Bone Mineral Accrual in Physically Active Girls

With Special Reference to Reduction in Physical Activity Level and Use of Oral Contraceptives

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ABSTRACT

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ANNALES UNIVERSITATIS TURKUENSIS
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Adolescence is an important time for acquiring high peak bone mass. Physical activity is known to be beneficial to bone development. The effect of estrogen-progestin contraceptives (EPC) is still controversial.

Altogether 142 (52 gymnasts, 46 runners, and 42 controls) adolescent women participated in this study, which is based on two 7-year (n =142), one 6-year (n =140) and one 4-year (n =122) follow-ups. Information on physical activity, menstrual history, sexual maturation, nutrition, living habits and health status was obtained through questionnaires and interviews. The bone mineral density (BMD) and content (BMC) of lumbar spine (LS) and femoral neck (FN) were measured by dual- energy X-ray absoptiometry. Calcaneal sonographic measurements were also made.

The physical activity of the athletes participating in this study decreased after 3-year follow-up. High-impact exercise was beneficial to bones. LS and FN BMC was higher in gymnasts than in controls during the follow-up. Reduction in physical activity had negative effects on bone mass. LS and FN BMC increased less in the group having reduced their physical activity more than 50%, compared with those continuing at the previous level (1.69 g, p=0.021; 0.14 g, p=0.015, respectively). The amount of physical activity was the only significant parameter accounting for the calcaneal sonography measurements at 6-year follow-up (11.3%) and reduced activity level was associated with lower sonographic values.

Long-term low-dose EPC use seemed to prevent normal bone mass acquisition. There was a significant trend towards a smaller increase in LS and FN BMC among long-term EPC users.

In conclusion, this study confirms that high-impact exercise is beneficial to bones and that the benefits are partly maintained even after a clear reduction in training level at least for 4 years. Continued exercise is needed to retain all acquired benefits. The bone mass gained and maintained can possibly be maximized in adolescence by implementing high-impact exercise for youngsters. The peak bone mass of the young women participating in the study may be reached before the age of 20. Use of low-dose EPCs seems to suppress normal bone mass acquisition.

Key words: adolescent women, bone mineral contents, bone mineral density, estrogen-progestin contraceptives, physical activity, quantitative ultrasound

TIIVISTELMÄ

Essi Pikkarainen

Liikuntaa harrastavien tyttöjen luun mineraalimassan kehittyminen liikunnan vähetessä ja ehkäisypillereitä käytettäessä

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Nuoruusikä on tärkeää aikaa huippuluumassan kehittymiselle. Liikunnan on todettu olevan hyödyksi kehittyville luille, mutta ehkäisyvalmisteiden käytön vaikutukset luustoon ovat tutkimustulosten mukaan jääneet epävarmoiksi.

142 (52 voimistelijaa, 46 juoksijaa ja 44 urheilua harrastamatonta kontrollia) nuorta naista osallistui tähän tutkimukseen, joka koostuu kahdesta seitsemän (n =142), yhdestä kuuden (n =140) ja yhdestä neljän vuoden (n =122) seurantatutkimuksesta. Tiedot fyysisestä aktiivisuudesta, elämäntavoista, terveydentilasta, kuukautishistoriasta, seksuaalisesta kypsymisestä sekä ravitsemuksesta hankittiin kyselylomakkein ja haastatteluilla. Lonkan ja reisiluun kaulan luun tiheys ja luumassa mitattiin kaksienergisellä röntgenabsorptiometrillä. Luun ominaisuuksia tutkittiin myös kantapäästä ultraäänimittauksin.

Tutkimukseen osallistuvien urheilijoiden liikunnan määrä väheni selvästi 3 vuoden seurannan jälkeen. Tärähdyksiä sisältävä liikunta on hyödyllistä luille. Lannerangan ja reisiluun kaulan luuntiheys oli suurempi voimistelijoilla verrokkeihin nähden koko seuranta-ajan. Liikunnan vähenemisellä oli negatiivinen vaikutus luuntiheysarvoihin. Lannerangassa ja reisiluun kaulassa luuntiheys kasvoi vähemmän liikunnan lopettaneella ryhmällä kuin liikuntaa jatkavalla ryhmällä (1.69 g, p=0.021; 0.14 g, p=0.015, vastaavasti). Liikunnan määrä oli ainoa todettu merkitsevästi selittävä tekijä kantaluun ultraäänimittauksissa kuuden vuoden seurannassa (11.3%) ja liikunnan väheneminen oli yhteydessä pienempiin ultraäänituloksiin.

Pitkäaikainen vähäestrogeenisten ehkäisyvalmisteiden käyttö näytti vaikuttavan epäedullisesti luun normaaliin kehitykseen. Pitkään ehkäisypillereitä käyttäneiden nuorten naisten luuntiheys suureni muita vähemmän.

Tämän tutkimusprojektin tulokset osoittivat, että tärähdyksiä sisältävä liikunta on hyödyksi luille ja sen lisääminen nuoruusiällä voi auttaa saavuttamaan suuremman maksimaalisen luumassan. Tähän tutkimukseen osallistuvilla nuorilla naisilla huippuluumassa saatetaan saavuttaa jo ennen 20 vuoden ikää. Vähäestrogeeniset ehkäisypillerit saattavat haitata luun normaalia kehitystä

Avainsanat: nuoret naiset, luuntiheys, luun mineraalimassa, estrogeeni-progesteroni ehkäisy, fyysinen aktiivisuus, kantaluun ultraääni

TABLE OF CONTENTS

ABBREVIATIONS

aBMD	Areal bone mineral density
ANOVA	Analysis of variance
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit
BUA	Broadband ultrasound attenuation
CI	Confidence interval
CV	Coefficient of variation
DMPA	Depot medroxyprogesterone acetate
DXA	Dual-energy X-ray absorptiometry
EE	Ethinyl estradiol
EPC	Estrogen-progestin contraceptives
FN	Femoral neck
LS	Lumbar spine
LTPA	Leisure time physical activity
MANOVA	Multivariate analysis of variance
MET	Ratio of work metabolic rate to resting metabolic rate
MRI	Magnetic resonance imaging
OC	Oral contraceptives
PBM	Peak bone mass
pQCT	Peripheral quantitative computed tomography
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound measurements
SD	Standard deviation
SOS	Speed of sound
SQMET	Square root of ratio of work metabolic rate to resting metabolic rate
vBMD	Volumetric bone mineral density

LIST OF ORIGINAL COMMUNICATIONS

The thesis is based on the following original communications, which are referred to in the text by their Roman numerals (I-IV).

I Pikkarainen E, Lehtonen-Veromaa M, Kautiainen H, Heinonen OJ, Viikari J, Möttönen T. Exercise-induced training effects on bone mineral content: a 7-year follow-up study with adolescent female gymnasts and runners. Published online in Scand J Med Sci Sports. 18-Feb-2008, doi: 10.1111/j.1600-0838.2008.00773.x

II Rautava E, Lehtonen-Veromaa M, Kautiainen H, Kajander S, Heinonen OJ, Viikari J, Möttönen T. The reduction of physical activity reflects on the bone mass among young females: A follow-up study of 142 adolescent girls. Osteop Int 2007;18:915-922

III Rautava E, Lehtonen-Veromaa M, Möttönen T, Kautiainen H, Heinonen OJ, Viikari J. Association of reduced physical activity and quantitative ultrasound measurements: A 6-year follow-up study of adolescent girls. Calcif Tissue Int 2006;79:50-56

IV Pikkarainen E, Lehtonen-Veromaa M, Möttönen T, Kautiainen H, Viikari J. Estrogen-Progestin Contraceptive Use during Adolescence Prevents Bone Mass Acquisition: A 4-year Follow-up Study. In press in Contraception.

1. INTRODUCTION

Osteoporosis is a significant public health issue. In osteoporosis, excessive bone loss leads to poor bone quality, weak bones and an increased risk of fractures. It is a major cause of disability especially in elderly women. There are two main causes of low bone mineral density (BMD) in older people: 1) inadequate attainment of BMD during growth and 2) failure to maintain bone mass during aging.

Puberty is the period of rapid bone growth. Recently, it has been generally accepted that the bulk of bone mass is achieved by late adolescence (Bonjour et al. 1991, Theintz et al. 1992, Haapasalo et al. 1996, Bailey 1997, Bass et al. 1998, Lin et al. 2003, Henry et al. 2004). Achieving high peak bone mass (PBM) during these years is protective against later-life fractures (Heaney et al. 2000). Although genetic factors determine 60-80% of PBM, environmental factors, such as physical activity, nutrition, and bone-affecting medications, are also important in bone mass development (Bonjour et al. 2007).

Physical activity and exercise have been demonstrated to have positive effects on growing bone before and during puberty and many studies have shown the beneficial effects of high-impact weight-bearing activity on the load-bearing sites of the skeleton (Heinonen et al. 1995, Robinson et al. 1995, Lehtonen-Veromaa et al. 2000, McKay et al. 2000, Shibata et al. 2003). The skeletal benefits of exercise have been shown to be greater when training is started prior to menarche (Kannus et al. 1995, Haapasalo et al. 1998, Heinonen et al. 2000, Kontulainen et al. 2001). Less is known about the maintenance of these benefits.

It has been suggested that physical activity is essential in sustaining the gained bone mass (Winters & Snow 2000, Gustavsson et al. 2003, Valdimarsson et al. 2005a). In an 8-year follow-up of young female soccer players, the players who retired during the follow-up lost bone at the femoral neck, whereas the controls, which did not play soccer, did not (Valdimarsson et al. 2005a). Nordström et al. (2006) reported only a partial loss of exercise-induced benefits in BMD after retirement from an active career.

Not all researchers agree with this view. In some studies, exercise-induced effects have been maintained after cessation of exercise (Bass et al. 1998, Kontulainen et al. 2001, Kudlac et al. 2004, Zanker et al. 2004). Former old male athletes had fewer fractures than their matched controls (Nordström et al. 2006).

The need for birth control often arises during youth and adolescence, and the use of oral contraceptives (OC) begins. Recently, there have been increasing concerns that the use of OCs during adolescent years alters the normal PBM development (Polatti et al. 1995, Cromer et al. 2004, Hartard et al. 2007). In a cross-sectional study, young women using low-dose estrogen OCs were found to have lower BMDs than their controls (Almstedt Shoepe & Snow 2005). In a 5-year follow-up, Polatti et al. (1995) found that long-term treatment with a monophasic pill prevented the physiologic occurrence

of PBM in 19-22-year-old adolescent women. Similar results have been obtained by Cromer et al. (2004) and Hartard et al. (2004).

By contrast, contraception was found to have no significant effect on bone in two follow-up studies with slightly older study populations (Berenson et al. 2004, Endrikat et al. 2004). In a population-based cross-sectional study, OC use ever had a positive association with lumbar spine BMD when compared with never users, showing increasing BMD with increasing duration of use (Pasco et al. 2000).

The purpose of this study was to determine the influence of physical activity on the development and maintenance of bone mineral density and content in healthy adolescent women with special emphasis on the effects of reduced physical activity and estrogen-progestin contraceptive (EPC) use. The vulnerable sites of the skeleton, the lumbar spine and the femoral neck, were examined by dual-energy X-ray absorptiometry and the calcaneus was examined by sonography.

2. REVIEW OF THE LITERATURE

2.1 Biology of bone

2.1.1 Structure of bone

The skeletal system is made up of cartilage and bone. Bones are stiff as they cannot bend under load, but they are also flexible and able to deform, as they must absorb energy. If bones deform too little or too much, structural failure may occur (Seeman 2008).

There are two kinds of bone structures, cortical and trabecular. Cortical bone, which is found in the shafts of long bones, accounts for approximately 80% of the skeletal mass in the human body. It is dense and forms a thin shell supporting the trabecular bone. The cortical wall is covered with a fibrous envelope, which is referred to as the periosteum. The inner surface of cortical bone is called the endosteum and it faces the bone marrow. The strength of cortical bone is determined by its thickness and porosity (Chappard 2006).

Cortical bone is arranged in Haversian systems (osteons) (Figure 1). Osteons consist of concentric lamellae of bone tissue surrounding a central Haversian canal. The Haversian canal contains blood vessels, nerves and a monolayer of bone lining cells (Chappard 2006).

Trabecular bone covers twice the surface area of cortical bone, but makes up only 20% of the skeletal mass. It is made up of trabeculae, which are rod- or plate-like structures. Trabecular bone acts as load-bearing tissue. It also transfers loads across joints, holds up compressive loads and acts as a shock absorber. In addition, it is involved in calcium metabolism. The strength of trabecular bone is influenced by its microarchitecture, i.e. the volume, number, thickness, orientation, connectivity, perforation and spacing of trabeculae (Borah & Dufresne 2006).

Long bones act as levers so they must be strong and able to resist lengthening and narrowing tensions as well as shortening and widening compressions (Seeman 2006). Long bones consist mainly of cortical bone and are thus stiff and resistant to bending and torsion (Chappard 2006). Bones must also be light to facilitate movement (Seeman 2006). Long bones are usually tubular, i.e. the marrow cavity is surrounded by bone.

In the axial skeleton, bone structure differs from tubular bones. Axial bones are organized as an open-celled porous structure. The vertebral bodies contain much trabecular bone and function as springs and shock absorbers. The structure is light yet strong and it can deform briefly without cracking (Seeman 2008).

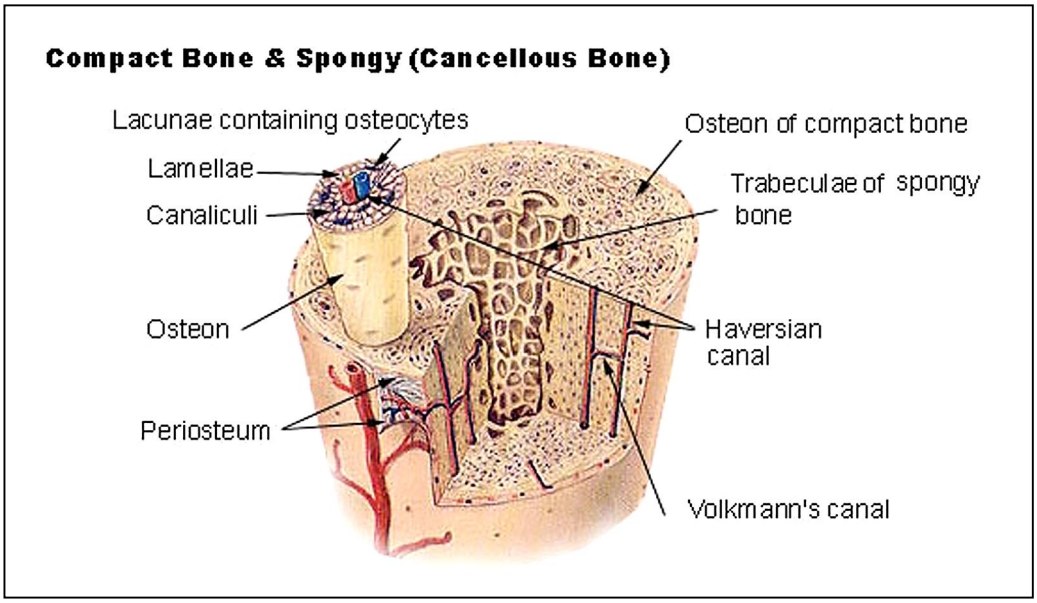

Figure 1. Structure of bone. Cortical bone = Compact bone, Trabecular bone = Spongy, cancellous bone (freely accessible on the internet)

2.1.2 Bone turnover

Bone is constantly being turned over. There are two processes of bone turnover, modeling and remodeling.

Modeling is the process that changes the shape, size and geometry of bone in childhood and adolescence and also in adult life in response to changed loading patterns. This is the process by which the skeleton is constructed. It involves deposition of a collagen matrix and mineralization of this matrix (Dempster 2006).

Remodeling is a continuous process that enables bone to repair damages, maintain structural integrity and fulfill mineral homeostasis. The remodeling cycle comprises 5 phases: resting, activation, resorption, reversal and formation (Figure 2). This is carried out by a coordinated group of different cell types, called basic multicellular unit (BMU) (Dempster 2006). In the resting state, no resorption or bone formation takes place. During activation, the resting bone cells, osteocytes, sense any mechanical changes and remodeling decreases or increases accordingly. Osteoclast precursors differentiate into osteoclasts, i.e. cells that resorb bone. In the reversal phase, osteoclasts undergo apoptosis and resorption stops. Any collagen uncleared by osteoclasts is removed by specific mononuclear cells, which then deposit a thin layer of proteoglycans to form the cement line. During the formation phase, new bone is formed by osteoblasts. In healthy bone, bone resorption is coupled with bone formation, so there is no bone loss (Dempster 2006). Several diseases, e.g. osteoporosis, are caused by abnormalities in bone remodeling.

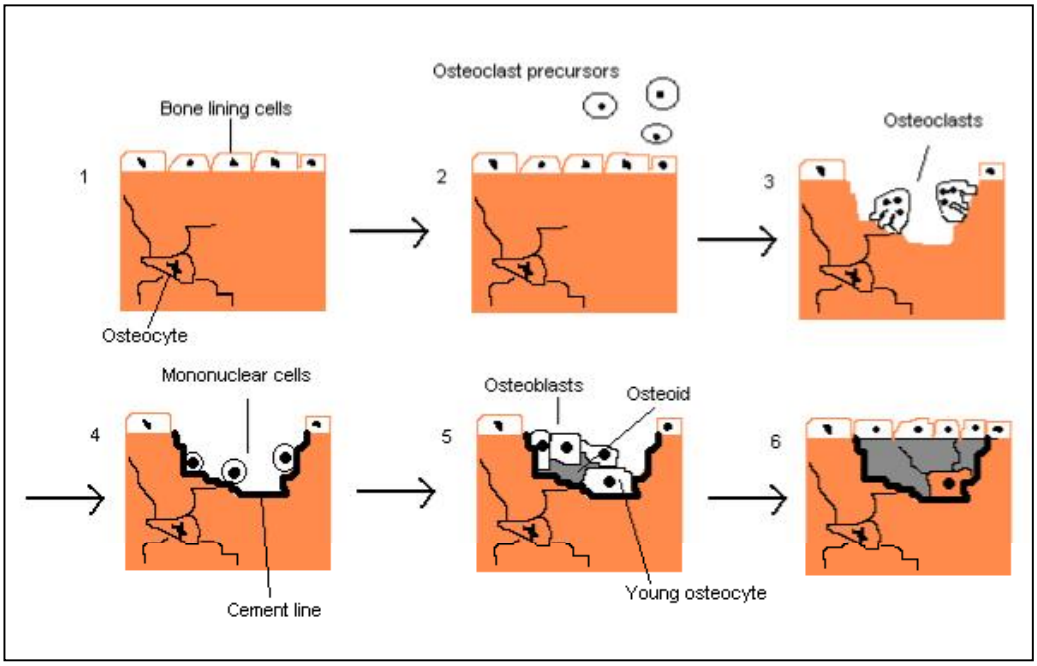

Figure 2. Bone remodeling cycle. 1. Resting state. 2. Activation. 3. Resorption. 4. Reversal. 5. Formation. 6. Completion of remodeling sequence.

2.1.3 Bone biomechanics

When considering bone strength, there are two important concepts. First, unlike most materials, bone is continuously adapting to its mechanical and hormonal environment. It is capable of self-renewal and repair. Second, the mechanical properties of bone are influenced by cellular, matrix and architectural factors (Bouxsein 2006).

The biomechanical properties describe the relationship between the forces applied to bone and the resulting deformations. Stress is the resistance that develops in response to the applied forces. Any local deformation that results from the applied forces is called strain. When forces are applied to bone, varied distribution of stresses and strains arises throughout bone structures (Bouxsein 2006). Depending on its orientation, stress and strain can be tensile, compressive, bending, shearing, torsion or combined (Figure 3). From a mechanical perspective, fracture is a structural failure when the forces applied to the bone exceed its capacity to bear loads (Bouxsein 2006).

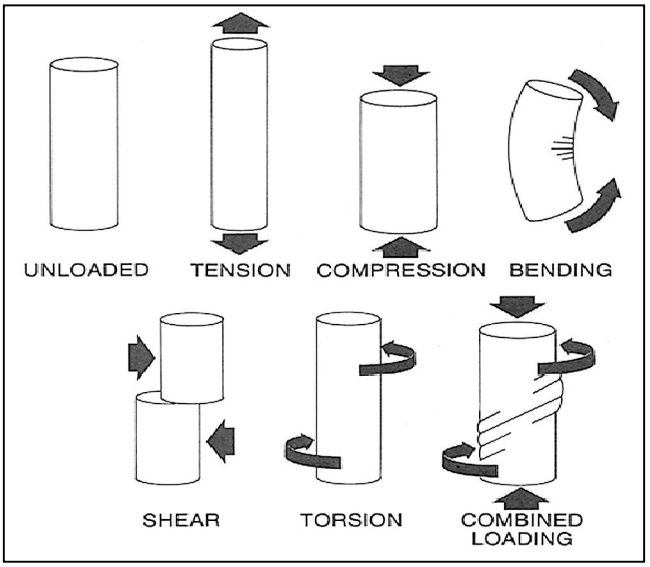

Figure 3. Schematic presentation of the basic types of stress (freely accessible on the internet)

Mechanical properties are best described by strength and stiffness. These two properties are shown graphically in the load-deformation curve in Figure 4. The elastic region before the yield point is the region where the structure would return to its original form if unloaded. If loading continues, some permanent deformation is likely to occur. This is called the plastic region. The transition from elastic to plastic region is called the yield point. The failure point, in turn, represents the load after which the structure fails (Bouxsein 2006). Strength is often described as the load at the yield point, while stiffness is the slope of the load-deformation curve.

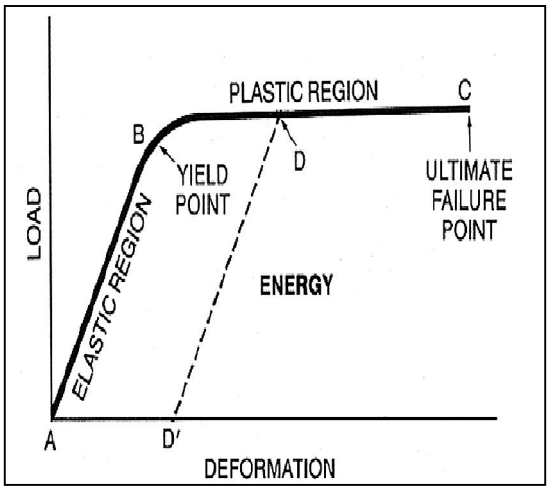

Figure 4. Load - Deformation curve (freely accessible on the internet)

2.1.4 Adaptation to loading

The skeleton's ability to adapt to functional demands was discovered a century ago, and has since then been referred to as Wolff's law (Wolff 1892). During growth, the skeleton responds to the changing environment by modeling. Bone modeling can determine and increase skeletal mass, but it seldom decreases it (Frost 1992, Frost 1997). In cortical bone, modeling becomes ineffective in adults, but in trabeculae it can continue throughout life. In the whole skeleton, modeling can add bone mass or leave it unchanged, but it usually does not reduce it (Frost 1992).

Bones adapt to changing mechanical loads also by remodeling by BMUs, which act on the periosteal, endosteal and trabecular surfaces throughout life. In the whole skeleton, remodeling can remove or conserve bone, but it usually does not add to it without the help of pharmacological agents (Frost 1992).

It has been suggested that when strain reaches a minimum magnitude, the modeling phase is switched on (Frost 1997). This strain is called the minimum effective strain of modeling and Frost suggests it to lie near 1000 μE (Frost 1997). If the strain stays below the minimum effective strain (50-100 μE), no remodeling takes place. This is the disuse mode, and it means increased bone loss (Frost 1997).

There is also a microdamage threshold. When loads exceed this threshold, the damage cannot be repaired by BMUs, resulting in a stress fracture. The microdamage threshold seems to lie near 3000 μE. For fracture strain, the threshold lies near 25 000 μE (Frost 1997).

The big muscles of athletes (e.g. weight lifters, gymnasts) exert high strains on bones. When training begins, the increase in muscle force makes the strains exceed the modeling threshold. This results in increased bone mass and strength. When muscle strength plateaus, the increase in bone mass tends to plateau too (Frost 1997).

2.2 Bone mass development in adolescence

2.2.1 Acquiring peak bone mass

The skeleton grows during childhood, puberty and adolescence. In general, there are two major growth spurts in skeletal development. The first occurs from 1 to 4 years of age and the second occurs during puberty (Ondrak & Morgan 2007). Figure 5 presents the development of bone mass and the risk factors of osteoporosis in normal, healthy individuals.

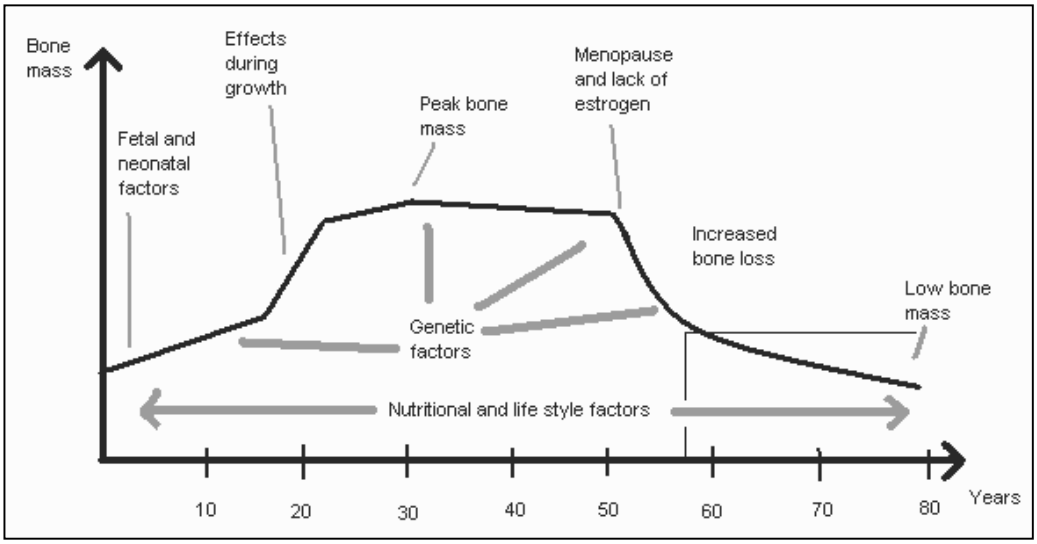

Figure 5. Factors that contribute to low bone mass and osteoporosis.

It is generally accepted that the bulk of bone mass accumulates at puberty during the latter growth spurt (Bonjour et al. 1991, Theintz et al. 1992, Slemenda et al. 1994, Haapasalo et al. 1996, Bass et al. 1998, Bailey et al. 1999, Lin et al. 2003, Henry et al. 2004). Although the exact timing of the fastest bone mass accrual remains under debate, it has been established that 32% of female BMC is accrued in the 2 years around peak BMC velocity (Bailey et al. 1999). Peak BMC velocity has been found to be within 12 months from peak height velocity (McKay et al. 1998, Bailey et al. 1999). There seems to be unanimity that peak BMD is attained by the end of the second decade of life (Heaney et al. 2000).

Many researchers have reported that bone growth tempo is region-specific (Bass et al. 1998, Lin et al. 2003, Henry et al. 2004). In these studies, PBM is reached earlier at the femoral neck than at the lumbar spine (Bass et al. 1998, Lin et al. 2003, Henry et al 2004). Bone growth is also sex- specific, i.e. females reach skeletal maturity earlier than males (Bonjour et al. 1991, Theintz et al. 1992, Bass et al. 1998).

Reports on the end of bone growth are also conflicting. Researchers have found skeletal mass growth to slow down dramatically both at the lumbar spine and femoral neck at 15-16 years of age in female adolescents (Bonjour et al. 1991, Theintz et al. 1992). Some studies report bone loss soon after the achievement of PBM at the age of 20 (Haapasalo et al. 1996). By contrast, in a 5-year follow-up, bone growth was complete by the age of 30 (Recker et al. 1992). It has also been shown that skeletal bone mass increases after cessation of linear growth (Henry et al. 2004).

2.2.2 Role of endogenous estrogen

Puberty is a period of profound physiological changes, involving a considerable increase in sex hormone production.

Sex steroids, predominantly estrogens in girls and androgens in boys, have major effects on skeletal growth and maturation. They are responsible for the sexual dimorphism of the skeleton, initiation of pubertal increase of growth velocity and the closure of the epiphyseal growth plates, resulting in cessation of linear growth of long bones (Turner et al. 1994). It is thought that estrogen is essential for normal epiphyseal maturation and skeletal mineralization in both sexes (Frank 1995).

In the female skeleton during puberty, there is 1) periosteal expansion and longitudinal growth, both largely controlled by growth hormone, although augmented by gonadal hormones in the pubertal growth spurt; and 2) growth arrest and increase in trabecular density largely due to an increase in estrogen levels (Heaney et al. 2000). Many cross-sectional studies have reported an inverse relationship between adult BMD and age at menarche, others, however, have found no association (Heaney et al. 2000). In girls, the menarche coincides with peak BMC velocity (McKay et al. 1998).

2.3 Environmental factors affecting skeletal development in adolescence

2.3.1 Physical activity

2.3.1.1 Physical activity during growth

Due to its mechanosensitivity, bone adapts its mass, size and architecture to changes in its loading environment. Growth is a dynamic process in which bones continually adapt to changes in bone length and muscle force.

Physical activity especially during growth and adolescence is one of the central determinants in the development of PBM (Heaney et al. 2000, Petit et al. 2006). There is evidence from an animal study that growing bone is more responsive to mechanical loading than mature bone (Forwood & Burr 1993). In human studies, too, the skeletal benefits of exercise have been shown to be greater when training is done during growth (Kannus et al. 1995, Bass et al. 1998, Haapasalo et al. 1998, Heinonen et al. 2000, Bass 2000, Kontulainen et al. 2001, Sundberg et al. 2002, Janz et al. 2007). Physical activity is a determinant of bone mineralization (Uusi-Rasi et al. 1997, Havill et al. 2007). In some studies, preadolescent gymnasts have had greater bone mineral values than sedentary controls (Dyson et al. 1997, Nickols-Richardson et al. 1999, Lehtonen-Veromaa et al. 2000, Nickols-Richardson et al. 2000).

It has been suggested that exercise at puberty may promote bone formation on the periosteal surface whereas exercise at late puberty and in early adulthood may enhance periosteal apposition in boys and endocortical apposition in girls (Daly 2007).

There is also evidence that habitual levels of physical activity influence bone mass (Hasselstrøm et al. 2007, Tobias et al. 2007). Nearly one third of the total lumbar mineral content of adult women accumulates in the 3 years around the onset of puberty. In prepubertal children, physical activity is associated with more rapid mineralization

(Slemenda et al. 1994). Physically active growing girls had a 17% greater BMC than their inactive peers one year after peak bone mineral accrual (Bailey et al. 1999).

The above findings have been supported by the results from exercise interventions in children. Fuchs & Snow (2002) found a 4% greater BMC at the femoral neck in a high-impact intervention group compared with controls. Heinonen et al. (2000) found a clear and large additional bone gain due to an exercise intervention in premenarcheal girls, but similar findings were not made in postmenarcheal girls. In a recent review of controlled exercise trials, all trials in early pubertal children and most trials in prepubertal children showed positive effects of exercise on bone (Hind & Burrows 2007).

2.3.1.2 Reduction in physical activity level

Physical activity has positive effects on bone. The maintenance of these benefits after a clear reduction in physical activity has been recently under intensive research, but the results diverge.

It seems that continued physical activity is needed to maintain all acquired benefits. Nordström et al. (2005a) reported that former male athletes lost some of the exercise-induced benefits in BMD in 4 years after cessation of their active careers. Still, after a 4-year pause in training, they had a higher BMD than the control group. Similar findings have been made with former gymnasts (Kudlac et al. 2004, Zanker et al. 2004). In the study of Zanker et al. (2004), the former gymnasts had a 6-11% higher areal BMD at all measurement sites than controls despite cessation of training. Kudlac et al. (2004) found site-specific declines in BMD in gymnasts after detraining, but the proximal femur BMD continued to be greater than in controls.

Exercise intervention-induced bone gains were retained in children for more than one year (Kontulainen et al. 2002). The BMD of gymnasts increases during training seasons, followed by declines during offseasons (Snow et al. 2001). Areal BMD of adult women improved in response to an 18-month exercise intervention. These gains were maintained for 3.5 years after the intervention (Kontulainen et al. 2004). The side difference in the humeral and radial BMDs of the playing and non-playing arms in racquet players remained with decreased training, which suggests good maintenance of exercise-induced bone gain (Kontulainen et al. 1999, Kontulainen et al. 2001).

In some studies, by contrast, all benefits gained were lost after detraining. In premenopausal women, the exercise-induced positive effects vanished in 6 months when training was stopped (Winters & Snow 2000). The same effect was seen in postmenopausal women (Dalsky et al. 1988). Reduced training has been associated with increased loss of BMD (Gustavsson et al. 2003, Nordström et al. 2005b, Valdimarsson et al. 2005a). In a study with an 8-year follow-up (Valdimarsson et al. 2005a), the femoral neck BMD of retired female soccer players fell.

2.3.1.3 Type of physical activity

The response of bone to mechanical loading depends on the bone site and the mode of exercise (Bennell et al. 1997). Weight-bearing activities including jumps seem to be beneficial to bones (Nordström et al. 1998, Pettersson et al. 2000). High strain rates and high peak stresses are more osteogenic than large amounts of low-force repetitions (Heinonen et al. 1995). In the study of Vainionpää et al. (2006), the intensity of exercise, measured as the acceleration level of physical activity, correlated significantly with BMD changes.

A few studies have compared impact loading sports, such as gymnastics and volleyball, to active loading sports, for example swimming (Grimston et al. 1993, Fehling et al. 1995, Cassell et al. 1996, Bellew & Gehrig 2006). In all these studies, the BMD of the impact loading group was higher than that of the active loading group. The BMD of swimmers did not differ from that of controls (Grimston et al. 1993, Fehling et al. 1995, Cassell et al. 1996, Bellew & Gehrig 2006). In growing children, exercise regimens combining resistance and impact training seem to provide a larger bone response than either one of them alone (Wang et al. 2007).

Gymnastics, in particular, is a sport with high-impact mechanical loads and strains. Some studies indicated that gymnasts had a higher BMD than their non-gymnastic controls (Robinson et al. 1995, Dyson et al. 1997, Nickols-Richardson et al. 1999, Nickols-Richardson et al. 2000). When compared with runners and controls, gymnasts had a higher increase in lumbar spine and femoral neck BMD in a 1-year follow-up study (Lehtonen-Veromaa et al. 2000). Robinson et al. (1995) found that gymnasts had a higher femoral neck BMD than runners and controls despite a higher prevalence of amenorrhea and oligomenorrhea.

2.3.1.4 Quantitative ultrasound examination and its reaction to physical activity

Quantitative ultrasound (QUS) measurements seem to be sensitive to changes in bone structure caused by physical activity. Two sonographic parameters, broadband ultrasound attenuation (BUA), and speed of sound (SOS), have been shown to reflect such bone characteristics as architecture and elasticity (Langton et al.1984, Glüer et al. 1994). Occupational physical activity, daily physical activity and sports participation have been associated with higher SOS and BUA values (Damilakis et al. 1999, Yamaguchi et al. 2000, Karlsson et al. 2001, Babaroutsi et al. 2005b, Yung et al. 2005, Falk et al. 2007, Robinson et al. 2007). In formerly sedentary women, brisk walking increased BUA values in a 1-year follow-up (Jones et al. 1991). Exercise was the most significant determinant of both BUA and SOS in Finnish young men (Välimäki et al. 2006). In soccer players, QUS values have been higher than in swimmers and controls in a cross-sectional study on Chinese male students (Yung et al. 2005).

Several studies suggest that SOS has higher sensitivity to changes than BUA. Yamaga et al. (1996) observed a significant decrease in SOS during pregnancy, whereas they

detected no significant change in BUA. Laugier et al. (2000) found a significant decrease in SOS during a 120-day bed rest, while the BUA values did not change significantly. SOS has been found to react more sensitively to physical activity than BUA (Lehtonen-Veromaa et al. 2001, Babaroutsi et al. 2005a, Välimäki et al. 2006).

High QUS values have been found in young female gymnasts (Lehtonen-Veromaa et al. 2001, Falk et al. 2003, Nurmi-Lawton et al. 2004). The QUS values of gymnasts were found to stay higher in a 3-year follow-up while training continued (Nurmi-Lawton et al. 2004). In a 1-year follow-up, the SOS values of retired athletes declined (Lehtonen-Veromaa et al. 2001).

2.3.2 Hormonal contraception

Postmenopausal estrogen therapy is known to be beneficial to bone (Komulainen et al. 1998, Dane et al. 2007). Perimenopausal use of combined oral contraceptives (OCs), even low-dose formulations, may prevent bone loss (Martins et al. 2006). The skeletal effects of OCs in premenopausal women are not as evident. In general, it is believed that OCs are not harmful and might even be beneficial to bone mass in premenopausal women (Kuohung et al 2000, Martins et al. 2006). However, there have recently been increasing concerns that hormonal contraception during adolescent years alters the normal PBM development (Polatti et al. 1995, Cromer et al. 2004, Hartard et al. 2007).

Family planning and the need for hormonal contraception often arise during the years of skeletal development. Approximately half of the Finnish female adolescent university students use hormonal contraception (Virtala et al. 2007). At present, the estrogen dose used in combined hormonal contraception is usually 35 µg or less. The estrogen amount has been reduced because of the risk of non-bone complications, such as thromboembolia.

Reduced circulating estrogen concentrations have been established to be the main cause for reduced bone mineral density in postmenopausal (Stevenson et al. 1989) and premenopausal (Sowers et al. 1998) hypoestrogenic women. Estrogen-progestin contraceptives (EPCs) modify the circulating estrogen level by maintaining constant concentrations low, similar to those measured during early follicular phase (Lloyd et al. 1989). This suppression of ovarian estrogen production might be the mechanism by which the estrogen-progestin and other hormonal contraceptive methods cause deficits in bone mass. These changes are, however, considered to be reversible after discontinuation of the therapy in young women (Clark et al. 2006). Table 1 presents the results of 9 studies investigating the effects of estrogen-progestin oral contraceptives on bone in healthy women.

It is known that disturbed menstrual cycles are associated with low BMD (Galuska et al.1999). Volpe et al. (1997) concluded that BMD can be preserved with OCs in almost all hypoestrogenic states observed from adolescence to menopause. Normalization of the menstrual cycle with hormonal contraception has been shown to improve BMD (Seeman

et al. 1992, Castelo-Branco et al. 2001). Elgán et al. (2003) reported that OC use seemed to moderate the negative effects of smoking on bones.

Table 1. Results of studies on the effects of oral contraceptives on bone measurements.

Reference	Design	Population	OC formulation	Bone measure	Results
Polatti et al. (1995)	5-year follow-up	n=200, n of OC users=76, aged 19-22	20 µg of EE + 0.150 mg desogestrel	LS BMD	The BMD of the control group increased 7.8%. the BMD of the OC group remained unchanged.
Lloyd et al. (2000)	8-year follow-up	n=62, n of OC users=28, aged 12	Not specified	TB BMD and BMC, FN BMD	The two groups did not differ from each other at any measurement point.
Pasco et al. (2000)	Cross-sectional	n=710, n of OC users=579, aged 20-69	Not specified	LS, FN, TB and distal forearm BMD	The OC users had a 3.3% higher spinal BMD.
Reed et al. (2003)	3-year follow-up	n=245, n of OC users=89, aged 18-39	80% used formulations containing 30-35 µg of EE	LS, FN and TB BMD	The OC group did not differ from non-users, 18-21-year-olds had a non-significant trend toward smaller BMD gains.
Hartard et al. (2004)	Retrospective analysis	n=69, n of OC users=31, aged 18-35	Not specified	LS and FN BMD	The OC users showed a significantly lower BMD at LS and FN, age at first OC use correlated with spine BMD.
Endrikat et al. (2004)	3-year double-blinded study	n=48, n of 20µg OC users=23, aged 20-35	20 or 30 µg of EE and levonorgestrel	LS BMD, AP, NTx	A small non-significant decrease in BMD in both OC groups.
Cromer et al. (2004)	12-month follow-up	n=215, n of OC users=79, aged 12-18	20 µg of EE, 100 µg of levonorgestrel	LS, FN, total hip, trochanter and Ward's triangle BMD	The mean percent change was significantly lower in the OC group than in the control group at LS (2.3% vs. 3.8%) and FN (0.3% vs. 2.3%).
Almstedt Shoepe & Snow (2005)	Cross-sectional	n=98, n of OC users=44, aged 18-25	20-35 µg of EE	LS, FN and TB BMD	The non-users had a 1.0-2.6% greater BMD at the spine, 0.8-1.8% greater at the hip and 1.1% greater at whole body.
Hartard et al. (2007)	Cross-sectional	n=248, n of OC users=201, aged 18-24	<50 µg of EE	LS and FN BMD, vBMD by pQCT	The young starters and long-time users had a 10% lower FN BMD than the non-users and the ever users had a 5% lower femoral neck BMD than the never users. The differences at LS were non- significant.

AP = bone alkaline phosphatase
BMC = bone mineral content
BMD = bone mineral density
EE = ethinyl estradiol
FN = femoral neck, proximal femur
LS = lumbar spine
NTx = cross-linked N-telopeptides
OC = oral contraceptives
pQCT = peripheral quantitative computed tomography
TB = total body
vBMD = volumetric bone mineral density

Progesterone contraception has also been examined. A growing body of literature suggests that long-term use of depot medroxyprogesterone acetate (DMPA) may have a negative impact on bone health during adolescence (Curtis & Martins 2006, DiVasta & Gordon 2006). Use of DMPA for 2 years or more had a significant adverse effect on BMD since there was a significant decrease in lumbar spine BMD when compared with non-users (Shaarawy et al. 2006). Other researchers have made similar findings in a one-year follow-up (Cromer et al. 2004). A double-blind randomized controlled trial has proven that estrogen supplementation is protective against these changes (Cromer et al. 2005). Estrogen therapy is not usually used, because bone mass increases after discontinuation of DMPA (Curtis & Martins 2006, DiVasta & Gordon 2006).

2.3.3 Other lifestyle factors

2.3.3.1 Nutrition, vitamin D and calcium

The production of bone matrix requires the synthesis and post-translational modification of collagen and an array of other proteins. The nutrients involved in such synthesis include protein, vitamins C, D and K as well as the minerals copper, manganese and zinc. In addition, the skeleton serves as a large nutrient reserve for phosphorus and calcium (Heaney et al. 2000). Calcium and vitamin D are the most studied of these important nutrients.

It is likely that variations in childhood calcium nutrition result in a difference of 5-10% in adult peak bone mass, which is sufficient to cause a 25-50% difference in hip fracture rate later in life (Heaney et al. 2000). Calcium has been described as a threshold nutrient, i.e. skeletal mass increases with increasing calcium until the intake reaches the level at which gain is constant. Calcium supplementation increased BMD in children in a twin study (Johnston et al. 1992). The number of daily dairy servings was associated with calcaneal BUA in adolescent girls (Novotny et al. 2004). By contrast, different calcium intakes did not appear to affect forearm bone mineral densities in girls and adolescent women in southern Italy and Europe (Kardinaal et al. 1999, Maggiolini et al. 1999). According to Finnish nutrition recommendations, young women aged 10-20 years should get 900 mg of calcium per day, and from the age of 21 onwards, 800 mg per day (Finnish nutrition recommendations 2005).

For calcium absorption, the most important nutrient is vitamin D, which is necessary for the active transport of calcium across the intestinal mucosa. Vitamin D promotes bone mineral accretion in adolescent girls (Lehtonen-Veromaa et al. 2002, Viljakainen et al. 2006). Bone mineral augmentation at the femur was 14.3% and 17.2% higher in the groups of 11-year-old girls receiving 5 and 10 µg of vitamin D, respectively, compared with the placebo group (Viljakainen et al. 2006). An intake of 7.5 µg of vitamin D is recommended for 3-60-year-old females (Finnish nutrition recommendations 2005).

There are two types of vitamin D. Vitamin D_3 is synthesized on the skin on exposure to sunlight. It is also found naturally in cod liver and oily fish like salmon (Holick 2007). Vitamin D_2 comes from the ultraviolet irradiation of ergosterol obtained from yeast (Holick 2007). The ingested vitamin D and the vitamin formed on the skin undergo metabolism in the liver to form 25- hydroxyvitamin D [25(OH)D] (Holick et al. 2007). Vitamin D_3 is more potent in raising serum 25(OH)D levels, because vitamin D_2 has faster metabolism and clearance from the organism (Armas et al. 2004).

2.3.3.2 Smoking, alcohol and coffee consumption

Pre- and perimenopausal smoking is associated with lower BMD measurements shortly after cessation of cyclic bleedings (Hermann et al. 2000). Women having smoked a pack of cigarettes per day have an average deficit of 5 to 10% in bone density at menopause (Hopper & Seeman 1994).

Smoking has been associated with lower bone density levels in adolescent and young adults in some, but not all studies (Heaney et al. 2000). Smoking was associated with lower BMD and reduced cortical thickness in young men (Lorentzon et al. 2007). Young women smokers had a negative BMD development in a 2-year follow-up (Elgán et al. 2003). Regularly smoking adolescents have an increased risk of fractures (Jones et al. 2004). Välimäki et al. (1994a) found that smoking during adolescence resulted in lower bone density levels in young men, but not in women, possibly because women smoked fewer cigarettes. Afghani et al. (2003) did not find a significant relationship between smoking and bone mass. This may be due to low levels of tobacco use in their study population.

Little is known about the effect of alcohol use on bone gain in adolescence. In adult men and premenopausal women, excess alcohol intake appears to have a modest adverse effect on the preservation of bone mass. Consequently, alcohol use can be presumed to have adverse effects on the skeletal development of adolescents. (Heaney et al. 2000)

Coffee consumption had no association with lumbar spine and femoral neck BMD in 45-65- year-old men (Saitoglu et al. 2007). Similarly, no association was found between coffee and BMD in a study on 200 postmenopausal women (Demirbag et al. 2006).

2.3.3.3 Medication

Adolescents sometimes have chronic diseases and are obliged to use medications. In this section, only bone-affecting medications are discussed.

Glucocorticoid treatment is associated with lower BMD at lumbar spine and femoral neck (Sambrook et al. 1990, Laan et al. 1993). A recent follow-up study found dose-dependence for all studied fractures, showing a 5-fold increase in hip and a 5.9-fold increase in vertebral fracture risk in long-term continuous users (Steinbuch et al. 2004). The results from a study on 62 glucocorticoid-treated juvenile idiopathic arthritis patients, mean age 11.8 years, showed a 10% prevalence of vertebral compression fractures,

although these findings did not correlate directly with glucocorticoid treatment duration or dose (Valta et al. 2007).

Anticonvulsants and opioids are associated with significantly reduced BMD in adults (Kinjo et al. 2005). Immunosuppressive medication has caused impaired bone health. More than half of the adolescents receiving immunosuppressive medication after liver transplantation had osteopenia and 18% had an asymptomatic vertebral fracture in a follow-up study (Valta et al. 2008).

Gonadotropin-releasing hormone analogue treatment has been associated with lower BMD in young women (Pasquino et al. 2008). At discontinuation, after complete resumption of gonadal activity, the BMD of the treated patients did not differ from controls.

Selective serotonin reuptake inhibitors have been associated with increased decline in BMD in elderly men (Haney et al. 2007) and with increased risk of hip fractures in elderly women (Diem et al. 2007). There are no results of increased fractures for young people.

Oral diabetes medications, glitazones, have been associated with greater bone loss at the whole body, lumbar spine and trochanter in older women (Schwartz et al. 2006).

2.4 Assessment of bone mass

2.4.1 T-score and definition of osteoporosis

In young healthy individuals, the distribution of bone mineral density is normal. Because of this normal distribution, bone mineral density values are often expressed in relation to a reference population in standard deviation (SD) (Kanis et al. 1997). The T-score is a comparison of a patient's BMD in standard deviation to that of a healthy thirty-year-old of the same sex and ethnicity. It is used in postmenopausal women and men aged over 50 because it predicts the risk of a future fracture.

For Caucasian women, there are two diagnostic thresholds of bone mineral density. A T-score of –2.5 or less is the diagnostic criterion for osteoporosis and defines the majority of individuals who might sustain a fracture in the future. A T-score between –1.0 and –2.5 defines those with low bone mass or osteopenia and who are likely to develop osteoporosis in the future. These thresholds apply to women only (Kanis et al. 1997).

2.4.2 Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) is the golden standard of bone mineral density measurements. DXA measurements can be made at the lumbar spine or femoral neck and at peripheral sites like the wrist and calcaneus. The x-ray used in imaging must have sufficient energy to pass through tissues and still be detectable by sensors after the passage. X-ray beam energy is reduced more or less depending on the thickness

and density of the tissues through which the ray passes. The principle of DXA is the measurement of the transmission of x-rays with high- and low-energy photons through the body. From these measurements, the DXA device calculates BMD. It also counts the scanned area and, by multiplying these two values, it calculates BMC (Crabtree et al. 2007).

Bone strength is a composite of structural (geometry/shape, size and microarchitecture) and material (collagen, mineral and microdamage) properties of bone. Bone remodeling influences all of these properties (Felsenberg & Boonen 2005). BMD measures only bone density, and does not represent the whole concept of bone strength. However, measurements of bone mineral density can predict fracture risk, but cannot identify individuals who will have a fracture (Marshall et al. 1996).

Assessment of bone mass accumulation with DXA has many strengths. First, it is quite accessible in many countries. Second, DXA has one of the lowest effective doses of all the ionizing radiation imaging techniques (Crabtree et al. 2007). Due to the wide availability and relatively low radiation dose, DXA data have been collected on samples of healthy infants, children, and adolescents in several countries (Crabtree et al. 2007). These data have been used clinically as reference values. Third, the DXA scan time is short. Forth, the precision of DXA is high: The average coefficient of variation for a spine DXA scan is 1.5% or less.

There are also limitations to the DXA technique. DXA provides only two-dimensional data (BMC and bone area) on a three-dimensional object (bone). Thus, BMD is not a measure of volumetric density (g/cm3), because it provides no information about bone depth (Crabtree et al. 2007). Consequently, children will have lower BMD than adults partly because of smaller size. Due to this confusing effect produced by bone size, several investigators have suggested that the use of BMC adjusted for body size is preferable to conventional units of areal BMD, especially in children (Crabtree et al. 2007). To adjust areal BMD to bone size and to calculate a DXA-derived volumetric bone mineral density, a method has been introduced and used with growing children (Kröger et al. 1992, Kotaniemi et al.1993). This method has been shown to be valid for BMD normalization in growing children when magnetic resonance imaging data are not available (Kröger at el. 1995).

DXA may introduce artifacts into the measurements of children and adults with abnormal body composition. Degenerative changes seen in osteoarthritis and ankylosing spondylitis and structural abnormalities, such as compression fractures and scoliosis may artificially elevate the measured lumbar spine BMD. At the femoral neck, BMD can be altered by degenerative arthritis, degree of internal rotation and overlying soft tissues (Agarwal & Camacho 2006). Osteomalacia, vascular calcification and for example overlying metal objects may also impair interpretation of DXA measurements (Kanis et al. 1997).

2.4.3 Quantitative ultrasound

Quantitative ultrasound (QUS) seems an ideal method to measure bone quality in children and adolescents because it is inexpensive, portable and free from ionizing radiation. It measures components of bone strength and structure (Glüer et al. 1994).

The use of calcaneal QUS has been extensively studied as a screening tool for osteoporosis. Since the heel is directly exposed to mechanical loads, the calcaneus is an attractive site for assessing the effect of exercise and physical activity on bone. The Sahara device used in the present study measures both BUA and SOS at a fixed region of interest in the midcalcaneus, and the results are combined to provide an estimate of heel BMD. The T-score is calculated from this estimation using the database of the device.

The sonographic parameters BUA and SOS have been shown to be sensitive indicators of hip fracture risk among postmenopausal women (Baran et al. 1988, Bauer et al. 1997). The QUS parameters and an estimated BMD value counted from them have predicted non-spinal fractures (Diez-Pérez et al. 2007).

The risk factors for low QUS and BMD values are the same (Gregg et al. 1997, Cheng et al. 1999) and both measurements have an equally strong association with the risk factors (Frost et al. 2001). Langton & Langton (2000) found the performance of BMD and calcaneal QUS statistically comparable for identification of osteoporotic subjects.

Conflicting results, however, have been reported (Fricke et al. 2005). In the study of Brukx & Waelkens (2003), the QUS method was not able to recognize children with low bone mass as determined by DXA. They state that sonometry is not useful in screening for low bone mass in children.

Frost et al. (2000) investigated the T-score threshold for diagnosing osteoporosis with different QUS devices. They found a slower rate of age-related decline in QUS T-score when compared with DXA. They suggest that a T-score of –1.80 might be appropriate for identifying postmenopausal women at risk of osteoporosis with the devices used (Frost et al. 2000). Similar results have been obtained by Damilakis et al. (2001).

2.4.4 Quantitative Computed Tomography

In computed tomography devises, a continuous spiral rotation of an X-ray tube and multiple rows of detectors permit rapid 3D scanning. In children, axial quantitative computed tomography (QCT) involves measurement of two adjacent vertebrae between L1-L3. The QCT technique is particularly useful in children because it measures volumetric density (g/cm3), which is not size-dependent (Ward et al. 2007).

The results of QCT are expressed as mean volumetric BMD (vBMD; mg/cm^3). vBMD is expressed as standard deviation (SD) from the mean of the appropriate age-, sex-, and race-matched reference data (i.e., a Z-score). QCT can measure cortical and trabecular bone separately, which is an advantage because trabecular vBMD is more sensitive to changes in BMD. The new QCT devices produce three-dimensional images rapidly and with high precision (coefficient of variation less than 3%) (Ward et al. 2007).

QCT has the potential to be used at peripheral skeletal sites. Peripheral measurements cause lower radiation exposure and lower costs than axial QCT measurements. These measurements are mainly used in research, e.g. when investigating the effect of exercise on bone (Heinonen et al. 2000, Ward et al. 2005).

There are also limitations to the QCT technique. Firstly, the ionizing radiation dose is approximately 10-12 times greater than with DXA. Secondly, fewer centers have QCT devices, so access to these may be problematic. Thirdly, the examination requires an experienced staff. Finally, there are fewer published pediatric reference data for QCT than for DXA (Ward et al. 2007).

2.4.5 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is the most recent technique for skeletal assessment in children. It is based on the resonance and relaxation of protons in lipids and water. Different tissues contain different amounts of these substances. MRI can measure trabecular and cortical bone separately. It provides volumetric measurements without radiation. It can image multiple anatomical planes without moving the subject.

The MRI technique also has its disadvantages. First, it requires a long scanning time; 20-30 minutes for one examination. Second, children often need sedation to keep still (Ward et al. 2007). Third, bone imaging with MRI requires high-resolution devices with advanced technology. Finally, the examination is more expensive than the traditional methods. To date, MRI has been used only in research protocols.

3. AIMS OF THE STUDY

The purpose of this study was to evaluate the association between physical activity and other lifestyle factors and skeletal development in adolescence.

In more detail, the aims were:

1) To assess the differences in the effects of the various types and amounts of physical activity (gymnastics, running and sedentary controls) on BMC and to compare the effects of reduced physical activity in these groups. (I)

2) To examine the maintenance of BMC and BMD in young women, especially after a clear reduction in physical activity level. (II)

3) To investigate the use of the QUS parameters BUA, SOS, and T-scores as measures of bone characteristics among adolescent girls. A special focus was put on the association between decreased physical activity and QUS values. (III)

4) To investigate whether estrogen-progestin contraception affects BMC acquisition in a population of Finnish adolescent women. (IV)

4. SUBJECTS AND METHODS

4.1 Study subjects

Initially, the study population comprised 191 healthy Caucasian girls aged 9-15 years at baseline. The present study is a long-term follow-up and since its beginning in 1997, 66 competing gymnasts, 65 competing runners, and 60 non-athletic controls have participated in it. The measurements were performed in 186 girls in the 1-year, 174 in the 2-year, 171 in the 3-year, and 142 girls in the 7-year follow-up. The 7-year follow-up population (n=142) comprised 52 gymnasts, 46 runners, and 44 controls. Table 2 shows the number of participants and the follow-up time in each substudy. Forty-nine girls of the initial 191 participants were lost to follow-up during the 7 years. Those girls who failed to attend were either living at a distance or could not find enough time for participation. The baseline data of these dropouts are presented in Table 3.

The subjects were recruited from local sports clubs and schools in the City of Turku and its vicinity. The athletes were volunteers and the controls were their classmates or children or relatives of the hospital personnel. None of the participants were diagnosed with any chronic disease affecting bone quality.

To expose any differences caused by different loading of the growing skeleton, the participants were assigned into three different activity groups at baseline: competitive gymnasts, competitive runners and controls. Gymnastics is a high-impact sport with jumps and body contacts with hard surfaces. Running, in contrast, is a more dynamic, repetitive type of sport. The group of runners consisted of long-distance and track-finding runners. The controls did not participate in any organized physical activity regularly. The intensity of exercise was considered competitive, if the subject participated regularly in competitive sport and competitions at local, provincial or national level for at least one year.

At baseline, the gymnasts were smaller and exercised more than runners or controls. Later growth was similar in all three groups. The participants who had used EPCs for more than 2 years had higher physical activity amount at baseline. More detailed subject characteristics are shown in Tables 2 (substudy I), 3 (substudy II) and 4 (substudy IV).

Table 2. Baseline (1997), 3-year and 7-year values in gymnasts, runners, and controls. Values are means (SD). The levels of physical activity (MET) at baseline are presented as medians (first and third quartiles). Substudy I.

	Gymnasts (G) n=52	Runners (R) n=46	Controls (C) n=44	P value between groups (multiple comparison)
Baseline				
Age (y)	13.0 (1.7)	13.0 (1.9)	13.0 (1.7)	0.89
Height (cm)	156 (9)	160 (10)	158 (8)	0.044 (G/R)
Weight (kg)	44.5 (9.5)	49.2 (10.2)	49.0 (9.0)	0.026 (G/R)
BMI (kg/m^2)	18.1 (2.2)	18.8 (2.4)	19.3 (2.5)	0.028 (G/C)
Age at menarche (y)	13.7 (1.9)	13.1 (1.1)	12.9 (1.1)	0.004 (G/R , G/C)
Calcium intake (mg)	1569 (552)	1715 (508)	1354 (489)	0.026 (R/C)
Vitamin D intake (µg)	3.9 (2.1)	5.1 (2.2)	4.2 (1.9)	0.024 (G/R)
MET (h/week)	80.0 (55.5, 90.0)	42.5 (16.0, 66.7)	10.0 (6.0, 19.0)	<0.001 (G/R, G/C, R/C)
Years of active training (y)	6.4 (2.9)	4.6 (2.0)	-	<0.001 (G/R)
3-year follow-up				
Height (cm)	162.7 (5.9)	166.6 (6.9)	164.8 (6.1)	0.011 (G/R)
Weight (kg)	53.0 (7.9)	56.7 (8.3)	56.6 (8.3)	0.039 (G/R)
Calcium intake (mg)	1546 (615.9)	1701 (546.0)	1309 (500.2)	0.005 (R/C)
Vitamin D intake (µg)	3.9 (2.0)	4.2 (2.1)	4.0 (2.6)	0.76
7-year follow-up				
Height (cm)	164.5 (5.7)	168.3 (6.7)	165.9 (6.2)	0.011 (G/R)
Weight (kg)	56.8 (7.2)	62.2 (8.7)	61.3 (9.4)	0.004 (G/R, G/C)
Calcium intake (mg)	1103 (429.5)	1259 (588.9)	984 (375.0)	0.024 (R/C)
Vitamin D intake (µg)	5.3 (2.8)	6.1 (2.7)	4.7 (2.1)	0.058

BMI=body mass index
MET= ratio of work metabolic rate

Table 3. Characteristics of the study population (n=142) at baseline, 3 years and 7 years. The results are expressed as unadjusted mean values (SD), medians (first and third quartile) for MET and frequencies (1=prepubertal girls, 2=pubertal girls, 3=mature girls) for Tanner stage. The baseline values of dropouts (n=49), which were originally enrolled but did not participate in the 7-year follow-up, are presented. No significant differences were observed between dropouts and study participants. (Substudy II)

	Baseline, Lost to follow-up (n = 49)	Baseline n = 142	3 years n = 142	7 years n = 142
Age, y	12.8 (1.7)	13.0 (1.8)	16.0 (1.8)	20.0 (1.8)
Height, cm	156.4 (10.2)	158.2 (9.2)	164.6 (6.5)	166.2 (6.3)
Weight, kg	45.1 (10.5)	47.4 (9.8)	55.3 (8.3)	60.0 (8.7)
BMI, kg/m^2	18.4 (2.7)	18.8 (2.5)	20.4 (2.5)	21.7 (2.8)
Tanner stage: 1	12 (24%)	23 (16%)	0	0
2	19 (39%)	86 (61%)	11 (8%)	0
3	18 (37%)	33 (23%)	131 (92%)	142 (100%)
MET index	32.0 (10.3, 68.0)	42.5 (12.4, 75.0)	25.5 (12.0, 75.0)	20.0 (8.8, 40.0)
Calcium intake, mg	1667 (573.3)	1550 (642)	1523 (577)	1116 (482)
Vitamin D intake, µg	4.3 (2.1)	4.4 (2.1)	4.0 (2.2)	5.4 (2.6)

MET=ratio of work metabolic rate. The MET index was calculated by multiplying frequency, mean duration and mean intensity of weekly physical activity and divided by 60.

Table 4. Baseline (at 3 years) values in the different groups of estrogen-progestin contraception use. Values are means (SD). The levels of physical activity (MET) at baseline are presented as medians (first and third quartiles). Substudy IV.

	No hormonal contraception, NC (n=52)	Use for 1-2 years, C1 (n=24)	Use > 2 years, C2 (n=46)	P value between groups (multiple comparison)
Age (y)	15.4 (1.8)	15.8 (1.7)	16.1 (1.6)	0.13
Height (cm)	164 (6)	166 (6)	165 (8)	0.32
Weight (kg)	53.8 (9.0)	58.4 (8.3)	54.8 (7.8)	0.90
Age at menarche (y)	13.4 (1.2)	13.1 (1.1)	13.4 (1.5)	0.78
Calcium intake (mg)	1599 (557)	1629 (658)	1489 (541)	0.52
Vitamin D intake (µg)	4.5 (2.6)	4.1 (2.1)	3.5 (1.8)	0.082
MET	26 (10, 67)	26 (10, 58)	63 (35, 90)	0.004 (NC/C2, C1/C2)

MET=ratio of work metabolic rate.

4.2 Study designs

The subjects of this study were followed for 7 years from 1997 to 2004. Follow-up visits were organized at four different time points: in 1997 (baseline), 1998 (1-year follow-up), 2000 (3-year follow-up) and in 2004 (7-year follow-up). Lumbar spine and femoral neck BMC were measured at baseline, 3 years and 7 years. Calcaneal quantitative ultrasound was measured in 1998, 2000 and 2004. Height and weight were measured at each follow-up and the Tanner stage was determined during the first 3 years. Data on the amount of physical activity, calcium and vitamin D intakes and use of medication were collected with questionnaires every 6 months for the first 3 years and then again at 7 years. Questions about menstruation were asked at each follow-up.

In substudy I, the participants (n =142) were divided into 3 different groups according to type of physical activity at baseline (gymnasts, runners or controls). The follow-up time in substudy I was 7 years.

In substudy II, the follow-up time was 7 years. The 142 participants were redivided into 3 groups according to the changes in physical activity (MET) observed during the last 4 follow-up years: 1) group D (for those discontinuing physical activity) consisted of girls whose physical activities decreased by at least 50% for at least one year, n = 30, 2) group R, (for reduced physical activity) consisted of girls who decreased their physical activities by 25-50% for at least one year, n = 30, and 3) group C, (for those continuing physical activity) consisted of girls who either continued their physical activity level or even increased it, n = 82. The baseline groups of different types of physical activity were thus mixed. The data were further divided into tertiles on grounds of the activity level during the whole follow-up period.

In substudy III, the follow-up time was 6 years from 1998 to 2004. The data on 140 girls were analyzed and divided into groups, as in substudy II, according to the changes observed in physical activity during the last 4 follow-up years (group D for discontinued physical activity, n = 30; group R for reduced physical activity, n = 29; and group C for continued physical activity, n = 81).

Substudy IV was a 4-year follow-up (from 2000 to 2004) with 122 girls, who were divided into 3 different groups according to length of hormonal contraceptive use: NC = no contraception, C1 = hormonal contraception for 1-2 years, and C2 = hormonal contraception for more than 2 years.

The study designs are illustrated in Figure 6 and Table 5.

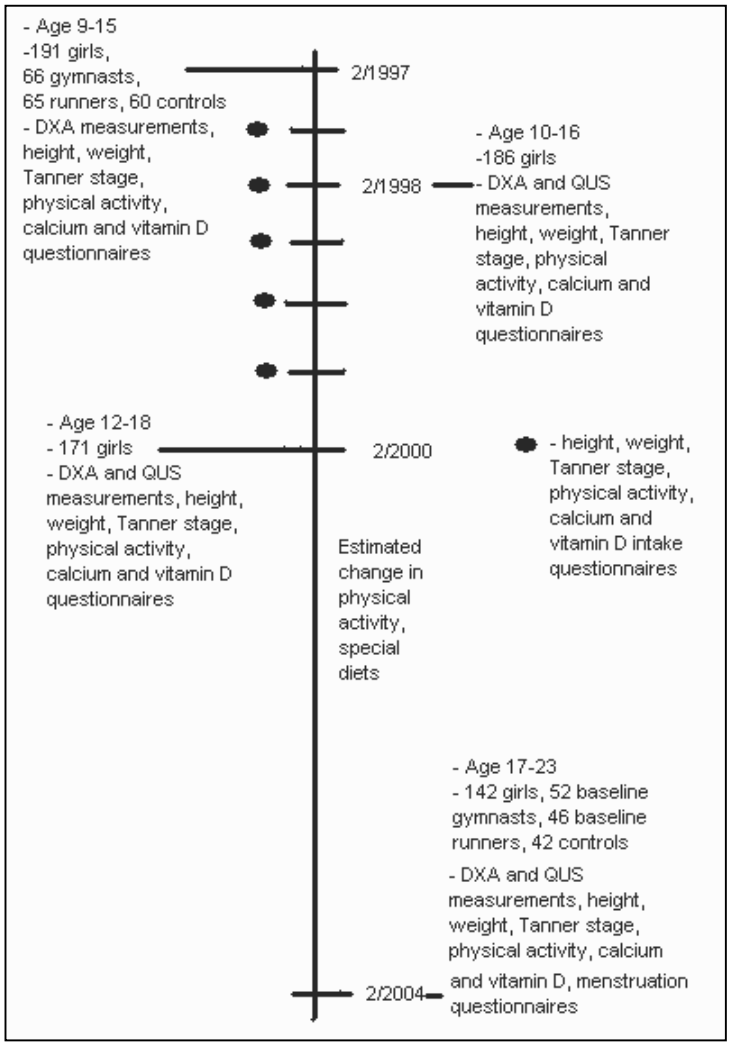

Figure 6. Study design.

Table 5. Study designs, participants, follow-up visits, and variables of the substudies. The baseline of the study was in 1997, the 3-year follow-up in 2000 and the 7-year follow-up in 2004. A follow-up visit was also organized in 1998, which was the baseline for substudy III.

Study	Study design	Subjects	Follow-up measurements	Variables and questionnaires
I	7-year follow-up	n = 142, 52 gymnasts, 46 runners, 44 controls	Baseline, 3- and 7-year	LS and FN BMC, height, weight, Tanner stage, physical activity, calcium and vitamin D intakes, medication questionnaires
II	7-year follow-up	n = 142, 82 continued, 30 reduced, 30 discontinued physical activity	Baseline, 3- and 7-year	LS and FN BMC and BMD, height, weight, Tanner stage, physical activity, calcium and vitamin D intakes, medication questionnaires
III	6-year follow-up	n = 140, 81 continued, 29 reduced, 30 discontinued physical activity	Baseline in 1998, follow-ups in 2000 and 2004	Calcaneal quantitative ultrasound, height, weight, Tanner stage, physical activity, calcium and vitamin D intakes, medication questionnaires
IV	4-year follow-up	n = 122, 52 no EPC, 24 EPC use for 1-2 years, 46 EPC use for > 2 years	Baseline in 2000 and 4-year follow-up in 2004	LS and FN BMC, height, weight, Tanner stage, physical activity, calcium and vitamin D intakes, medication and menstruation questionnaires

LS=lumbar spine
FN=femoral neck
BMC=bone mineral content
BMD=bone mineral density
EPC = estrogen-progestin contraception

4.3 Physical examinations, interviews and questionnaires

At each visit, height was measured with a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Crymych, UK) to the nearest 0.1 cm and weight recorded with an electronic scale (EKS exclusive, EKS International, Sweden) to the nearest 0.1 kg in light clothing. BMI was calculated as kg/m^2. The stages of pubertal development were evaluated with the Tanner stage method (Tanner 1962) during the first 3 follow-up years.

All study subjects were interviewed before the measurements. Information was recorded on history of physical activity, previous and present diseases, medications, fractures, family history of osteoporosis, smoking, alcohol consumption, menarche, menstrual status (number of bleeding days, number of intermenstrual days and annual number of cycles), menstrual cycle history since menarche, and use of oral contraceptives. This information was collected by questionnaires and interviews.

During the first 3 years, the participants completed detailed questionnaires on their physical activities every 6 months. Their competitive athletic history and leisure time physical activities (LTPA) were reported for the last six months (weekly mean frequency, mean duration in minutes and mean intensity of all bouts of physical activity (Raitakari et al. 1996). At the 7-year follow-up visit, the same detailed questionnaires were used, together with a questionnaire on the physical activity habits between 3 and 6.5 years. The physical

activity level during the last 4 follow-up years was compared with the activity level at the 3-year follow-up (an estimated activity change in % and the years since the change). On the basis of this information, LTPA was calculated as MET (ratio of work metabolic rate) hours per week by multiplying frequency, mean duration in minutes, and mean intensity of physical activity each week (Raitakari et al. 1996). The mean LTPA was calculated for the first three years and for the last six months (6.5-7 years). The mean LTPA was estimated for 3-6.5 years from the estimated amount of physical activity during that time.

4.4 Bone measurements

4.4.1 Dual-energy X-ray absorptiometry

The BMD (g/cm^2) and the bone area (cm^2) of the hip in the non-dominant leg and of the lumbar spine (L2-L4) were measured with DXA (Hologic QDR 4500C, Waltham, Massachusetts, USA, software version 12.3) at baseline, at 1 year, at 3 years and at 7 years. The same scanner was used for every participant. The same three trained radiographers performed all measurements and analyzed the data. They performed quality assurance daily by calibrating the device with a spine phantom supplied by the manufacturer. The coefficients of variation for 2 consecutive measurements of 10 girls were 1.3% for the spine, 0.8% for the hip, and 0.4% for the phantom for the total study period.

The participants were told their DXA results. Girls with exceptionally low values were further examined in the Turku University Central Hospital.

4.4.2 Quantitative ultrasound of the calcaneus

Both calcanei were always measured using the same sonographic densitometry (Hologic Sahara Clinical Bone Sonometer, Waltham, Massachusetts, USA). The results of the non-dominant calcanei were used for further analysis. The non-dominant lower extremity was established on the same side of the body as where the non-dominant hand was (Lehtonen-Veromaa et al. 2000a). The measurements of the os calcis consisted of SOS values expressed in meters per second (m/s) and BUA in decibels per megahertz (dB/MHz). The Sahara device measures both BUA and SOS at a fixed region of interest in the midcalcaneus and the results are combined to provide an estimate of heel BMD. The T-score is calculated from this estimate by using the database of the device. Quality assurance was performed daily by calibrating the device on a dedicated phantom supplied by the manufacturer. With 40 participants, the intraclass correlation coefficients of 2 consecutive measurements were 0.99 (0.98-1.00) for BUA and 0.99 (0.98-1.00) for SOS.

4.5 Assessment of vitamin D and calcium intakes

During the first 3 years of study, intakes of vitamin D and calcium were estimated at 6-month intervals using a questionnaire (Välimäki et al. 1994b, Lehtonen-Veromaa et al. 1999). Another validated questionnaire involving calcium intake was filled out in the last

follow-up measurement (Uusi-Rasi et al. 1994). The questionnaire on vitamin D-intake had to be revised and up-dated because vitamin D supplementation was increased in Finnish milk products in 2003.

The participants were told the amount of their calcium and vitamin D intake yearly. They were also told the recommended intake amounts.

4.6 Statistical analyses

The anthropometrical variables of all substudies (I-IV) were reported as mean values and standard deviations (SD). The amount of physical activity (MET) was reported as median and interquartile ranges in all substudies (I-IV). The dietary intakes were reported as mean values (SD) in all substudies. The normality of the variables was tested by using the Shapiro-Wilk W test.

In substudy I, statistical comparisons between groups were made by using analysis of variance (ANOVA) with covariates when appropriate or using the Kruskal-Wallis test with Hommel's adjusted Mann-Whitney U test as a post-hoc test (Monte Carlo p-values). Repeated measures were analyzed using generalized linear mixed models**.** The fixed effects were group, time, and group-time interactions as well as covariates of height, weight, and years from menarche in the 7th year measurement. Participant effects were assumed to be random. Regression analysis was used to calculate the effect of change (1 MET hour/week) at the level of physical activity.

In substudy II, statistical comparisons between groups were made by using ANOVA with covariates when appropriate. Repeated measures were analyzed using generalized linear mixed models. The fixed effects were group, follow-up, and group-follow-up interactions as well as covariates of age, height, and weight. Participant effects were assumed to be random.

In substudy III, statistical comparisons between groups were made by using the t-test or ANOVA with Tukey's honestly significant test. When the variables did not have a normal distribution, descriptive values were expressed as medians and interquartile ranges. Statistical comparisons between groups were made by using the Kruskal-Wallis test with the Bonferroni adjusted Mann-Whitney U test. The distribution of the MET values was not normal; the square root of MET (SQMET) was used in the statistical analyses. The most important descriptive values were expressed as 95% CI. Stepwise linear regression analyses were performed to assess the effect of independent variables (calcium, vitamin D intake, age, weight and physical activity) on the variation of the values of T-scores, SOS, and BUA. The changes in T-scores of 3 different activity groups during the follow-up were tested using the repeated MANOVA (multivariate analysis of variance) test. Paired samples t-tests were used to determine changes in the SOS and BUA values in 3 different physical activity groups between the 2-year and 6-year follow-up measurements

In substudy IV, statistical comparisons between groups were made by using ANOVA with covariates when appropriate or the Kruskal-Wallis test (Monte Carlo p-values). Either Tukey's honestly significant difference test or Hommel's adjusted Mann-Whitney U test was used for post- hoc comparisons. Repeated measures were analyzed by using generalized linear mixed models. No adjustment was made for multiple testing.

4.7 Ethics

This study was approved by the Ethics Review Committee of the Hospital District of Southwestern Finland and it was carried out in accordance with the declaration of Helsinki. All study participants were volunteers and were informed on the study before accepting the invitation to participate. Written informed consent was obtained from each participant and, if minor, from her parent or guardian. The study subjects were also informed about the findings of the examinations and the study results by mail.

5. RESULTS

5.1 Amount of physical activity during follow-up

The amounts and habits of physical activity changed during the follow-up. During the first three years, the gymnasts and runners were very active, but during the last 4 years, their physical activity reduced markedly. Some of the physically most active girls retired from their careers. On the other hand, some of the inactive controls started active training. The changes in physical activity during the follow-up are presented in Figures 7 and 8.

Most participants (82/142) continued to exercise as earlier. However, 21% (30/142) of the girls gave up physical activity and 20% (30/142) reduced markedly their physical activity level. The reduction in physical activity was significant in the group of gymnasts, even thought their activity level was still higher when compared with controls at 7 years.

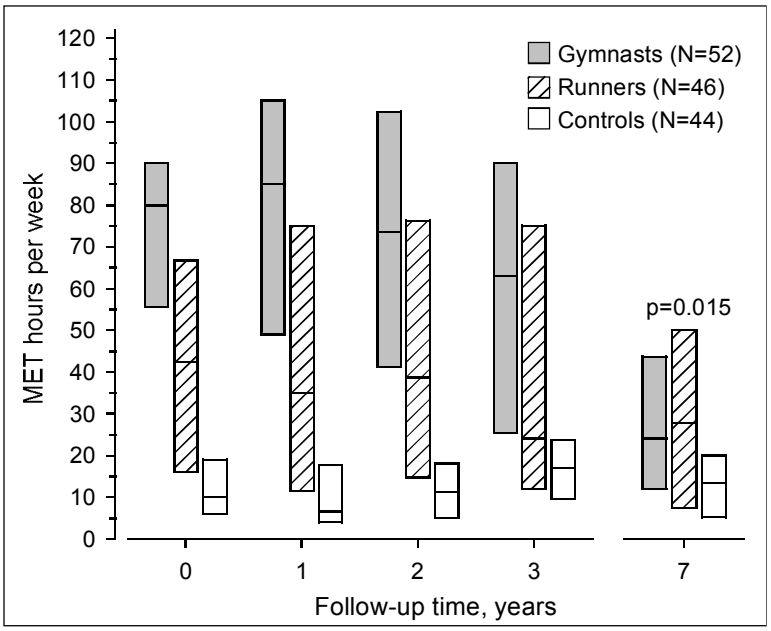

Figure 7. Levels of physical activity in gymnasts, runners, and controls at baseline, 2 years, 3 years and 7 years, expressed as MET hours/week. The horizontal lines across the bars indicate medians and the boxes the first and third quartiles. (Substudy I)

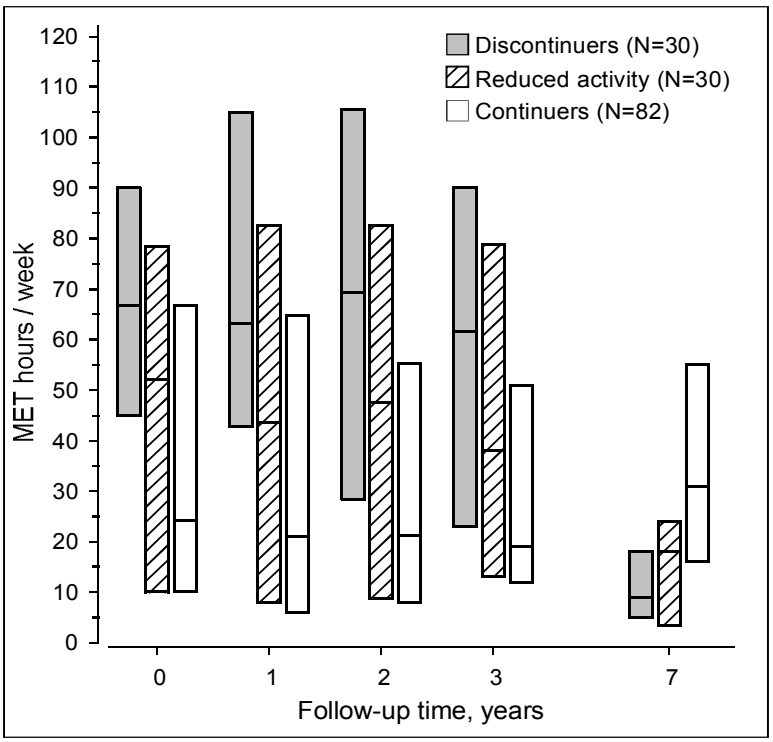

Figure 8. Median weekly MET hours (denoted by horizontal lines across the bars) in groups D (discontinued physical activity or discontinuers in the chart), R (reduced physical activity) and C (continued physical activity or continuers in the chart). The boxes indicate the first and third quartiles. (Substudy II)

5.2 Bone mineral accrual

Bone mineral accrual was determined by DXA at baseline, at 1 year, 3 years and 7 years. The QUS measurements were made at 1 year, 3 years and 7 years.

At 3 years, bone mineral measurements correlate with age. Older participants have higher BMC and BMD values (Figure 9a). At 7 years, this correlation does not exist anymore. The bone mineral accrual at the lumbar spine and femoral neck of the different physical activity groups is shown in Figures 9b and 9c. It seems that the effects of juvenile growth and development on bones have mainly been reached by the age of 16-17. There was no correlation between SOS values and age at 3 years or at 7 years. The correlation coefficients are shown in Table 6.

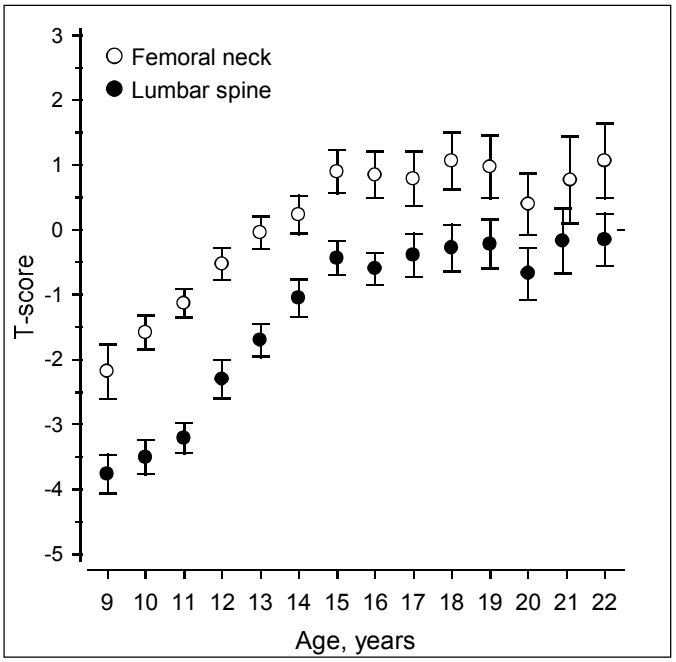

Figure 9a. Development of lumbar spine and femoral neck T-scores during follow-up. At each follow-up, all girls of the same age were taken into account.

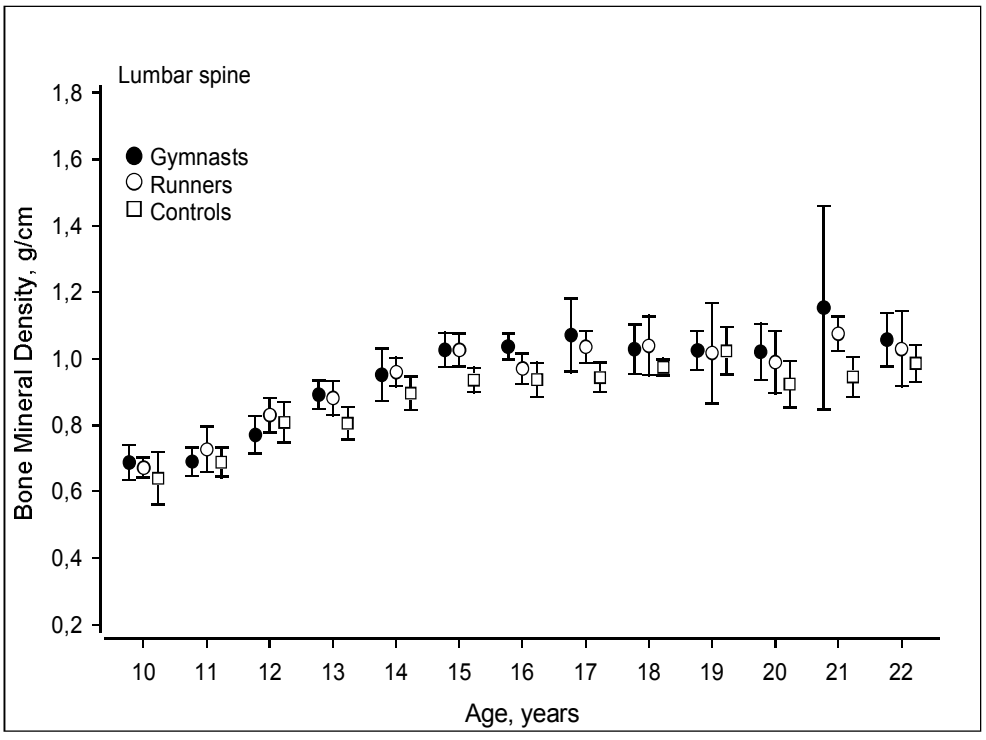

Figure 9b. Development of lumbar spine BMD in baseline gymnasts, runners and controls during follow-up. At each follow-up, all girls of the same age were taken into account.

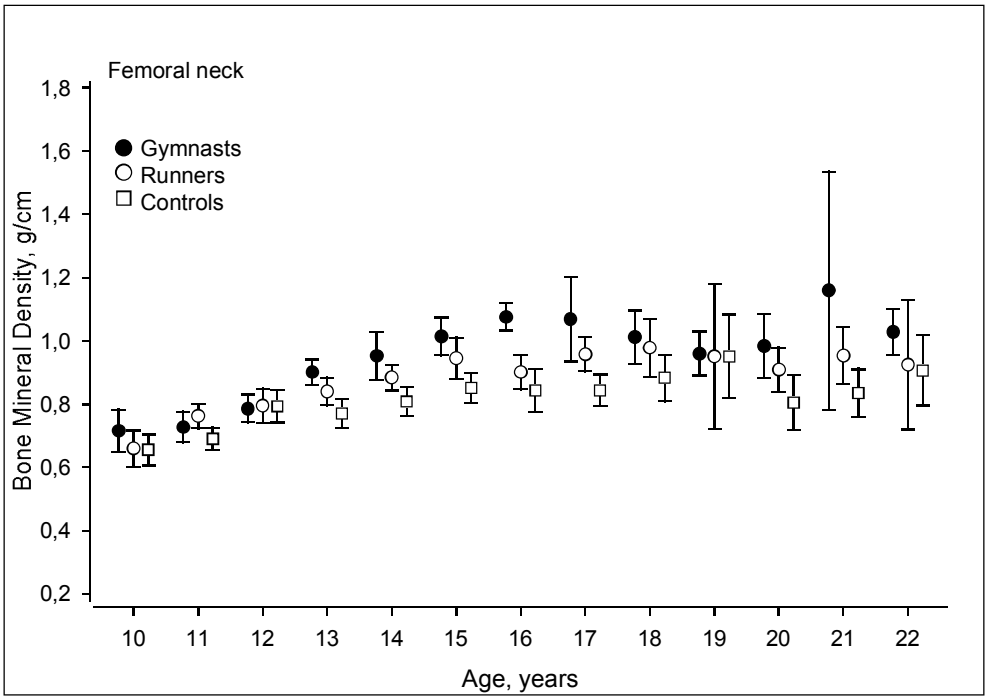

Figure 9c. Development of femoral neck BMD in baseline gymnasts, runners and controls during follow-up. At each follow-up, all girls of the same age were taken into account.

Table 6. Correlation between DXA parameters (BMC and BMD), sonographic parameter (SOS) and age at 3-year or 7-year follow-up (Spearman's correlation coefficients)

	Age at follow-up
BMC LS 3 years	0.481**
BMC FN 3 years	0.423 **
BMC LS 7 years	NS
BMC FN 7 years	NS
SOS 3 years	NS
SOS 7 years	NS

** p< 0.001
NS=not significant

5.3 Long-term effects of different types and amounts of physical activity

(Substudies I and III)

The gymnasts, runners and controls were followed for 7 years and screened for any long-term effects of different types of physical activity. The development of the mean BMC at the lumbar spine and femoral neck (adjusted for height, weight, and the years from menarche in the 7th year follow-up measurement) is described in Figure 10. The

unadjusted area and BMC values are shown in Table 7. The increase in the mean adjusted BMC at lumbar spine during the total follow-up period was similar in all groups. The mean adjusted LS BMC of the gymnasts was higher than that of the runners or controls throughout the follow-up period. The group-by-time interaction was significant only at the femoral neck (p=0.048). During the first three years, the increase in the adjusted FN BMC was 4.6% (95% CI 0.41% to 9.1%) greater among gymnasts than among controls (p=0.028). Therefore, in comparison with the lumbar spine, the development of the mean adjusted FN BMC values was different among the athletes during the last 4 years (Figure 10). The mean increase in BMC from baseline to 3-year follow-up was 0.745 g, 0.638 g, and 0.568 g in gymnasts, runners and controls, respectively, when change in height, change in weight, and baseline femoral neck BMC were used as covariates. The difference was significant between gymnasts and controls (p=0.014).

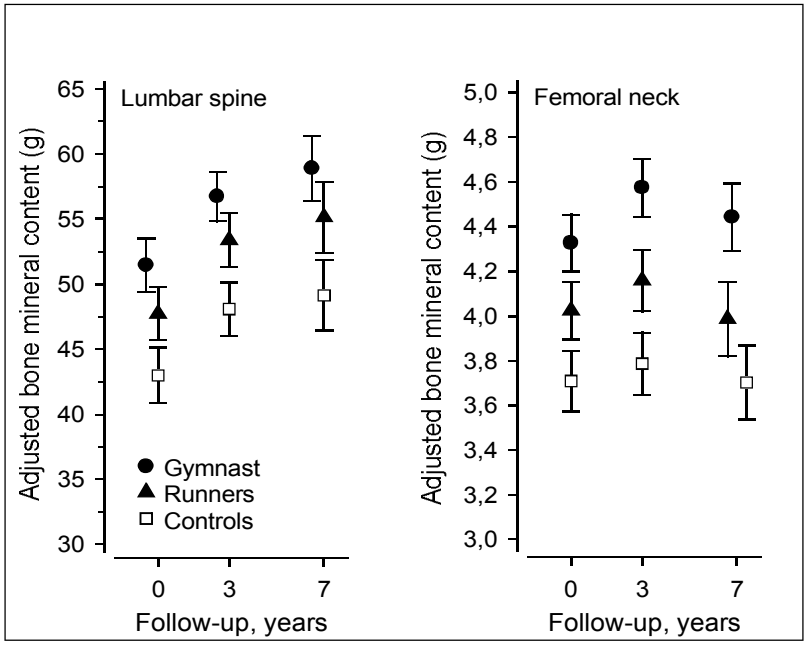

Figure 10. Lumbar spine and femoral neck BMC (adjusted for height, weight and years from menarche at 7-year follow-up) among gymnasts, runners, and controls at baseline, 3 years and 7 years. The dots/triangles/squares indicate mean values and the whiskers show 95% confidence intervals. (Substudy I)

Table 7. Unadjusted lumbar spine and femoral neck area and BMC values in gymnasts, runners and controls. The values are presented as mean (SD). (Substudy I)

	Baseline Gymnasts (G) n=52	Baseline Runners (R) n=46	Baseline Controls (C) n=44	P value between groups (multiple comparison)
Baseline				
LS Area (cm^2)	46.8 (8.1)	50.2 (8.7)	47.3 (8.1)	0.10
FN Area (cm^2)	4.2 (0.4)	4.6 (0.4)	4.4 (0.4)	<0.001 (G/R , G/C)
BMC_{LS} (g)	41.0 (15.5)	44.0 (15.1)	38.2 (12.3)	0.17
BMC_{FN} (g)	3.7 (0.9)	3.8 (0.9)	3.4 (0.7)	0.082
3-year follow-up				
LS Area (cm^2)	54.9 (5.3)	58.4 (6.1)	55.5 (5.3)	0.006 (G/R, R/C)
FN Area (cm^2)	4.5 (0.3)	4.8 (0.3)	4.7 (0.4)	<0.001 (G/R, G/C)
BMC_{LS} (g)	55.2 (11,4)	57.3 (12.2)	51.4 (8.4)	0.002 (R/C)
BMC_{FN} (g)	4.5 (0.8)	4.4 (0.8)	4.0 (0.6)	0.001 (G/C, R/C)
7-year follow-up				
LS Area (cm^2)	58.0 (6.5)	61.6 (5.7)	57.6 (5.7)	0.003 (G/R, R/C)
FN Area (cm^2)	4.5 (0.3)	4.8 (0.3)	4.8 (0.4)	<0.001 (G/R, G/C)
BMC_{LS} (g)	60.1 (11.6)	62.8 (10.6)	55.2 (8.7)	0.002 (R/C)
BMC_{FN} (g)	4.5 (0.7)	4.4 (0.7)	4.1 (0.6)	0.002 (G/C, R/C)

BMC = bone mineral content
LS = lumbar spine
FN = femoral neck

The data were divided into tertiles according to the amount of physical activity (the MET index) of the participants during the whole follow-up period. A positive association was found between physical activity and adjusted BMC (Figure 11). During the whole follow-up period, the means of LS and FN BMC (adjusted for age, height, and weight) were significantly higher in the highest physical activity tertile than in the lowest tertile ($p < 0.001$). No significant interaction was found between the tertiles and the follow-up period for LS and FN BMC.

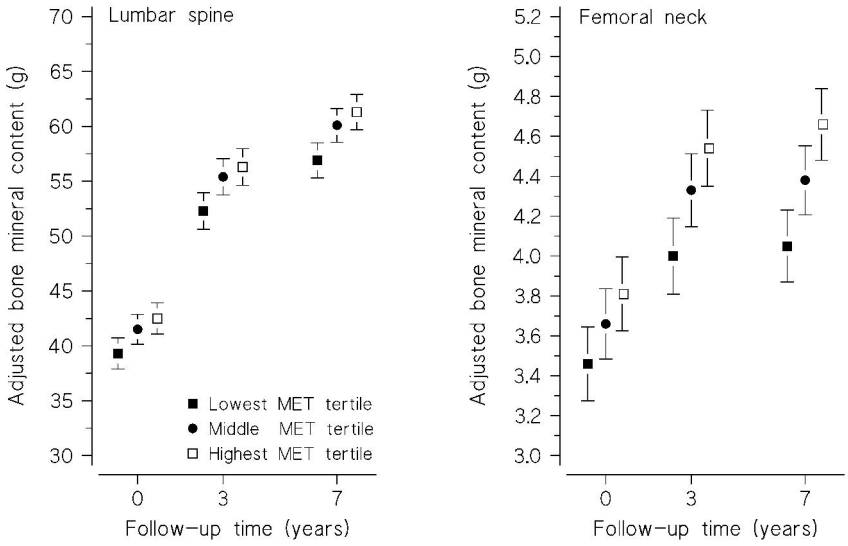

Figure 11. Lumbar spine and femoral neck BMC adjusted for age, height and weight at all follow-up points. The participants were divided into tertiles according to the amount of physical activity during the whole follow-up. The dots/squares indicate mean values and the whiskers show 95% confidence intervals. □ =Highest tertile of physical activity (n=46) ● =Middle tertile of physical activity (n=50) ■ =Lowest tertile of physical activity (n=46) (Substudy II)

The amount of physical activity was a good predictor of sonographic T-scores. When age, body weight, calcium and vitamin D intakes, and LTPA (as SQMET) were set in the models of stepwise linear regression analysis, age and physical activity were the two significant parameters accounting for the variation in T-scores at baseline in 1998 and at 2 years.

For older girls in the 6-year follow-up measurement in 2004, physical activity was the only significant parameter and accounted for 11.3% of the variation in the calcaneal T-scores (Table 8). When the independent variables of the 6-year follow-up measurement were set in the model, physical activity was the only significant factor for the SOS and BUA values and accounted for 13.4% and 6.9% of the variation, respectively.

Table 8. Association between calcaneal T-scores and physical activity (SQMET), age, weight and calcium and vitamin D intakes at baseline, 2 years and 6 years by stepwise linear regression analysis (n = 140) and time-to-time analysis. (Substudy III)

	Baseline	2 years	6 years
SQMET	0.061	0.136	0.113
Age (yrs)	0.106	0.028	NS
Weight (kg)	NS	NS	NS
Calcium intake (mg)	NS	NS	NS
Vitamin D-intake (µg)	NS	NS	NS
Total R^2	16.7%	16.4%	11.3%

SQMET = square root of the counted MET
NS = not significant

5.4 Reduction in physical activity level

5.4.1 Dual-energy X-ray absoptiometry measurements

(Substudies I and II)

The participants (n = 142) were followed for 7-years. During that time, many of them reduced their physical activity levels clearly. According to the level of reduction during last 4 follow-up years, the participants were redivided into three groups: 1) discontinued physical activity (D) 2) reduced physical activity (R), and 3) continued physical activity (C).

When the mean 4-year changes in adjusted (age, increase in height and weight) LS and FN BMC were examined in the above groups, an evident negative effect was observed. The development of bone mineral measurements is shown in Table 9.

Table 9. Unadjusted BMC and BMD values of the study population (n =142) at baseline, 3 years and 7 years. The baseline values of dropouts (n=49), who were originally enrolled but did not participate in the 7-year follow-up, are presented. The values are presented as means (SD). (Substudy II)

	Baseline, Lost to follow-up (n = 49)	**Baseline n = 142**	**3 years n = 142**	**7 years n = 142**
BMC_{LS}, g	38.9 (12.6)	41.11 (14,55)	54.67 (11.01)	59.45 (10.82)
BMD_{LS}, g/cm^2	0.808 (0.129)	0.832 (0.168)	0.965 (0.122)	1.002 (0.107)
BMC_{FN}, g	3.46 (0.75)	3.64 (0.85)	4.29 (0.74)	4.36 (0.70)
BMD_{FN}, g/cm^2	0.790 (0.116)	0.824 (0.153)	0.918 (0.147)	0.927 (0.143)

BMC = bone mineral content
BMD = bone mineral density
LS = lumbar spine
FN = femoral neck

At the lumbar spine, the mean of the 4-year change of the adjusted BMC was 1.69 g greater (3%) (95% CI 0.26 g to 3.12 g, p=0.021) for those whose activity level was maintained than for those who had reduced their activities. The increase in the mean adjusted LS BMC was greater (5.59 g vs. 2.43 g, p=0.001) in the group that maintained the previous activity level (group C) than in the group that had reduced its activity more than 50% (group D) (Figure 12).

The mean 4-year change in adjusted FN BMC was 0.14 g greater (4.6%) (95% CI 0.02 g to 0.26 g, p=0.015) among those whose physical activity level was maintained than among those who had reduced their activities. The mean adjusted FN BMC increased in the group that continued to exercise, but decreased in most girls that discontinued (Figure 12).

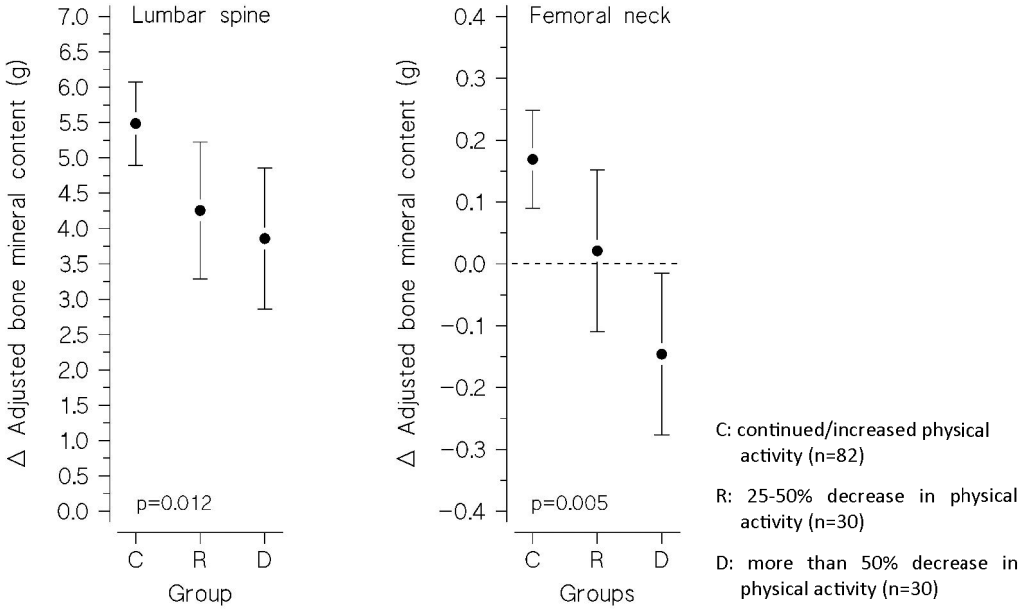

Figure 12. Change in LS and FN BMC (adjusted for age, increase in height and weight) in groups C, R and D during the whole follow-up period. The filled circles indicate mean values and the whiskers show 95% confidence intervals. (Substudy II)

At the lumbar spine, the mean 4-year change in adjusted BMD was 0.029 g/cm^2 greater (95% CI 0.013 g/cm^2 to 0.046 g/cm^2, p=0.004) among those whose physical activity level was maintained than among those who had reduced their activities. The percentage increase was greater in group C than in group D (5.8% vs. 0.4%). At the femoral neck, the mean 4-year adjusted change in BMD was 0.051 g/cm^2 greater (95% CI 0.017 g/cm^2 to 0.083 g/cm^2, p=0.004) in group C than in group D. There was a decrease in FN BMD in group D (-0.029 g/cm^2; -2.9%), whereas the BMD value of group C increased (0.021 g/cm^2; 2,3%).

The mean adjusted areas of the lumbar spine (adjusted for height, weight, age and area of lumbar spine at 3-year measurement) and femoral neck (adjusted for height,

weight, age, and area of femoral neck at 3-year measurement) in the 7-year follow-up measurement are shown in Table 10.

Table 10. Mean areas of the lumbar spine (adjusted for height, weight, age, and area of lumbar spine at 3-year measurement) and femoral neck (adjusted for height, weight, age, and area of femoral neck at 3-year measurement). The values are mean.

	Continued activity, C	Reduced activity, R	Discontinued activity, D	p-value
LS area (cm^2)	59.6	59.1	58.2	0.042
FN area (cm^2)	4.7	4.6	4.7	NS

NS = not significant

The reduction in physical activity was also studied in the different physical activity groups. On average, a change of 1 MET hour/week from the 3-year to the 7-year measurement reflected a change in LS BMC and indicated a reduction in BMC of 0.17% (95% CI 0.08% to 0.26%) among gymnasts, 0.19% (0.07% to 0.30%) among runners, and 0.13% (-0.04% to 0.30%) among controls. The impact of the one MET hour/week change on LS BMC was similar in the groups. The corresponding FN values for gymnasts, runners, and controls were 0.16% (0.09% to 0.22%), 0.10% (0.04% to 0.17%), and 0.02% (–0.09% to 0.13%), respectively. The impact of the change in exercise level on BMC among gymnasts was significantly greater than among controls. The relationship between the change in the level of exercise and the percentage change in BMC at the lumbar spine and femoral neck is illustrated in Figures 13a and 13b for the last four follow-up years in the gymnasts and runners.

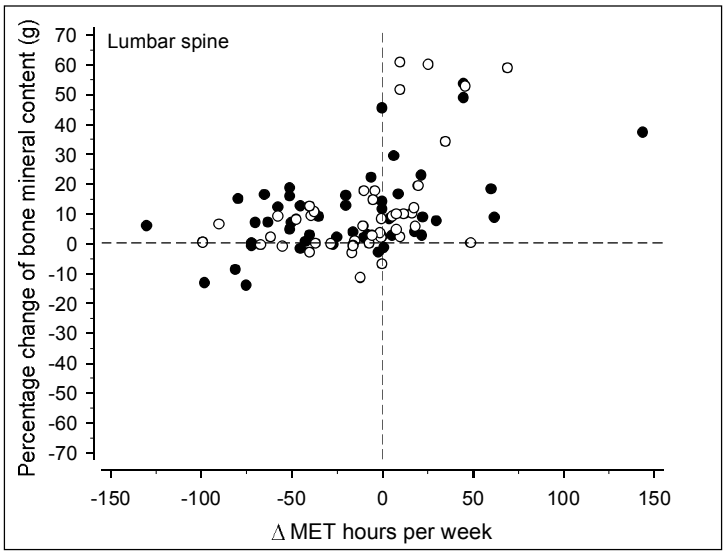

Figure 13a. Change (%) in crude LS BMC in relation to the change in MET (ΔMET) hours/week from the 3-to 7-year measurements. ○ =gymnasts, ● =runners (Substudy I)

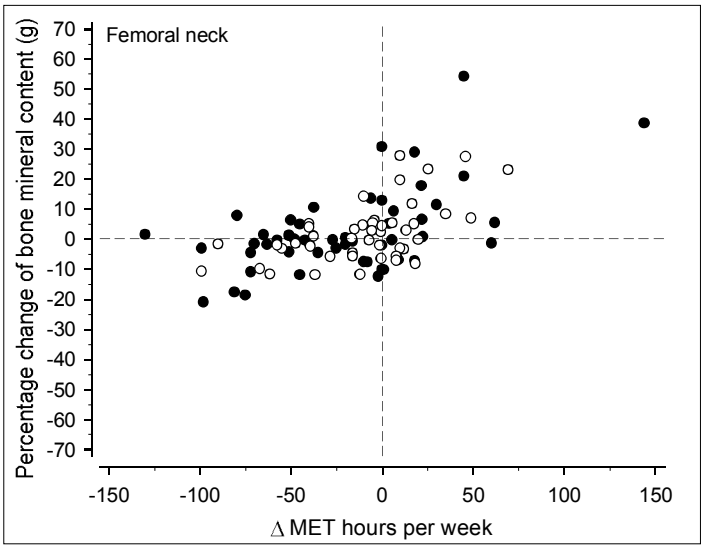

Figure 13b. Change (%) in crude FN BMC in relation to the change in MET (ΔMET) hours/week from the 3- to 7-year measurements. ○ =gymnasts, ● =runners (Substudy I)

5.4.2 Calcaneal quantitative ultrasound values

(Substudy III)

The effects of reduced physical activity were also examined in relation to sonographic measurements (n = 140). Thirty girls (21.4%) reduced their physical activity by more than 50% (group D) and 29 girls (20.7%) by 25-50% (group R) as compared with the level of frequency in the 2-year follow-up measurement. The majority of the girls (81/140) maintained their previous level of physical activity (group C). The development of the sonographic measurements of the whole study population is shown in Table 11. The baseline values of age, weight, BMI, and calcium and vitamin D intakes were similar in the 3 physical activity groups (Table 12). There were no statistically significant differences between the groups, except for MET.

Table 11. Development of the sonographic measurements of the study population (n = 140). The values are presented as means (SD).

	Baseline	2 years	6 years
SOS (m/s)	1587.9 (37.2)	1587.5 (39.9)	1584.3 (37.8)
BUA (dB/MHz)	77.3 (78.9)	81.7 (27.0)	87.9 (22.7)
T-score	1.1 (1.9)	0.6 (1.5)	0.6 (1.4)

Table 12. Baseline values of groups C, R and D assigned according to change in exercise habits between study years 2-6. The results are expressed as mean values (SD). The levels of physical activity (MET) at baseline are presented as medians (first and third quartiles). (Substudy III)

D: more than 50% decrease in physical activity
R: 25-50% decrease in physical activity
C: continued/increased physical activity

	Group D	Group R	Group C
n	30	29	81
Age (years)	14.5 (1.2)	14.3 (1.6)	13.7 (1.9)
Calcium intake (mg)	1462 (520)	1577 (572)	1578 (548)
Vitamin D intake (µg)	3.8 (1.8)	4.1 (2.2)	3.9 (2.2)
Weight (kg)	48.7 (9.0)	50.0 (8.0)	49.0 (10.0)
BMI (kg/m^2)	18.8 (2.2)	18.9 (2.2)	19.2 (2.5)
MET hours/week	63.3* (42.8, 105.0)	43.5* (8.0, 82.5)	21.0* (6.0, 64.8)

***P = 0.001**
BMI = body mass index
MET=ratio of work metabolic rate

The changes in the mean calcaneal T-scores of groups D, R, and C are shown in Figure 14. The decline in T-scores was significant (the repeated MANOVA test, $p<0.001$) in group D. The changes in all 3 groups were statistically significantly different from each other ($p = 0.003$) (Figure 14). The T-scores differed significantly in groups C and D at baseline ($p = 0.004$) and at 2 years ($p = 0.006$). In the 6-year measurement, the difference between the groups was not significant.

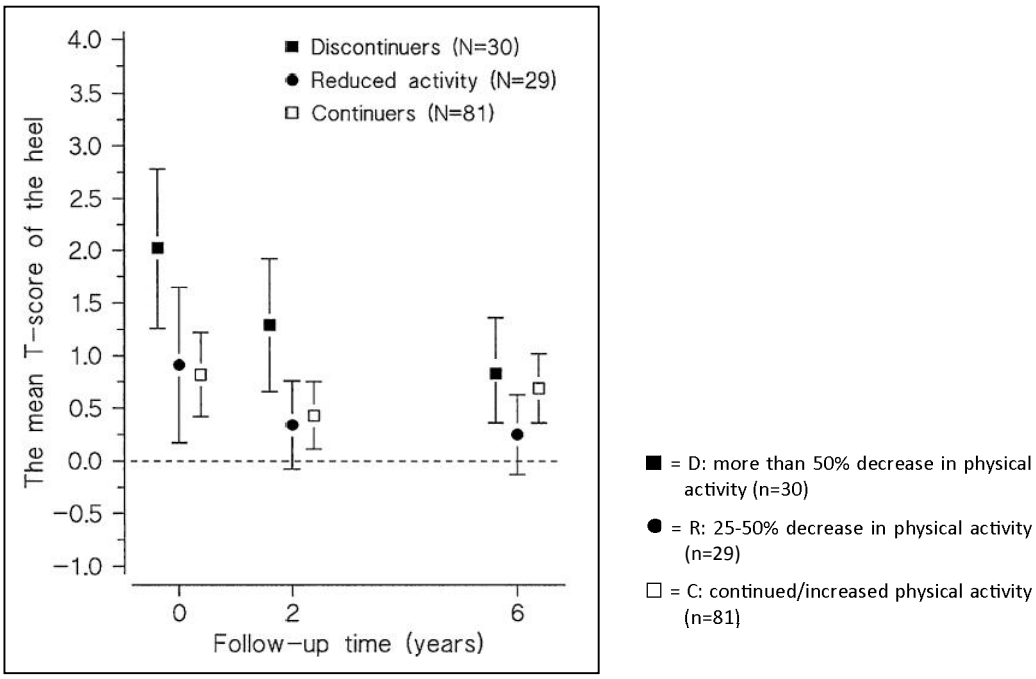

Figure 14. Mean calcaneal T-scores at baseline, 2 years and 6 years in groups D, R, and C. The dots/squares indicate mean values and the whiskers show 95% confidence intervals. (Substudy III)

The relationship between change in level of physical activity and change in SOS values is shown in Figure 15. Most of the participants who increased their physical activity also had an increase in SOS values. Those who decreased their physical activity were more likely to have a negative percentage change in SOS.

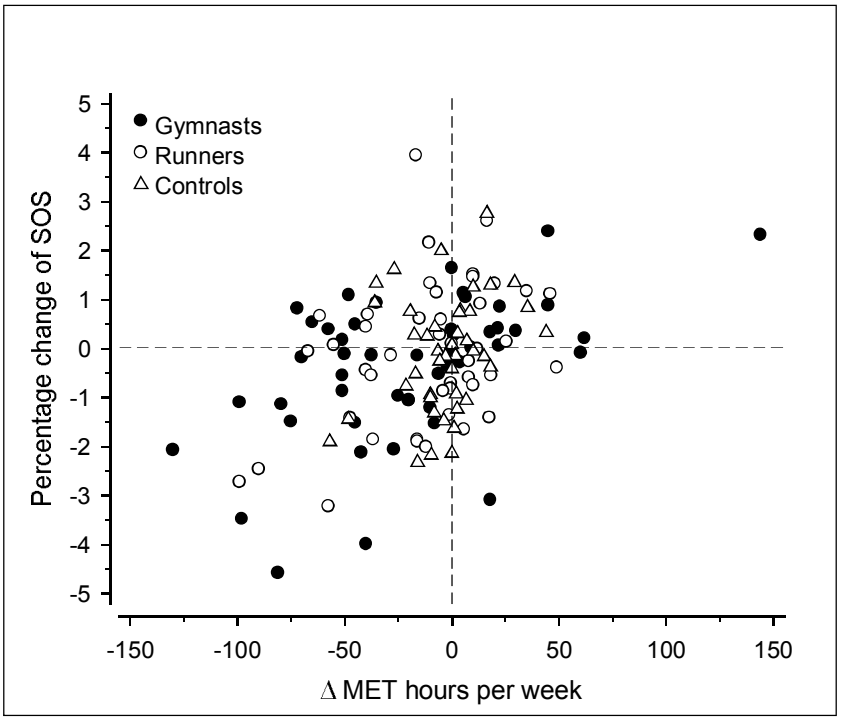

Figure 15. Change (%) in crude SOS values in relation to the change in MET (ΔMET) hours/ week from the 3- to 7-year measurement.

5.5 Estrogen-progestin contraception and bone growth

(Substudy IV)

There were no differences in the BMC measurements between the users of different estrogen-progestin contraceptive (EPC) formulations. One participant used a vaginal ring containing 15 μg of ethinyl estradiol and 120 μg of etonogestrel. One participant used triphasic pills. All the other participants using hormonal contraception used monophasic formulations with 35 μg or less of estrogen, and in 57/70 (81.4%) preparations the estrogen amount was 30 μg or less. The used contraceptive formulations are shown in Table 13.

Table 13. Doses of ethinyl estradiol (EE) and progesterone in the formulation used by groups C1 and C2. (Substudy IV)

Number of users	EE dose (µg)	Progesterone dose (mg)	Type of progesterone
25	20	0.075	gestodene
11	30	3.0	drospirenone
11	35	2	cyproterone acetate
9	20	0.15	desogestrel
5	30	0.075	gestodene
3	30	0.15	levonorgestrel
3	30	0.15	desogestrel
1	15	0.12	etonogestrel
1	35	0.25	norgestimate
1	30/40/30	0.05/0.075/0.125	levonorgestrel

Some study participants did not use hormonal contraception (group NC). Group C1 had used EPCs for 1.8 (SD 0.9) years and group C2 for 3.5 years (SD 0.7). The participants of this study did not have significant menstrual disturbances during the follow-up time. The menstrual irregularities did not differ between the 3 groups. The alcohol and tobacco use did not differ between groups during the whole follow-up.

The change in mean LS BMC (adjusted for years from menarche, height, weight, baseline $BMC_{LS,}$ and amount of physical activity during the study) is shown in Figure 16. There was a significant trend of a lesser increase in LS BMC in the group that had used contraception for more than 2 years as compared with the two other groups (p for linearity = 0.0046).

A similar trend was seen for the femoral neck. The change in mean FN BMC (adjusted for age from menarche, height, weight, baseline $BMC_{FN,}$ and amount of physical activity during the study) is shown in Figure 16. The development of the FN BMC values was different between the two EPC groups. The longer duration of EPC use seemed to suppress normal BMC development (p for linearity = 0.038). The results remained unchanged when the girls under 13 were left out of the analyses. The long duration of EPC use seemed to suppress BMC increase in this case, too.

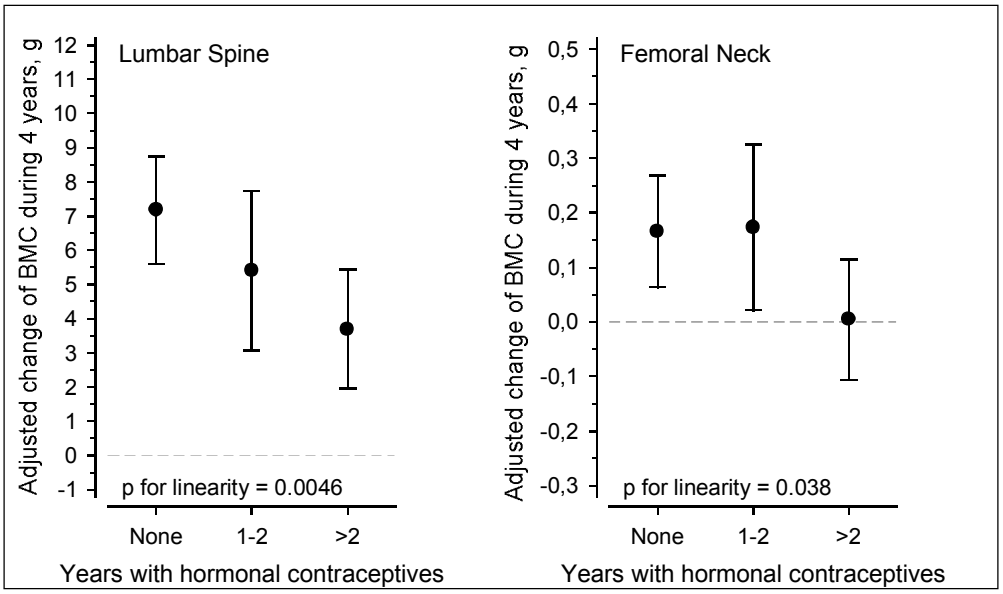

Figure 16. Change in LS and FN BMC (adjusted for years from menarche, height, weight, baseline BMC, and the amount of physical activity during the study) by group: None =no estrogen-progestin contraception, 1-2 =estrogen-progestin contraception for more than one year, but less than two years, >2 =estrogen-progestin contraception for more than two years. (Substudy IV)

6. DISCUSSION

6.1 Methodology

6.1.1 Subjects

With the aim of studying spine, hip and calcaneal bone density in healthy adolescent females, 142 girls were followed for 7 years. At 7-year follow-up, 98 original athletes and 44 original controls remained. The athletes were volunteers from local sports clubs and the controls were either classmates of the athletes or children or relatives of the hospital personnel. There was a clear difference in the physical activity habits between athletes and controls, but the sample was not truly population-based.

There were 29 (17%) dropouts during the last 4 follow-up years. The girls who failed to be present in the measurements were either living at a distance or could not find adequate time for participation. For girls of this age, a 17% dropout rate is quite acceptable.

All study participants were Caucasian, thus representing the same ethnic group, which facilitates interpretation of the results.

6.1.2 Bone measurements

Dual-energy X-ray absorptiometry is generally regarded as a safe, accurate and precise method for the determination of BMD in adults. It is widely used with pediatric and adolescent subjects due to its relatively low radiation, low cost and provision of information with high evaluative value. However, the use of DXA to evaluate children has highlighted its limitations. BMC and BMD are highly size-dependent. DXA systematically underestimates BMD in a smaller individual because it is an areal (g/cm2), not a volumetric (g/cm3), measure (Crabtree et al. 2007). It has been suggested that calculated apparent volumetric BMD values should be used with growing individuals (Kotaniemi et al. 1993, Kröger et al. 1995).

In the present study, the same DXA scanner (Hologic QDR 4500C, Waltham, Massachusetts, USA, software version 12.3) was used to measure the bone mineral density (g/cm^2) and bone area (cm^2) of the hip in the non-dominant leg and of the lumbar spine (L2-L4) at baseline as well as in the 1-, 3- and 7-year follow-up measurements. The same scanner was used with every participant. The same trained radiographers performed all measurements and analyzed the data. They performed quality assurance daily by calibrating the device with a spine phantom supplied by the manufacturer. The coefficients of variation (CV) of 2 consecutive measurements of 10 girls were 1.3% for the spine, 0.8% for the hip, and 0.4% for the phantom for the total study period. The mean age of these girls was 12.1 years (range 10.2-15.0 years). The CV values were in line with previous studies (Kudlac et al. 2004, Nordström et al. 2005b, Valdimarsson et al. 2005b).

Sonographic bone measurements are attractive because the ultrasound device is portable, inexpensive and non-ionizing. In the present study, sonography was always performed on both calcanei and using the same sonographic densitometry (Hologic Sahara Clinical Bone Sonometer, Waltham, Massachusetts, USA). At 7-year follow-up, the measurements were performed either by the author or another investigator and during the first three years, a third researcher was involved. Quality assurance was performed daily by calibrating the device on a dedicated phantom supplied by the manufacturer. The inter-observer kappa coefficient was not counted, but the intraclass correlation coefficients 0.99 (0.98-1.00) for BUA and 0.99 (0.98-1.00) for SOS were excellent and well in line with a previous study by Vignolo et al. (2006).

6.1.3 Questionnaires

The amount and intensity of physical activity was assessed by questionnaires. The questionnaires used to estimate the level of leisure-time physical activity in the present study included commonly used questions on frequency, intensity and duration of exercise (Raitakari et al. 1996). These questionnaires are used for children or adolescents (Telama et al. 1985). The study group answered the same questions 8 times during the study. The questionnaires have been used successfully with adolescents earlier (Raitakari et al. 1996).

In the 7-year follow-up the participants filled an additional questionnaire about the changes in their physical activity habits during the last 4 follow-up years. The information on the reduction of the physical activity level in the present study was collected retrospectively with a questionnaire between the 3rd and 7th year follow-up measurement

The intakes of vitamin D and calcium were assessed be semi-quantitative questionnaires. For the first 3 years, intakes of vitamin D and calcium were estimated at 6-month intervals (Välimäki et al. 1994b, Lehtonen-Veromaa et al. 1999). Another validated questionnaire on calcium intake was completed in the 7-year follow-up measurement (Uusi-Rasi et al. 1994). The questionnaire on vitamin D intake was revised and up-dated because vitamin D supplementation was increased in Finnish milk products in 2003. The questionnaires on vitamin D and calcium intakes were not specifically validated for children and adolescents.

In the case of younger children, all questionnaires were filled in by one of the parents together with the child. The participants completed the same questionnaires repeatedly and thus became familiar with them, which probably cuts down reporting errors.

6.2 Peak bone mass attainment

It is possible that the participants of the present study reach their peak bone mass (PBM) before the age of 20. In previous studies, the fastest bone mass accumulation happened around the age of 15-16 (Bonjour et al. 1991, Theintz et al. 1992, Bailey et al. 1999).

Bone mass accumulation clearly slowed down after the age of 15-16 (Bonjour et al. 1991, Theintz et al. 1992). McKay et al. (1998) found that BMC accrual is fastest during the time of menarche at the age of 12-13 years.

The results of the present study agree quite well with those of Haapasalo et al. (1996) who found that peak BMC values were reached around the age of 20. In their study, bone loss seemed to start soon after PBM was reached. This was, however, not seen in the present study, because the follow-up ended when the oldest participants were only 23 years old.

Other researchers have found that bone gain may also occur during the third decade. In a study of Recker et al. (1992), the estimated time when bone mineral acquisition ceased ranged from 28.3 to 29.5 years.

In the present study, PBM seemed to be attained simultaneously at the lumbar spine and femoral neck. In some other studies (Bass et al. 1998, Lin et al. 2003, Henry et al. 2004), PBM was reached earlier at the femoral neck.

When assessing the results of the present study, it should be taken into account that the number of girls per age group is quite small in the study population, which causes variation in the results.

6.3 Reduction in physical activity level and bone mass

6.3.1 Effects of different types of physical activity

According to the findings of substudy I, the amount of physical activity and type of exercise have a significant effect on BMC in adolescent women. The BMC means at the lumbar spine and femoral neck were significantly higher among gymnasts than among runners or controls at baseline and in all follow-up measurements. The differences persisted for 7 years even when the amount of training was markedly reduced following the 3-year follow-up measurement. Although the mean increase in BMC was quite similar between the groups at the lumbar spine, it was greater at the femoral neck in gymnasts than in controls during the first three years.

Gymnasts were included in this study, because gymnastics is a weight-bearing sport involving floor contacts, great impacts, and strains, and it is thought to be especially beneficial to the developing bones. Premenarcheal, pubertal, and preadolescent female gymnasts have been found to have greater BMD as compared with controls (Cassell et al. 1996, Dyson et al. 1997, Lehtonen-Veromaa et al. 2000b, Nickols-Richardson et al. 2000).

Robinson et al. (1995) found that gymnasts have a higher femoral neck BMD when compared with that of runners and controls despite a higher prevalence of amenorrhea and oligomenorrhea. This is in agreement with our findings, although the participants of the present study did not have significant menstrual irregularities despite the later menarche of the gymnasts. In the present study, the runners had greater BMC values at the measured sites when compared with controls. In the study of Robinson et al. (1995),

LS and FN BMD were smaller in runners than in controls. This might be explained by the high prevalence of amenorrhea and oligomenorrhea in that group.

Regular weight-bearing activity has been found to be strongly connected with BMD in youngsters (Grimston et al. 1993, Pettersson et al. 2000). In earlier studies, the type of physical activity has been an important determinant of bone density (Nordström et al. 1998). Grimston et al. (1993) found that in 10-16 year old children participating in such weight-bearing activity that it produced ground reaction forces greater than 3 times the body weight of the child, BMD was greater than in children participating in competitive swimming.

The decrease in the exercise level reduced the mean values of FN BMC. The calculated effect of the decrease in the level of physical activity, while using the indicator of 1 MET hour/week, on FN BMC was 0.16% for gymnasts, whereas the corresponding change was significantly lower (0.02%) for controls. It is worth mentioning that the level of weekly activity was very high for gymnasts as compared with runners during the first three years and that the duration of active training was 1.8 (mean) years longer among gymnasts than among runners. The baseline 80 MET hours/week of the gymnasts correspond to 8 hours of running or 20 hours of walking each week (Fogelholm 1991). The 42 MET hours/week of the runners correspond to 4.2 hours of running, and the 10 MET hours/week of the controls correspond to one hour of running each week (Fogelholm 1991).

At the beginning of the present study, the gymnasts were at the top of their careers and their frequency of physical activity was at a high level. The frequency of training was markedly reduced after the 3-year follow-up measurement. Only a few gymnasts maintained the same activity level for the total follow-up period. It would have been interesting to study the bone measurements of the gymnasts if their training had continued with the similar intensity. As discussed earlier, it is known that a clear reduction in physical activity will cause a reduction in BMD and BMC (Winters & Snow 2000, Gustavsson et al. 2003, Nordström et al. 2005b, Valdimarsson et al. 2005a).

In some recent studies, gymnasts had a higher BMD after retirement from active career when compared with controls (Bass et al. 1998, Zanker et al. 2004). The present results from substudy I support these findings: the BMC of the gymnasts remained greater despite a clear reduction in physical activity level. The mean adjusted BMC of the lumbar spine and femoral neck was significantly greater among gymnasts compared with runners and controls during the whole follow-up period. The difference persisted even after a clear reduction in the activity level of the gymnasts. Partial maintenance of acquired benefits has also been reported for former young male athletes (Nordström et al. 2005a).

6.3.2 Reduction in physical activity level and dual-energy X-ray absorptiometry measurements

In substudy II, the mean 4-year changes in the adjusted (age and increase in height and weight) BMC of the lumbar spine and femoral neck were examined in the groups assigned

according to reduction in physical activity. An evident negative effect was observed with reduced activity levels. The mean BMC and BMD were maintained or improved among those girls whose physical activity levels did not go down, but the increases in BMC and BMD declined among most of those girls who had markedly reduced their physical activity. The benefits attained by physical activity were lost to some extent. BMC and BMD did not decrease below the values of sedentary participants at any point.

The results confirm that exercise-induced bone gain is not permanent without a sustained physical activity level during later life. Others have made similar conclusions. Ice-hockey players had a significantly higher BMD compared with controls. The players lost significantly more BMD from the weight-bearing sites within 3 years of cessation compared with controls (Gustavsson et al. 2003, Nordström et al. 2005b). In 18-year-old female soccer players, reduced training was associated with increased loss of BMD in an 8-year follow-up (Valdimarsson et al. 2005a). Nordstöm et al. (2005a) reported a partial loss of exercise-induced skeletal benefits after 4 years' retirement from an active athletic career. They also reported former old male athletes to have fewer fractures than age-matched controls. The difference in fracture incidence might be partly caused by other factors than the effects of previous sports participation on bone, such as genetic factors, better neuromuscular functioning and differences in lifestyle factors (Nordström et al. 2005a).

In premenopausal women, the skeletal benefits attained by 12-month training were reversed in 6 months when the training was withdrawn. Similar findings have been made with postmenopausal women (Dalsky et al. 1988).

Controversial results have also been presented. Follow-up studies on racquet sport players have shown good maintenance of exercise-induced bone gain (Kontulainen et al. 1999, Kontulainen et al. 2001). Lack of negative response in these studies might be partly explained by the fact that most players were still active enough to maintain the exercise-induced bone gain. It may be that decreased but still active racquet training was able to produce sufficient stimuli. Former female gymnasts aged 20-32 years had a 6-11% higher BMD when compared with sedentary controls in a cross-sectional study (Zanker et al. 2004). These women had trained competitive aerobics between ages 6-14. In a study by Kudlac et al. (2004), former gymnasts had significantly higher proximal femur BMD than controls, even after a similar decline in the values in both groups. In their study, only gymnasts had a significant decline in lumbar spine BMD (Kudlac et al. 2004).

Premenopausal women gained BMD in an 18-month high-impact exercise intervention (Kontulainen et al. 2004). Both trainees and controls showed subsequent BMD loss at measured sites, but the changes in exercise and control groups were similar. The exercise-induced BMD benefits still existed at the follow-up 3.5 years later (Kontulainen et al. 2004).

Gains in hip bone resulting from high-impact training intervention are maintained in children after detraining for 7 months (Fuchs & Snow 2002). Similar findings have been made with growing girls followed for 20 months (Kontulainen et al. 2002).

During the whole 7-year follow-up period, higher physical activity level was significantly associated with higher values of bone measurements with DXA. Other researchers have reported similar results (Heinonen et al. 1995, Madsen et al 1998, Lloyd et al. 2002, Havill et al. 2007). Hours of weekly exercise and other exercise measures are positively associated with total body BMC and BMD and with spinal BMD (McLean et al. 2001). An association has been reported between hip BMD of 20-year-old adolescent women and their previous physical exercises (Lloyd et al. 2002). College-aged women involved in weight-bearing collegiate sports had significantly greater BMD than their sedentary controls (Madsen et al. 1998).

Contrary to the findings above, two studies reported no positive relationship between physical activity and bone mineral measurements in females (Bakker et al. 2003, Afghani et al. 2003). Bakker et al. (2003) concluded that after reaching peak bone mass, physical activity should be promoted in order to reduce bone re-absorption and/or stimulate bone formation. In a study on Chinese adolescents, the physical strain produced by leisure time activity may not have been strong enough to influence bone modeling (Afghani et al. 2003). Only a few participants of the study reported being members of sports teams.

6.3.3 Reduction in physical activity level and calcaneal quantitative ultrasound

In the 6-year follow-up measurement of substudy III, physical activity was the only significant parameter and accounted for 11.3% of the variation of the calcaneal T-scores. Physical activity has been associated with QUS measurements (Damilakis et al. 1999, Yamaguchi et al. 2000, Karlsson et al. 2001, Yung et al. 2005, Babaroutsi et al. 2005b, Falk et al. 2007, Robinson et al. 2007). When the independent variables of the 6-year follow-up measurement were set in the model, physical activity was the only significant factor for the SOS and BUA values and accounted for 13.4% and 6.9% of the variation, respectively. This agrees with previous results on Finnish young men (Välimäki et al. 2006). In addition, some have found a relationship between calcium intake and QUS values (Yamaguchi et al. 2000, Robinson et al. 2007)

The decline in T-scores was significant in the group whose physical activity amount reduced more than 50%. The mean SOS values decreased most in the same group while the decline was smaller in the group of reduced physical activity and no change was observed in the group that continued to exercise. The BUA values of the group that discontinued physical activity did not change, while the values of the two other groups increased. These results are in line with the previous results of the study population, in which SOS values decreased after a reduction in training levels (Lehtonen-Veromaa et al. 2001).

The present results also show that SOS is sensitive to changes. Physical activity was the only predictor of the calcaneal SOS value in the 6-year follow-up measurement of substudy III, in which it accounted for 13.4% of the variation. Physical activity accounted for 6.9% of the variation in the obtained BUA values. Others have made similar findings

(Lehtonen-Veromaa et al. 2001, Babaroutsi et al. 2005a, Välimäki et al. 2006). Laugier et al. (2000) reported a significant 1.1-1.7% decrease in SOS for both heels during a 120-day bed rest of male participants, but the BUA values did not change. Yamaga et al. (1996) found a decrease in SOS values during pregnancy, but there was no change in BUA.

Not all findings on SOS are similar. Daly et al. (1999) did not find any increases in the SOS values of gymnasts during their 18-month follow-up study, but the SOS values of gymnasts were higher as compared with those of controls during the whole follow-up time period. They proposed that an equilibrium point may have been reached among the gymnasts, and for further bone accrual, physical activity may need to be even more intense. SOS values have decreased during a 10-week military training (Etherington et al. 1999). This was speculated to be due to increased trabecular separation and microfractures.

6.4 Hormonal contraception and bone

After the study of Vessey at al. (1998), increasing concerns about the use of oral contraceptives (OC) have been raised. Vessey and coworkers reported a minor, but highly significant trend of increased risk of hospital referral for fractures with long duration of OC use in a large cohort study (Vessey et al. 1998). In contrast, Vestergaard et al. (2006) found no relationship between OC use and fracture risk. In their unadjusted values, however, low-dose OC use was associated with a small increase in overall fracture risk. Evidence has been found in a population-based case-control study (Michaelsson et al. 1999) that oral contraceptive use late in reproductive age may decrease the risk of postmenopausal fractures. In another study (Barad et al. 2005), however, past OC use did not protect 50-79-year-old women from fractures.

The main finding of substudy IV was a trend of smaller increase in LS and FN BMC in the group of adolescent women who had used EPCs for more than two years. Oral contraceptive use during adolescence is common, approximately half of the Finnish female adolescent university students use hormonal contraception (Virtala et al. 2007). Recently, there have been increasing concerns that hormonal contraception especially during adolescent years alters the normal PBM development (Polatti et al. 1995, Cromer et al. 2004, Hartard et al. 2007). This agrees with the results of substudy IV. Low-dose EPCs suppress the normal estrogen production by reducing the concentration of circulating estrogen to the level measured during early follicular phase (Lloyd et al. 1989).

In a cross-sectional study, the 18-24 years old young women, who had ever used OCs had lower BMD at femoral neck when compared with never users (Hartard et al. 2007). In that study, there was no significant difference in lumbar spine BMD between the two groups. Others have concluded that OC use in late adolescence may compromise bone health, as controls had significantly higher BMD at the lumbar spine and femoral neck

compared with OC users in a cross sectional setting (Almstedt Shoepe & Snow 2005). The study population was 18-25 years old and the amount of ethinyl estradiol in the pills was 20-35 µg. Young starting age (Hartard et al. 2004, Hartard et al. 2007) and long duration of treatment (Hartard et al. 2007) have been associated with lower BMD measurements.

The BMD of young oral contraceptive users did not change in a 5-year follow-up, whereas the control group demonstrated an increase of 7.8% (Polatti et al. 1995). The increase in femoral neck BMD was significantly smaller in 12-18-year-old OC users than in non-users in a one-year follow-up (Cromer et al. 2004). Similarly, Reed et al. (2003) found a trend toward a smaller increase in BMD in 18-21-year-old OC users.

Similar findings have been made with vaginal ring users. The BMD of 18-35 years old users of vaginal ring did not change in a 2-year follow-up, while the BMD of controls increased (Massai et al. 2005).

The results are, however, conflicting. Two studies have not found any significant changes in BMD after OC use (Berenson et al. 2004, Endrikat et al. 2004). Both of these studies included women older than 30 years. Lloyd at al. (2000) did not find any difference in the bone mass measurements between previous OC users and non-users in an 8-year follow-up study. The study population comprised 62 girls aged 12 at baseline. The amount of ethinyl estradiol in the used pills is not specified in the article (Lloyd et al. 2000).

Oral contraceptive use at older age seems to protect against low bone mass. The summary of evidence in a review article suggests that combined oral contraceptives have no deleterious effect on BMD in perimenopausal women and that low-dose formulations may prevent menopausal bone loss (Martins et al 2006). Pasco et al. (2000) found OC exposure to be associated with higher lumbar spine BMD in a random sample of 20-69 years old women. Similar results were obtained by Tuppurainen at al. (1994), although in their study the OC users and non-users differed in many behavioral characteristics.

Disturbed menstrual cycles are associated with low BMD (Galuska & Sowers 1999). OCs can preserve bone mineral density in almost all hypoestrogenic states from adolescence to menopause (Volpe et al. 1997). Normalization of menstrual cycle with hormonal contraception has been shown to improve BMD in patients with hypothalamic oligoamenorrea and anorexia nervosa (Seeman et al. 1992, Castelo-Branco et al. 2001). Elgán et al. (2003) reported results in which OC use seemed to moderate the negative effects of smoking on bones. In substudy IV, the participants did not have significant menstrual irregularities and smoking habits did not affect bone.

Some studies have reported reductions in bone resorption markers after exposure to OCs (Mais et al. 1993, Paoletti et al. 2000, Nappi et al. 2003, Rome et al. 2004). However, none of these studies showed changes in BMD.

In premenopausal OC use, an estrogen dose of 25-35 µg seemed to be optimal for preventing or delaying postmenopausal bone loss (DeCherney 1996). In substudy IV, all

participants used formulations containing 35 µg or less of estrogen, 81% (57/70) even 30 µg or less. We did not find a difference between the effects of various amounts of EPCs on BMC because there were not many users per formulation and all participants used low dose formulations. No differences were found in the effect of two low-dose pills containing either 20 µg or 30 µg of ethinyl estradiol on bone mineral density in a randomized controlled study of Endrikat et al. (2004).

6.5 General discussion and future perspectives

The study population consisted of 142 Finnish adolescent women in substudies I and II, 140 in substudy III, and 122 in substudy IV. At baseline, they were grouped as gymnasts, runners and controls. The dropout rates of 25.2% for the athletes and 26.7% for the controls were moderate for a 7-year follow-up period. Those participating in the last follow-up study represented the original population well. Dropout analysis revealed no differences in the baseline values of the attending and non-attending participants**.**

Initially, the study population consisted of athletes and sedentary controls. There is always a possibility of selection bias in a study that investigates athletes, i.e. athletes participating in a certain sport might be genetically suitable for that sport.

The participants of this study were informed on their bone measurement results. Those who had low bone mineral values were referred to further examinations in Turku University Central Hospital. The girls were also informed on the importance of adequate calcium and vitamin D intakes. Consequently, the girls in the present study might have been more aware of the recommended daily intake of these nutrients than the average Finnish girl. This might have affected the results.

During follow-up, the groups were mixed, which might have affected the findings on the effects of physical activity. For the first 3-years of the present study, the gymnasts were at the top of their careers and the frequency of their physical activity was at a high level. The frequency of training was markedly reduced after the 3-year follow-up measurement. Only a few gymnasts maintained the same activity level for the total follow-up period. It would have been interesting to study the bone measurements of the gymnasts if their training had continued with similar intensity. In addition, many of the inactive controls started to exercise, and at the end of the follow-up period, many of these young women exercised more than the original athletes.

In the present study, the information on the reduction of physical activity was collected retrospectively with a questionnaire between the 3- and 7- year follow-up measurements and not in the follow-up measurement every 6 months, as was the case during the first three years. The information on the use of oral contraceptives was also collected retrospectively.

When investigating bone mass in growing adolescents, BMC is known to be a better measurement than BMD, because it is not affected by bone size (Heaney et al. 2000). The young women participating in this study reached menarche during the study period

and most of the participants were still growing during follow-up. Consequently, we used BMC in all DXA studies.

The present results confirm that exercise is beneficial to bones in adolescent women. However, it cannot be concluded whether the benefits are maintained into old age. It would be interesting to see whether the active group will have fewer fractures in the future.

The age when peak bone mass is reached remains a matter of debate. The present results seem to indicate that peak bone mass may be reached before the age of 20. To confirm this, another follow-up study with DXA would be needed to establish whether bone density increases after the last follow-up. Another interesting aspect would be to compare the QUS values with the DXA measurements.

Physical activity has a clear association with increased bone mineral measurements (Kannus et al. 1995, Bass et al. 1998, Haapasalo et al. 1998, Bass 2000, Heaney et al. 2000, Heinonen et al. 2000, Kontulainen et al. 2001, Sundberg et al. 2002, Petit et al. 2006, Janz et al. 2007). Maintenance of physical activity is important in preserving exercise-induced benefits (Gustavsson et al. 2003, Nordström et al. 2005a, Nordström et al. 2005b, Valdimarsson et al. 2005a). The benefits induced by physical activity cannot be used as mere therapeutic agents in diseases. For example, glucocorticoid-induced osteoporosis cannot be treated with exercise alone. However, the possible negative effects of excessive coffee and alcohol consumption might be reversed with physical activity.

In conclusion, this study shows that bones are meant to be used and that their normal development requires physical activity.

7. CONCLUSIONS

1. Physical activity, especially gymnastics training, during prepuberty and puberty is positively associated with BMC. In the present study, this association may be due to the younger starting age and higher training intensity of the gymnasts compared with the two other groups. Reduction in physical activity seems to have a more marked effect on the BMC of gymnasts than that of sedentary controls.

2. Regular physical activity is important in preserving the acquired bone mass in adolescent women and thus contributes to the prevention of osteoporosis. Reduced physical activity was reflected negatively on BMC and BMD even in 20-year-old women.

3. Detraining decreases the calcaneal SOS value. In addition, the calcaneal SOS value is a sensitive indicator of changes in bone.

4. Long-term use of low dose oral contraceptives may slow down the normal development of bone mineral content in adolescent women.

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10. ORIGINAL PUBLICATIONS