). The scan location was at 60% of lower leg length up from the lateral malleolus of the fibula. The in-plane pixel size was 0.59 × 0.59 mm. Total bone cross-sectional area (tCSA, mm^2), cortical cross-sectional area (cCSA, mm^2), total BMC (tBMC, mg/mm), cortical volumetric BMD (cBMD, mg/cm^3), and polar cross-sectional moment of inertia (CSMI, mm^4) were analyzed by Stratec software. The threshold for tCSA was 280 mg/cm^3 and for cCSA and cBMD was 710 mg/cm^3. The muscle CSA (mCSA, mm^2) was analyzed using validated software (Geanie 2.1, GeanieCE; Commit, Espoo, Finland). A contour was drawn manually along the outer boundary of muscle to eliminate the subcutaneous adipose tissue before analysis. The threshold for muscle was 10–279 mg/cm^3. The CV of two repeated measurements on the same subject on the same day was on average 1% for tCSA, cCSA, mCSA, and tBMC and <1% for cBMD.

The tibial length (TL, mm) was measured from DXA scans (Prodigy; GE Lunar, Madison, WI, USA). TL was defined as the distance between the proximal edge of tibia (middle point of the line from medial to lateral condyle) and distal border of tibia. The CV of three repeated measurements of TL was <1%.

Bone–muscle strength index (BMSI) was calculated as BSI/(mCSA × TL), where bone strength index (BSI) was the product of area moment of inertia and cBMD.[10,11] The product of mCSA and TL represented the bending moment exerted on the tibia by muscle on the lever length of the tibia.[12]

Statistical analysis

All data were checked for normality using Shapiro-Wilk test in SPSS 15.0 for Windows. Student's *t*-test was used for comparison between girls and adults. Hierarchical nonlinear models with random effects were used to depict the growth of bone and muscle parameters (MLwiN 2.02 software; Institute of Education, University of London, London, UK). The hierarchical model allows inclusion of the data from every subject regardless of irregularity of temporally spaced follow-up or missing data. Time relative to menarche (TRM), instead of age, was entered as the explanatory variable in the form of polynomial functions to explain the growth of target variables. The time of menarche itself was selected as a shift knot for the function curve, which means that the coefficients of independent variables could be different on either side of this time point, but the function curve remains smooth and continuous. Up to fifth-order polynomial functions were used in the models. The best model was determined by three criteria: the largest reduction in deviance test ($-2 \times$ log-likelihood by iterative generalized least squares [IGLS]), the lowest within-individual variance, and the necessary parsimony of the model.[13]

To make variables of different scales comparable, the original values of TL, tCSA, cCSA, mCSA, tBMC, and cBMD were transformed into T-scores [(girl's value − mean of adult values)/(SD of adult values)]. Growth velocities of TL, tCSA, cCSA, tBMC, cBMD, and mCSA, in terms of T-score change per month, were calculated from the predictive equation for each variable. The growth velocity was computed as the difference between predicted values at two adjacent time points divided by the time span between these two measurements. The growth velocity was plotted against TRM, and the peak velocity time (PVT) was determined from the maximum in the growth velocity curve.

RESULTS

The mean age of the subjects was 11.2 ± 0.7 yr at baseline and 17.8 ± 1.0 yr at 7-yr follow-up. The mean duration of follow-up was 7.5 yr. The mean age at menarche was 12.9 yr. At 18 yr of age (7-yr follow-up), girls already had similar body height and TL as adults, whereas the tibial tCSA, cCSA, tBMC, cBMD, and mCSA were still significantly lower (all were $p < 0.01$) than adults (Table 1).

The growth patterns of tibial length, tCSA, cCSA, tBMC, volumetric cBMD, and mCSA are shown in Fig. 1. All these parameters increased before or around menarche. Whereas tibial length ceased to increase at ~24 mo after menarche (15 yr old), the tibial tCSA, cCSA, tBMC, cBMD, and mCSA kept increasing throughout the whole follow-up period, but at a lower velocity 24 mo after menarche (Fig. 1).

Growth velocities of TL and tCSA peaked 20 mo before menarche. In contrast, the growth velocities of tBMC and mCSA peaked ~1 yr later, at 5 and 8 mo before menarche, respectively. The growth velocity of cCSA continued to accelerate despite the slowing down of tCSA expansion and peaked at 7 mo before menarche, which was 2 mo earlier than tBMC (Fig. 2).

BMSI, quantifying the balance of bone strength to the load on it from muscle contraction, was independent of age (Fig. 3) and remained relatively constant from prepuberty to early adulthood. BMSI was 1.78 ± 0.30 at baseline and 1.84 ± 0.25 at 18 yr old. No significant difference was found between BMSI at baseline and 7-yr follow-up ($p = 0.075$).

DISCUSSION

In this 7-yr longitudinal study from prepuberty to early adulthood, we found that the growth velocity of mCSA peaked 1 yr later than tibial size (TL and tCSA) but 1, 3, and 8 mo earlier than cCSA, tBMC, and cBMD,

TABLE 1. Physical Characteristics of Participants at Baseline and 7-yr Follow-Up Compared With Adults

	Baseline	*7-yr follow-up*	*Adults*	*p**	*T-score*†
N	258	102	70		
TRM (mo)	−21.1 ± 12.9	59.2 ± 15.7			
Age (yr)	11.2 ± 0.8	17.9 ± 1.0	41.5 ± 2.9		
Height (cm)	145.6 ± 8.0	165.2 ± 6.1	165.8 ± 6.1	0.527	−0.09 ± 1.00
Weight (kg)	39.2 ± 8.7	58.9 ± 10.3	71.3 ± 15.1	<0.001	−0.82 ± 0.68
TL (mm)	333.5 ± 23.7	372.8 ± 17.7	370.8 ± 19.9	0.490	0.10 ± 0.89
tCSA (mm^2)	372.5 ± 54.7	455.2 ± 53.6	482.5 ± 62.5	0.003	−0.44 ± 0.86
cCSA (mm^2)	198.5 ± 30.8	266.0 ± 33.5	288.1 ± 37.8	0.001	−0.58 ± 0.89
tBMC (mg/mm)	246.9 ± 36.0	342.8 ± 40.0	374.9 ± 45.8	<0.001	−0.70 ± 0.87
cBMD (mg/cm^3)	1043 ± 28	1141 ± 23	1163 ± 24	<0.001	−0.91 ± 0.94
mCSA (mm^2)	4311 ± 833	6174 ± 1024	6800 ± 1091	<0.001	−0.57 ± 0.94

Data are given as mean ± SD.

* *p* for comparison between 7-yr follow-up and adult values by Student's *t*-test.

† T-score at 7-yr follow-up calculated as (girl's value − mean of adult values)/(SD of adult values). Data for the group of mothers were used to represent adult values.

respectively. Despite the asynchrony in growth velocities, BMSI, which takes into consideration both bone structural and material properties, remained relatively constant throughout the whole growth period, suggesting a well-coordinated, although asynchronous, development of the bone–muscle unit. In addition, whereas the growth of TL was essentially completed 2 yr after menarche, tCSA, cCSA, tBMC, cBMD, and mCSA continued to increase, and at the age of 18 yr, were still significantly lower than adults' values.

The growth of bone length takes place by endochondral ossification in the growth plates, whereas the increase in diaphyseal width is driven by periosteal apposition.[14] Bone lengthening tends to undermine bone mechanical competence, whereas widening augments it.[14–16] Our results showed marked similarity between the growth patterns of bone length and CSA in which their growth velocities peaked simultaneously at 20 mo before menarche. The mechanism underlying the concerted growth of bone length and width is unknown. Bone lengthening and widening are possibly controlled by the same set of genes controlling body size or controlled by different genes, which became well coordinated during evolution. However, mechanical loading may play an important role that cannot be neglected, as shown by studies of children with cerebral palsy in which the growth in length and width is lower than healthy controls.[17,18] Furthermore, nutrition, stress, and other factors also play a role in the growth of bone length and width. For a given mass, the bending strength of bone is proportional to the fourth order of its diameter. A more slender tibia, as indicated by the ratio between long bone width and length,[19,20] has been shown to be a major predictor of stress fracture risk and fragility in certain populations.[21–23] Bearing this in mind, the synchrony between bone lengthening and widening may be significant in avoiding the possible biomechanical disadvantage of relative bone slenderness during rapid growth. Furthermore, our data confirm that, after bone elongation had ceased, bone width continued to increase, although at a slower speed, into early adulthood, increasing bone strength, coinciding with the wane in fracture risk in late adolescence.

The accrual of bone mass lags behind the growth of bone dimension by >1 yr. This asynchrony may contribute to lower mineralization of bone tissue or more porous cortex associated with rapid growth in bone size,[24] a view that is supported by our observation that cortical BMD increased rapidly coinciding with BMC accrual but not with bone size. The rapid growth in bone dimensions without similar increase in BMC and BMD during early puberty is considered one of the reasons underlying the elevated risk of fracture.[13,25,26]

The mechanical loads from muscles are believed to dominate the postnatal development of whole bone strength and mass.[27] However, at present, bone strength can not be directly measured in human in vivo, but is usually estimated from the measurable bone properties, including mainly the properties of bone material and arrangement of this material in space—the size and shape of the bone. If muscle force is the primary driving force of bone development, we would expect the peak in growth velocity of mCSA, which has been widely accepted as a surrogate for muscle strength, to precede that of those bone strength determinants. However, we found the opposite: the growth peak of bone length and width preceded that of muscle area. On the other hand, the growth velocities of tBMC and cBMD peaked a little later than that of muscle area, consistent with an early report by Rauch et al.[6] The distribution of bone mass further away from its neutral axis is more important than the amount of mass in determining the bone strength, and precedence is a necessary condition for discerning a cause-and-effect relationship; hence, our study indicates that bone growth in size is relatively independent of muscle strength increment.

The growth velocity of cCSA continued to accelerate despite the slowing down of tCSA expansion. The slight precedence of PVT of cCSA over that of tBMC indicated that continuing bone mineral accrual after the peak of periosteal bone apposition is not only caused by endosteal bone formation but also increased mineralization of bone

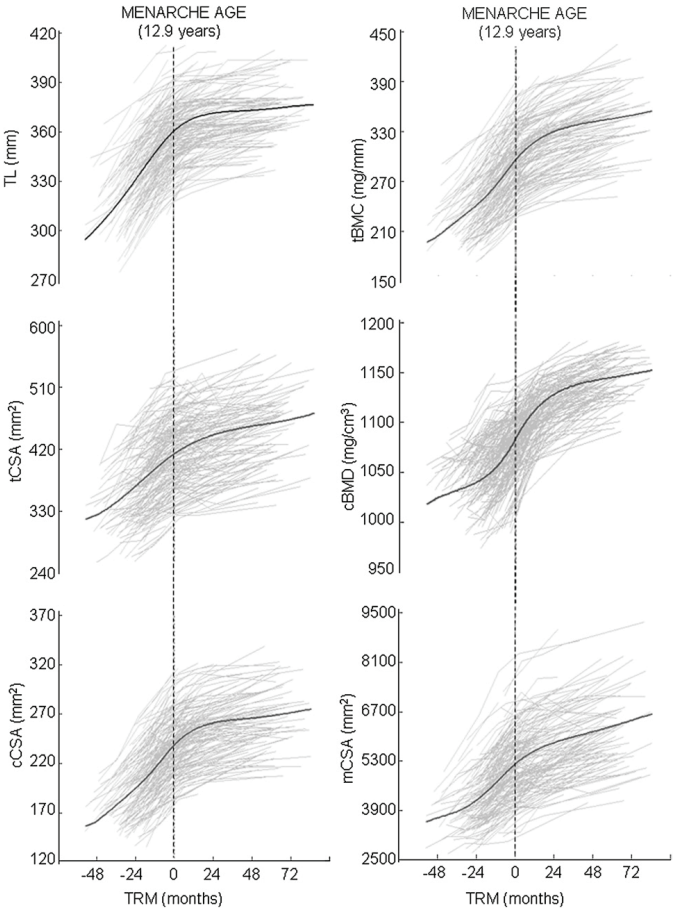

FIG. 1. Growth patterns of TL, tCSA, cCSA, mCSA, tBMC, and cBMD. Data for TL, tCSA, cCSA, mCSA, tBMC, and cBMD are plotted against time relative to menarche (TRM). Gray lines represent longitudinal change of each individual's values and the black lines are the best fitting lines derived from hierarchical models.

tissue. Because cCSA and BMC growth peaked a little later than mCSA, the possibility can not be ruled out that increasing muscle strength exerts a positive effect on mineral accrual of growing bone through enhancing endosteal bone formation and bone tissue mineralization.

The view that muscle is the primary source of stress applied on bone may oversimplify the bone–muscle relationship. For example, for a given muscle strength, the bone mass is 30% greater in the lower than the upper limbs in pubertal girls.[28] After space flight, the loss of bone mass in the weight-bearing sites is tremendous, whereas no or very little loss occurs in the upper limbs.[29,30] Although muscle strength is maintained by strength training in the lower limb during spaceflight, bone mass is lost dramatically.[31] These findings imply that, in addition to muscle forces, mechanical loads from the external environment such as the constant compression from gravitational forces and impact forces during normal ambulation, even including the inertial forces during dynamic motion, may also influence bone strength to a significant extent.

Despite the asynchrony in growth velocities of bone dimension, mineral accrual, and muscle CSA, it is interesting to note that the BMSI, which takes into account all these parameters, remained relatively constant over the 7-yr period, suggesting that the overall mechanical competence of the bone–muscle unit is maintained, in a well-coordinated fashion, from prepuberty to early adulthood. In comparison with muscle, bone growth is a more complex process, with various structural and material traits developing differently. In early puberty, bone diameter is

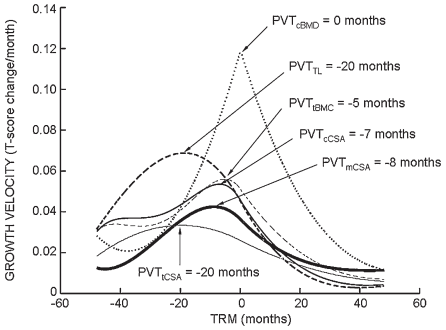

FIG. 2. Growth velocities of bone and muscle variables in terms of T-score change per month. T-scores of TL, tCSA, cCSA, tBMC, cBMD, and mCSA were calculated as (girl's value − mean of adult values)/(SD of adult values). Data for the group of mothers were used to represent adult values.

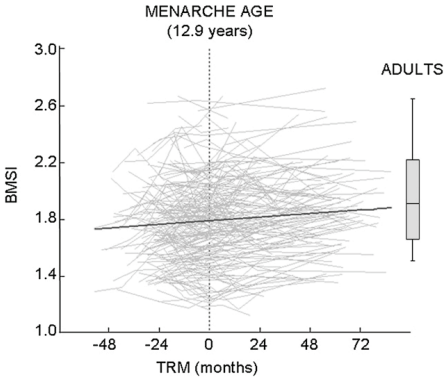

FIG. 3. BMSI during growth. BMSIs were modeled using hierarchical nonlinear models with random effects. Adult values are indicated by box with error bars (mean and 95% CI). Gray lines represent longitudinal change of each individual's BMSI, and the black lines are the best fitting lines derived from a hierarchical model.

increased by rapid periosteal apposition, which slows down in later puberty. However, endocortical bone formation ensues continuing to increase cortical thickness and bone mass under the influence of increased estrogen exposure.[13,32,33] Furthermore, the increased degree of bone tissue mineralization in late puberty also increases the bone strength. Hence, the constancy of bone–muscle strength index is achieved by different growth processes of bone at different stages of growth.

This study was subject to some limitations. Results obtained in the lower leg cannot necessarily be applied to other skeletal sites. Bone growth does not take place at a uniform rate throughout the skeleton,[34] and the relationship between bone and muscle may also be site specific. It is important to note that the timing of the peak growth rates cannot prove cause-and-effect between variables, but it can disprove such relationships. Precedence is a necessary precondition for causality; hence, the fact that growth in bone size preceded muscle growth confirms that muscle growth is not the cause of bone growth. Our use of T-scores implies an assumption that the girls values will ultimately equal their mothers' values, which may not be the case given secular changes. However, we considered T-scores to be the most appropriate way to normalize the girl's growth data and compare growth trends among very different properties. Use of T-scores will not have affected our results concerning the timing of peak growth.

In summary, we found that the growth of muscle lags behind growth in bone size in pubertal girls. This contradicts the hypothesis that muscle force drives the growth of bone size, although the possibility remains that muscle exerts an effect on bone mass accrual.

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II

CONCERTED ACTIONS OF INSULIN-LIKE GROWTH FACTOR-1, TESTOSTERONE AND ESTRADIOL ON PERIPUBERTAL BONE GROWTH - A 7-YEAR LONGITUDINAL STUDY

by

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Concerted Actions of Insulin-Like Growth Factor 1, Testosterone, and Estradiol on Peripubertal Bone Growth: A 7-Year Longitudinal Study

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ABSTRACT

A better understanding of how bone growth is regulated during peripuberty is important for optimizing the attainment of peak bone mass and for the prevention of osteoporosis in later life. In this report we used hierarchical models to evaluate the associations of insulin-like growth factor 1 (IGF-1), estradiol (E_2), and testosterone (T) with peripubertal bone growth in a 7-year longitudinal study. Two-hundred and fifty-eight healthy girls were assessed at baseline (mean age 11.2 years) and at 1, 2, 3.5, and 7 years. Serum concentrations of IGF-1, E_2, and T were determined. Musculoskeletal properties in the left lower leg were measured using peripheral quantitative computed tomography (pQCT). Serum levels of IGF-1, E_2, and T increased dramatically before menarche, whereas they decreased, plateaued, or increased at a lower rate, respectively, after menarche. IGF-1 level was positively associated with periosteal circumference (PC) and total bone mineral content (tBMC) throughout peripuberty but not after adjustment for muscle cross-sectional area (mCSA). On the other hand, IGF-1 was associated with tibial length (TL) independently of mCSA before menarche. T was positively associated with TL, PC, tBMC, and cortical volumetric bone mineral density, independent of mCSA, before menarche but not after. E_2 was associated with TL positively before menarche but negatively after menarche. These findings suggest that during puberty, circulating IGF-1 promotes bone periosteal apposition and mass accrual indirectly, probably through stimulating muscle growth, whereas the effects of sex steroids on bone growth differ before and after menarche, presenting a biphasic pattern. Hence the concerted actions of these hormones are essential for optimal bone development in peripuberty. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: IGF-1; ESTRADIOL; TESTOSTERONE; BONE LENGTH; PERIOSTEAL CIRCUMFERENCE; BONE MASS

Introduction

Bone traits track from the position established early in life, and hence the risk of fragility fractures in old age has its origin during growth.[1,2] Bone growth from the onset of puberty to young adulthood is critical because bone mass approximately doubles during this period.[3] Understanding how bone growth is regulated by mechanical and hormonal factors is important for optimizing the attainment of peak bone mass and to prevent or postpone the occurrence of fragility fracture.[4]

Muscle force is considered the primary driving force for bone development[5–10] because it produces the dominant mechanical loads to which the bone adapts its structure and mass.[9,11,12] Bone growth is also under the strong influence of the hormonal milieu.[1,13–16] Impaired bone growth has been observed in patients with growth hormone (GH) deficiency[17] and in transgenic mice lacking insulin-like growth factor 1 (IGF-1).[18] Estrogen deficiency in females and androgen deficiency in males adversely affect endosteal and periosteal apposition, reducing bone size and density.[19] However, IGF-1 and sex steroids also play a role in muscle hypertrophy.[20,21] It remains unclear whether the effects of these hormones on bone development are mediated through their effects on muscle.

The timing of puberty depends on the concerted function of the hypothalamic-pituitary-ovarian and other endocrine systems.[22] Peripuberty is a critical stage of secondary sexual

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dimorphism, and both bone size and bone mass grow rapidly during this period.[23] In females, menarche is a landmark in bone growth: Bone grows vigorously before but slows down rapidly after it,[24] presenting a biphasic pattern.

This pattern of bone growth in peripuberty approximately synchronizes with the changes of growth/sex hormone levels. IGF-1 concentration rises progressively through childhood, peaks around 12 to 14 years of age, and then decreases sharply after puberty, reaching a plateau in early adulthood.[25] A similar phenomenon in the changes of estradiol (E_2) concentration in relation to the time of menarche also has been shown in cross-sectional studies.[26]

These phenomena lead to the following questions: What is the relationship between biphasic bone growth and alterations in IGF-1 and sex steroids during peripuberty? Do the effects of these hormones on bone growth differ before and after menarche? Do the hormones affect peripubertal bone growth independently or through stimulating muscle growth?

To answer these questions, we explored the patterns of longitudinal changes of serum IGF-1, E_2, and testosterone (T) concentrations in adolescent girls in a 7-year prospective study using hierarchical models. The associations between these hormones and bone lengthening, widening, and mass accrual, with or without adjustment for muscle growth, were evaluated before and after menarche, respectively.

Materials and Methods

Subjects

The adolescent participants of this study are part of the CALEX study, which has been described elsewhere.[24,27–29] Briefly, the participants were recruited from local schools in the city of Jyväskylä and its surroundings in central Finland. They participated in the laboratory tests one to eight times over a maximum period of 8 years (mean duration of total follow-up was 7.5 years). To be eligible for the study, the participants had to have no history of medical conditions or medications known to affect bone metabolism. Of the eligible subjects, 258 girls participated in the baseline measurement, and 200 girls were present at 1-year follow-up bone measurements, 221 at 2 years, 87 at 3.5 years, and 102 at 7 years. The main reasons for dropout were loss of interest, lack of time, and relocation. The age at menarche was defined as the first onset of menstrual bleeding and was determined by questionnaire or phone call during the follow-up. Time relative to menarche (TRM, in months) was defined as the difference between the age at measurement and the age at menarche. The mean age at menarche was 12.9 years. Thus 99%, 81%, 38%, 11% and 0% of the girls were premenarcheal at baseline and 1, 2, 3.5, and 7 years of follow-up, respectively.

Written informed consent was obtained from all participants and their parents prior to the study. The study protocol was approved by the Ethics Committee of the University of Jyväskylä, the Central Finland Health Care District, and the Finnish National Agency of Medicine.

Anthropometric and bone measurements

Body weight and height were measured with subjects wearing light clothes and on bare feet. The left lower leg was scanned using peripheral quantitative computed tomography (pQCT; XCT 2000, Stratec Medizintechnik, Pforzheim, Germany). The scan location was at 60% of the lower leg length up from the lateral malleolus of the fibula. The thickness of the slice was 2 mm, and in-plane pixel size was 0.59×0.59 mm. Total bone mineral content (tBMC, mg/mm), cortical volumetric bone mineral density (vBMD, mg/cm^3), and periosteal circumference (PC, mm) were the outcome variables. The threshold for bone edge detection was 280 mg/cm^3. The muscle cross-sectional area (mCSA, cm^2) was analyzed using validated software (Geanie 2.1, GeanieCE, Espoo, Finland). A contour was drawn manually along the outer boundary of muscle to eliminate the subcutaneous adipose tissue before analysis. The threshold for muscle was 10 to 279 mg/cm^3. The coefficient of variation (CV) of two repeated measurements on the same subject on the same day was, on average, less than 1% for PC, mCSA, tBMC, and vBMD. The tibial length (TL, mm) was measured from dual-energy X-ray absorptiometry (DXA) scans (Prodigy, GE Lunar, Madison, WI, USA). TL was defined as the distance between the proximal edge of tibia (middle point of the line from the medial to the lateral condyle) and the distal border of tibia. The CV of three repeated measurements of TL was, on average, 2.7%.

Hormone determination

Blood samples were collected in the morning between 7:00 and 9:00 am after an overnight fasting at each time point. If the girls began menstruation, the blood samples were collected between 2 and 5 days after menstrual bleeding started. Serum was extracted from blood by centrifugation and stored immediately at -80°C until analyzed. The samples from different time points were analyzed by one technician using the same kits and instrument. IGF-1 was assessed using time-resolved fluoroimmunoassays (IMMULITE, Siemens Healthcare Diagnostics, Deerfield, IL, USA). E_2 and T were determined using ELISA (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany). Inter- and intraassay CVs were 6.1% and 3.1% for IGF-1, 3.2% and 5.4% for E_2, and 3.9% and 6.2% for T, respectively.

Statistical analysis

All data were checked for normality using a Shapiro-Wilk test in SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Because E_2 and T concentrations were not normally distributed, their natural logarithms (ln E_2 and ln T) were used in further analyses. A hierarchical (multilevel) model with random effects (MLwiN 2.02 software, Institute of Education, University of London, UK) was used to explore the patterns of longitudinal changes of IGF-1, E_2, and T in order to provide a basis for understanding of their associations with musculoskeletal variables in growing subjects. The hierarchical model allows inclusion of the data from every subject regardless of irregularity of temporally spaced follow-up or missing data.[30,31] Time relative to menarche (TRM), instead of age, was entered as the explanatory variable in the form of polynomial functions to explain the pattern of change of target

variables. The peak time (PT) of hormone concentration, if any, was determined from the maximum in the prediction curve.

The associations between longitudinal changes in bone parameters and the changes in hormonal variables before and after menarche were assessed by the following hierarchical model:

$$\text{Model 1}: y_{ij} = \beta_{0ij} + f(\text{time}_{ij}) \times dumi\_1 + f(\text{time}_{ij}) \times dumi\_2 + \beta_1 \times \text{IGF-1}_{ij} \times dumi\_1 + \beta_2 \times \text{IGF-1}_{ij} \times dumi\_2 + \beta_3 \times \ln \text{E}_{2ij} \times dumi\_1 + \beta_4 \times \ln \text{E}_{2ij} \times dumi\_2 + \beta_5 \times \ln \text{T}_{ij} \times dumi\_1 + \beta_6 \times \ln \text{T}_{ij} \times dumi\_2 \text{ with } \beta_{0ij} = \beta_0 + u_{0j} + e_{0ij}$$

where the outcome variable y_{ij} is TL, PC, tBMC, or vBMD of the *i*th measurement occasion from the *j*th individual, respectively. The predictor variables are IGF-1, ln E_2, and ln T. The term *time* denotes TRM, and *f*(time) denotes the curvilinear function comprised of the TRM, TRM^2, and TRM^3 components. The introduction of the *f*(time) function is to control for TRM, and it also has the effect of capturing the nonlinear characteristics of growth. β_0 is the intercept, and both u_{0j} and e_{0ij} form the random portion of the model, whose means are equal to zero.

The terms *dumi_1* and *dumi_2* are the dummy variables introduced to denote the respective period before or after menarche: *dumi_1* = 1 and *dumi_2* = 0 stand for measurements done before menarche, and *dumi_1* = 0 and *dumi_2* = 1 stand for those after menarche. Here, the time of menarche itself is selected as a shift knot for the model, which means that the coefficients of the independent variables could be different on either side of this time point. Thus the associations between bone parameters and each hormone can be assessed by regression coefficients β_1, β_3, and β_5 for before menarche and by β_2, β_4, and β_6 for after menarche, respectively, adjusting for other hormones.

With the a priori assumption that these hormones might interact with each other in regulating bone growth, the multiplication interaction terms of IGF-1–ln E_2, IGF-1–ln T, and ln E_2–ln T were included previously in the equation. However, unexpectedly, none of these interaction terms was significant, and thus they were removed from the model because inclusion of nonsignificant interaction terms made it difficult to estimate the main effects of these hormones in the model.

To further analyze whether the associations between hormones and tibial bone size expansion and mass accrual were independent of muscle growth, mCSA, a surrogate for muscle strength, was introduced to the model as follows:

$$\text{Model 2}: y_{ij} = (\text{equation of Model 1}) + \beta_7 \times \text{mCSA}_{ij} \times dumi\_1 + \beta_8 \times \text{mCSA}_{ij} \times dumi\_2$$

in which the associations between bone size/mass and a certain hormone are evaluated by corresponding betas before and after menarche, respectively, after adjusting for muscle growth. A *t* test was used to assess whether the betas were statistically different from 0.

In addition, Pearson correlation coefficients were used to assess the associations of baseline levels of IGF-1, T, and E_2 with bone variables at 7-year follow-up in order to address whether premenarcheal hormone levels predict bone traits in early adulthood. A *p* value less than .05 was considered statistically significant.

Results

Descriptive statistics for anthropology, bone traits, and hormones are shown in Table 1. The values of bone properties and sex hormone levels were higher at 7-year follow-up than those at baseline (all $p < .001$), whereas there was no difference in IGF-1 level ($p = .459$).

Serum IGF-1 level increased and reached a peak at around 6 months after menarche (13.5 years of age) and then decreased until the age of 18 years (Fig. 1*A*). Both E_2 and T increased before menarche. E_2 reached a plateau slightly prior to menarche and thereafter remained at a relatively high level with large between-individual variations (Fig. 1*B*), whereas T kept increasing at a lower rate after menarche up to 18 years of age (Fig. 1*C*).

IGF-1 was associated with TL independent of muscle CSA before menarche ($p < .001$) but not after. IGF-1 was positively associated with PC and tBMC both before and after menarche (all $p < .05$), but these associations disappeared after controlling for muscle CSA (all $p > .05$). IGF-1 level did not associate with cortical vBMD throughout peripuberty.

E_2 level was positively associated with TL before menarche ($p = .014$) but negatively after menarche ($p = .005$). No associations between E_2 and PC, tBMC, or vBMD were found throughout growth after controlling for IGF-1 and T (all $p > .05$).

Table 1. General Characteristics, Left Lower Leg Bone and Muscle Parameters, and Hormone Concentrations at Baseline Measurement

Variables	Baseline	7-Year follow-up
n	258	102
General characteristics		
Age (years)	11.2 ± 0.8	17.9 ± 1.0[b]
TRM (months)	−21.1 ± 12.9	59.2 ± 15.7[b]
Height (cm)	145.6 ± 8.0	165.2 ± 6.1[b]
Weight (kg)	39.2 ± 8.7	58.9 ± 10.3[b]
Left lower leg		
TL (cm)	33.4 ± 2.4	37.3 ± 1.8[b]
PC (mm)	63.0 ± 5.0	73.3 ± 4.9[b]
tBMC (mg/mm)	246.9 ± 36.0	342.8 ± 40.0[b]
vBMD (mg/cm^3)	1043 ± 28	1141 ± 23[b]
mCSA (cm^2)	43.1 ± 8.3	61.7 ± 10.2[b]
Hormones		
IGF-1 (nmol/L)	32.5 ± 12.7	31.5 ± 7.9
E_2 (nmol/L)[a]	0.09 (0.04–0.24)	0.22 (0.03–1.50)[b]
T (nmol/L)[a]	0.41 (0.05–1.38)	3.25 (0.70–15.2)[b]

Note: Data are given as mean ± SD. TRM = time relative to menarche; TL = tibial length; PC = periosteal circumference; tBMC = total bone mineral content; vBMD = cortical volumetric bone mineral density; mCSA = muscle cross-sectional area; IGF-1 = insulin-like growth factor 1; E_2 = estradiol; T = testosterone.

[a]For E_2 and T, the median and its 5th through 95th percentiles were given.

[b]$p < .001$ compared to baseline by Student's *t* test or by Wilcoxon signed-ranks test (for E_2 and T only).

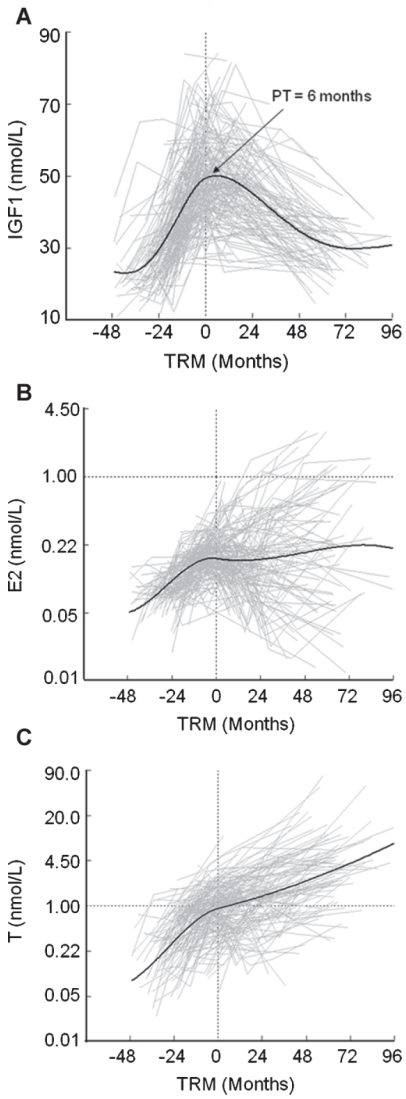

Fig. 1. Longitudinal change patterns of serum levels of IGF-1 (*A*), E_2 (*B*), and T (*C*) from prepuberty to early adulthood. The concentrations of IGF-1, E_2, and T were plotted against time relative to menarche (TRM). Gray lines represent longitudinal change of individual's value, and the black lines are the best-fitting lines derived from hierarchical regression models. The concentrations of E_2 and T along the *x* axis are back-transformed from ln E_2 and ln T, respectively.

Before menarche, T was positively associated with TL, PC, tBMC, and vBMD independent of muscle CSA (all $p < .05$). After menarche, T was not associated with TL, tBMC, and vBMD. The only significant association of T with PC ($p = .01$) found after menarche disappeared after controlling for muscle CSA ($p = 0.06$; Table 2).

The baseline level of IGF-1 was significantly associated with the tBMC and vBMD at 7-year follow-up (all $p < .05$). No association was found between baseline E_2 level and bone variables at 7-year follow-up, whereas baseline T was associated with TL and vBMD at early adulthood (all $p < .05$; Table 3).

Discussion

In this 7-year longitudinal study, we found that circulating IGF-1 promotes longitudinal bone growth independently, but its effect on radial bone growth depends on muscle size expansion. The effects of E_2 and T on peripubertal bone growth are biphasic—they differ before and after menarche. T in girls has a strong independent effect on bone longitudinal and radial growth during the early pubertal years (premenarche) but not later (postmenarche). E_2 stimulates longitudinal bone growth before menarche but inhibits it after menarche; it is not associated with periosteal circumference and bone mass throughout puberty. These results are largely but not totally in agreement with current views on the relationships between bone growth and related hormones.[32–35]

Bone lengthening

Bone elongation takes place by endochondral ossification in the growth plates.[36] IGF-1 regulates the physiology of the growth plate via endocrine, paracrine, or autocrine regulation.[37–39] The IGF-1 produced locally in bone is considered more important than circulating IGF-1 in the regulation of chondrogenesis in the growth plates of tibias in rats.[40] However, we found a strong positive association between serum IGF-1 level and tibial length. This agreed with recent reports that maintaining long-term elevations in serum IGF-1 in the absence of tissue *Igf1* gene expression is sufficient to establish and maintain skeletal architecture during growth.[41,42]

E_2 level increased dramatically and was positively associated with bone elongation before menarche, in accordance with reports that the estradiol concentration was significantly associated with growth velocity in pubertal girls.[43,44] However, the association of E_2 with tibial length became negative after menarche. This finding explains in part the observation that earlier age at menarche is associated with shorter stature in adulthood[45,46] because an earlier age at menarche, as shown in this study, corresponds to an earlier plateau of E_2 concentration at a high level, which decelerates bone lengthening. Our results echo the findings in animal studies that the effect of estrogen on longitudinal bone growth is biphasic: At low levels, estrogen stimulates growth,[13,47] whereas higher levels of estrogen have potent inhibitory effects on longitudinal growth by accelerating epiphyseal closure.[48]

The positive association between T level and tibial length before menarche suggests that androgen promotes bone

Table 2. Associations Between Hormones and Bone Variables at Left Lower Leg

	Outcome (before menarche)				Outcome (after menarche)			
Predictors	TL	PC	tBMC	vBMD	TL	PC	tBMC	vBMD
Model 1								
IGF-1	0.014 (0.003)[a]	0.023 (0.009)[b]	0.167 (0.066)[b]	NS	0.008 (0.004)[c]	0.024 (0.010)[b]	0.194 (0.074)[b]	NS
ln E_2	0.178 (0.072)[b]	NS	NS	NS	−0.122 (0.043)[b]	NS	NS	NS
ln T	0.344 (0.048)[a]	0.535 (0.136)[a]	2.914 (0.977)[b]	2.422 (1.180)[c]	NS	0.380 (0.148)[b]	NS	NS
Model 2								
IGF-1	0.014 (0.004)[a]	NS	NS	NS	NS	NS	NS	NS
ln E_2	0.158 (0.078)[c]	NS	NS	NS	−0.108 (0.044)[c]	NS	NS	NS
ln T	0.339 (0.049)[a]	0.398 (0.111)[a]	2.364 (0.829)[b]	2.786 (1.188)[c]	NS	NS	NS	NS
mCSA	0.015 (0.007)[c]	0.249 (0.017)[a]	0.014 (0.001)[a]	NS	0.020 (0.007)[c]	0.223 (0.016)[a]	0.014 (0.001)[a]	NS

Note: Data presented are the regression coefficients (betas) and their SE values (in parentheses) obtained from hierarchical regression model 1 and 2. Model 1 regresses bone variables (TL, PC, tBMC, or vBMD) on TRM and its square and cube, IGF-1, ln E_2. and ln T, with dummy variables indicating the respective period before or after menarche. Model 2 is in the form of introduction of mCSA with dummy variables into Model 1. Thus the associations between bone variables and hormonal factors were assessed by betas before and after menarche with or without controlling for mCSA, respectively. NS indicates $p > .05$.

[a] $p < .001$.

[b] $p < .01$.

[c] $p < .05$.

lengthening in women. This inference is supported by the findings in animal models that lack of androgen leads to an increase in apoptosis and a decrease in the proliferation of chondrocytes in the growth plate.[49,50] However, no association was found after menarche, although the increasing T level was found up to age of 18 years, when peripheral bone growth ceased.[24] This suggests that the effect of T on longitudinal bone growth is also biphasic—T promotes endochondral bone growth before menarche, but this effect is arrested by estrogen-related growth plate closure after menarche. Despite this, the stimulatory effects of T on long bone lengthening before menarche contribute considerably to final bone length, and hence height, as shown by the significant association of baseline T level with tibial length at 18 years of age.

Bone widening

Bone accrual on periosteal surfaces leads to an increase in diaphyseal circumference, which is essential for increasing bone strength. Periosteum is abundant in receptors for IGF-1,[51] providing a stage for circulating IGF-1 to exert its anabolic effects on the periosteal surface. Studies have shown that circulating IGF-1 stimulates periosteal bone growth along the cortex,[41,42,52] whereas reduced serum levels of IGF-1 in mice lacking liver-specific IGF-1 were associated with impaired periosteal apposition, leading to the development of slender bones during growth.[42] When subjected to a loading regimen, periosteal bone formation was substantially elevated in the IGF-1-over-expressing mice but not in wild-type littermates,[53] suggesting that circulating IGF-1 enhances bone response to mechanical loading.

Table 3. Correlations Between Baseline Hormone Levels and Bone Variables at 7-Year Follow-Up

	Bone variables at 7-year follow-up			
Baseline hormones	TL	PC	tBMC	vBMD
IGF-1	0.059	0.129	0.247[b]	0.201[b]
ln E_2	−0.131	−0.080	−0.010	0.070
ln T	0.269[a]	0.163	0.191	0.233[b]

Note: Data presented are Pearson correlation coefficients.

[a] $p < .01$.

[b] $p < .05$.

However, evidence that negates the direct effect of systemic IGF-1 on periosteal apposition has been reported. In animal studies, liver-derived IGF-1 could not substitute as a bone anabolic stimulus in mice after the removal of whole leg muscles,[54] suggesting that the anabolic effects of circulating IGF-1 on bone probably depend on muscle. This suspicion is reasonable because IGF-1 potently stimulates muscle hypertrophy[21] and hence increases the muscle force to which the bone periosteal apposition is particularly responsive.[55,56] It was further confirmed by our findings that the association between IGF-1 and bone periosteal circumference disappeared after adjustment for muscle CSA. We infer that during growth, the circulating IGF-1 promotes periosteal apposition indirectly to a large extent through stimulating muscle growth and hence increasing mechanical loading from growing muscle.

Based on the findings in rodents, it was assumed that estrogen and androgen have different endocrine effects on bone widening: Androgen stimulates periosteal bone formation,[32,57] whereas estrogen inhibits it.[32,58,59] This mechanism was regarded as being responsible for the development of a sexually dimorphic skeleton (ie, male bones are wider than female bones).[32–34] Our finding that T was significantly associated with periosteal circumference throughout peripubertal growth supports the view drawn from animal models.[58,59] However, this study does not support the idea that estrogen inhibits periosteal apposition. The smaller bone size in females is more likely due to their shorter prepubertal and pubertal growth periods[3] and earlier estrogen-related epiphyseal closure.[48]

T stimulates muscle hypertrophy and hence increases the mechanical loading on bone independent of IGF-1.[20,60] However, in contrast to IGF-1, the association of T with bone periosteal circumference before menarche remained significant after controlling for muscle CSA, suggesting that the stimulatory effect of T on periosteal apposition is independent of growing muscle in early puberty. This is consistent with previous reports in rats.[58,59] However, after menarche, the stimulatory effect of T on periosteal apposition depends on muscle.

Bone mass accrual

Bone mass at the diaphysis increases through periosteal apposition,[57] as well as through the increased degree of tissue mineralization.[61] In this study, we did find a positive association between serum IGF-1 level and total BMC increment at the tibial shaft throughout peripubertal growth. However, the association disappeared after adjustment for muscle CSA, similar to the pattern of the relationship between IGF-1 and periosteal circumference. In addition, IGF-1 was not associated with cortical vBMD. Taken together, the results suggest that IGF-1 promotes bone mass accrual probably by adding more bone on the periosteal surface, not by increasing the degree of tissue mineralization. Again, however, the effect of circulating IGF-1 on bone mass accrual is not independent of muscle growth.

Unexpectedly, E_2 level was not associated with either total BMC or vBMD after controlling for IGF-1, T, and muscle CSA. It is possible that estrogen plays a permissive role in bone mass accrual via modifying the modeling and/or remodeling thresholds[62] and hence limiting or augmenting the anabolic effects of other hormonal or mechanical stimuli on bone.

T was positively associated with both BMC and cortical vBMD before menarche but not after, indicating that T promotes bone mass accrual in women at an early age by both enhancing periosteal bone formation[32,57] and increasing tissue mineralization.[63] The total bone CSA and cortical vBMD are the two most important factors determining bone strength.[64] During fast growth, the dissociation between rapid bone size expansion and lagging mineral accrual leads to a deficit in bone mineralization relative to bone size that may contribute to the elevated risk of fracture during early puberty.[65–67] The actions of T on bone may compensate for this defect because they not only increase the total amount of bone by enhancing periosteal apposition, leading to a larger bone cross section, but they also pack more bone into a given volume, resulting in a denser bone. From this point of view, T is of considerable significance for bone consolidation and reducing fracture risk during fast growth in females.

The associations between baseline IGF-1 and T levels and bone mass at early adulthood suggest that premenarcheal hormone levels may have considerable influence on bone traits, at least until early adulthood, and probably affect the attainment of peak bone mass in later years.

Limitations

This study has a few limitations. First, there was considerable attrition at 7-year follow-up. The high dropout rate among the cohort of girls potentially may introduce bias. However, adding another 134 girls who participated only in the 7-year follow-up did not change the pattern of results in the study (data not shown). Second, additional influences of other factors that may contribute to bone growth, such as exercise and intake of vitamin D, calcium, protein, and other nutrients were not taken into consideration. Therefore, the study is limited in its ability to provide a comprehensive view of peripubertal bone growth regulation. Third, the serum level of E_2 showed a large variation after menarche, although the timing of menstruation had been strictly controlled for throughout the study, indicating that one snapshot measurement cannot reflect the real hormonal exposure. This limitation makes the predictive power of E_2 for bone parameters weaker than expected. Fourth, since less bone growth occurs after menarche,[24] the possibility remains that the lack of association between T and bone traits after menarche is, to some extent, due to a loss of statistical power. In addition, care should be taken if seeking to generalize from our results to other bone sites or to boys because the effects of these hormones, especially the sex steroids, on bone may be site-, level-, and sex-specific.

In conclusion, circulating IGF-1 promotes peripubertal bone growth largely in a muscle-dependent fashion. The sex steroids strongly stimulate bone growth before menarche, but the stimulatory effects wane or become inhibitory after menarche, resulting in a biphasic pattern of bone growth during this critical period. Our findings imply that the timing of menarche is critical to peripubertal bone growth, and the concerted actions of IGF-1, E_2, and T on bone before menarche, when the bone grows fast, are essential for optimal bone growth in the early life of women.

Disclosures

All the authors state that they have no conflicts of interest.

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III

FAT MASS ACCUMULATION COMPROMISES BONE ADAPTATION TO LOAD IN FINNISH WOMEN: A CROSS-SECTIONAL STUDY SPANNING THREE GENERATIONS

by

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Fat Mass Accumulation Compromises Bone Adaptation to Load in Finnish Women: A Cross-Sectional Study Spanning Three Generations

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ABSTRACT

Body weight and lean mass correlate with bone mass, but the relationship between fat mass and bone remains elusive. The study population consisted of 396 girls and 138 premenopausal mothers and 114 postmenopausal grandmothers of these girls. Body composition and tibial length were assessed using dual-energy X-ray absorptiometry (DXA), and bone traits were determined at the tibia using peripheral quantitative computed tomography (pQCT) in the girls at the ages of 11.2 ± 0.8, 13.2 ± 0.9, and 18.3 ± 1.0 years and in the mothers (44.7 ± 4.1 years) and grandmothers (70.7 ± 6.3 years). The values of relative bone strength index (RBSI), an index reflecting the ratio of bone strength to the load applied on the tibia, were correlated among family members (all $p < .05$). The mean values of RBSI were similar among 11- and 18-year-old girls and premenopausal women but significantly lower in 13-year-old girls and postmenopausal women. However, in each age group, subjects in the highest BMI tertiles had the lowest RBSI values (all $p < .01$). RBSI was inversely associated with body weight (all $p < .01$), indicating a deficit in bone strength relative to the applied load from greater body weight. RBSI was inversely associated with fat mass (all $p < .001$) across age groups and generations but remained relatively constant with increasing lean mass in girls and premenopausal women (all $p > .05$), indicating that the bone-strength deficit was attributable to increased fat mass, not lean mass. Moreover, the adverse effect of fat mass was age-dependent, with every unit increase in fat mass associated with a greater decrease in RBSI in pre- and postmenopausal women than in girls (all $p < .001$). This is largely due to the different capacity of young and adult bones to increase diaphyseal width by periosteal apposition in response to increased load. In summary, increasing body weight with fat accumulation is accompanied by an age-dependent relative bone-strength deficit in women because the beneficial effects of increased fat mass on bone, if any, do not compensate for the mechanical burden that it imposes.

KEY WORDS: BODY COMPOSITION; LEAN MASS; FAT MASS; BONE STRENGTH

Introduction

For a given stature, bone mineral content (BMC) is usually greater with increased body weight in children, adults, and the elderly.[1–3] Total-body lean mass is a consistent predictor of bone mass regardless of the gender and age of the studied cohorts.[4–7] However, the relationship of fat mass, another major component of body weight, to bone mass or density remains controversial[8–15] largely owing to the different approaches used in statistical analysis. For example, a positive bivariate correlation between fat and bone mass may become negative after controlling for weight,[8–10] suggesting inadequate skeletal adaptation to the load applied on it from fat mass.[16] However, for a given body weight, a higher fat mass percentage usually corresponds to a relatively lower lean mass percentage, which also may be linked to decreased bone mass, and this type of analysis cannot differentiate which of these is responsible.

The relationship between fat mass and fracture risk is also elusive. Obese children have a higher risk of fracture,[17,18] whereas it has been suggested that fat tissue plays a protective role against bone loss and fracture in postmenopausal women by increasing the load in normal daily activities and cushioning the impact applied on bone when a fall occurs.[19] However, one recent study casts doubts on this view with the observation of a

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high prevalence of low-trauma fracture in obese postmenopausal women, the majority of whom had normal bone mineral density (BMD).[20]

There are strong reasons to expect a pathophysiologic linkage between fat and bone tissue. Osteoblasts and adipocytes in bone marrow originate from the same mesenchymal stem cells,[21] normal aging is associated with a high incidence of both osteoporosis and bone marrow adiposity,[22,23] and both bone remodeling and adiposity are regulated by the hypothalamus and sympathetic nervous system.[22] Fat and bone are also linked biomechanically. Bone adapts to the mechanical loads imposed on it, and these loads ultimately arise from supporting or moving body or limb mass against gravity and inertial forces. With greater fat mass causing larger load on bone, fat mass is likely to be an important factor affecting bone health.

The accumulation of adipose tissue may compete with the development of bone in the use of mesenchymal stem cells.[24] The passive loading from fat mass is less anabolic to bone than the active dynamic loads from muscle contraction,[25,26] and thus a bone may not adapt fully to a given body mass comprised of higher fat mass. Therefore, we hypothesized that higher fat mass adversely affects bone mechanical competence. To test our hypothesis, we investigated the association between fat mass and bone traits and relative bone strength index (RBSI), the ratio of estimated bone strength to the applied load, at the tibial shaft in girls from peripuberty to early adulthood and in their mothers and grandmothers, with a focus on how RBSI varies with body weight, lean mass, and fat mass, respectively.

Subjects and Methods

Subjects

The recruitment of the study population is presented schematically in Fig. 1. The adolescent subjects of this report are a part of the CALEX (calcium and exercise) study, which has been described elsewhere.[27–29] Briefly, the girls were first contacted via class teachers in grades 4 to 6 (age 9 to 13 years) in 61 schools in the city of Jyväskylä and its surroundings in central Finland (96% of all the schools in these areas). Of those eligible, 396 girls participated in laboratory tests one to eight times over a maximum period of 8 years (mean duration of total follow-up was 7.5 years). Of the 396 girls, 258 (mean age at baseline 11.2 years) participated in a calcium and vitamin D intervention trial during the first 2 years ($n = 221$ at 2-year follow-up, mean age 13.2 years). A total of 235 girls participated in both body composition and bone assessment in the 7-year follow-up (mean age 18.3 years). Since no intervention effects on bone mass were found,[27–29] data were pooled in this analysis.

In addition, biological mothers and grandmothers (maternal and paternal) of the girls were invited to participate in the bone measurements in the years 2003, 2004, 2007, and 2008 (Fig. 1). Of the mothers, 108 were measured in 2003 and 2004 and 214 in 2007 and 2008. Sixty-five mothers had two bone measurements, but only the first bone measurement was included in this report. This resulted in 257 mothers with first-time bone measurement, but of these, 119 were excluded owing to being postmenopausal or having medications or diseases known to affect bone metabolism. Thus 138 premenopausal mothers (mean age 44.7 years) were included in the final analysis. Of the grandmothers, 75 were measured in 2003 and 2004 and 120 in 2007 and 2008. Among them, 41 had two bone measurements but again, only the first bone measurement was included. Forty grandmothers were using hormone-replacement therapy (HRT) or other medications affecting bone and therefore were excluded, leaving 114 grandmothers (mean age 70.7 years) included in this report. In total, 138 daughter-mother and 107 daughter-grandmother pairs and 44 daughter-mother–maternal grandmother trios were included in this report.

The study protocol was approved by the Ethics Committee of the University of Jyväskylä, the Central Finland Health Care

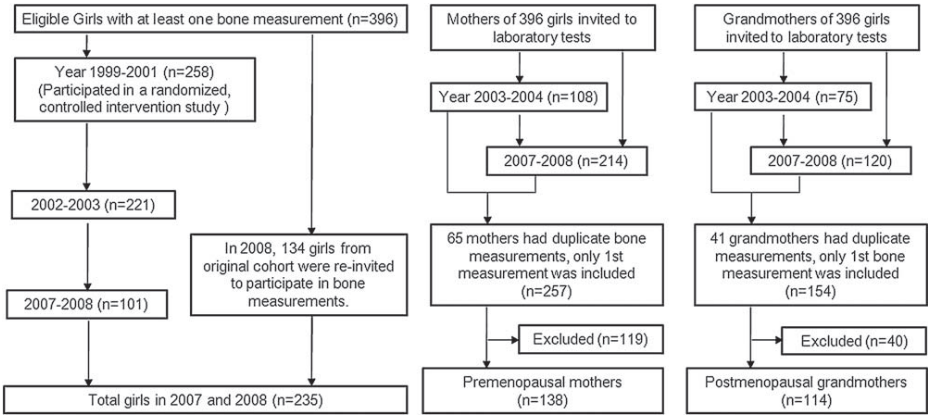

Fig. 1. Flowchart of the CALEX study population. The girls were recruited from the fourth to sixth grades (age 9 to 13 years old) in 61 schools in the city of Jyväskylä and its surroundings in central Finland (96% of all schools in these areas). The pre- and postmenopausal women were recruited from the mothers and grandmothers of 396 girls who had at least one bone measurement.

District, and the Finnish National Agency of Medicine. Informed consent was given by all subjects prior to the assessments.

Body composition and bone measurements

Body composition was assessed using dual-energy X-ray absorptiometry (DXA Prodigy, GE Lunar Corp., Madison, WI, USA). Total body weight (TW, kg) was the sum of bone mass (BM, kg), lean tissue mass (LM, kg), and fat mass (FM, kg). Body mass index (BMI) was calculated as weight (kg)/height^2 (cm). The coefficient of variation (CV) of two repeated measurements on the same day was, on average, 1.0% for LM and 2.2% for FM.

The left tibia was scanned using peripheral quantitative computed tomography (pQCT; XCT 2000, Stratec Medizintechnik, Pforzheim, Germany). The scan location was at 60% of the lower leg length up from the lateral malleolus. The in-plane pixel size was 0.59×0.59 mm. Total bone cross-sectional area (CSA, mm^2), cortical CSA, total bone mineral content (BMC, mg/mm), cortical BMC and volumetric bone mineral density (vBMD, mg/cm^3), and polar cross-sectional moment of inertia (CSMI, mm^4) were determined using the Stratec software. The threshold for bone edge detection was 280 mg/cm^3, and it was 710 mg/cm^3 for cortical bone. The CV of two repeated measurements on the same subject on the same day was, on average, 1% for total CSA, cortical CSA, cortical BMC, and total BMC and less than 1% for cortical vBMD.

Tibial length (TL, cm) was measured from the DXA scans. TL was defined as the distance between the proximal edge of the tibia (middle point of the line from the medial to the lateral condyle) and distal border of the tibia (ankle joint surface). The CV of three repeated measurements of TL was 2.7%.

Bone strength index and relative bone strength index

Bone strength index (BSI) was the product of polar cross-sectional area moment of inertia and cortical vBMD. This BSI has been validated by its close correlation with the actual, mechanically tested bending/breaking force of all bones.[30,31] Furthermore, we calculated a new parameter, relative bone strength index (RBSI), in order to reflect the relationship between tibial bone strength and the load applied on it. RBSI was computed as BSI/(tibial length $\times$ body weight). The product of body weight and tibial length, reflecting the bending moment of body weight acting on the lever length of the tibia, was used as a surrogate for load.[32,33] Raw RBSI was multiplied by 10^7 for presentational convenience. Note that the higher the RBSI value, the stronger is the bone relative to the applied load.

Statistical analysis

All data were checked for normality using the Shapiro-Wilk *W* test in SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Since fat mass was not normally distributed, its natural logarithm was used. Analysis of variance with a least significant difference (LSD) post hoc test was used to compare the differences in bone traits between age groups and in RBSI among BMI tertiles within each age group. The correlations between body weight and lean mass or fat mass in each age group and the correlation in RBSI among family members were evaluated using Pearson correlation coefficients. Fisher's *Z*-transformation was adopted to assess whether the correlation of body weight with fat mass was stronger than with lean mass. Partial correlation coefficients were used to assess the relationship between bone variables and fat mass, controlling for lean mass and tibial length, for each age group separately. Simple linear regression was performed in each age group to evaluate the associations of RBSI and other bone variables with body weight, lean mass, and fat mass, respectively. Also, *t* tests with dummy variables were used to test whether the regression slopes (β) in adult women were different from those in girls. The slope represents the change in RBSI or other bone variables associated with a unit change in body weight, fat mass, or lean mass. Differences were considered significant if $p < .05$.

Results

At 18 years of age, the girls already had similar body heights and tibial lengths as the premenopausal adults, whereas values for body weight, BMI, lean mass, fat mass, tibial total CSA, cortical CSA, total BMC, cortical BMC, cortical vBMD, CSMI, and BSI were still significantly lower (all were $p < .05$) than the values of premenopausal women (Table 1). Postmenopausal women had higher BMIs and fat mass but shorter body heights and tibial lengths and lower values for cortical CSA, total BMC, cortical BMC, and cortical vBMD than premenopausal women (all $p < .01$). No significant differences in body weight, total CSA, and BSI were found between pre- and postmenopausal women. Girls at the ages of 11 and 18 years and premenopausal women had similar RBSIs ($p > .05$), whereas 13-year-old girls and postmenopausal women had lower RBSIs than other groups (all $p < .05$; Table 1). Significant correlations in RBSI values were found among the family members spanning three generations (all $p < .05$; Table 2).

Comparison of RBSI among BMI tertiles in each age group showed that those in the highest BMI tertile had lower RBSIs than those in the middle or lowest BMI tertiles in all age groups (all $p < .01$; Fig. 2).

The correlations of fat mass with weight were $r = 0.87$, 0.91, 0.90, 0.94, and 0.94 for 11-year-old girls through to postmenopausal women, respectively, and were stronger than the corresponding correlations of lean mass with weight ($r = 0.83$, 0.78, 0.70, 0.74, and 0.75, respectively) in all age groups (all $p < .01$) except in 11-year-old girls ($p > .05$).

Fat mass was positively correlated with tibial total CSA, total BMC, cortical CSA, and cortical BMC in growing girls after controlling for tibia length and lean mass (Table 3). However, fat mass was inversely associated with RBSI in all age groups (Fig. 3*A*). The slopes of the regression lines were steeper in pre- and postmenopausal women than in girls (Fig. 3*A*), indicating that every unit increase in fat mass was associated with a greater decrease in RBSI in adult women than in girls. By contrast, RBSI remained relatively constant with increasing lean mass in girls and premenopausal women (all $p > .05$), whereas in postmenopausal women the association became negative ($p < .001$; Fig. 3*B*). In addition, RBSI was negatively associated with body weight across age groups and generations, with steeper regression lines in pre- and postmenopausal women than in girls (Fig. 3*C*).

Table 1. General Characteristics and DXA and pQCT Measurements of Finnish Women in Different Age Groups

	11-Year-old girls	13-Year-old girls	18-Year-old girls	Premenopausal mothers	Postmenopausal grandmothers
n	258	221	235	138	114
Age (years)	11.2 ± 0.8	13.2 ± 0.7	18.3 ± 1.1	44.7 ± 4.1	70.7 ± 6.3
Height (cm)	145.6 ± 8.0	157.9 ± 7.0	165.8 ± 5.7	165.3 ± 5.7	159.2 ± 5.2
Weight (kg)	39.1 ± 8.5	50.0 ± 10.5	60.0 ± 10.0[a]	69.7 ± 13.4	71.6 ± 11.3
BMI (kg/m^2)	18.3 ± 2.9	20.0 ± 3.5	21.8 ± 3.2	25.5 ± 4.5[b]	28.2 ± 4.2
Lean mass (kg)	27.3 ± 4.3	34.6 ± 4.5	38.1 ± 4.2	42.0 ± 4.8[b]	40.0 ± 4.1
Fat mass (kg)	10.5 ± 5.4	13.4 ± 6.9	19.4 ± 7.5	25.0 ± 10.2[b]	29.3 ± 8.4
Tibial length (cm)	33.3 ± 2.4	36.0 ± 1.9	37.2 ± 1.7	36.9 ± 1.8	35.9 ± 1.7
Total CSA (mm^2)	372.5 ± 54.7	413.6 ± 55.2	471.7 ± 56.0[a]	487.8 ± 59.1	490.3 ± 49.8
Total BMC (mg/mm)	246.9 ± 36.0	298.7 ± 39.3	354.2 ± 42.0	376.6 ± 48.0[b]	337.3 ± 40.8
Cortical CSA (mm^2)	198.5 ± 30.8	240.6 ± 32.0	271.1 ± 34.7	288.8 ± 39.4[b]	260.2 ± 34.6
Cortical BMC (mg/mm)	207.0 ± 33.1	263.5 ± 36.9	310.9 ±39.5	334.8 ± 45.4[b]	287.9 ± 44.4
Cortical vBMD (mg/cm^3)	1042.7 ± 28.1	1095.5 ± 30.8	1134.6 ± 20.9	1160.3 ± 21.6[b]	1103.6 ± 43.9
CSMI (mm^4)	24749.8 ± 7420.9	30696.4 ± 8286.2	40267.9 ± 9407.8[a]	43362.9 ± 10840.3	43480.9 ± 9251.6
BSI (mg × cm)	2578.9 ± 771.8	3358.2 ± 901.7	4562.7 ± 1040.1	5019.9 ± 1214.8[b]	4786.1 ± 901.1
RBSI	19.6 ± 3.4	18.7 ± 3.3[c]	20.3 ± 3.3	19.9 ± 4.6	18.8 ± 3.5[c]

Note: Data are given as mean ± SD. BMI = body mass index; CSA = cross-sectional area; BMC = bone mineral content; vBMD = volumetric bone mineral density; CSMI = cross-sectional moment of inertia; BSI = bone strength index; RBSI = relative bone strength index.

[a] $p < 0.01$ versus values of pre- or postmenopausal women.

[b] $p < .01$ versus values of other age groups.

[c] $p < .05$ versus values of 11- and 18-year-old girls or premenopausal women.

We further explored the associations of the bone parameters, especially those used in the calculation of RBSI, with weight, lean mass, and fat mass, respectively (Table 4). A significant association of cortical vBMD with body composition variables was found only with lean mass in 13-year-old girls and postmenopausal women ($p = .002$ and .03, respectively). Positive associations of weight, lean mass and fat mass with total CSA, CSMI, and BSI were found in girls (all $p < .001$). However, the slopes of regression models for total CSA, CSMI, or BSI against weight, lean mass, or fat mass all were significantly reduced in adult women compared with girls (all $p < .05$), indicating that every unit gain in weight, lean mass, or fat mass was associated with less increase in these bone variables in adult women. In particular, total CSA, CSMI, and BSI were not associated at all with fat mass in postmenopausal women (all $p > .05$).

Discussion

In this study based on family members spanning three generations, we found that the mean value of relative bone strength index (RBSI), a ratio of bone strength to load from body weight, was similar in 11- and 18-year-old girls and premenopausal women. This is consistent with the idea that bone adapts to load to maintain an adequate safety margin without extra bulk. Lower RBSI implies a reduced ability of bone to sustain the imposed load and thus a greater vulnerability to fracture. Indeed, we found that girls aged around 13 years and postmenopausal women had a significantly lower RBSI compared with other age groups. This is in accordance with the known peaks of the peripheral limb fracture incidence in peripubertal girls[34–36] and in postmenopausal women.[37,38] Besides, the significant correlation in RBSI values among the family members indicated that the relationship between bone strength and the applied load was, to some extent, inheritable.

In women, RBSI was the lowest in the highest BMI tertile and was inversely associated with body weight across age groups and generations, demonstrating a relative bone-strength deficit associated with greater load from body weight. Consequently, being overweight would be expected to be a risk factor for fracture when a fall occurs, which is consistent with reports that overweight children have a higher risk of fractures than those of average body weight,[17,18] especially in fast-growing children, in whom the dissociation of mineral deposition and rapid growth in bone size[39–41] leads to lower mineralization of bone tissue or more porous cortex.[42] We also found that the association

Table 2. Correlations of RBSI Values Among Three Generations of Finnish Families

	11-Year-old girls			18-Year-old girls			Maternal grandmothers		
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*
Mothers	84	0.49	<.001	100	0.38	<.001	44	0.33	.04
Grandmothers	55	0.31	.02	80	0.33	.002			

Note: Data are Pearson correlation coefficients (*r*) and corresponding *p* values.

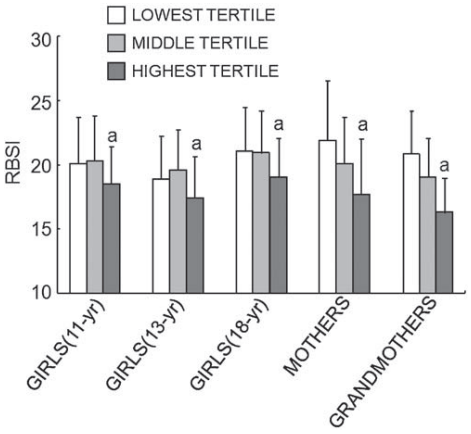

Fig. 2. Comparison of relative bone strength index [RBSI, calculated as CSMI × cortical vBMD/(tibial length × body weight)] across BMI tertiles in Finnish females at different ages. The cutoff values between lowest and middle tertiles and between middle and highest tertiles were, respectively, 16.8 and 19.1 for 11-year-old girls, 17.9 and 20.8 for 13-year-old girls, 20.2 and 22.6 for 18-year-old girls, 23.3 and 25.9 for premenopausal mothers, and 26.1 and 30.1 for postmenopausal grandmothers. [a] $p < .01$ compared with values in either middle or lowest tertiles.

between RBSI and body weight was age-dependent: Every unit increase in body weight was associated with a greater decrease in RBSI in pre- and postmenopausal women than in girls. We may infer from this that overweight and obese adult women should have a higher risk of fracture, which contradicts the observation of lower fracture rates at the hip and central body[37,38] but agrees with the reported higher extremity fracture rates, especially in obese postmenopausal women.[37,38] The discrepancy may be attributable to the different padding effects of fat tissue on bones at different sites.

In our study population, the correlation of body weight with fat mass was higher than with lean mass except in 11-year-old girls, indicating that the weight gain from puberty onward in women is largely due to fat mass accumulation.[29] However, RBSI decreased with increasing fat mass in all age groups but remained relatively constant with increasing lean mass in girls and premenopausal women. This agrees with the existing paradigm that bone adaptation is driven primarily by changes of mechanical load,[43] which are generated largely by muscle force (surrogated here by lean mass), not fat mass.[32,44] Since body weight is composed principally of lean and fat mass, and since fat mass accumulation contributes more than lean mass to weight gain in women,[45,46] we can conclude that it is the underlying negative relationship between RBSI and fat mass that gives rise to the negative association between RBSI and body weight. Therefore, it is fat mass that compromises the mechanical competence of the bone. Furthermore, the relationship between RBSI and fat mass differed in girls and adults, which explaines the age-dependent association between RBSI and body weight.

Further analyses showed that cortical vBMD was not associated with weight and fat mass across age groups. By contrast, total CSA and hence the cross-sectional moment of inertia of the tibia were strongly positively associated with weight, lean mass, and fat mass in girls. But these associations were weaker or became nonsignificant in pre- and postmenopausal women, indicating that the same amount of gain in body weight and its components was associated with less or even no total CSA expansion in adult women compared with young girls. The total CSA expansion and diaphyseal widening are driven by periosteal apposition,[47] and the distribution of bone mass further away from its neutral axis is more important than the amount of bone mass in determining bone strength.[48] Thus it is largely the different capacity of periosteal apposition of young and adult bones in response to increased load that resulted in an age-dependent relationship between RBSI and body weight and fat mass.

We found positive correlations between fat mass and bone variables, in agreement with other studies.[11–13] However, after adjusting for tibial length and lean mass, positive correlations were found only in girls, not adults. In growing children and adolescents, it is difficult to interpret the relationship between fat and bone because both grow during normal development. Hence positive associations between accumulating fat mass and increasing bone mass do not necessarily imply a cause-and-effect relationship. On the other hand, increasing fat mass may

Table 3. Relationships Between Tibial Bone Variables and Fat Mass in Finnish Women in Different Age Groups

	Fat mass[a]				
	11-Year-old girls	13-Year-old girls	18-Year-old girls	Premenopausal mothers	Postmenopausal grandmothers
Total CSA	0.24[b]	0.24[b]	0.23[b]	−0.05	0.09
Total BMC	0.28[b]	0.27[b]	0.24[b]	−0.01	0.09
Cortical CSA	0.29[b]	0.25[b]	0.21[b]	0.03	0.10
Cortical BMC	0.24[b]	0.20[b]	0.22[b]	−0.01	0.06
Cortical vBMD	−0.10	−0.10	0.04	−0.12	−0.10

Note: Data shown are partial correlation coefficients for tibial bone variables with fat mass controlling for lean mass and tibial length. CSA = cross-sectional area; BMC = bone mineral content; vBMD = volumetric bone mineral density.

[a] Fat mass was natural logarithm transformed.

[b] $p < .001$.

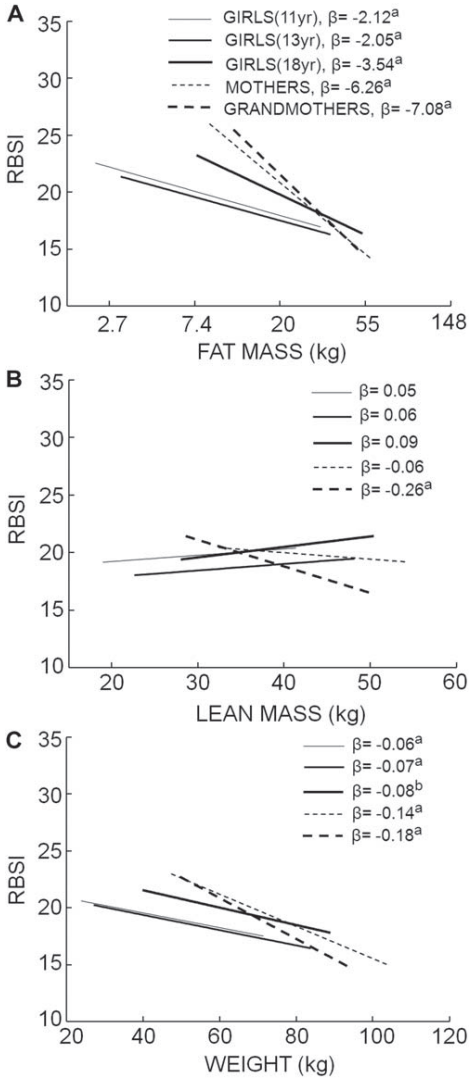

Fig. 3. Relative bone strength index [RBSI, calculated as CSMI × cortical BMD/(tibial length × body weight)] as a function of fat mass (*A*), lean mass (*B*), and weight (*C*) in Finnish females at different ages. Linear regression was performed separately in each age group. Solid and dashed lines are the best-fitting lines of the linear regression models. β is the regression slope. The fitting lines in panel *A* are derived from model RBSI versus ln FM (natural logarithm transformed FM); fat mass along the *x* axis was backtransformed ln FM. The slopes of the fitting lines in panels *A* and *C* are higher in mothers and grandmothers than in girls ($p = .045$ to $p < .001$). $^{a}p < .001$. $^{b}p < .01$.

be accompanied by a reduction in bone formation owing to the switching of stem cell differentiation in bone marrow to favor adipocytes over osteoblasts.[24] In addition, the influence of fat on bone is likely to be hormonal, involving a web of interrelated regulatory pathways. Fat tissue secretes bone-active hormones such as estrogens and leptin, which can have both stimulatory and inhibitory effects on bone formation.[49,50] Therefore, the influence of fat mass on bone is likely to be the product of a range of both negative and positive effects.

It is widely accepted that bone adapts to muscle force,[43,44,51] and therefore, a balance between lean mass and bone biomechanical competence could be expected. Indeed, RBSI remained relatively constant with increasing lean mass in girls and premenopausal women, suggesting that muscle is playing its expected role in maintaining bone strength.[52,53] Interestingly, RBSI was negatively associated with lean mass and, inferably, muscle strength in postmenopausal women. Postmenopausal bone loss is related to estrogen deficiency.[54] However, the loss of muscle strength follows a more gradual course and is not affected significantly by a sudden hormonal decline, as is the case with bone loss.[55] Consequently, the exponential loss of bone at the postmenopausal stage is not accompanied by an incremental loss of muscle strength.[55] Further, a senile bone lacking hormonal support would not adapt sufficiently to increased load.[56] Hence the dissociation of bone and muscle loss and the weakened bone adaptation to load act in tandem, producing a negative association between RBSI and lean mass in postmenopausal women. This may have considerable clinical significance in that the ability of bone to adapt to loading should be taken into consideration when planning exercise interventions for elderly women. Low- to moderate-intensity exercises may be appropriate without posing a danger of fracture.

A limitation of the study was that RBSI may not reflect all the factors that determine bone stability because the calculation of BSI included only the cortical vBMD and cross-sectional moment of inertia without taking into consideration the material and structural traits of the trabecular compartment and collagen characteristics, which also have substantial influence on bone strength.[57] However, the agreement of the timing of the trough in RBSI in girls with the observed peak in childhood fractures and in postmenopausal women who had a higher risk of fracture indicates that RBSI captures important aspects of the bone biomechanical competence. Moreover, the consistency of RBSI among 11- and 18-year-old girls and premenopausal women is in accordance with the evolutionary view that bone is designed by nature to meet the biomechanical needs of a species. In addition, while the results found here in the tibia may apply to other peripheral long bones, caution should be taken when extrapolating to the axial skeleton, where different bone structure and mechanical properties exist.

This study was complicated by its mixed longitudinal and cross-sectional study population and the possibility that the high dropout rate among the cohort of girls potentially could introduce bias. However, when we compared the physical characteristics of the girls who remained in the 7-year follow-up and those who dropped out (data not shown), there were no significant differences. Furthermore, including or removing the data for the 134 girls who participated only in the 7-year

Table 4. Associations of Body Weight, Lean Mass, and Fat Mass With Tibial Bone Variables in Females in Different Age Groups

	11-Year-old girls		13-Year-old girls		18-Year-old girls		Premenopausal Mothers		Postmenopausal Grandmothers	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Cortical vBMD *vs*. Weight	0.04	.84	0.32	.13	−0.12	.42	−0.23	.08	0.43	.20
Lean mass	0.63	.13	1.42	.002	−0.51	.14	−0.51	.15	1.95	.03
ln FM	−2.73	.43	3.03	.46	−0.53	.89	−5.97	.20	4.60	.74
Total CSA vs. weight	4.83	<.001	3.46	<.001	3.42	<.001	1.66[a]	<.001	0.89[a]	.01
Lean mass	10.21	<.001	8.78	<.001	8.86	<.001	6.81	<.001	2.35[a]	.01
ln FM	56.36	<.001	49.81	<.001	57.41	<.001	27.37[a]	.04	25.12	.07
CSMI vs. weight	671.89	<.001	537.87	<.001	602.78	<.001	316.73[a]	<.001	150.16[a]	.01
Lean mass	1407.72	<.001	1357.73	<.001	1504.27	<.001	1270.01	<.001	403.95[b]	.01
ln FM	7844.29	<.001	7738.12	<.001	9816.09	<.001	5404.20[a]	.03	4010.52	.09
BSI vs. weight	70.22	<.001	59.60	<.001	65.70	<.001	34.08[a]	<.001	18.30[a]	.004
Lean mass	148.76	<.001	152.26	<.001	169.01	<.001	142.71	<.001	49.67[b]	.003
ln FM	810.55	<.001	850.93	<.001	1101.42	<.001	546.16[a]	.04	466.94	.07

Note: Data are regression slopes (β) and corresponding *p* values obtained from linear regression models with bone parameters as dependent variables and weight, lean mass, and LnFM as independent variables, respectively. vBMD = volumetric bone mineral density; CSA = cross-sectional area; CSMI = cross-sectional moment of inertia; BSI = bone strength index; ln FM = natural logarithm transformed fat mass.

[a] $p < .05$ vs. β in 11-, 13-, or 18-year-old girls by *t* test.

[b] $p < .05$ vs. β in other age groups by *t* test.

follow-up did not change the pattern of results in the study (data not shown), which again suggests that the findings are robust.

In conclusion, bone does not strengthen adequately to maintain equilibrium with the load from greater body weight, leading to an age-dependent relative bone-strength deficit in women from peripuberty to postmenopause. This is largely due to the fact that the weight gain in women from puberty onward is, for the most part, attributable to fat mass accumulation, and the beneficial effects of increased fat mass on bone, if any, do not compensate for the mechanical burden that it imposes.

Disclosures

The study sponsors played no role in the study design; data collection, analysis, or interpretation; writing of the report; or the decision to submit the article for publication. The authors are solely responsible for writing and submitting the manuscript for publication. All the authors state that they have no conflicts of interest.

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IV

IS BONE LOSS THE REVERSAL OF BONE ACCRUAL? - EVIDENCE FROM A CROSS-SECTIONAL STUDY IN DAUGHTER-MOTHER-GRANDMOTHER TRIOS

by

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Is Bone Loss the Reversal of Bone Accrual? Evidence From a Cross-Sectional Study in Daughter-Mother-Grandmother Trios

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ABSTRACT

Bone adapts to mechanical loads applied on it. During aging, loads decrease to a greater extent at those skeletal sites where loads increase most in earlier life. Thus, the loss of bone may occur preferentially at sites where most bone has been deposited previously; ie, bone loss could be the directional reversal of accrual. To test this hypothesis, we compared the bone mass distribution at weight-bearing (tibia) and non-weight-bearing (radius) bones among 18-year-old girls, their premenopausal mothers, and their postmenopausal maternal grandmothers. Bone and muscle properties were measured by pQCT, and polar distribution of bone mass was obtained in 55 girl-mother–maternal grandmother trios. Site-matched differences in bone mass were compared among three generations. The differences between girls and mothers and between mothers and grandmothers were used to represent the patterns of bone mass accrual from early adulthood to middle age and bone loss from middle to old age, respectively. Compared to the mothers, 18-year old girls had less bone mass in the anterior and medial-posterior regions of the tibial shaft, while the grandmothers had less bone in the anterior and posterior regions. In contrast, the bone mass differences in the radial shaft between girls and mothers and mothers and grandmothers were relatively uniform. We conclude that both bone accrual and loss are direction-specific in weight-bearing bones but relatively uniform in non-weight-bearing bones. Bone loss in old age is largely, but not completely, a reversal of the preferential deposition of bone in the most highly loaded regions during early life. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: BONE MASS DISTRIBUTION; TIBIA; RADIUS; LOAD; MECHANOSTAT

Introduction

Bone must be strong for bearing load, yet light for facilitating mobility.[1,2] During growth, optimal bone strength is achieved by modifying mass distribution rather than by increasing mass alone.[3] The diversity of bone mass distribution is attributable to the different degrees of focal modeling around the periosteal perimeter (periosteal apposition) and remodeling at the corresponding point on the endocortical surface (endocortical resorption).[4,5] Bone modeling deposits bone mineral where it is needed and remodeling removes it from where it is not, to optimize strength while minimizing mass.[1,4,6]

Bone is designed to meet mechanical demand and hence the optimization of bone mass distribution is driven by the load applied on it. As a result, the pattern of bone mass accumulation resembles the strain distribution.[2,7] For example, we previously demonstrated that in pubertal girls, significantly more bone mass was deposited at the anterior and posterior periosteal surfaces than at the medial and lateral surfaces at the tibial shaft during a 2-year follow-up.[8]

After puberty, the optimization of bone mass distribution probably continues, in response to the change of the strains from youth through adulthood to old age. Indeed, periosteal apposition subsides but continues at a slower rate,[9] while the endocortical resorption increases gradually.[10] During aging, loads decrease to a greater extent at those skeletal sites where loads increase most from youth to adulthood.[11,12] Thus, the loss of bone may occur preferentially at sites where more bone is

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deposited in earlier life. In other words, the bone loss in old age is possibly a directional reversal of bone accrual in early life. This hypothesis that "bone loss is the reversal of accrual" is attractive and in accordance with the principles of mechanostat theory. However, it lacks supportive evidence because of the infeasibility of longitudinal cohort studies from youth to old age.

In this study, instead of longitudinal data we used cross-sectional data spanning three generations to test this hypothesis by comparing the bone mass distribution of weight-bearing (tibia) and non-weight-bearing bone (radius) among 18-year-old girls, their premenopausal mothers, and their postmenopausal maternal grandmothers. The differences in bone mass distribution among girl-mother-grandmother trios were used to represent the bone accrual from early adulthood to middle age and the bone loss from middle to old age. The family-based design was intended to minimize the differences in genetic background and lifestyle among subjects, and it may be the best alternative to impractical long-term longitudinal studies.

Subjects and Methods

Subjects

The study population of this report is a part of the CALEX family study that has been described elsewhere.[13,15] Briefly, 396 girls recruited in the city of Jyväskylä and its surroundings in Central Finland participated in the laboratory tests 1 to 8 times from 2000 to 2008 (mean duration of total follow-up was 7.5 years) and the data of girls at the early adult stage (mean age 18.1 yrs) measured in 2007 and 2008 were used in this study. In addition, 257 biological mothers and 111 maternal grandmothers of these girls were invited to participate in the bone measurements in the years 2003, 2004, 2007, and 2008. In total, 106 girl-mother–maternal grandmother trios were found in this population. Further, only premenopausal mothers (mean age 43.6 years) and postmenopausal grandmothers (mean age 68.0 years) who had no history of medication (such as hormone replacement therapy or antiosteoporotic drugs) or diseases known to affect bone metabolism were included, which resulted in the exclusion of 51 trios. Hence, 55 girl-mother–maternal grandmother trios were used in this report.

The study protocol was approved by the ethical committee of the University of Jyväskylä and the Central Finland Health Care District. Informed consent was given by all subjects prior to the assessments.

Bone and muscle measurements

The left tibial and radial shafts were scanned using peripheral quantitative computerized tomography (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany). The scan locations were at 60% of lower leg length up from the lateral malleolus and 30% of forearm length proximal to the wrist joint surface. Image processing and calculations of bone parameters were done using the Geanie 2.1 (BonAlyse Oy, Jyväskylä, Finland). The bone and muscle parameters included total and cortical bone mineral content (BMC, mg/mm), bone cross-sectional area (CSA, mm^2), volumetric bone mineral density (vBMD, mg/cm^3), and muscle CSA (mCSA, mm^2). A threshold of 280 mg/cm^3 was used for the detection of the outer bone border and 11–279 mg/cm^3 for the determination of mCSA. The coefficient of variation (CV) of two repeated measurements on the same subject on the same day was on average 1% for total CSA, cortical CSA, mCSA, cortical BMC, and total BMC and $< 1\%$ for vBMD.

The principle of polar bone mass distribution analysis has been described elsewhere.[16] Briefly, an orthogonal coordinate system was established on the cross-section of the tibial and radial shaft. The *y*-axis was defined to coincide with the direction of the greatest width of the tibial or radial shaft, passing through the mass center of the cross-section. The *x*-axis was defined as perpendicular to the *y*-axis through the mass center. Then, the total cross-section was divided into 72 sectors, each having an angle of 5°. The BMC within the area of each sector was given by the Geanie 2.1 software (Bonalyse Oy, Jyväskylä, Finland). The differences in the mean values of BMC in each sector (ie, the site-matched difference in BMC) between girls and mothers and between mothers and grandmothers were calculated.

Physical activity assessment

Leisure time physical activity (LTPA) level was evaluated using a self-administrated physical activity questionnaire, which has been described elsewhere.[17,18] Briefly, the intensity of each activity was calculated on the basis of the energy expenditure per minute.[19] Bone loading was based on whether the activity was weight bearing or not. This LTPA score took into account the frequency, intensity, duration, and loading of certain exercises.

Statistical analysis

All data were checked for normality using the Shapiro-Wilk W test in SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Because the LTPA score was not normally distributed, its natural logarithm was used in the comparisons. Repeated-measures analysis of variance (ANOVA) was used to evaluate the differences of all parameters among the girl-mother-grandmother trios. In this analysis, the measurements of the members of each trio were matched and treated as if they had been in one woman at three time points (ie, young adulthood, middle age, and old age, respectively). The BMC values in each of the 72 sectors of tibial and radial cross-sections were compared in site-matched and pairwise fashion among trios with Sidak correction. The differences in estimated marginal mean BMC between girls and mothers and between mothers and grandmothers were, respectively, used to represent the bone accrual from young adulthood to the middle age and bone loss from middle to old age in specific sites. Differences were considered significant if $p < .05$.

Results

Girls' and mothers' height did not differ and both were taller than grandmothers, whereas girls weighed less than mothers and grandmothers. Girls were the most physically active and grandmothers were the most inactive as measured by LTPA score (Table 1).

In the tibial and radial shaft, mothers had the highest cortical CSA, total and cortical BMC, and BMD (p all $<.05$). However, the

Table 1. Basic Characteristics of Girls, Their Mothers, and Their Grandmothers

	Girls	Mothers	Grandmothers
n	55	55	55
Age (yr)	18.1 ± 1.0	43.6 ± 3.1*,†	68.0 ± 4.4‡
Height (cm)	165 ± 5	166 ± 7†	161 ± 5‡
Weight (kg)	60.1 ± 9.4	70.5± 13.1*	71.6 ± 13.2‡
BMI (kg/m^2)	22.0 ± 3.2	25.6 ± 4.5*,†	27.6 ± 4.6‡
LTPA score	58.2 (0.21, 449)	36.7 (0.30, 302) *,†	28.8 (0.30, 244)‡

All data were presented as mean ± SD except LTPA score, which was presented as median (range). BMI, body mass index, calculated as weight (kg)/(height [m])^2; LTPA score, leisure time physical activity score.
*Mothers compared with girls, $p < .05$.
†Mothers compared with grandmothers, $p < .05$.
‡Grandmothers compared with girls, $p < .05$.

total CSA remained similar between mothers and grandmothers (p all $>.05$). In the left lower leg, the mothers had larger muscle CSA than girls and grandmothers (p all $<.05$), whereas in left forearm, the mothers' muscle CSA was larger than that of the girls ($p < .001$) but similar to that of the grandmothers ($p = .234$) (Table 2).

The analysis of bone mass distribution showed that, in the tibial shaft, more bone mass was located in the anterior and posterior regions, with less in the lateral and medial regions in all subjects (Fig. 1*A*, *C*). Compared with mothers, 18-year-old girls had significantly less bone mass in the anterior and medial-posterior regions, whereas no difference was found in the lateral-posterior region (Fig. 1*B*). The site-matched differences in BMC between mothers and grandmothers showed that the bone mass reduction in the older women mainly occurred in the anterior and posterior regions (Fig. 1*D*).

In the radial shaft, the girls' bone mass distribution was very similar in shape to that of their mothers (Fig. 2*A*). The difference of bone mass between the girls and the mothers was relatively homogenous except in the ulnar-lateral direction (Fig. 2*B*). Compared with the mothers, the grandmothers' radial shafts had a similar mass in the ulnar region but uniformly less in other regions (Fig. 2*C*, *D*).

Discussion

In this study, we compared the difference in bone mass distribution among family members spanning three generations to infer the longitudinal patterns of bone accrual and loss in weight-bearing (tibia) and non-weight-bearing (radius) long bones. The results showed that in the anterior side of the tibial cross-section and the majority of the radial cross-section, the changes in bone mass distribution between mothers and grandmothers were almost the mirror image of the pattern of accrual observed between girls and mothers. However, in some

Table 2. Bone and Muscle Traits of Girls, Their Mothers, and Their Grandmothers

	Girls	Mothers	Grandmothers
Tibial shaft	*n* = 55	*n* = 55	*n* = 55
BMC_{tot} (mg/cm)	3572 ± 388	3806 ± 422*,†	3474 ± 444
CSA_{tot} (mm^2)	386 ± 41	402 ± 46*	395 ± 42
BMD_{tot} (mg/cm^3)	927 ± 48	948 ± 36*,†	879 ± 55‡
BMC_{co} (mg/cm)	3101 ± 359	3323 ± 396*,†	2973 ± 444
CSA_{co} (mm^2)	292 ± 33	305 ± 35*,†	287 ± 39
$vBMD_{co}$ (mg/cm^3)	1061 ± 33	1090 ± 25*,†	1034 ± 41‡
mCSA (mm^2)	6514 ± 1107	7152 ± 1077*,†	6642 ± 978
Radial shaft	*n* = 52	*n* = 48	*n* = 38
BMC_{tot} (mg/cm)	948 ± 100	1042 ± 124*,†	920± 124
CSA_{tot} (mm^2)	94.5 ± 8.8	99.9 ± 11.0*	99.8 ± 9.8‡
BMD_{tot} mg/cm^3)	1003 ± 37	1042 ± 44*,†	920 ± 73‡
BMC_{co} (mg/cm)	816 ± 96	906 ± 121*,†	763 ± 123‡
CSA_{co} (mm^2)	75.8 ± 7.6	81.0 ± 9.7*,†	72.7 ± 9.7
$vBMD_{co}$ (mg/cm^3)	1076 ± 26	1118 ± 31*,†	1046 ± 45‡
mCSA (mm^2)	1509 ± 205	1723 ± 234*	1678 ± 267‡

All data presented as mean ± SD. Tot, total bone; co, cortical bone. BMC, bone mineral content; CSA, cross-sectional area of bone; vBMD, volumetric bone mineral density; mCSA, muscle cross-sectional area.
*Mothers compared with girls, $p < .05$.
†Mothers compared with grandmothers, $p < .05$.
‡Grandmothers compared with girls, $p < .05$.

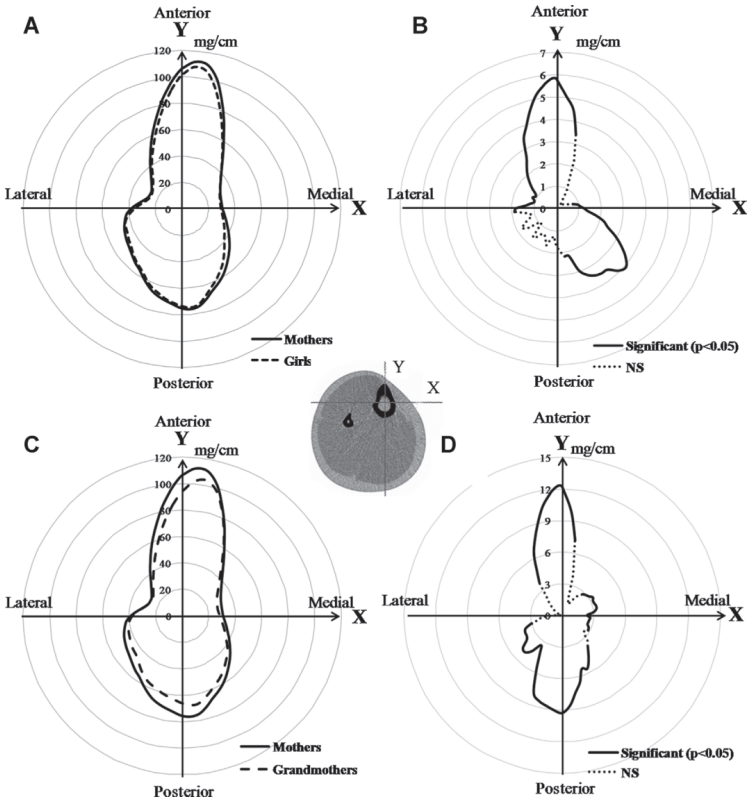

Fig. 1. Polar distribution of bone mineral content (BMC) and the site-matched differences of estimated marginal mean BMC followed the anatomic shape of tibial shaft in three generations. The *y*-axis was defined to coincide with the direction of the greatest width of tibial shaft, which goes through the mass center of the cross section. The *x*-axis was defined as perpendicular to the *y*-axis through the mass center. *(A)* Polar distribution of BMC at girls' and their mothers' tibial shaft *(B)* Site-matched differences of estimated marginal mean BMC between girls and their mothers at tibial shaft *(C)* Polar distribution of BMC in mothers' and grandmothers' tibial shaft *(D)* Site-matched differences of estimated marginal mean BMC between mothers and grandmothers at tibial shaft.

regions, such as the posterior side of tibial shaft and part of the ulnar side of radial shaft, the patterns of bone accrual and loss did not exactly follow the "reversal" paradigm.

Our previous study demonstrated that more bone was deposited along the anterior and posterior directions at the tibial shaft in pubertal girls.[8] This different focal periosteal apposition continued through the adolescent years, producing a bone cross-sectional shape that deviated more strongly from circularity. After 18 years of age, the gain of body weight, as well as the expansion of muscle CSA of the lower leg, continued. As a result, the loads applied on the tibial shaft increased, with larger strains on the anterior side than elsewhere.[20,21] Such mechanical change can be sensed by osteocytes and osteoblasts,[22,23] which initiate periosteal apposition preferentially where the highest strains exist.[24,25] Accordingly, in this analysis the most significant difference in bone mass between 18-year-old girls and their mothers was found in the anterior region. This indicated that bone mass accrual from early adulthood to middle age is direction specific, corresponding with the change of strains. This observation is consistent with the direction-specific changes of bone geometry and mass distribution in people subjected to different exercise loading,[26,27] and it corroborates the mechanostat paradigm.[28]

During aging physical activity level dropped, as demonstrated by the difference in LTPA score between mothers and grandmothers. In addition, muscle CSA also reduced, indicating the weakening of muscle strength. As a result, the customary loads on the tibial shaft are lower. According to the mechanostat paradigm, when loading of bone is insufficient to produce bone strains that are above a minimum level of effective strain, bone mass and architecture are remodeled until bone strains are within the minimal effective strain range.[29] The bone mass

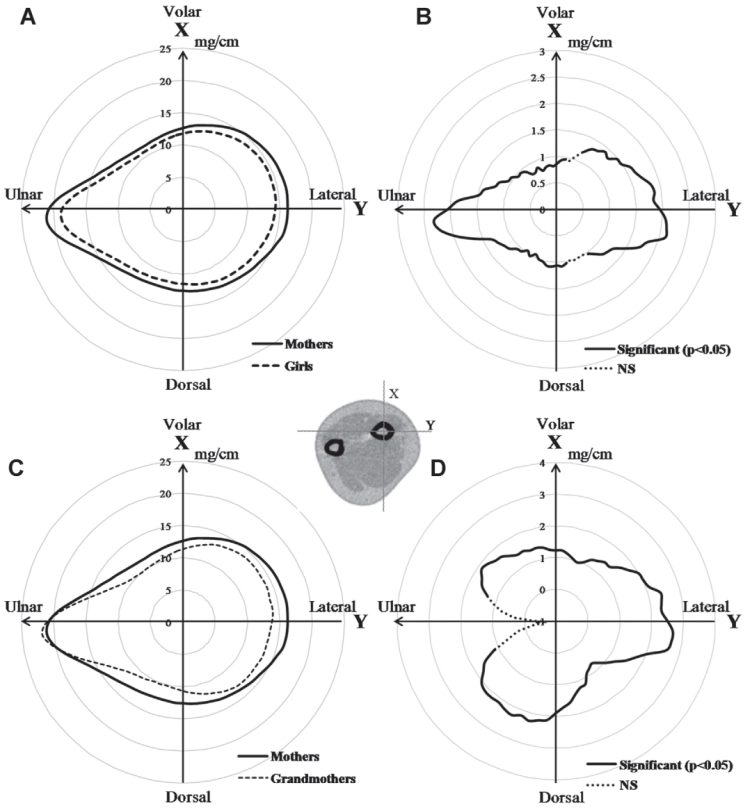

Fig. 2. Polar distribution of bone mineral content (BMC) and the site-matched difference of estimated marginal mean BMC followed the anatomic shape of radial shaft in three generations. The *y*-axis was defined to coincide with the direction of the greatest width of radial shaft, which goes through the mass center of the cross section. The *x*-axis was defined as perpendicular to the *y*-axis through the center of mass. *(A)* Polar distribution of BMC at girls' and their mothers' radial shaft *(B)* Site-matched differences of estimated marginal mean BMC between girls and their mothers at radial shaft *(C)* Polar distribution of BMC at mothers' and grandmothers' radial shaft *(D)* Site-matched differences of estimated marginal mean BMC between mothers and grandmothers at radial shaft.

reduction in grandmothers occurred mainly in the anterior and posterior regions, indicating that bone strain declined most pronouncedly in the anterior-posterior direction.

In contrast to the tibia, the loads on the radius largely come from the muscles responsible for motions of hand and fingers,[30] and the forces from these muscles are usually multidirectional and of mild intensity. As a result, the bone mass accumulation or loss in the radial shaft was relatively uniform compared to that in the tibial shaft.

In some regions of tibial and radial shaft, the patterns of bone accrual and loss were more complicated. For example, bone mass in the lateral-posterior region of tibial shaft did not differ between girls and mothers, indicating that at age 18 years the BMC in this region has already approximately reached its peak value. On the other hand, in the medial-posterior region bone mass still increased pronouncedly from early adulthood to middle age but decreased less from middle to old age. The frequent direct stimuli to this region from the powerful soleus muscle[30] may induce bone accrual during growth and may also slow bone loss during aging.

A similar pattern was observed in the ulnar region of the radial shaft. This region corresponds to the sharp and prominent interosseous crest that gives attachment to the interosseous membrane functioning to preclude the oversupination of the forearm.[30] The direct mechanical stimuli from the dragging of muscles or the interosseous membrane may help prevent the bone loss, consistent with the previous finding that increased muscle gain during growth affects modeling of muscle attachment sites.[31] These findings suggested that the change of strains along the tibial and radial shaft was not homogeneous

from site to site throughout life. Despite the complex biomechanical surroundings, the possibility cannot be ruled out that bone mass accrual and loss patterns are largely genetically determined.

The directional modification of bone mass distribution during growth and adulthood has important biomechanical advantages. By depositing more bone where it is mechanically needed the most and less bone where it is needed the least, bone strength relative to the customary load increases efficiently without excessive bulk. The directional bone loss may also have important implications. On one hand, the loss from strategic regions will have a large effect on bone strength that is not fully reflected in the decrease in total bone mass measured by DXA or other densitometric methods. On the other hand, the results indicate that lifestyle modification by increasing physical activity level may preferentially preserve bone at strategic regions.

This study has some limitations. The difference in bone mass distribution among girl-mother–maternal grandmother trios did not perfectly represent the true longitudinal pattern of bone accrual and loss in individuals. However, the genetic link and the resemblance in living environment among family members may help minimize possible biases, because the variance in bone properties in the population is largely the result of individual differences in genetic makeup, confounded by lifestyle.[32,33] Indeed, replacing the 55 biological mothers with unrelated age-matched premenopausal women led to more variable results (see the supplemental data). The distribution of BMC differences between girls and unrelated premenopausal women was irregular and different from that between girls and their biological mothers. In addition, although the distribution of BMC differences between unrelated premenopausal women and grandmothers was similar to that obtained from mother-grandmother pairs, especially at the tibia shaft, the differences were much less statistically significant because of the larger variance. This extra finding suggested that the genetic influence is more pronounced for bone accrual, whereas bone loss is probably a more age-dependent process. However, it also indicated that, for a given sample size, the three-generation setting is superior to other designs comprising unrelated populations in reducing variation. Another limitation of this study is that the age of the mothers (around 43 years old) in this study was older than that of peak bone mass (30 to 39 years).[34,35] Thus, the continued modification of bone mass distribution (either increase or decrease) may be underestimated.

In conclusion, the distribution of bone mass undergoes modification from early adulthood through middle age into old age. Both bone accrual and loss are direction specific in weight-bearing bone but relatively uniform in non-weight-bearing bone. The bone loss in the old age is largely, but not completely, a reversal of the preferential deposition of bone during early life.

Disclosures

All the authors state that they have no conflicts of interest.

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