STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
110

Qingju Wang

Bone Growth in Pubertal Girls

Cross-Sectional and Longitudinal Investigation of the Association of Sex Hormones, Physical Activity, Body Composition and Muscle Strength with Bone Mass and Geometry

JYVÄSKYLÄN YLIOPISTO

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Editors
Harri Suominen
Department of Health Sciences, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
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Read about it extensively, inquire it thoroughly, reflect it carefully, judge it prudently, and practice it earnestly.
Let a man proceed in this way, though dull, He will surely become intelligent; though weak, he will surely become strong.

----- « Doctrine of the Mean - 20th »

博學之, 審問之, 慎思之, 明辨之, 篤行之。果能此道矣, 雖愚必明, 雖柔必強.

----- «中庸• 二十»

To my family and mentors
獻給我的家人及良師益友們

ABSTRACT

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Strong bones are essential to overall health and quality of life. Optimizing peak bone mass during growth is a key strategy in preventing fragility fractures in later life. Understanding the biological process of bone growth and its regulators assumes the greatest importance in realizing this strategy. This study aimed to investigate bone growth in terms of bone size, bone mineral content (BMC) and volumetric mineral density (vBMD) and its relationship with sex hormones, physical activity (PA), body composition and muscle strength in pubertal girls. A total of 258 healthy girls aged 10-13 years were included in the 2-years observational study at baseline. The PA level was assessed using a questionnaire. Body composition and BMC were assessed by dual-energy x-ray absorptiometry. Bone geometry and vBMD at the distal radius (DR) and tibial shaft (TS) were measured by peripheral quantitative computed tomography. Muscle strength of elbow flexors and knee extensors was determined by a dynamometer. Sex hormones were assessed by fluoroimmunoassay. The results showed that the timing of peak growth velocity of BMC lags behind that of bone size by more than 6 months in pubertal girls. This growth asynchrony resulted in vBMD of DR (the metaphyseal trabecular bone site) decreasing slightly from prepuberty up to 1 year before menarche. The cross-sectional area (CSA) of marrow cavity at TS (the diaphyseal cortical bone site) did not change prior to menarche but decreased thereafter. 17β-Estradiol (E2) was not correlated with total bone CSA, but was positively correlated with cortical CSA and cortical thickness (Cth) at TS. On the other hand, testosterone (T) was positively correlated with total bone CSA, but not with cortical CSA and Cth. PA level was positively associated with BMC in early pubertal girls. Total body FM correlated significantly higher with leg BMC than with arm BMC. BMC of arm and leg was highly correlated with muscle strength of elbow flexors and knee extensors, respectively. However, BMC per unit of muscle strength was significantly higher in leg than in arm. The observational study suggests that in pubertal girls, the growth of bone size precedes that of bone mass, and this phenomenon may responsible for the elevated fracture risk during pubertal growth spurt. E2 might be important to counteract this transitory elevated fracture risk, because it makes bone stronger by inhibiting bone resorption and even promoting bone formation at the endocortical surface. E2 may also interact with mechanical loading to facilitate bone mass accretion during pubertal growth. In addition, muscle strength together with body composition may play an important role in determining bone mass.

Key words: Puberty, girls, bone, physical activity, sex hormones, body composition, muscle strength.

Author's address

Qingju Wang
Department of Health Sciences
University of Jyväskylä
40014 Jyväskylä
Finland

Supervisors

Sulin Cheng, Ph.D., Professor
Department of Health Sciences
University of Jyväskylä

Markku Alén, M.D., Ph.D., Professor
Department of Health Sciences
University of Jyväskylä

Harri Suominen, Ph.D., Professor
Department of Health Sciences
University of Jyväskylä

Reviewers

Ego Seeman, M.D., Professor
Department of Endocrinology and Medicine
Austin and Repatriation Medical Centre
University of Melbourne
Australia

Eckhard Schönau, M.D., Professor
Children's Hospital
University of Cologne
Germany

Opponent

Pekka Kannus, M.D., Ph.D., Professor
Accident & Trauma Research Center
UKK Institute for Health Promotion Research
Tampere
Finland

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Jyväskylä, November 2005 Qingju Wang

王 慶 聚

LIST OF ORIGINAL ARTICLES

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

I Wang Q, Alén M, Nicholson PHF, Lyytikäinen A, Suuriniemi M, Helkala E, Suominen H, Cheng S 2005. Growth patterns at distal radius and tibia shaft in pubertal girls – a 2-year longitudinal study. J Bone Miner Res 20: 954-61

II Wang Q, Alén M, Nicholson PHF, Suuriniemi M, Lyytikäinen A, Helkala E, Suominen H, Cheng S 2004. Relationship of sex hormones to bone geometric properties and mineral density in early pubertal girls. J Clin Endocrinol Metab 89: 1698 – 1703

III Wang Q, Alén M, Nicholson PHF, Halleen JM, Alatalo SL, Ohlsson C, Suominen H, Cheng S. Differential effects of sex hormones on peri- and endocortical bone surfaces in pubertal girls. J Clin Endocrinol Metab published on-line first October 25, 2005 as doi:10.1210/jc.2005-1608

IV Wang Q, Suominen H, Nicholson PHF, Zou L, Alén M, Koistinen A, Cheng S 2005. Influence of physical activity and maturation status on bone mass and geometry in early pubertal girls. Scand J Med Sci Sports 15: 100-6

V Wang Q, Alén M, Nicholson PHF, Suominen H, Koistinen A, Kröger H, Cheng S. Bone mineral content in relation to body composition and muscle strength in upper and lower limbs of pubertal girls – 2-year longitudinal study (submitted for publication).

ABBREVIATIONS

ANOVA	Analysis of variance
BA	Bone area
BMC	Bone mineral content
BMD	Bone mineral density,
aBMD	Areal bone mineral density
vBMD	Volumetric bone mineral density
CSA	Cross-sectional area
Cth	Cortical thickness
CV	Coefficient of variation (expressed as %)
DR	Distal radius
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
DXA	Dual energy X-ray absorptiometry
E1	Estrone
E2	17β-estradiol
E3	Estriol
EC	Endocortical circumference
FM	Fat body mass
FN	Femur neck
GH	Growth hormone
LS	Lumbar spine
LS2-4	Lumbar spine 2-4
LM	Lean body mass
MVC	Maximal voluntary contraction
PA	Physical activity
PBM	Peak bone mass
PHV	Peak height velocity
PC	Periosteal circumference
pQCT	Peripheral quantitative computed tomography
QCT	Quantitative computed tomography
SD	Standard deviation
SE	Standard error
SHBG	Sex hormone binding globulin
T	Testosterone
TB	Total body
TF	Total femur
TS	Tibial shaft

CONTENTS

1 INTRODUCTION

A skeleton with adequate material and structural strength is essential to overall health and quality of life. However, osteoporosis and consequent fragility fractures are major clinical and public health problems in aging populations (Wasserman & Barzel 1987; Melton 1997). Despite advances in therapy, reversal of established osteoporosis is a formidable challenge, and deformity and disability due to fractures often persist. Bone mineral content (BMC) in old age is the result of peak bone mass (PBM) achieved at skeletal maturity and subsequent age-related bone loss (Seeman et al. 1993). Thus, optimizing PBM by promoting bone mass accretion in childhood is a clinically-attractive approach to prevent fragility fractures in later life.

To be able to achieve abovementioned goal, a proper understanding of the biological process of bone growth is a first step. Puberty is characterized by rapid anthropometric growth and emergence of secondary sexual characteristics. Approximately 50% of PBM is accreted during this period (Bass et al. 1999; Bailey et al. 2000). This rapid growth period represents a prime time for intervention to promote bone health. Rapid growth also provides a window for unfavorable factors, such as smoking, alcohol using, malnutrition, and physical inactivity, to predispose bone development to a suboptimal level. Furthermore, rapid growth itself also is accompanied by elevated fracture risk, especially in the upper limbs. Thus, as Parfitt claimed: "Growth confers risks as well as benefits to the skeleton" (Parfitt 1994).

Sex hormones together with growth hormone induce the pubertal growth spurt. Since both estrogens and androgens are present in each sex, it is difficult to discern the exact role that each sex steroid plays on skeletal physiology. A growing body of evidence indicates that estrogen is the major hormone responsible for the acquisition and maintenance of bone in growing and aging bones and for normal pubertal development and epiphyseal maturation (Smith et al. 1994; Morishima et al. 1995). On the other hand, the effects of estrogen and androgen on the bone periosteal and endocortical surfaces are less clear. This study aimed to clarify the roles of sex hormones

in the development of bone mass and bone geometric properties during pubertal growth. The results of this study will hopefully further our understanding of various bone disorders associated with sex hormones during growth.

The skeleton is remarkably adapted to provide adequate strength and mobility so that bones do not break when subjected to substantial forces. Elite athletes usually possess higher bone mass than non-athlete controls (Suominen 1993). Most people do not participate in intensive physical training, and hence the effect of normal leisure-time PA on bone size and mass is of great significance for advocating and promoting life-long active and healthy lifestyles. Local muscle contraction provides the largest voluntary bone loads and strains, without which bone mass will not fully develop, as evidenced by paralyzed limbs (Henderson 1997). However, the relative importance of muscle strength and weight-bearing in bone mass accretion has not been addressed quantitatively. This is important issue in order to formulate an exercise regimen to efficiently promote bone mass accretion during growth. In addition, the role of body fat and its effect on bone mass accretion during puberty remain unclear. To clarify this question has great significance in order to understand why fracture risk is higher in overweight or obese children even though they possess more bone mass than their normal counterparts.

2 REVIEW OF THE LITERATURE

2.1 Growth and development of girls during puberty

After a relative stable growth process during childhood, children enter a dynamic development process marked by rapid changes in body size, shape and composition as well as the emergence of secondary sexual characteristics (Rogol et al. 2002). The clock that turns on puberty is still unknown (Apter & Hermanson 2002). The serum levels of sex hormones increase progressively during puberty. Largely due to the differential sex hormone exposure between pubertal girls and boys, complete sexual differentiation and maturation is achieved at the end of puberty: girls become women and boys become men.

According to the development of breast and pubic hair, puberty is subdivided into 5 developmental stages according to Tanner (Tanner Stage I-V) (Tanner 1969). The adolescent growth spurt, one of the hallmarks of puberty, typically occurs at Tanner breast stage III, corresponding to 11-12 years-of-age in girls. Girls average a peak height velocity (PHV) of 9 cm/year at age 12 and a total gain in height of 25 cm in the pubertal period (Marshall & Tanner 1969; Sheehy et al. 1999). The growth tempos of the axial and appendicular skeleton differ. Before puberty, the legs grow more rapidly than the trunk. During puberty, the growth spurt focus shifts to the trunk (Bass et al. 1999).

Significant weight gain occurs during puberty. In girls, peak weight velocity reaches 8.3 kg/year at about 12.5 years and lags behind PHV by approximately 6 months (Barnes 1975). Moreover, body composition also undergoes dramatic change. Girls and boys have similar amounts of FM in the prepubertal period, and the percentage of FM is slightly higher in girls (Taylor et al. 1997; Mast et al. 1998; Lohman et al. 1999). During puberty, sexual dimorphism in body composition, including the regional distribution of body fat, develops under the effects of sex hormones. Girls accumulate fat

mass (FM) by more than 1 kg/year and their percentage of body fat also increases during puberty (Wheeler 1991; Bitar et al. 2000). Importantly, FM during puberty in girls is distributed more preferentially to the peripheral and subcutaneous sites than in childhood. This results in the typical gynoid pattern of fat distribution in the older female adolescents (de Ridder et al. 1990; Fox et al. 2000).

Peak velocity of lean body mass (LM) reaches more than 5 kg/year at about 12 years in pubertal girls. The peak velocity of BMC lags behind that of LM by 6 months and can reach more than 310 g/year (Bass et al. 1999; Rauch et al. 2004). At age 18, more than 90% of PBM has been accrued in healthy female adolescents (Bonjour et al. 1991; Theintz et al. 1992; Matkovic et al. 1994).

During the prepubertal stage, growth hormone (GH), in addition to thyroid hormones, is the major determinant of growth (Patel & Clayton 2005). Preceding the onset of puberty by about 2 years, increased amount of androgens, such as DHEA and DHEAS, are excreted by adrenal cortex (Sklar et al. 1980). These adrenal androgens are responsible for the mid-childhood growth spurt and the appearance of axillary hair and, in part, for the appearance of pubic hair (Voutilainen et al. 1983; Parker 1991). As puberty initiates and progresses, the frequency and amplitude of gonadotropin secretary pulses increase (Corley et al. 1981). The rising levels of gonadotropins stimulate ovaries to enlarge and produce 17β-estradiol (E2) (Motta et al. 1968). E2 is responsible for the development of the breasts and reproductive organs, fat redistribution and bone maturation (Dattani & Hindmarsh 2005). In Tanner stage III-IV, the endometrium of the uterus is sufficiently proliferated to allow the first withdrawal bleeding, the menarche, to occur. The mean age at menarche in Europe varies from 12.5 ± 1.5 years in Italy to 13.6 ± 1.5 years in Nordic countries (Hoey et al. 1986; Onland-Moret et al. 2005). Androgen and estrogen both increase GH secretion (Patel & Clayton 2005). The interplay of sex hormones and GH triggers the pubertal growth spurt (Rogol 2004).

There is a large between-individual variation in tempo of growth, timing and rate of sexual development, and skeletal maturation even among children of the same sex and ethnic background (Rogol et al. 2002). Figure 1 demonstrates the normal variation in the timing of PHV, menarche age and Tanner stage development.

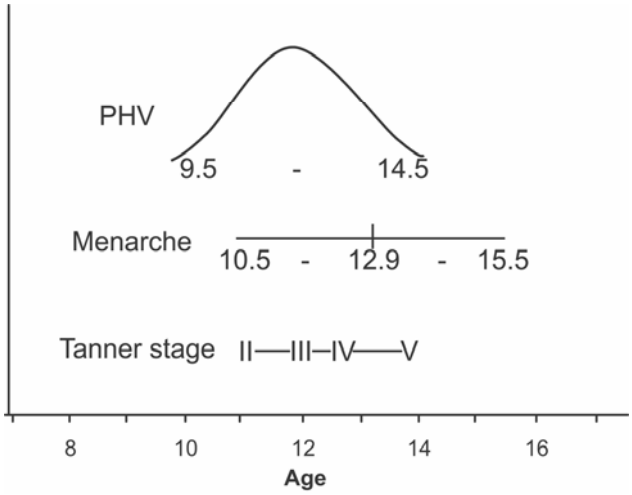

FIGURE 1 Normal variation of timing of peak height velocity (PHV), menarche age, and Tanner stage development (Marshall & Tanner 1969). The mean menarche age (12.9 years) in our study is also given.

2.2 Bone structure and functions

Bone is a highly specialized form of connective tissue in which the extracellular organic matrix is mineralized. This confers bones marked rigidity while still maintaining some degree of elasticity (Martin 1998). It is an ever-active organ and possesses remarkable abilities of adaptation and regeneration to better carry out its functions and repair any damage to it (Rubin & Rubin 1999). The skeleton serves both a structural function - providing mobility, support, and protection for the body - and a reservoir function, as the storehouse for essential minerals. In addition, bone is the primary site of hematopoiesis (Buckwalter 1995).

Crystalline salts make up approximately 65% of the wet weight of the bone, and those are primarily calcium and phosphate in the form of hydroxapatite. The organic matrix usually constitutes a little more than 20% of the wet weight, approximately 90% of which is type I collagen; the remaining 10% is composed of proteoglycans and numerous noncollagenous proteins. Compared to adults, each unit of bone tissue contains more organic matrix in children. This results in differential fracture patterns in various age groups (Quelch et al. 1983). Water contributes approximately 10% of the wet weight of bone (Marks & Einhorn 2002).

Three mature cell types are found in bone: osteoblasts and osteoclasts are present on bone surfaces, whereas osteocytes are embedded in the mineralized interior. Osteoblasts are responsible for the production of the bone organic matrix. Osteocytes are mature osteoblasts trapped within the bone matrix and their function is not yet entirely clear. Osteoclasts are large, multinucleated cells that resorb bone tissue (Puzas 1996; Mundy 1999).

The skeleton is composed of two parts: the axial skeleton including the vertebrate, ribs, sternum and skull, and the appendicular skeleton which includes pectoral girdle (shoulder), pelvic girdle (pelvis) and the bones attached to them (arms and hands, legs and feet). Bones can be classified into one of four types based on their shape: long, short, flat, and irregular. Morphologically, there are two forms of bone: cortical (compact or dense) and trabecular (spongy or cancellous) (Figure 2). Differences between cortical and trabecular bone are both structural and functional.

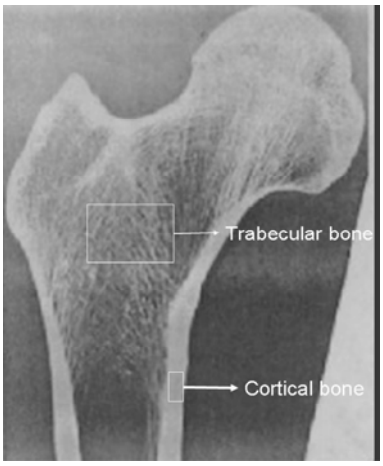

FIGURE 2 Proximal femur structure shows that bone tissue is categorized into two types: trabecular and cortical bone.

2.2.1 Cortical bone

Cortical bone represents approximately 80% of the total skeletal mass, and is found primarily in the diaphyses of long bones and forms the outer shell around trabecular bone at the end of long bones and the vertebrae (Mundy 1999). Cortical bone is relatively dense with a porosity ranging from 5% to 10% (Buckwalter 1995). It has a slower turnover rate and a higher resistance to bending and torsion than trabecular bone.

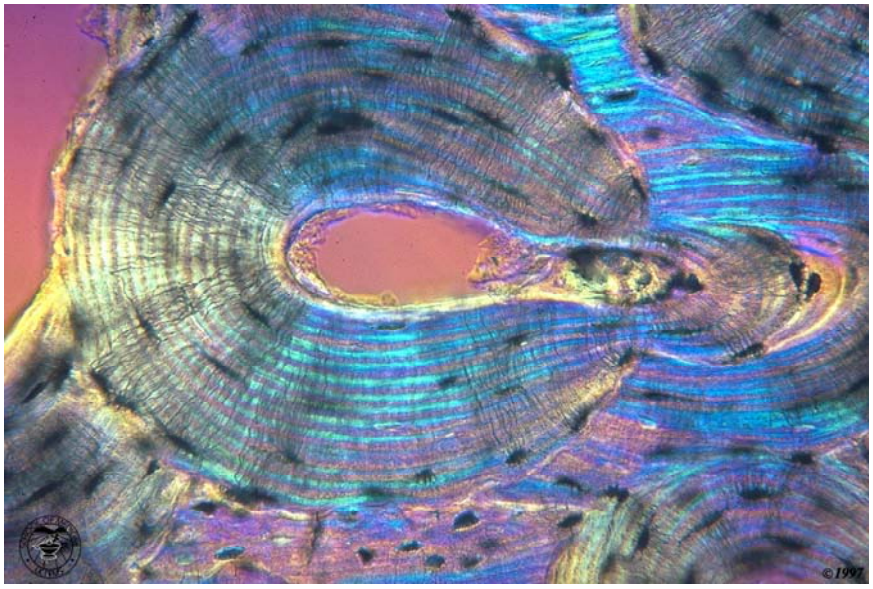

FIGURE 3 Interference contrast micrograph of a Haversian system in cross-sectional view. It demonstrates that a number of concentric lamellae are involved in making a Haversian system. The central canal, Haversian canal, encloses small vessels and nerve. Osteocyte lacunae are linked by web-like canaliculi. At the perimeter of the structure is the cement line (Buckwalter, 1987). Reproduced with permission.

The basic structure unit of cortical bone is the osteon, or Haversian system (Figure 3). Five key elements describe the structure of an osteon: Haversian canal, lamellar, lacunae, canaliculi and cement lines (Jee 1983). Inside the Haversian canal run blood vessels and nerves. Densely packed collagen fibrils form concentric lamellae, and the fibrils in adjacent lamellae run in perpendicular planes as in plywood. Osteocytes lie in the lacunae being connected with each other through canaliculi.

2.2.2 Trabecular bone

Trabecular bone represents 20% of the skeletal mass. It is found in the epiphyseal and metaphyseal regions of long bones. In addition, short, flat and irregular bones are all made of trabecular bone covered with a thin layer of cortical bone. Trabecular bone is less dense than cortical bone, with porosity ranging anywhere from 50% to 90% (Buckwalter 1995). These features are well designed to absorb mechanical energy and provide a large bone surface for mineral exchange.

The basic structure unit of trabecular bone is the trabecula (Figure 4). Trabeculae are rod- or plate-like struts which branch and intersect to form a three-dimensional sponge-like meshwork. The struts are orientated preferentially in the principal direction of stress to which the bone is subjected. The matrix of trabeculae is also deposited in the form of lamellae. However, trabecular lamellae do not form Haversian systems, but are deposited on preexisting trabeculae. Osteocytes, lacunae, and canaliculi in

trabecular bone resemble those in cortical bone. Because of the larger porosity in trabecular compared to cortical bone, the apparent BMD (BMC in a projected area or volume including bony tissue and marrow cavity) at trabecular bone sites is far less than that of cortical bone sites (typically 150 vs. 1100 mg/cm^3) (Arnold et al. 1966). At the material level where only bony tissue is included, however, the BMD of trabecular bone is very similar to that of cortical bone (Arnold 1960; Gong et al. 1964).

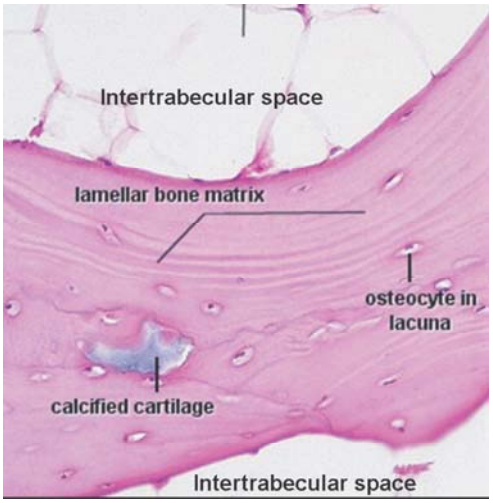

FIGURE 4 Trabecular bone histology shows that trabeculae are deposited in the form of lamellae with osteocytes embedded inside. Reproduced with permission from Slomianka L (2004).

2.2.3 Bone mechanical features

Undoubtedly, several hundred million years of evolution has made bones three-dimensional masterpieces of biomechanical engineering that are strong but also light (Seeman 2003). These two indispensable features are conferred by its special design at the material level and the structural level.

The material properties of bone refers to the intrinsic properties of bone as a material, regardless of its structure or geometry (Einhorn 1992). The organic phase bestows bones with flexibility, allowing bone to deform but not break under stress. The inorganic phase, however, provides bone with stiffness when subjected to stress (Cullinane & Dinhorn 2002). The material properties of bone can be assessed by performing standardized mechanical tests on uniform bone tissue samples under specific loading conditions. This test yields the stress-strain curve (Einhorn 1992) (Figure 5).

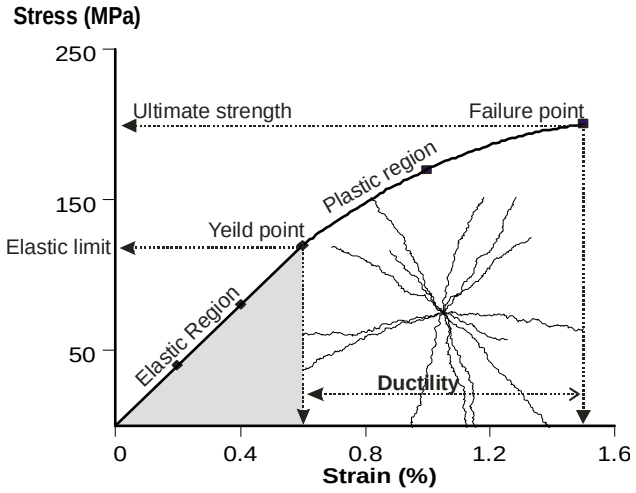

FIGURE 5 Schematic illustration of a typical stress-strain curve of cortical bone specimen loaded in compression. The linear portion of the curve represents the elastic region; loading in this region will result in nonpermanent deformation. The nonlinear portion of the curve represents the plastic region in which damage is induced in the bone with a consequent degree of permanent deformation.

Stress, defined as the force per unit area (Pascal, Newton/m^2) = force (Newton) / area (m^2), can be classified into three basic types: compression, tension, and shear. Alone or in combination, the three basic stress types produce four types of load on bone in nature: compression, tension, bending and torsion (Einhorn 1992). The deformation of a bone while subjected to stress is called strain, defined as the ratio of change in length versus the initial length (strain (%) = $\Delta L/L$). There is a linear relationship between the stress and the resultant deformation. The slope of the linear portion of the stress-strain curve in Figure 5, called Young's Modulus (elastic modulus), denoted as E (Pascal) = stress (Pascal)/strain (%), is a measure of bone's stiffness or rigidity in that particular loading direction. In the elastic region, a material will return to its original shape and dimensions when an applied load is removed. At the point where the curves become nonlinear, the elastic region gives way to the plastic region. The point on the curve where this occurs is known as the yield point. Further loading beyond the yield point will cause permanent deformation of the material. The strength of a bone or specimen of bone tissue is determined by calculating the maximum stress at the failure point. The area under the curve reflects the energy stored by the material prior to failure and is known as the toughness of the specimen. Ductility, which is reflected in the strain from yield point to failure point, describes the extent to which the material can deform plastically before catastrophic failure (Cullinane & Einhorn 2002).

Bone exhibits different mechanical properties in different loading directions (Figure 6) (Turner et al. 1995). The strength and rigidity of a bone are typically greater in the direction of customary loading (Turner & Pavalko 1998; Carter 2000; Martin 2000). A compressive load that is easily sustained by a bone axially can result in fracture if the same load applied from a

transverse direction. This anisotropy results from the preferential orientation of osteons in cortical bone and trabeculae in trabecular bone in the principal loading direction.

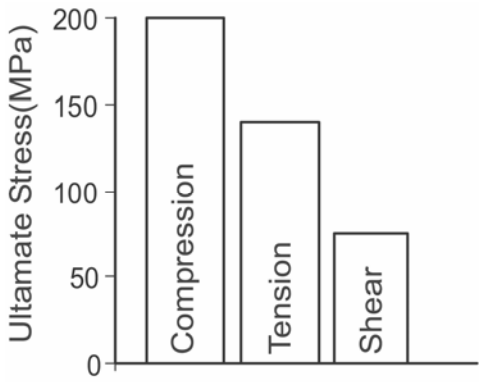

FIGURE 6 Ultimate stress for human adult cortical bone specimens tested in compression, tension and shear. Data was from Reilly (1975).

The bone, when regarded as an anatomic organ with intact structure, possesses specific size, shape, and distribution of its bony tissue in space affecting its strength and conferring it with the properties of lightness. The bone structure is designed to counteract the customary stress it experiences (Wolff 1892). The primary stress at the ends of long bones is compressive. Because of the specific orientation of bone's micro-architecture in favor of sustaining compression, less bony tissue at this site is needed and preferred in order to absorb impact loads by allowing greater deformation than at the shaft of long bones where high bending and torsion stresses are present (Pearson & Lieberman 2004). Expanding the bone diameter while keeping the same amount of bone in the transverse plane so the cortex becomes thinner, bone strength resistance to bending and torsion increases to the fourth power of the increase in diameter (Turner & Burr 1993). Hence, bony tissue at the diaphysis is preferred to be distributed as far away from the neutral axis of the load as possible, because the bone next to marrow contributes little to the bending strength. The tube-like structure makes bone light but strong enough to resist daily voluntary loads (Frost 1990).

The geometric parameter used to describe the resistance to bending is the area moment of inertia (I_x and I_y, Figure 7), defined as the moment of bone area around a given axis (the neutral plane in bending situation) of the rectangular coordinate system in the bone cross-section ($I_x = \int_A y^2 dA$, $I_y = \int_A x^2 dA$, in which x and y are the distance of bone area element dA from the Y and X axis, respectively, and A the area of bone tissue). In the case of torsion, the relevant parameter is the polar moment of inertia (I_p), defined as moment of bone area around the longitudinal bone axis Z (Figure 7) ($I_p = I_x + I_y$) (Turner & Burr 1993).

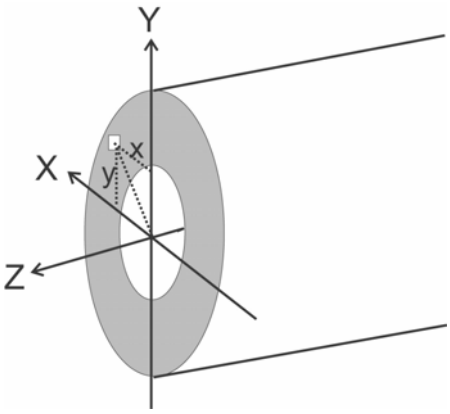

FIGURE 7 Illustration of the bone coordinate system used in the calculation of area and polar moment of inertia.

2.2.4 Definition of bone mineral content/density

Clarifying the definition of BMC and different BMDs is important for interpreting the biological meaning of different densitiometric measurements. BMC is defined as the mass of mineral in a given bone or part of the bone. It is measured by different absorptiometric modalities through the attenuation of the photons during their transmission through the bone (Seeman 2001). Areal BMD is the BMC per unit projected bone area of the bone in the coronal plane (Seeman 2001). In a precise sense, it is not a true density measurement. The thickness of the bone in the unmeasured dimension confounds the interpretation of this measurement. The measurement of vBMD, however, overcomes this limitation. Since bone is a hierarchical structure, the vBMD can be at the total bone level, compartment level, and material level (Rauch & Schönau 2001). The different definition of total bone vBMD, compartment vBMD and material vBMD is illustrated in Figure 8.

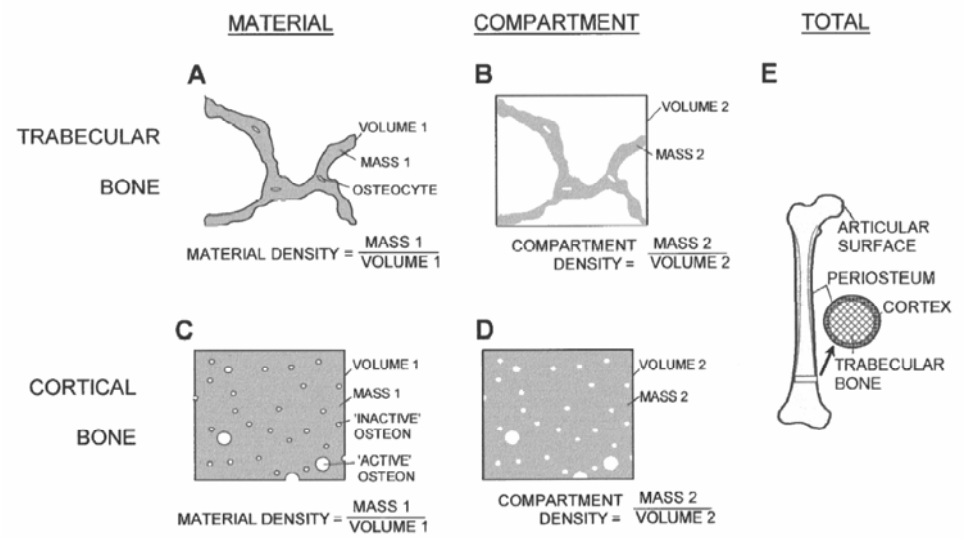

FIGURE 8 Definitions of the various types of mineral density. Material BMD and compartment BMD in trabecular (A and B) and in cortical bone (C and D). The mass of mineral (in gray) determining material BMD and compartment BMD is identical (mass 1 = mass 2), but the volume (encircled by black lines) differs (volume 2 > volume 1). Therefore, material BMD is higher than compartment BMD. (E) Total BMD is defined as the mass of mineral divided by the volume enclosed by the periosteal envelope. This definition can be applied to the entire bone, part of the bone (e.g., the distal or proximal end), or a section through the bone, as shown (Rauch & Schönau 2001). Reproduced with permission of the American Society for Bone and Mineral Research.

2.3 Bone development and growth

There are three distinct types of ossification: endochondral, intramembranous and appositional. Bone formation arising from a cartilaginous template is referred to as endochondral ossification. Intramembranous ossification is achieved by the direct transformation of mesenchymal cells into osteoblasts in the absence of a cartilaginous scaffold (Karaplis 2002). Appositional bone formation occurs during periosteal enlargement of bones and during bone modeling and remodeling. This is strictly true only for the diaphyses of long bones (Marks & Einhorn 2002). All three types of bone formation occur throughout life and can contribute to the restoration of the skeleton after injury or disease (Marks & Einhorn 2002).

2.3.1 Prepubertal development

There is approximately 30 grams of calcium in a fetus at term (Givens & Macy 1933), of which around 80% is accumulated during the third trimester when the fetal skeleton is rapidly mineralized (Givens & Macy 1933; Trotter & Hixon 1974). In the first year of life, the rate of bone turnover approaches 100% per year (Avioli & Krane 1990) and the mean daily balance of calcium

is approximately +400 mg/d (Matkovic 1991). This is achieved by the highest fractional absorption of calcium in infancy (40-60%) and small urine loss (Matkovic 1991; Abrams et al. 1997). As a result, average TB BMC increases by 400% during infancy (Koo et al. 1998). With rapid gain in bone mass, however, the total bone vBMD of long bones decreases by 30% during the first few months of life (Trotter & Peterson 1970). This is followed by a rapid increase until about 2 years of age and a slower increase thereafter (Rauch & Schönau 2001). The postnatal drop in total bone vBMD mainly reflects the larger expansion of marrow cavity relative to total bone volume (Bonnard 1968), which results from the rapid redistribution of bone tissue from the endocortical to the periosteal surface (Vinz 1970; Rodriguez et al. 1992; Rauch & Schönau 2001). The postnatal drop of material vBMD and compartment vBMD also contribute to the drop of total bone BMD (Dickerson et al. 1962; Vinz et al. 1970; Hangartner & Gilsanz 1996; Rauch & Schönau 2001). Toddlers are at highest risk for nutritional rickets; the incidence peaks from 6 to 24 months of age (Abrams 2002). Vitamin D deficiency and low calcium intake represent its two primary causes (Pettifor 2004).

In childhood, TB BMC and aBMD increase steadily with age, for which body weight and height serve as strong predictors (Ponder et al. 1990; Moro et al. 1996; Nelson et al. 1997; Bailey et al. 1999; Bass et al. 1999; Molgaard et al. 1999). At the onset of puberty, boys (10-year-old) and girls (9-year-old) have accumulated approximately 40% of their adult bone mass (3100-3500g for young men and 2300-2700g for young women) (Gotfredsen et al. 1987; Rico et al. 1992; Bass et al. 1999; Horlick et al. 2000). During this period, bone expands radially and elongates longitudinally; bone tissue is deposited at the periosteal surface and resorbed at the endocortical surface.

2.3.2 Pubertal growth

Tremendous skeletal growth occurs during puberty. The total body BMC doubles in girls between the onset of puberty (9-year-old) and menarche (12.7-year-old) (from 40% to 80-85% of adult value) (Bass et al. 1999). Larger gain is achieved in boys than girls because of the longer period of puberty in males than in females (Boot et al. 1997; Martin et al. 1997). Consequently, sex differences in bone mass emerge after puberty (Figure 9).

FIGURE 9 Bone mass development in boys and girls (Anderson 2001). Reproduced with permission.

The aBMD measured by dual-energy X-ray absorptiometry (DXA) has similar growth trends to that of BMC for whole body or sub-sites (Lu et al. 1994). Because of the two-dimension nature of this type of measurement, the growth in bone size, not the mineralization of bone tissue, explains the majority of aBMD gain during puberty (Lu et al. 1996; Seeman 2001). The change of vBMD of either trabecular or cortical bone measured by quantitative computed tomography (QCT) or reanalyzed from DXA measurements is much smaller than that of aBMD (Lu et al. 1996; Neu et al. 2001; Rauch et al. 2001; Schönau et al. 2002).

Bone modeling activity is different between sexes as well as between bone surfaces during puberty. Bone is formed at the periosteal surface both in male and female adolescents, but occurs to a larger extent in males than in females (Neu et al. 2001). At the endocortical surface, bone is resorbed in males. However in females there is no such resorption at the endocortical surface, and there may even be some bone formation (Libanati et al. 1999; Neu et al. 2001). Consequently, males have larger bone size and marrow cavity size than females but have similar cortical thickness (Neu et al. 2001). As a result, female adults possess higher total bone vBMD than males. There is some evidence that cortical vBMD is higher in females than in males (Schönau et al. 2002).

It is suggested that the peak height velocity precedes the timing of peak bone mass gain during pubertal growth by at least half a year (Bonjour et al. 1991; Fournier et al. 1997). This phenomenon may be responsible for the transiently elevated fracture risk in adolescents (Landin 1983; Bailey et al. 1989). By the cessation of puberty the growth velocity of bone size declines while consolidation of bone mass continues, and fracture risk reduces (Alffram & Bauer 1962). Peak bone mass is achieved at the end of skeletal maturation.

2.3.3 Peak bone mass

Peak bone mass (PBM), defined as the amount of bony tissue present at the end of skeletal maturation (Bonjour et al. 1994), has long been widely accepted as an important factor in determining risk of fragility fracture in later life (Seeman et al. 1993; Heaney et al. 2000). This association arises for two reasons: firstly bone mass is a surrogate for bone strength, and secondly the tracking properties of bone mass throughout life.

Observational studies have suggested that, other things being equal, bone mass tracks throughout life (Newton-John & Morgan 1970; Matkovic et al. 1979; Ferrari et al. 1998). For instance, if an individual is on the high end of the population distribution at age 30 years, he or she will likely be on the high end at age 70 years. For one specific individual, if he or she accumulates the bone mass of his or her full hypothesized potential by adopting healthy lifestyle, such as participating in exercise frequently and having balanced nutrient intake, he or she is protected more against late-life osteoporotic fracture by accumulating more 'bone strength' during growth. This view is depicted schematically in Figure 10.

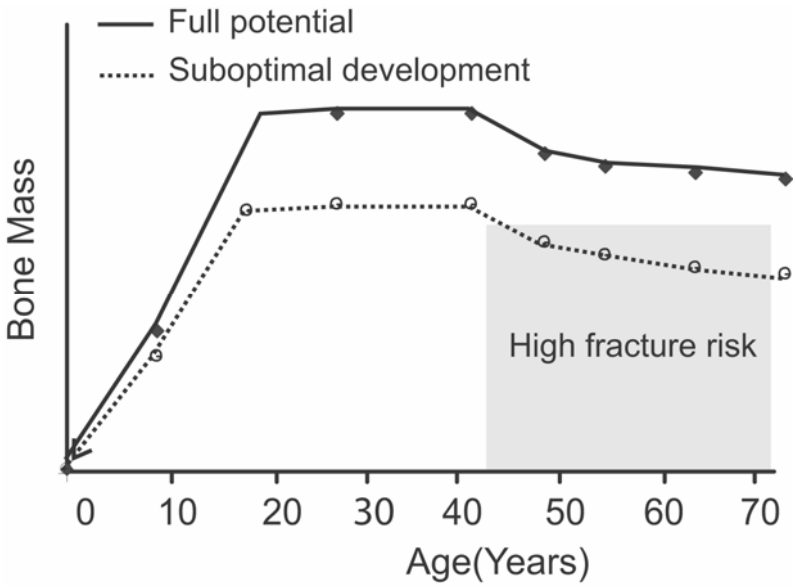

FIGURE 10 Schematic representation of the bone mass-age curve in individuals who achieve their full hypothesized potential for bone mass in optimal environment and those who do not in suboptimal environment. Modified from Heaney (1999). Reproduced with permission.

Although heredity exerts powerful effects on PBM (Seeman et al. 1989; Ferrari et al. 1998), the modifiable factors, such as calcium and vitamin D intakes, PA and muscle strength, constitute the major focus of interest in seeking to optimize the bone health of individuals.

2.4 Factors related to bone mass accretion

2.4.1 Sex hormones

Sex hormones are derived from cholesterol and synthesized mainly by endocrine glands (testis and ovary) (Granner 2000; Gruber et al. 2002). They are released into circulation and transported to target tissues (Figure 11). Their major functions include controlling sexual differentiation and maturation, promoting growth during development, maintaining the reproductive system and controlling or modulating sexual behavior in adulthood (Lipsett 1986; Sherwin 1998; McEwen & Alves 1999). Albright et al. (1941; 1948) related the causation of osteoporosis to estrogen deficiency in postmenopausal women and androgen deficiency in elder men. More recently, receptors for estrogens and androgens have been found in bone cells (Eriksen et al. 1988; Komm et al. 1988; Colvard et al. 1989; Oursler et al. 1991; Pederson et al. 1999). The skeletal system has become another research frontier for exploring the function of these ubiquitous hormones.

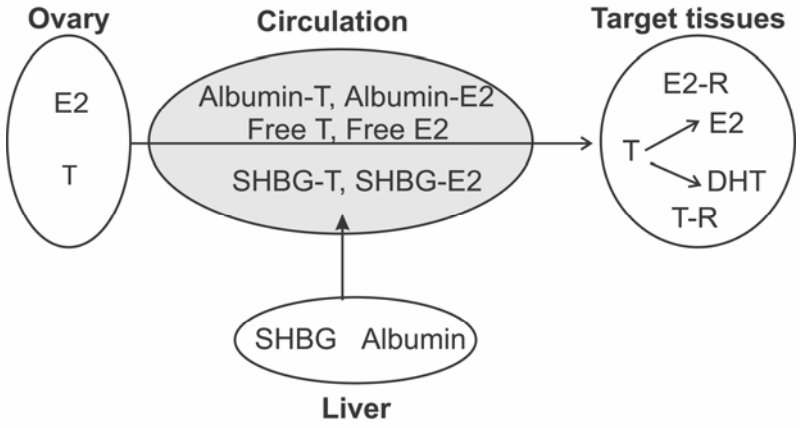

FIGURE 11 Schematic illustration of the sex hormones transportation system. E2 and T are synthesized mostly in ovaries in pubertal girls and then released into circulation. In blood, most of E2 and T molecules are bound to sex hormone binding globulin (SHBG) or albumin, which are secreted from liver. Only the free, unbound E2 and T can enter target cells to interact with their receptors (R). T can be converted to E2 and dihydrotestosterone (DHT) locally.

Estrogens

The most important endogenous estrogens in human are 17β-estradiol (E2), estrone (E1), and estriol (E3). The fetus is exposed to very high estrogen concentration due to the enormous E3 production by the placenta (Casey & Macdonald 1992). The association of estrogen exposure with bone mass accretion in this stage or bone mass of neonates is not known. In neonates, a

sudden decline of serum estrogen levels coincides with a short-term decline in BMD in the first 6 months of life. During infancy and childhood, estrogen concentrations are usually at or below the detection limit (Dotsch 2001). Whether estrogens have significant influences on bone mass accretion as well as bone radial or longitudinal growth in this period is not known.

During puberty, estrogens, mainly E2, play a major role in bone growth. The increase of E2 in pubertal girls is strongly associated with the gain of TB BMC and TB BMD (Cadogan et al. 1998). Female adolescents with estrogen deficiency show low BMC, BMD and Cth; and estrogen replacement therapy is effective to reverse these abnormalities (Emans et al. 1990). The importance of estrogens is further demonstrated by the reduced BMC and delayed epiphyseal closure in male patients with estrogen deficiency due to mutations in the aromatase gene and estrogen resistance due to disruptive mutations in the estrogen receptor gene (Smith et al. 1994; Morishima et al. 1995). Based on evidence from animal studies and sexual dimorphism, it is postulated that estrogens inhibit periosteal bone formation and endocortical resorption, which is realized via the suppressive effect of estrogens on bone turnover (Riggs et al. 2002). This current study investigated the influence of estrogens on bone periosteal and endocortical surface in children and adolescents.

Estrogens may also affect the bone sensitivity to mechanical loading. The ratio of BMC to LM (BMC/LM) is higher in reproductive women than in prepubertal children, postmenopausal women or men (Ferretti et al. 1998). Frost and Schiessl (1998) have proposed that estrogens lower the bone remodeling threshold, thus the previous remodeling-dependent bone loss (bone tissue adjacent to marrow cavity) decreases from the onset of puberty, while the modeling-dependent addition of bone continues normally.

Androgens

The association of androgens with bone mass/geometry in early childhood is not clear. Androgens derived from the adrenal cortex are associated with Cth, cortical area, BMC and bone strength index, but not periosteal circumference of the proximal diaphysis of the radius in prepubertal children (older than 6 years). However, these findings cannot be extended to pubertal adolescents or to the distal metaphysis of the radius (Remer et al. 2003). Prepubertal girls with premature adrenarche possess significantly more BMC and BMD than controls (Sopher et al. 2001).

During puberty, testosterone is an important factor for BMC accretion in pubertal boys. A highly significant correlation has been reported between the time of maximal increase in T concentrations (Tm-T) and that of BMC (Tm-BMC), with a mean difference between Tm-T and Tm-BMC of 4.7 months (Krabbe et al. 1984; Riis et al. 1985). In male patients with constitutional delay of puberty, BMC and BMD of the distal third of the radius were reduced according to chronological age, stature age, and bone

age (Bertelloni et al. 1995). T supplementation increased BMC, BMD, and Cth after 6 months treatment in comparison to patients without supplementation (Bertelloni et al. 1995). In animal studies, androgen-resistant male rats had a female skeletal phenotype, but were not osteoporotic (Vanderschueren et al. 1993). On the other hand, male and female mice with aromatase knockout (E deficiency) are osteoporotic (Oz et al. 2001). These animal studies suggested that androgens promote periosteal bone formation independent of E2, but maintain bone integrity depending on local aromatization of androgens into estrogens (Vanderschueren et al. 2004).

The role of androgens in the female skeleton has not been established in humans. Androgens may contribute in a clinically significant way to the bone phenotype in women. This is demonstrated by the higher PBM in hirsute women with polycystic ovary syndrome than age-matched controls (Buchanan et al. 1988; Dagogo-Jack et al. 1997). However, whether the androgens have a significant influence on bone growth in normal pubertal girls is unclear.

2.4.2 Physical activity

Physical activity (PA) is defined as any body movement that is produced by the contraction of skeletal muscle, which substantially increases energy expenditure (Services 1996). PA is a multi-dimensional factor which can be characterized by a range of features including type, intensity in term of energy expenditure, and the duration and frequency of each activity session.

Regulation of bone biology by PA begins as early as prenatal life. Intermittent skeletal stresses caused by involuntary muscular contractions in utero play an important role in modulating cartilage growth, ossification, and bone modeling and remodeling (Carter et al 1987; 1991; Wong & Carter 1990). This is manifested by the abnormally low skeletal mass and strength in newborn with neuromuscular disease-induced fetal immobility (Ralis et al. 1976; Rodriguez et al. 1988; Rodriguez et al. 1988).

Very few studies have been performed to investigate the effect of PA on bone mass in infants and toddlers. In one randomized pilot study, preterm infants in the exercise group (experiencing a range of motion with passive resistance at all extremities) had significantly more gains in bone width, BMC and aBMD of radius than the control groups over a 4 week study period (Moyer-Mileur et al. 1995; Litmanovitz et al. 2003). However, in another study, toddler and 3-5-year-old children exhibited no beneficial skeletal effect from increased PA (Specker et al. 1999; 2003).

A great deal of evidence has been collected concerning PA and bone development in late childhood and adolescence. Two sources of evidence can be identified: observational and interventional. In observational studies, PA is found generally, but not uniformly, to be associated positively with bone mass. For example, in children aged 5-14 years PA was significantly related to BMD of radius and hip, independently of age and sexes (Slemenda et al. 1991). Active girls and boys accreted 10-40% more bone (dependent on the

site) during the two years surrounding the peak of BMC accretion than inactive children (Bailey et al. 1999). Weight-bearing PA was more highly associated with BMC of hip, spine and TB than non-weight-bearing activity (Matkin et al. 1998). This is confirmed by the observation that higher BMD at femur neck (FN) and lumbar spine (LS) as well as radius in pubertal gymnast girls than in swimmers and controls (Grimston et al. 1993; Cassell et al. 1996; Courteix et al. 1998; Lehtonen-Veromaa et al. 2000). On the other hand, no such difference was found between swimmers and controls (Courteix et al. 1998).

The timing of PA appears to be of great importance in promoting bone strength. Haapasalo et al. (1996) reported that training started in childhood clearly increases bone size, BMC and Cth; but on the other hand, increasing physical loading on mature bone only marginally increases BMC and Cth. Furthermore, the dominant-nondominant difference between players and controls does not become evident until the pubertal growth spurt or Tanner Stage III (Haapasalo et al. 1998). However this is not supported by another report in which the dominant-nondominant difference in bone size and mass was found to be fully developed in prepubertal players and did not increase further (Bass et al. 2002). Greater periosteal expansion at the dominant arm explains the dominant-nondominant difference in player started in childhood, and marrow contraction explained the difference in players who started postmenarche (Kannus et al. 1995; 1998; Haapasalo et al. 2000; Bass et al. 2002).

Beneficial effects are also demonstrated in intervention trials. After 8 months exercise, children (7.5-years-old) gained significantly more BMC of LS and FN (3.1% and 4.5%, respectively) than controls (Fuchs et al. 2001). In peripubertal girls (10-years-old), 5.5-8.3% more BMC and 2.3-10.3% more aBMD at TB, LS and FN were gained in the exercise group than in the control group over 10 months to 2 years intervention (Morris et al. 1997; Bradney et al. 1998; MacKelvie et al. 2003). In another study, the effect of high-impact exercise was examined in pre- and post-menarcheal girl; about 3.5% more BMC was accreted at LS and FN in intervention group over controls in premenarcheal girls (Heinonen et al. 2000). In older female adolescents, no intervention study has revealed a beneficial effect of PA on bone mass accretion (Blimkie et al. 1996; Heinonen et al. 2000; Witzke & Snow 2000; Sundberg et al. 2001). In contrast to impact exercise, resistance training has not shown skeletal benefits in children (Blimkie et al. 1996).

In summary, PA exerts a positive effect on bone mass accretion during childhood and puberty, and the effect is site-specific. Weight-bearing PA is more efficient than non-weight-bearing PA. The period of rapid bone growth provides the best opportunity for exercise to promote bone health.

2.4.3 Body composition and muscle strength

Body composition

Although a large part of the variation in body composition is determined by genetic preprogramming (Nguyen et al. 1998), LM and FM may exert osteogenic effect independent of genetic preprogramming by specific mechanisms, such as static and mechanic loading, production of hormones, and metabolic effects (Reid 2002).

The relationship of TB BMC to FM has been examined extensively in various cohorts. In prepubertal and adolescent boys and girls, FM was found to be significantly and independently associated with BMC, and the association was similar in boy and girls (Ferretti et al. 1998; Pietrobelli et al. 2002). During the reproductive period, however, the fat-bone correlation is higher in women than in men (Tsutsumi et al. 1993; Ferretti et al. 1998). In men, the correlation is rather weak (Kirchengast et al. 2001; Lim et al. 2004). Moreover, the fat-bone correlation is stronger in postmenopausal than premenopausal women (Compston et al. 1992; Tsutsumi et al. 1993; Khosla et al. 1996; Chen et al. 1997; Douchi et al. 2000). Regional bone-fat correlation has also been examined for different skeletal regions. In premenopausal women, the correlation seems much weaker in arm than in leg ($r = 0.19$ vs. 0.37), while in postmenopausal women, the limb difference in fat-bone correlation is rather smaller (Douchi et al. 2001). In a longitudinal analysis, the change of FM in pubertal girls was not correlated with the change of total body BMC (Nelson et al 1997; Cadogan et al. 1998). In late adolescents and young adults, however, the change of FM, not the change of LM, is the predominant predictor of change in TB BMC (Chen et al. 1997; Young et al. 2001). After 25 years-of-age an increase in body weight is associated with low hip fracture risk in white women (Cummings et al. 1995).

On the other hand, Bolotin et al (1998; 2001; 2003) has found that in vivo DXA assessment of bone mineral is subject to sizeable inherent patient-specific inaccuracies. The ratio of FM to LM within the scanned region and the ratio of the intraosseous yellow to red marrow affect the accuracy of the measurement. They argued that the positive relationship between aBMD and FM may be more due to such methodological effects rather than reflecting a real biological relationship.

Lean mass (LM) has been recognized as the most powerful factor associated with BMC at different age stages. The correlation coefficient between LM and BMC in children is of the order of 0.90 (Manzoni et al. 1996; Pietrobelli et al. 2002). The strength of the lean-bone correlation in males remains constant from prepuberty to old age ($r = 0.82$- 0.90), while in females, the highest lean-bone correlation appears in prepubertal girls ($r = 0.82$); the correlation coefficient drops to 0.42-0.48 during the reproductive stage, and is 0.51-0.61 in postmenopausal women (Ferretti et al. 1998; Capozza et al. 2004). The change of LM is strongly correlated with change of TB BMC in pubertal girls($r = 0.68$) (Nelson et al. 1997; Cadogan et al. 1998). In late adolescents and

young adults, however, the change of LM is a significant predictor, but secondary to the change of FM, for the change of TB BMC (Young et al. 2001). It is suggested that the strong correlation between BMC and LM is established during growth and may be predetermined by heredity (Seeman et al. 1996; Nguyen et al. 1998).

Muscle strength

More than one century ago Wolff (1892) formulated the classical theory of bone adaptation in response to the loads it experiences, and this was restated in modern terms by Bassett (1968): "The form of the bone being given, the bone elements place or displace themselves in the direction of the functional pressures and increase or decrease their mass to reflect the amount of functional pressure". John C. Koch reasoned that the stresses from body weight are so much greater than the tensions being normally produced by the muscles and therefore, the effect of muscular action is of relatively little importance in determining the architecture of the bones (Koch 1917). However, now it is found that the maximal bone deformation is derived from peak momentary muscle contraction (Martin et al. 1998). This is due to the fact that muscles work against such bad lever arms that it takes well over 2 kg of muscle force on bones to move each kg of body weight around on earth (Crowninshield et al. 1978; English & Kilvington 1979; Lu et al. 1997). However, to date there has been no quantitative evidence regarding the relative importance of muscle strength vs. weight-bearing on bone in weight-bearing skeletons.

The relationships between muscle strength and bone mass/density have been examined in different groups of population using different modalities. Schönau has reported that grip strength correlated strongly with bone strength index of distal radius in 6-13 year-old children ($r = 0.9$) (Schönau et al. 1996; 1998). The changes in Cth and cross-sectional area (CSA) of bone, not the volumetric BMD (vBMD), represent the most important adaptation in response of the mechanical usage (Schönau et al. 1996; 1998). Using a similar approach, Hasegawa confirmed these findings in adult males and females, and reported that maximal grip strength is better than muscle CSA in predicting bone strength, as indicated by the so-called "stress-stain index" (Hasegawa et al. 2001). The strength of knee extensors but not flexors was weakly but significantly associated with aBMD of total body, lumbar spine and leg ($0.33 < r < 0.44$) (Duncan et al. 2002). This weak association between muscle strength and aBMD was also reported in female sedentary adolescents and young soccer players (Soderman et al. 2000).

In intervention trials, however, generally no effect or only a marginal effect of strength training on BMC, BMD or bone mechanical competence (through geometric change) has been demonstrated in children or young adult women (Peterson et al. 1991; Snow-Harter et al. 1992; Vuori et al. 1994; Blimkie et al. 1996; Heinonen et al. 1996). Using biomechanical analysis, Heinonen et al. (1996) found that high-intensity strength training did not

create sufficient strain magnitude to initiate bone formation and adaptation in bone geometry in young women. In postmenopausal women, however, it was reported that strength training is a feasible means to preserve bone density while improving muscle mass, strength, and balance (Pruitt et al. 1992; Ryan et al. 1994; Nelson et al. 1994; Kerr et al. 2001).

2.4.4 Other factors

Genetic factors may account for up to 60-85% of inter-individual variations of bone mass (Pocock et al. 1987; Seeman et al. 1996). The rest of the variation is related to environmental factors, such as nutrient intake, exercise, alcohol use and smoking, etc (Kelly et al. 1990). Adequate calcium intake during childhood and adolescence is necessary for maximizing PBM (Johnston et al. 1992). Other nutrients, such as vitamin D, vitamin K, sodium, phosphorus, vitamin A, vitamin C, potassium and magnesium may modify the utilization of calcium and also exert independent effects on bone metabolism (Matkovic 1991; Lehtonen-Veromaa et al. 2002; Kobayashi et al. 2004; Marwaha et al. 2005). Many traumas, diseases (eating disorders and juvenile rheumatoid arthritis, etc) and medications (glucocorticoids) also affect bone mass accretion in children (Johnston & Slemenda 1993).

2.5 Summary of literature review

Osteoporosis has been described as "a pediatric disease with geriatric consequences", because PBM attained at the end of maturity provides the baseline value protecting against the occurrence of low bone mass at old age (Chesnut 1989; Matkovic 1992; Seeman et al. 1993). About half of the PBM is accumulated during the few years of rapid pubertal growth (Bailey et al. 2000). However, rapid growth confers risks as well as benefits to the skeleton (Parfitt 1994). The large increase in incidence of upper extremity (particularly distal forearm) fractures is coincident with the adolescent growth spurt in both sexes (Cooper et al. 2004). A transient osteopenia induced by growth asynchrony of body size and bone mass may be responsible for the phenomenon (Parfitt 1994).

According to the literature to date, multiple factors are involved in bone mass accretion during puberty. Two factors play a central and interactive role: sex hormones (especially E2) and mechanical loading (Schiessl et al. 1998). Few studies have been done so far to elucidate the specific influence of sex hormones on bone geometry and density in pubertal girls. PA and muscle strength represent different aspects of mechanical loading applied to skeletons. Although muscles are the major source of mechanical load applied on bones, weight-bearing may also contribute to a large part of BMC accretion in weight-bearing bones. This is evidenced by the observation that,

in the absence of weight-bearing, a larger fraction of bone loss occurs in the legs than in the arms even when resistance training is undertaken (Whalen 1993; Collet et al. 1997; Vico et al. 2000; Rittweger et al. 2005).

Short-term intervention trials have revealed that impact exercise facilitates bone mass accretion in children and adolescents, and the benefit of exercise is larger in pre- and early pubertal subjects than in older counterparts (Blimkie et al. 1996; Heinonen et al. 2000). The skeleton responds to increased PA by enlarging its size if the exercise training starts in childhood and early puberty, but when PA starts in later adolescence and adulthood the skeletal reaction is a decrease in marrow cavity size (Haapasalo et al. 1996). FM is associated with BMC in various populations (Douchi et al. 2000). Simply comparing BMC between normal and overweight subjects does not provide pertinent information about the influence of body fat on bone health, because it has been reported that fracture risk in the upper extremities was higher in overweight girls although they had higher BMC than normal girls (Goulding et al. 1998; Goulding et al. 2001; Skaggs et al. 2001). LM is the strongest predictor of bone mass (Schönau et al. 1996; Capozza et al. 2004). This LM-BMC covariance is largely predetermined by genetic factors.

3 PURPOSE OF THE STUDY

Understanding the growth of bone and its modulators during puberty is of great importance for clinical practice and public health in order to promote bone health in children as well as to prevent osteoporosis in later life. Therefore, the main purpose of the observational study was to investigate bone growth in terms of bone radial geometry and mineral mass/density and to study its association with sex hormones, physical activity, body composition and muscle strength in pubertal girls. The investigation made use of both cross-sectional and longitudinal study designs.

More specifically, the objectives were the following:

1) To describe the growth patterns of bone geometry and mineral density at diaphysis (tibial shaft) and metaphysis (distal radius) of long bones in pubertal girls using a longitudinal study design (I).
2) To study the influence of sex hormones on bone geometry and mineral density in pubertal girls using cross-sectional and longitudinal study designs (II and III).
3) To investigate the association of leisure-time physical activity with BMC in growing girls using cross-sectional (IV) and longitudinal designs (unpublished data).
4) To study the relationship of BMC with muscle strength, fat body mass and lean tissue mass in non-weight-bearing and weight-bearing bones in pubertal girls using a longitudinal study design (V).

4 MATERIALS AND METHODS

4.1 Subjects and study design

This observational study was a part of the Calex study, which is a 2-year intervention study evaluating the effects of calcium, vitamin D and milk product on bone mass accretion in pubertal girls. A detailed description of participant recruitment is given elsewhere (Cheng et al. 2005). In brief, the girls of 4th to 6th grades in 61 schools were first contacted through their teachers in the city of Jyväskylä and its surroundings in Central Finland (96% of the total schools in these areas). Of the 1367 girls in these schools, 258 healthy girls aged 10-13 years passed through the screening procedure and agreed to participate in the study at baseline. 198 and 221 girls presented in the 12-months' and 24-months' follow-up measurements respectively. The reasons for dropout were loss of interest in taking the supplementation and loss of contact. The date of the first menstruation bleeding was recorded using a questionnaire (I, III, V). Eight girls (3%) already had menarche at baseline measurement. There were 25 girls (10%) who had not menarche and another 26 girls (10%) who did not provided information on menarche at the end of this study. The Tanner grading system (Marshall & Tanner 1969) was used to determine the physical development by a public health nurse (I).

4.2 Measurements

4.2.1 Anthropometry

Body height and weight were determined using an electronic scale and stadiometer, respectively. Height and weight were used to calculate body mass index (BMI), expressed as weight (kg)/height (m)^2.

4.2.2 Bone mineral density and geometric properties

Total body (TB), lumbar spine (LS2-4) and proximal femur were scanned using DXA (Prodigy, GE Lunar Corp., Madison, WI USA). BMC, aBMD and BA were the outcome variables (Table 1) (IV and V). The detailed description of DXA measurement was given in previous report (Cheng et al. 2005). The precision of repeated measurements for different region of interest expressed as coefficient of variation (CV) was 0.7% to 1.4% for BMC, 0.9% to 1.3% for aBMD.

TABLE 1 Scanned sites and obtained variables using DXA.

	Scanned sites	Analyzed sites	Variables
0-mo	LS, left Femur	LS2-4, FN, and TF	FM, LM, BMC, BA and aBMD (IV)
	TB	TB, left arm, left leg	FM, LM, BMC, BMC/LM (V)
12-mo	LS, left femur	LS2-4 and FN	BA, BMC and BMD
24-mo	TB	TB, left arm, left leg	FM, LM, BMC, BMC/LM (V)

BMC/LM: ratio of BMC to LM; -mo: months' follow-up

A detailed description of the usage of peripheral quantitative computed tomography (pQCT) has been described previously (I, II and III). In brief, the distal radius (DR) and tibial shaft (TS) were scanned using a pQCT device (XCT-2000, Stratec Medizintechnik, GmbH, Pforzheim, Germany). DR was scanned at 4% of the forearm length proximal to the wrist joint surface using the standard mode, and TS was scanned at 60% of lower leg length up from the lateral malleolus using the research mode (Figure 12). The DR and TS were selected because they are representatives of the metaphyseal (trabecular bone) and diaphyseal (cortical bone) sections of long bones in the upper and lower limbs, respectively. Image processing and calculation of bone parameters were done using the manufacture's software package (version 5.40) and Geanie 2.1 (Bonalyse Oy, Jyväskylä, Finland). The CV was 1% for CSA and BMC, and less than 1% for vBMD. The analyzed variables from DR and TB are shown in Table 2.

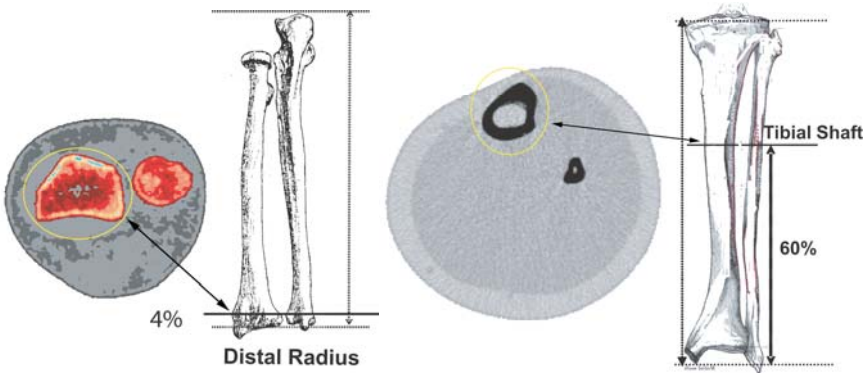

FIGURE 12 Scanned bone sites and their transverse planes at the left distal radius and tibial shaft, respectively, using pQCT.

TABLE 2 Scanned sites and obtained variables using pQCT at 0-, 12- and 24-months.

Scanned sites	Analyzed sites	Variables
Distal radius	Total bone	BMC, CSA and vBMD (I, IV)
Tibial shaft	Total bone	BMC, CSA and vBMD (I, II, III, IV), PC (III)
	Cortical bone	CSA, vBMD and Cth (I, II, III, IV), proportion (I, II, III)
	Marrow cavity	CSA (I), proportion (I, II, III), EC (III),

PC: periosteal circumference; EC: endocortical circumference, proportion: ratio of cortical or marrow CSA to total bone CSA.

4.2.3 Sex hormones

Blood samples were collected in the morning between 7:30 and 9:00 after overnight fasting at baseline, 12-months' and 24-months' follow-up. Serum was extracted from blood by centrifugation and stored immediately at –70°C until analyzed.

E2, T, and sex hormone binding globulin (SHBG) were assessed using time-resolved fluoroimmunoassays (Delfia, Wallac Oy, Turku). The detection limits of the E2 and T assays were 0.05 nmol/L (13.6 pg/ml) and 0.3 nmol/L (0.09 ng/ml), respectively. Inter- and intra-assay coefficients of variation (CV) were 5.2% and 5.1% for E2, 9.2% and 9.4% for T, and 1.1% and 1.1% for SHBG, respectively. Free E2 and T were calculated using the equation from Ekins (Ekins 1990) (II, III).

4.2.4 Physical activity

General PA level was evaluated using a self-administrated PA questionnaire. Detailed information about the calculation of PA score is given in the original report (IV).

For the cross-sectional analysis using the baseline data, the subjects were assigned into low, moderate, and high PA groups according to their PA

scores. In addition, the subjects were categorized into low-impact and high-impact PA groups according to their favorite physical activity, independent of its duration, intensity and frequency (IV).

For the longitudinal analysis, the girls were categorized into three groups: the consistently inactive group (the girls being in the low PA group both at baseline, 12- and 24-months' follow-up assessments), the inconsistent PA group (the girls being in different PA groups at baseline, 12- and 24-months' follow-up), and the consistently active group (the girls being in the high PA group both at baseline, 12- and 24-months' follow-up). The bone properties were then compared between the consistently inactive and consistently active groups.

4.2.5 Body composition

Body composition of total body and regional sites was assessed by DXA (V). The obtained variables are shown in Table 1.

4.2.6 Muscle performance

Isometric strength of the left elbow flexors and leg extensors was measured in a maximal voluntary contraction (MVC) test in a sitting position with an adjustable dynamometer chair (Good Strength, Metitur Oy, Jyväskylä, Finland). A detailed description of the measurement procedures is presented in the original report (V).

4.3 Statistical analysis

All data were checked for normality before further analysis. A $p<0.05$ with 2-tail was considered to be statistically significant. In cross-sectional analyses, Pearson's correlation was used to explore the relationship between sex hormones and bone variables (II). Analysis of covariance (ANCOVA) was used to compare bone variables between PA groups controlling for body weight and height (IV). Student's t-test was used to compare the ratio of BMC to MVC (BMC/MVC) between arm and leg (V). In longitudinal analyses, a hierarchical liner model with random effects was used to explore the growing patterns of bone variables (I), the association of bone variables with sex hormones (III), and BMC with body composition or MVC (V). Cross-sectional data analyses were performed using SPSS and longitudinal data using MLwiN 2.0 ((Multiple Project, Institute of Education, University of London, UK). The comparison of the correlation coefficients of FM with BMC/LM between the upper and lower limbs was analyzed using Fisher's z-transformation.

5 RESULTS

5.1 General characteristics

Table 3 presents the results of the variables used in this study at baseline and 24-months' follow-up. The mean menarche age was 12.9 years in our study population. Over the 2-year period, body height and weight increased 12cm (8%) and 11kg (28%), respectively; LM, FM and BMC of TB increased 7.3kg (27%), 2.7kg (29%) and 481g (34%), respectively. LM-adjusted BMC (BMC/LM) of TB, leg or arm also increased significantly ($p<0.001$). Areal BMD of TB and sub-sites showed substantial increases. On the other hand, vBMD of DR did not change over time in this period. The total vBMD of TS increased 9%, and the cortical vBMD of TS increased only 5%. Isometric strength (MVC) of elbow flexors and knee extensors increased 23% and 32%, respectively. Serum levels of E2 nearly doubled and T nearly tripled; at the same time, SHBG decreased significantly ($p<0.001$).

TABLE 3 Anthropometry, bone variables, sex hormones and muscle strength. Variables at baseline and 24-mo' follow-up are presented as mean (95% CI).

Modalities and sites			*Variables*	*0-mo(n=258)*	*24-mo(n=221)*
Anthropometry			Age (y)	11.2 (9.8-12.6)	13.2 (11.8-14.6)
			Height (cm)	146 (130-162)	158 (144-172)
			Weight (kg)	39.2 (22.1-56.3)	50.0 (29.4-70.6)
			BMI (kg/m^2)	18.3 (12.6-24.0)	20.0 (13.1-26.9)
			Tanner Stage (I-V) (%)	52/40/8/0/0	1/24/47/27/1
			Menarche age (y)	12.9 (11.5-14.3)	
Physical activity			Duration (h/week)	2.3 (1.1-5.3)	2.5 (1.2-5.2)
DXA	TB		LM (kg)	27.3 (18.9-35.7)	34.6 (25.8-43.4)
			FM (kg)	9.4 (3.5-25.0)	12.1 (4.4-33.6)
			BMC (g)	1412 (879-1945)	1893 (1219-2567)
			BMC/LM (g/kg)	51.6 (43.0-60.2)	54.8 (43.8-65.8)
			BA (cm^2)	1484 (1064-1903)	1812 (1379-2245)
			aBMD (g/cm^2)	0.95 (0.83-1.06)	1.04 (0.89-1.19)
	LS2-4		BMC (g)	23.5 (12.1-34.9)	34.6 (18.7-50.5)
			BA (cm^2)	28.0 (20.4-35.6)	34.5 (26.3-42.7)
			aBMD (g/cm^2)	0.83 (0.63-1.03)	0.99 (0.73-1.26)
	FN		BMC (g)	3.3 (2.3-4.3)	4.0 (2.8-5.2)
			BA (cm^2)	3.9 (2.9-4.9)	4.3 (3.5-5.1)
			aBMD (g/cm^2)	0.82 (0.64-0.10)	0.93 (0.70-1.15)
	Left arm		LM (kg)	1.3 (0.91-1.69)	1.7 (1.1-2.3)
			FM (kg)	0.4 (0.1-1.5)	0.5 (0.1-1.9)
			BMC (g)	73 (38-108)	107 (64-150)
			BMC/LM (g/kg)	56.5 (43.6-69.4)	63.3 (48.6-78.0)
	Left leg		LM (kg)	4.7 (3.1-6.3)	6.1 (4.3-7.9)
			FM (kg)	2.1 (0.9-5.0)	2.7 (1.2-6.4)
			BMC (g)	280 (162-398)	378 (241-515)
			BMC/LM (g/kg)	59.0 (47.6-70.4)	61.4 (50.4-72.4)
pQCT	DR		BMC (mg)	64.5 (43.3-85.7)	79.4 (53.1-105.7)
			CSA (mm^2)	227 (143-311)	272 (168-376)
			vBMD (mg/cm^3)	288 (215-361)	296 (204-388)
	TS	Total	BMC (mg)	247 (176-318)	299 (223-375)
			CSA (mm^2)	373 (267- 479)	414 (306-522)
			vBMD (mg/cm^3)	664 (570-758)	724 (616-832)
			PC (mm)	68.2 (58.4-78.0)	71.9 (62.5-81.3)
		Cortical	CSA (mm^2)	199 (138-260)	240 (177-303)
			vBMD (mg/cm^3)	1043 (988-1098)	1095 (1034-1156)
			Cth (mm)	3.5 (2.7-4.3)	4.1 (3.3-4.9)
			Proportion (%)	53.4 (44.8-62.0)	58.3 (49.3-67.3)
		Marrow	EC (mm)	46.6 (38.0-55.2)	46.4 (37.4-55.4)
			Proportion (%)	24.3 (15.9-32.7)	22.3 (13.3-31.3)
Muscle	Elbow flexors		MVC (Newton)	123 (80-166)	151 (100-202)
	Knee extensors		MVC (Newton)	296 (169-423)	392 (233-551)
Sex hormones			E2 (pmol/L)	89 (39-242)	161 (43-293)
			Free E2 (pmol/L)	1.9 (0.6-5.6)	3.8 (1.2-9.7)
			T (nmol/L)	0.41 (0.05-1.38)	1.15 (0.17-2.95)
			Free T (pmol/L)	3.6 (0.4-23.2)	11.3 (1.7-54.8)
			SHBG (nmol/L)	83 (14-151)	65 (8-122)

5.2 Body and bone growth

The growth curves of body height and weight are shown in Figure 13. Their growth velocities peaked at 16 and 2 months before menarche, respectively. Body height and weight of these girls at menarche were 158cm (95% CI: 145-170) and 49.4kg (95% CI: 32.1-66.6), respectively.

The timings of peak growth velocity of bone properties at DR and TS are summarized in Figure 14. These results indicate that the bone size growth velocity peaked at least 6 months earlier than bone mass. The growth patterns of vBMD were different at DR and TS. At DR, vBMD decreased until 11 months before menarche and increased dramatically thereafter. At TS, however, total bone vBMD and cortical vBMD increased monotonically (I). The changing pattern of EC of TS was rather interesting: EC did not change before menarche and decreased thereafter (Figure 15).

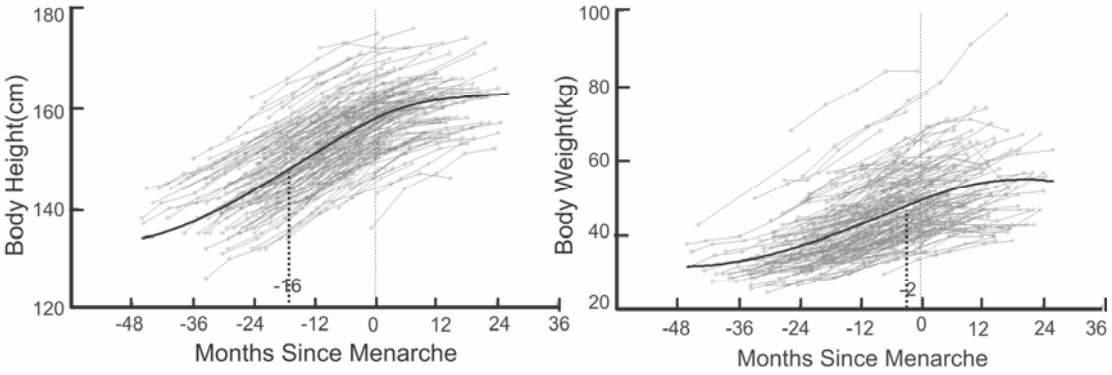

FIGURE 13 Growth curves of body height and weight in pubertal girls. The growth velocities of height and weight peaked at 16 and 2 months before menarche, respectively (indicated by dotted line).

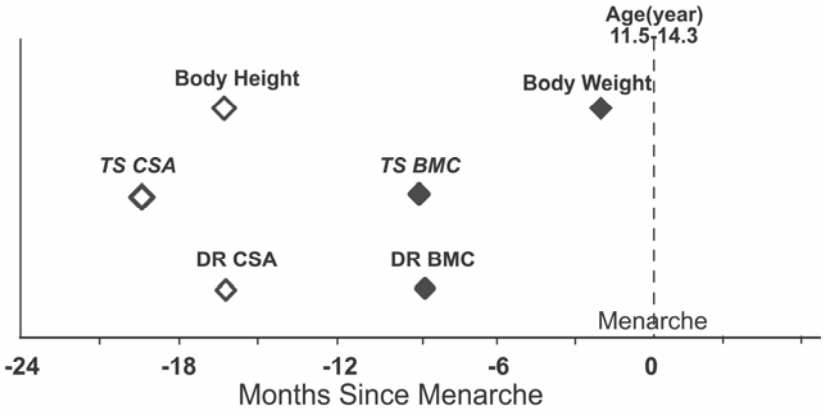

FIGURE 14 Timing of peak growth velocity of body height and weight, BMC and CSA of distal radius and tibial shaft. The 95% CI of menarche age in these girls was 11.5-14.3 years-of-age.

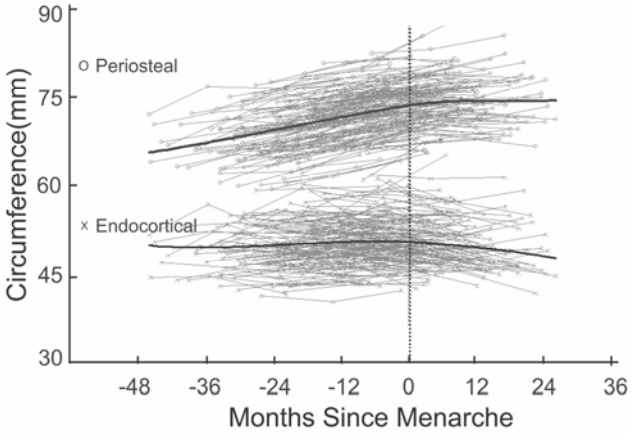

FIGURE 15 Growth patterns of the periosteal and endocortical circumference of the tibial shaft in pubertal girls.

5.3 Sex hormones

The serum concentrations of sex hormones and SHBG are shown in Table 3. The correlation of E2, T and SHBG with bone geometry and density of TS in multivariate model is summarized in Table 4. Both E2 and T were positively correlated with total bone BMC, but not with cortical vBMD. E2 and T had different patterns of association with bone geometry. Increased E2 was associated with a smaller bone marrow cavity and higher Cth, but not with total bone CSA; on the other hand, increased T was associated with larger total bone CSA and marrow cavity, but not with Cth. The differential association of E2 and T with marrow cavity size explained the differential association of E2 and T with total bone vBMD. The association of SHBG with bone properties of TS was in the opposite direction to that of E2 (in most cases) and T (in total bone CSA) (II, III).

TABLE 4 Correlation of sex hormones with bone variables of tibial shaft in multivariate model with time relative-to-menarche as covariate. '↑', '↓' and '↔' indicate positive, negative and no significant correlation, respectively.

Bone variables at tibial shaft		**E2**	**T**	**SHBG**
Total bone	BMC	↑	↑	↓
	CSA	↔	↑	↓
	vBMD	↑	↓	↓
	PC[a]	↔	↑	↓
Cortical	CSA	↑	↔	↓
	vBMD	↔	↔	↔
	Cth	↑	↔	↓
	Proportion	↑	↓	↓
Marrow	EC[b]	↓	↑	↔
	Proportion	↓	↑	↑

[a] and [b] represent periosteal and endocortical circumferences, respectively.

5.4 Physical activity

In cross-sectional analyses using the baseline data, the difference in bone properties of TB, LS2-4, and TF between low, moderate and high PA groups is shown in Figure 16. At Tanner stage I, girls in the high PA group had 5.4%, 8.9%, 8.1% higher BMC of TB, LS2-4 and TF than girls in the low PA group, respectively ($p<0.01$). At Tanner stage II, only BMC of LS2-4 was found to be significantly (6.5%) higher in the high compared to the low PA group ($p=0.02$).

Within Tanner Stage I, the high-impact group had 5.8%, 10.8% and 5.6% higher BMC of TB, LS2-4, and TF than the low-impact group, respectively ($p<0.01$). However, at Tanner Stage II only BMC of LS2-4 was found to be significantly (6.7%) higher in the high-impact group compared to the low-impact group ($p=0.005$) (Figure 17).

In longitudinal analyses, significant differences in bone properties between consistently inactive and active groups were found. At the time of menarche, BMC of TB, LS2-4, and FN were 7.6%, 6.0% and 7.0% higher ($p=0.012$, 0.011 and <0.001, respectively) in consistently active girls than their consistently inactive counterparts. However, the growth velocity of BMC (slope) at these sites did not differ between the two groups (Figure 18).

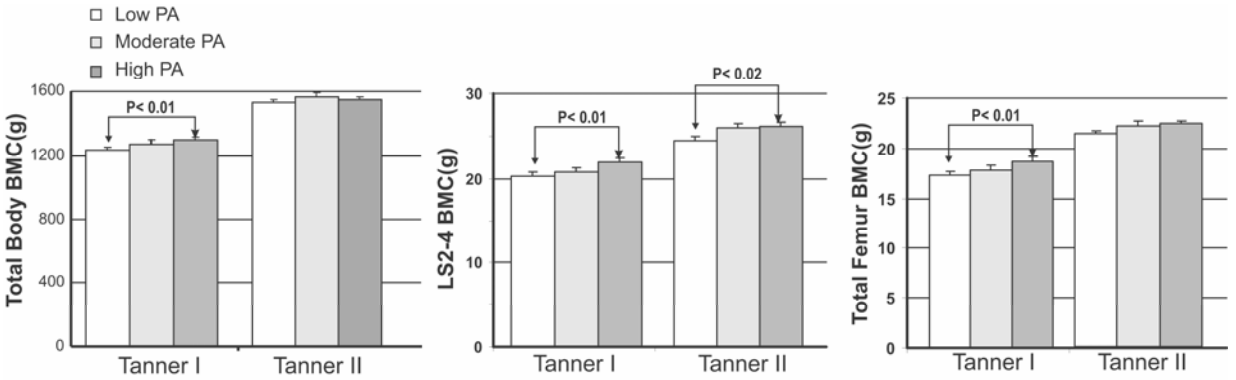

FIGURE 16 Comparison of BMC of total body, lumbar spine 2-4 (LS2-4) and total femur between low, moderate and high physical activity (PA) groups in different maturational stages with body height and weight as covariates. Error bars show SEs. At Tanner Stage I, High PA groups had significant higher BMC in all the three sites ($p<0.01$). At Tanner Stage II, only LS2-4 BMC was higher in high than low PA group ($p=0.02$).

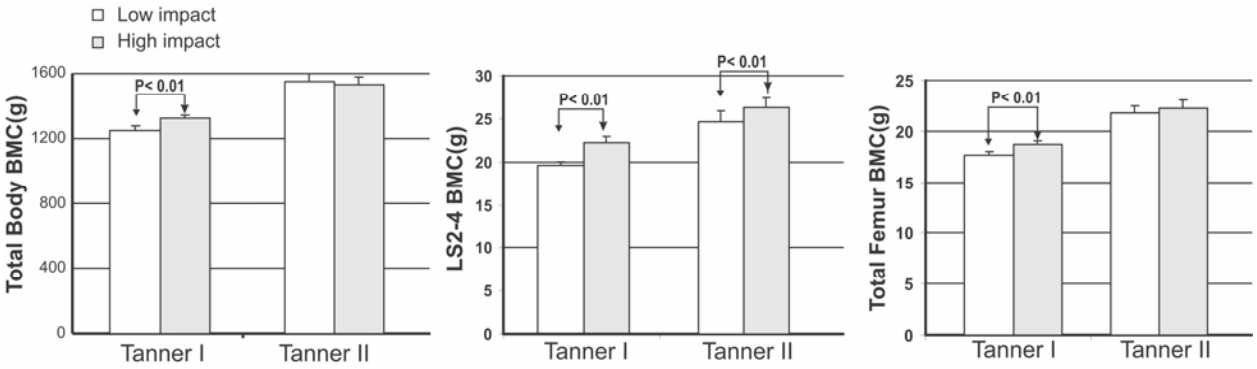

FIGURE 17 Comparison of BMC of TB, LS2-4 and TF between low- and high-impact PA groups in different maturational stages with body height and weight as covariates. Error bars show SEs. At Tanner Stage I, girls in high-impact PA groups had significant higher BMC in all the three sites ($p<0.01$). At Tanner Stage II, only LS2-4 BMC was higher in high- compared to low-impact PA group ($p < 0.01$).

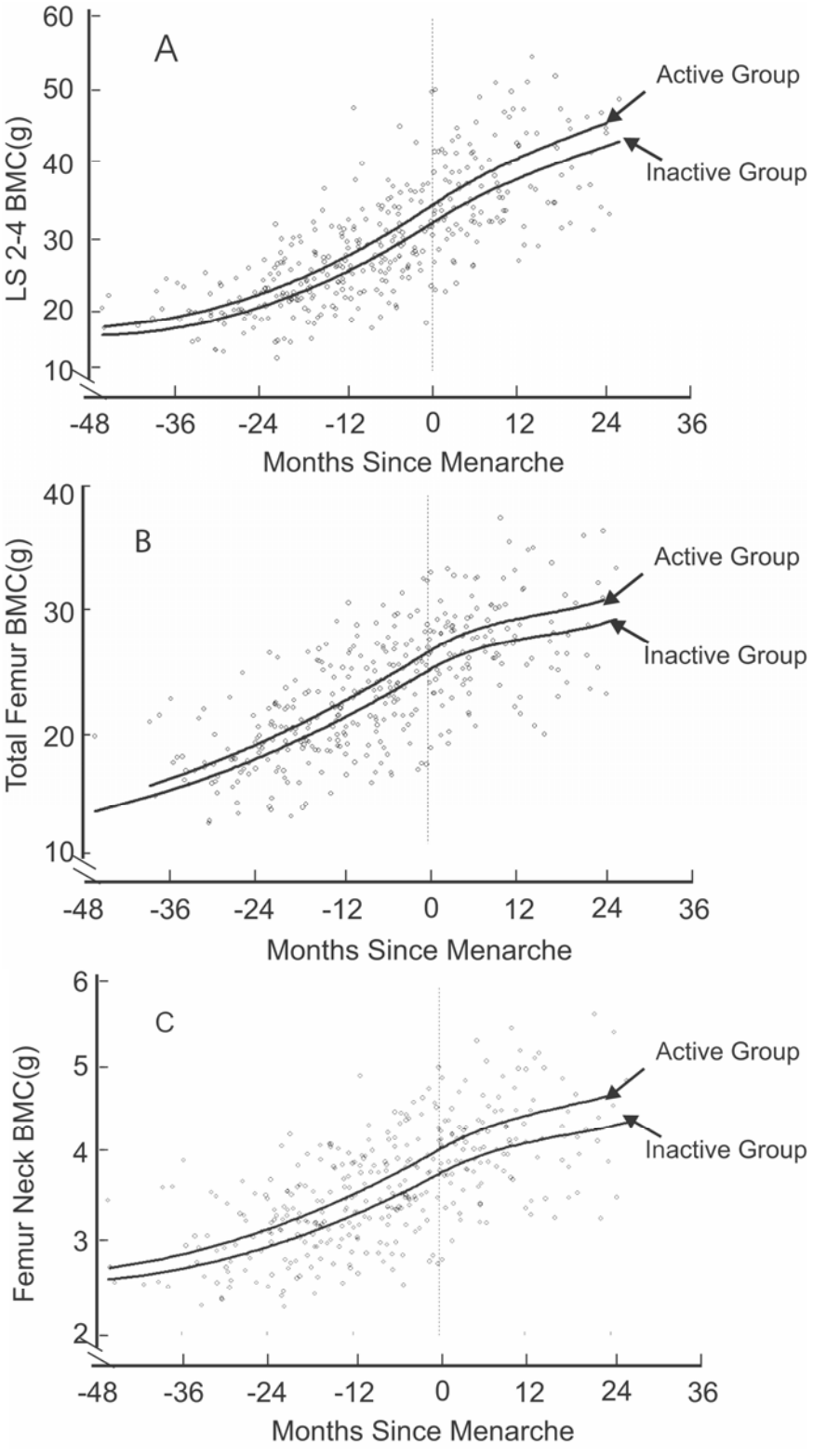

FIGURE 18 Comparison of the growth in BMC of LS2-4 (A), total femur (B) and femur neck (C) between consistently active and inactive groups. There was no difference in growth velocities (slope). At menarche, girls in the consistently active group were significantly higher in BMC at the three sites than their inactive counterparts ($p < 0.012$).

5.5 Body composition and muscle strength

Body composition

BMC, LM and FM of TB and regional sites are shown in Table 3. FM and LM were significantly correlated with BMC of TB (r=0.597 and 0.913, respectively) (Figure 19). The strength of the association between FM and BMC decreased considerably after controlling for LM (r = 0.184). FM of TB correlated with BMC of the upper and lower limbs differently, being significantly higher in leg than in arm after controlling for limb-matched LM (r=0.212 vs. 0.089, p<0.01).

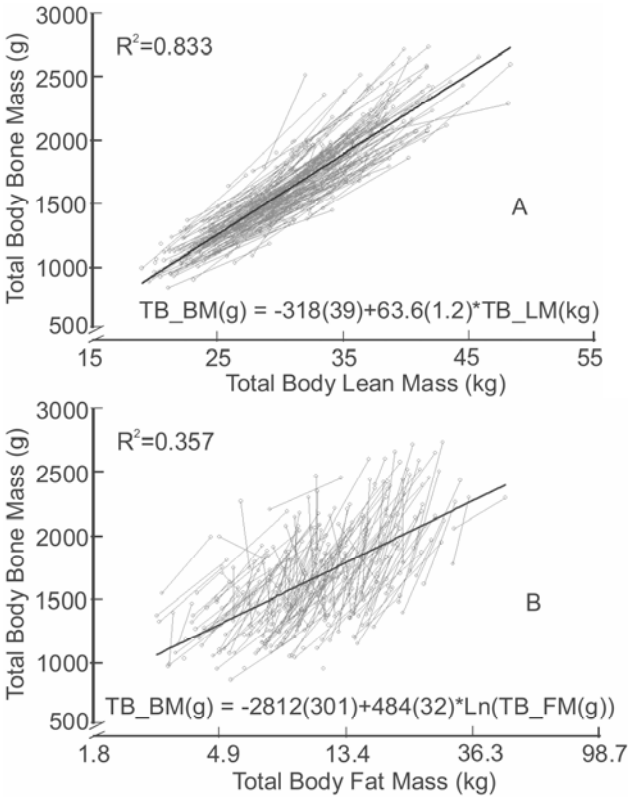

FIGURE 19 Regression of total body BMC against total body lean mass (A) or fat mass (semi-log plot, B).

The correlation of FM with limb BMC/LM was also investigated. FM of TB was significantly correlated with BMC/LM of both arms and legs. However, the correlation was significantly higher in legs than in arms (0.538 vs. 0.322 p<0.01). This result is summarized in Table 6.

TABLE 6 Correlation coefficients (r) between FM of TB and BMC/LM of TB, upper and lower limbs, with or without adjusting for time relative-to-menarche.

	TB-FM	
	Univariate model	Adjusting for time relative-to-menarche
TB BMC/LM	0.504	0.425
Arm BMC/LM	0.322	0.166
Leg BMC/LM	0.538	0.412

Muscle strength

Isometric strength (MVC) of left elbow flexors and knee extensors is shown in Table 3. The MVC of elbow flexors and knee extensors was positively and highly associated with BMC of arm and leg, respectively (r^2 = 0.544 and 0.501). The regression of BMC against MVC in arm and leg did not differ significantly (b=0.76 vs. 0.70, p=0.16). On the other hand, the ratio of leg BMC to MVC of knee extensors was 30% higher than the ratio of arm BMC to MVC of elbow flexors at both baseline and 24-months' follow-up (0.97 vs. 0.60 and 0.97 vs. 0.70, respectively).

6 DISCUSSION

This cross-sectional and longitudinal investigation of pubertal girls had three components. *Firstly*, the growth patterns of bone geometry and mineral density at the distal radius and tibial shaft during a period from 4 years before menarche to 2 years after were modeled. *Secondly*, the influence of sex hormones on bone geometry and density at the tibial shaft was studied. *Finally,* the association of physical activity, body composition and muscle strength with bone mass was investigated.

6.1 Bone growth

The growth velocities of bone radial size of DR and TS peaked simultaneously (or nearly so) with that of body height. This suggests that bone radial growth is proportional to bone longitudinal growth during puberty. The accretion of BMC at the two bone sites peaked at the same time, more than 7 months later than that of bone radial size. These results confirmed previous reports, which used a different bone measurement modality, that there is a growth asynchrony between bone size and bone mass during puberty (pQCT vs. DXA) (Bonjour et al. 1991; Fournier et al. 1997). Moreover, we found that the asynchrony induced a slight decrease in vBMD of DR, but not in vBMD of TS in pubertal girls.

The suggested growth asynchrony between bone size and mass, especially at bone sites where trabecular bone predominates, such as DR, may play an important role in the elevated fracture risk in pubertal children. As long as the growth rate of bone size exceeds the mineralization rate during rapid growth, bone mass relative to bone size (BMD at organ level) will decrease ("transient osteopenia" or "pubertal mineral debt"). Therefore, the fracture risk of pubertal girls will be at the highest level around one year

prior to menarche, when the growth rate of bone mass just crosses that of body size. This phenomenon is highlighted in Figure 20.

We did not find any signs of a transient increase in porosity at the cortex of TS, but rather saw that the cortical vBMD increased slightly but monotonically during puberty. This finding is in agreement with most previous studies that have reported increases in cortical vBMD at the diaphysis during puberty, presumably due to decreased porosity (Schönau et al. 2002; Remer et al. 2003).

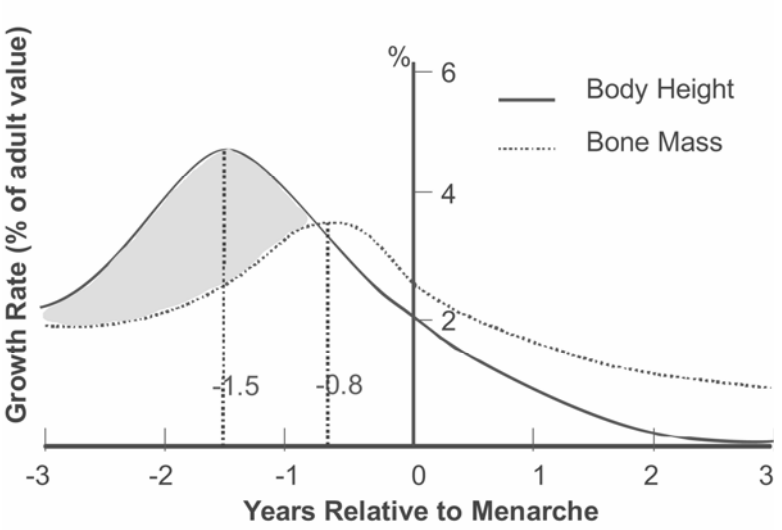

FIGURE 20 Illustration of growth rates of body height and total body bone mass with respect to time relative-to-menarche, which peaked at about 1.5 and 0.8 years before menarche, respectively. The growth rate was expressed as the percentage of adult value. To make them comparable, the cubic root of the growth rate of bone mass was taken because body height is a one-dimensional variable and bone mass a three-dimensional variable. The area in the gray can be called "pubertal mineral debt", caused by the growth asynchrony of body height and bone mass, analogous to the "oxygen debt" that occurs after vigorous exercise. Fracture risk during puberty is at the highest level when the two curves cross, around 1 year before menarche in girls (average 11-12 years old). The "mineral debt" will be paid back progressively afterward (based on data from Sabatier (1999) and Bass (1999)).

Two processes may contribute to the linear increase of Cth of TS in pubertal girls: periosteal expansion before menarche and both periosteal expansion and endocortical contraction after menarche (Figure 21). Our finding was consistent with previous reports in which the second metacarpal or mid-femoral marrow diameter decreased in girls with advanced puberty (Bass et al. 1999; Libanati et al. 1999). In a cross-sectional study, Neu et al. (2001) did not find a significant contraction in medullary area at the radial shaft from prepuberty to adulthood in females. Nonetheless, they reported that the growth of the total bone CSA ceased but the cortical CSA continued to increase in girls after 15 years of age, which does imply a decrease in marrow cavity area.

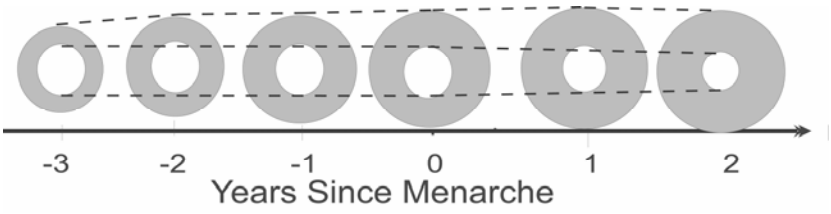

FIGURE 21 Schematic illustration of the bone geometric growth at tibial shaft in pubertal girls.

6.2 Sex hormones

At organ level, E2 was positively associated with BMC and vBMD of TS. However, E2 was not correlated with cortical vBMD, a measurement at tissue level. This result is supported by ovariectomized young animal studies in which the cortical vBMD at long bone shaft did not change over the experimental period (Andersson et al. 2002; Surve et al. 2002). Our further analyses showed that it was via associations with bone geometry that E2 was related with total bone BMC and vBMD (II, III).

E2 was not associated with total bone CSA of TS. Instead, it was associated with the geometric configuration of CSA. There are three compartments in the total bone CSA: cortex, marrow cavity and the transitional area between the two main compartments. It was found in our reports (II and III) that high E2 was associated with a smaller marrow cavity. These results, together with the decreased endocortical circumference after menarche in pubertal girls, indicate that E2 may inhibit bone resorption during rapid growth and later, after menarche, acts at higher concentrations to promote bone formation on the endocortical bone surface. As a result of the action of E2 on this surface, E2 is an important determinant of Cth at TS. This view is in agreement with the findings that E2 deficiency is correlated with thin cortex in girls, women and even men (Takahashi et al. 1996; Bilezikian et al. 1998).

Our findings do not support the view that E2 inhibits bone formation at the periosteal surface in pubertal girls. The sexual dimorphism in bone morphology might be due to the fact that E2 is a major determinant of the fusion of growth plate (Smith et al. 1994; Bilezikian et al. 1998). As long as the bone elongates, its radial size increases proportionately. Thus, the sexual dimorphism in bone size is more likely due to the combination of earlier fusion of bone growth plate in girls than boys and the stronger pro-formative effect of androgens on the periosteal surface in boys than in girls due to the sex difference in T concentration.

Similar to E2, T was positively associated with total bone BMC of TS, but not with cortical vBMD. Thus, it is conceivable that, as for E2, T may also

affect BMC by its influence on bone geometric properties. High T was associated positively with larger total bone CSA and marrow cavity. This result was in agreement with the view that T promotes bone formation at the periosteal surface (Kim et al. 2003). Since high T was associated with larger marrow cavity, it is comprehensible that T level is negatively associated with total vBMD of TS. This is in agreement with the reports that the total BMD at the long bone shaft measured by pQCT was higher in women than men (Schönau et al. 2002) (III).

The association of SHBG with bone properties of TS was in the opposite direction to that of E2 (in most cases) and T (in total bone CSA) (II and III). SBHG modifies the bioavailability and metabolism of E2 and T (Figure 11). Thus, it is conceivable that SHBG exerts inhibitive influences on the osteogenic effect of both E2 and T indirectly. Furthermore, because the time-to-time variation of sex steroids is much higher than that of SHBG, the concentration of this binding globulin should be a more stable indicator for sex hormone exposure than the level of sex steroids themselves. With a smaller variation of SHBG, the association will be easier to detect. These results provide an explanation for the previous finding that SHBG was superior to sex hormone in predicting the occurrence of postmenopausal fracture (van Hemert et al. 1989).

6.3 Physical activity

Based on our results, prepuberty is likely more sensitive period for PA to exert or exhibit its beneficial effect on bone development than early puberty. It is highly possible that the physically active girls at an early developmental stage will remain active at later developmental stages. Thus, other factors with stronger osteogenic effect, such as sex hormones, may have masked the positive but slight beneficial effect of PA at the later maturational stages. This view is consistent with previous reports that PA intervention did not induce significant bone change in late adolescents or young adults (Peterson et al. 1991; Snow-Harter et al. 1992; Blimkie et al. 1996; Heinonen et al. 1996), but in postmenopausal women with E2 depletion, significant bone gain following exercise intervention can be demonstrated (IV) (Pruitt et al. 1992; Nelson et al. 1994; Ryan et al. 1994).

Our results support the view that impact PA was more efficient than non-impact PA in inducing BMC accretion (IV). To initiate a positive adaptive response in bones, mechanical strain must exceed a minimal effective level (Frost 1987; Turner 1991). The strain induced by local muscle contraction has been analyzed in young women, and found that although strength training induced a significant increase in muscle strength, the strains remained within the customary mechanical range (Heinonen et al. 1996). On the other hand, during impact PA, the strain of weight-bearing

bones is the combination of local muscle contraction and function of body weight, thus the strains induced by impact PA is much higher than non-impact PA. Our result that FM of TB was more strongly correlated with leg BMC than with arm BMC, and that BMC per unit of muscle strength was significantly higher in leg than arm, corroborates this finding.

Girls who were physically active in their leisure time during puberty had higher BMC of LS2-4, FN and TF than those who were inactive. This difference, however, was already present as early as our study was able to detect (Figure 15). Continued PA during puberty played an additive role but could not make any further significant difference (the slopes of the growth curves did not differ significantly between groups). It is possible that the difference had already been formed in early lifetime, such as in infancy or early childhood. Because no exercise or training intervention was conducted in this study, any conclusion concerning the effect of leisure-time PA on BMC are speculative.

6.4 Body composition and muscle strength

BMC was correlated moderately with FM, and highly with LM. After controlling for LM, the FM-BMC correlation decreased dramatically to a very weak level (r=0.184). This indicated that the increased LM associated with overweight is the major explanation for the positive correlation between FM with BMC. These results were consistent with previous reports (Khosla et al. 1996; Capozza et al. 2004).

FM may correlate with BMC via mechanism(s) independent of LM. Systemic influences, such as genetic/endocrine, nutrient and life-style factors, are exactly the same for weight-bearing and non-weight-bearing sites, whilst the physical loading regime is different. In essence, the legs have to support FM of TB, but the arms do not. Our observation that FM of TB was correlated significantly higher with leg BMC than with arm BMC indicates that static loading from FM plays a major role in determining the FM-BMC relationship. Comparing the correlation of FM with BMC/LM in the upper and lower limbs may be minimally affected by the confounding effect of any methodological problems associated with DXA measurement, since the measurement uncertainty is much smaller in limbs than in trunk (Cheng, et al. 2005). Our result was in line with previous reports that obese children possessed 46% more BMC than their normal counterparts in the leg, but only 21% more in the arm (Manzoni et al. 1996). Since the mechanical loading applied to the forearm is proportional to body weight during a fall, and the compensatory response of bone strength to increased FM is more concentrated in the weight-bearing bones, overweight or obesity should be a risk factor of forearm factures. This view is in line with previous reports that

forearm fracture risk is higher in overweight than normal children (Goulding et al. 1998; 2001; Skaggs et al. 2001).

The MVC of elbow flexors and knee extensors was highly associated with BMC of arm and leg, respectively. There was no difference in the correlation or regression of BMC with/against MVC in arms and legs. This indicates that muscle strength has similar osteogenic efficiency (change in BMC in response to per unit change in muscle strength) in arm and leg. On the other hand, the ratio of limb BMC to MVC (BMC/MVC) was significantly higher (30%) in leg than in arm. These two results suggest that other factor(s), most likely weight-bearing, not muscle strength *per se*, are responsible for the difference in BMC/MVC between limbs. This result is in line with reports that in spaceflight or analogous situations, a significant fraction of bone mass is lost in the legs, but very little or none is lost in the arm (Whalen 1993; Collet et al. 1997; Vico et al. 2000).

Whether the difference in BMC/MVC between the upper and lower limbs is predetermined by genetics or arised during development and growth is unknown. Ruff (2003) found that there was a small but distinct growth spurt in femoral strength in late infancy (1-2 years of age). However, the growth of humeral strength showed a peak velocity around 1-year-old followed by a precipitous decline thereafter. Human femoral/humeral length ratio in human infants was close to those of adults, while the femoral/humeral diaphyseal strength ratio only developed after the adoption of bi-pedalism at about 1 year of age; while in baboons, much smaller age changes in the femoral/humeral strength ratio were observed (Ruff 2003). These observations reflect the effect of change in mechanical environment on bone strengths in arms and legs during the transitory period from quadri-pedalism to bi-pedalism. Thus, it is conceivable that the difference in BMC/MVC between the upper and lower limbs is initiated during the period of late infancy and fully constructed progressively thereafter.

6.5 Limitations and perspectives

This study is an observational investigation using a sample of an intervention study (the Calex study). Pooling of the girls regardless of the intervention brings with it the risk that the supplementation of calcium and vitamin D might modify the growth tempo of bone mineral accrual (Bonjour et al. 1997; Chevalley et al. 2005)(I, III, V), although no such effect was found in our study (Cheng et al. 2005). The drop-out rate was about 14% in the last measurement, which may have influenced the results. The various correlations of bone properties with sex hormones (II and III), physical activity (IV), body composition and muscle strength (V) are in line with

previous reports. However, causality cannot be inferred from observational studies.

In study I, the construction of the growth models of bone properties using polynomial functions was based on data collected from pubertal girls at the period between 4 years before and 2 years after menarche. The times when the velocities of these variables peaked might shift slightly when using different models to those used in this study. These models cannot be used beyond this developmental stage and nor can they be generalized to pubertal males. Furthermore, the reliability of these models at the two ends of this period may be in doubt because less data was available in these regions.

In the present study, the serum levels of sex steroids are only one snapshot measurement during the low level phase of hormonal activity, therefore may not reflect the real hormonal exposure. This limitation makes the calculated association of sex hormones with bone parameters much weaker than the expected value. It would be valuable if objective and stable indicators for sex hormone exposure, as the glycosylated hemoglobin used in monitoring long-term blood glucose, could be defined in the future (II and III).

There is currently no accepted 'gold standard' or truly validated measure of physical activity. The questionnaire as an approach to assess physical activity level is subject to participants' reporting errors and other errors associated with the chosen formula used to produce a PA score. Another arguable point concerns the method for assessing impact and non-impact exercise. Only the type of favorite exercise was taken into account in assessing this, regardless of its duration, frequency, and intensity or of other exercise types also engaged in. There may be better ways of making the distinction between impact and non-impact activities (for example, looking at all three types of exercise listed by the subjects), but again there is no established consensus, and the approach adopted has the benefit of being straightforward (IV).

In study V, we made an attempt to understand the relative importance of muscle strength and weight-bearing on bone accretion in lower limbs of healthy girls. The two factors are so entangled in the weight-bearing bones that quantitative evidence addressing this question is scarce. Comparing the BMC/MVC between arms and legs might be subject to serious methodological questioning. Firstly, the relative importance (in terms of mechanical loading in all bones of a certain limb) of elbow flexors in arms and knee extensors in legs might be rather different. In other words the force generated by elbow flexors and knee extensors may represent a different proportion of the mechanical load in the bones of arm and leg, respectively. The knee extensors may be the strongest muscle group in the lower extremity or even in the whole body. With a relatively higher denominator of BMC/MVC in leg than in arm, the effect of weight-bearing on BMC accretion in legs, however, was underestimated. Secondly, local muscle contraction is not only a source of mechanical loading, but also a protective factor for bones

(Currey 1984). Muscle contraction during physical movement compensates efficiently for bending load in bones produced by gravitational and external forces (Munih et al. 1992). Therefore, simply comparing BMC/MVC between arm and leg without considering the entire biomechanical environment might have resulted in a distorted picture of the bone-muscle relationship.

To prevent fragility fractures in later life by optimizing the development of PBM, identifying those children with risk of suboptimal bone mass accretion assumes great importance for early cost-effective intervention. In the future, using longitudinal studies spanning from early childhood to young adulthood, plausible indicator(s) may be defined. Fracture occurrence is an event determined by a multitude of factors. The elevated fracture risk of forearm during puberty could be significantly decreased if the risk factors were identified and counteracted. Our result regarding the influence of E2 on total bone radial size at long bone shaft in pubertal girl is not in line with animal studies. To elucidate this controversy, bone length should be controlled for when comparing bone radial size between ovariectomized animals and controls. The small effect of physical activity on bone mass may under-represent its protective effect against fracture. Better balance and increase muscle strength induced by exercise may be more important (Suominen 1988; Karlsson 2004). Comparing the effect of different exercise types, for instance, between strength and balance training, may shed light on this question. Although our study corroborates previous reports that excess weight or obesity is related to higher risk of forearm fracture in children (Goulding et al. 1998; 2001; Skaggs et al. 2001), a population-based prospective study is needed. The results regarding the relative importance of muscle strength and weight-bearing on bone strength raise the old hypothesis again: weight-bearing may contribute to a significant amount of bone strength in legs. A well-designed animal study may answer this question. Comparing the BMC/LM between arm and leg in infants may also provide useful information.

7 MAIN FINDINGS AND CONCLUSIONS

On the basis of this observational study, we conclude that:

1. The suggested asynchronous growth between bone size and bone mass during puberty was confirmed. The growth velocity of bone size peaks at least half years earlier than that of bone mass, in pubertal girls.
2. Volumetric BMD of the distal radius decreased slightly up to one year before menarche; on the other hand, total and cortical volumetric BMD of the tibial shaft increased progressively. Marrow cavity size of the tibial shaft did not change before menarche, and decreased after menarche, in pubertal girls.
3. At the tibial shaft, both 17β-estradiol and testosterone were not associated with cortical volumetric BMD. 17β-estradiol did not correlate with total bone cross-sectional area, but was negatively associated with the size of marrow cavity. With smaller marrow size, high 17β-estradiol level was positively associated with total bone volumetric BMD and BMC. On the other hand, testosterone was positively related to total bone cross-sectional area and marrow cavity size.
4. In prepubertal girls, high level physical activity was associated with higher BMC of total body, lumbar spine (LS2-4) and total femur; in early pubertal girls, the positive association was only found in LS2-4. On the other hand, the difference in BMC between consistently active and inactive girls during puberty was present as early and late as our study could detect.
5. Total body fat mass was related significantly more with BMC in legs than with BMC in arms. This indicates that physical loading is an important mechanism by which fat mass is related to BMC. Lean tissue mass was highly correlated with BMC. Muscle strength correlated to BMC similarly in arms and legs. However, BMC per unit of muscle strength was significantly higher in legs than arms. This study provided quantitative evidence for the importance of weight-bearing in BMC accretion.

TIIVISTELMÄ

Kasvun ja kehityksen aikana kertynyt luumassa on tärkeä tekijä, kun tarkastellaan luiden lujuutta ja osteoporoosin liittyviä ongelmia ikääntyvässä väestössä. Luiden kasvuun sisältyvien biologisten prosessien ymmärtäminen onkin tärkeää, kun suunnitellaan väestötasoisia toimenpiteitä osteoporoottisten murtumien ehkäisyssä. Tässä tutkimuksessa tutkittiin luun kasvua tarkastelemalla luun koon, mineraalimäärän (BMC) ja volymetrisen mineraalitiheyden (vBMD) yhteyttä sukuhormonituotantoon, fyysiseen aktiivisuuteen, kehon koostumukseen ja lihasvoimaan 10–13-vuotiailla tytöillä (n=258). Tyttöjen kasvua ja kehitystä seurattiin toistuvin mittauksin kahden vuoden ajan. Fyysinen aktiivisuus määritettiin kyselylomakkeen avulla ja kehon koostumus ja BMC röntgenabsorptiomenetelmällä (DXA). Luun geometria ja vBMD mitattiin värttinäluun distaaliosasta (hohkaluu) ja sääriluun varsiosasta (putkiluu) kvantitatiivisella tietokonetomografialla (pQCT). Kyynärnivelen koukistajalihasten ja polven ojentajalihasten isometrinen voima mitattiin dynamometrilla. Seerumin sukuhormoni-pitoisuudet määritettiin fluoroimmunometrisesti. Mittaukset osoittivat, että luun mineraalimäärän suurin kasvunopeus ajoittuu yli puoli vuotta myöhemmäksi kuin luun koon. Kasvun asynkronian seurauksena värttinäluun distaaliosan vBMD väheni jonkin verran varhaispuberteetissa. Sääriluun ydinontelon poikkipinta-ala ei muuttunut menarkea edeltävän kasvun aikana, mutta alkoi pienetä sen jälkeen. Seerumin estradiolipitoisuus ei korreloinut sääriluun kokonaispoikkipinta-alaan, mutta sillä oli positiivinen yhteys kortikaaliseen poikkipinta-alaan ja luun kuorikerroksen paksuuteen. Toisaalta testosteronipitoisuus korreloi positiivisesti sääriluun kokonaispoikkipinta-alaan, muttei kuorikerroksen poikkipinta-alaan eikä paksuuteen. Fyysisen aktiivisuuden ja luiden BMC:n välillä oli positiivinen yhteys. Koko kehon rasvamassa korreloi voimakkaammin alaraajan kuin yläraajan BMC -arvoihin. Ylä- ja alaraajan luiden BMC korreloi merkitsevästi niistä mitattuihin lihasvoimiin. Lihasvoimaan suhteutettu BMC oli kuitenkin merkitsevästi suurempi ala- kuin yläraajassa. Tutkimuksen tulokset viittaavat siihen, että murrosikäisillä tytöillä luun koon kasvu tapahtuu aiemmin kuin luun massan kasvu, mikä saattaa selittää muissa tutkimuksissa todetun murtumariskin lisääntymisen kasvukirin aikana. Estradiolin merkitys luun lujuudelle korostuu tässä kehitysvaiheessa, koska se hillitsee luun resorptiota ja mahdollisesti jopa lisää luun endokortikaalista muodostusta. Estradiolin luumassaa lisäävä vaikutus saattaa edellyttää sopivaa mekaanista rasitusta. Myös lihasvoimalla ja kehon koostumuksella näyttää olevan merkitystä luun lujuuden kehitykselle.

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