Vitamins, Fatty Acids, Physical Activity and Peak Bone Mass

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To my beloved children,

Gabriel, Gustav and Hedvig

This is not the end, but it is the road.
Martin Luther

Magnus Högström, *Vitamins, Fatty Acids, Physical Activity and Peak Bone Mass*

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Abstract

Osteoporosis is a disease characterized by low bone mineral density, deteriorated bone microstructure and increased fracture risk. About 50% of all women and 25% of all men will have an osteoporotic fracture. Given that there is no effective cure in established osteoporosis, prevention is of high importance. Bone mineral density (BMD) is accumulated during childhood and adolescence with a peak at about 20 years of age. Peak BMD has been suggested to explain at least half of the variation in BMD up to old age. Thus, to increase peak BMD could decrease the risk of later fractures. The purpose of the present thesis was to investigate the influence of physical activity, vitamins A and D, and fatty acids on peak bone mass in men.

The influence of physical activity on bone accrual was studied in two cohorts. In the first cohort 46 ice hockey players, 18 badminton players and 27 controls, all 17 years of age at baseline, were followed for four years. During the follow up the badminton players gained more bone mass at the hip compared to both the ice hockey players and controls. In the second cohort the associations between physical activity and BMD were investigated in 62 female and 62 male young medical students. The estimated high impact activity per week was associated with bone mass at all sites in the male medical students (r=0.27-0.53, $p<0.05$). In the female cohort different estimates of physical activity were not related to bone mass at any site. In both males and females correlations between bone mass and body constitution parameters were observed.

Levels of vitamin D_3, vitamin D_2, retinol, retinol-binding-protein-4 (RBP-4) and fatty acids were measured in 78 young men with a mean age of 22.6 years. BMD at various sites were measured using Dual-Energy X-ray absorptiometry. Levels of vitamin D_3 showed a significant positive association with all BMD sites and also lean body mass (r=0.23-0.35, $p<0.05$). Levels of vitamin D_2, however, showed a significant negative correlation with BMD of the total body (r=-0.28, p=0.01) and spine (r=-0.27, p=0.02). There was also a significant negative relationship between levels of vitamin D_3 and D_2 (r=-0.31, p=0.006). Concentrations of n-3 (omega-3) fatty acids showed a positive association with BMD at the total body (r=0.27, p=0.02) and spine BMD (r=0.25, p=0.02). There was also a positive association between levels of n-3 fatty acids and changes in BMD of the spine between 16 and 22 years of age (r=0.26, p=0.02). The significant associations found seemed to be related mostly to the concentration of the n-3 fatty acid docosahexaenoic acid. Levels of retinol and RBP-4 were not related to BMD but to levels of osteocalcin, which is a marker of bone formation. This association disappeared when adjusting for the influence of abdominal fat mass.

In summary, the present thesis suggests that many modifiable factors may influence the accumulation of peak bone mass in males, such as physical activity, vitamins, and fatty acids. Further studies are needed to investigate whether optimizing these factors in youth may decrease the risk of osteoporosis later in life.

Key words: physical activity, vitamin A, vitamin D, fatty acids, peak bone mass, males.

Preface

This thesis is based upon the following papers, referred to in the text by their Roman numerals:

I. Nordstrom A, Hogstrom M, Nordstrom P. Effects of Different Types of Weight-Bearing Loading on Bone Mass and Size in Young Males: A Longitudinal Study. Accepted for publication in Bone, Nov 8th 2007.

II. Hogstrom M, Nordstrom A, Alfredson H, Lorentzon R, Thorsen K, Nordstrom P. Current Physical Activity is Related to Bone Mineral Density in Males but not in Females. Int J Sports Med 2007;28(5):431-6.

III. Hogstrom M, Nordstrom A, Nordstrom P. Relationship between Vitamin D Metabolites and Bone Mineral Density in Young Males: a Cross-Sectional and Longitudinal Study. Calcif Tissue Int 2006;79(2):95-101.

IV. Hogstrom M, Nordstrom A, Nordstrom P. Retinol, Retinol-Binding-Protein-4, Abdominal Fat Mass, Peak Bone Mineral Density and Markers of Bone Metabolism in Men: The NO_2-study. Conditionally accepted for publication Eur J Endocrinol Oct 22nd 2007.

V. Hogstrom M, Nordstrom P, Nordstrom A. n-3 Fatty Acids are Positively Associated with Peak Bone Mineral Density and Bone Accrual in Healthy Men: the NO2 Study. Am J Clin Nutr 2007;85(3):803-7.

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Abbreviations

aBMD	Areal bone mineral density
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
BAP	Bone specific alkaline phosphatase
BMC	Bone mineral content
BMD	Bone mineral density
BMU	Bone multicellular unit
BUA	broadband ultrasound attenuation
CTX	C-telopeptide of crosslinked collagen type 1
CV	Coefficient of variation
DXA	Dual-energy X-ray absorptiometry
DEXA	Dual-energy X-ray absorptiometry
DPYR	Deoxypyridinoline cross-links
FSH	Follicle stimulating hormone
GH	Growth hormone
GnRH	Gonadotropin releasing hormone
HPLC	High-performance liquid chromatography
IGF1	Insulin-like growth factor 1
LH	Luteinizing hormone
MRI	Magnetic resonance imaging
NTX	N-telopeptide of crosslinked collagen type 1
OC	Osteocalcin
pDXA	Periferal dual-energy X-ray absorptiometry

PICP	Procollagen I carboxyterminal propeptide
PINP	Procollagen I aminoterminal propeptide
pQCT	Periferal quantitative computerized tomography
PUFA	Polyunsaturated fatty acid
PYR	Pyridinoline cross-links
QCT	Quantitative computerized tomography
QUS	Quantitative ultra sound
RBP-4	Retinol binding protein 4
SD	Standard deviation
SOS	Speed of sound
ucOC	Undercarboxylated osteocalcin
vBMD	Volumetric bone density
WHO	World Health Organization

Osteoporosis

Osteoporosis is a bone condition characterized by reduced bone mineral density and structural changes that lead to increased fracture susceptibility. In recent years, osteoporosis has emerged as a major public health concern: the number of osteoporotic fractures reported in Sweden and in other industrialized countries has increased dramatically since the second World War [1-4], and projections indicate that further increases are expected worldwide, especially in Asia (Figure 1) [3, 5].

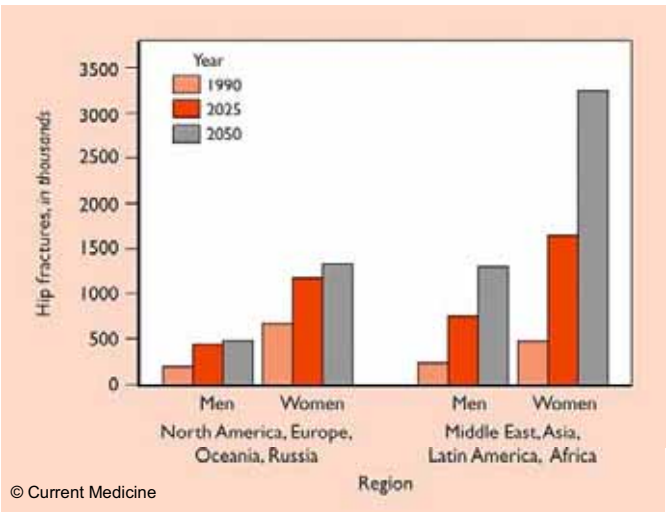


Figure 1. Hip fracture in 1990 and projections for the future[5]

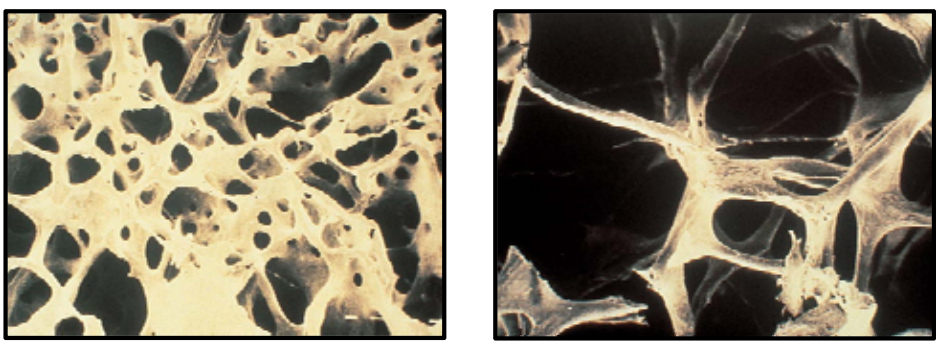
Osteoporosis and osteoporotic fractures are of particular interest to Swedish scientists, as Scandinavian countries have some of the world's highest incidences of osteoporotic fractures [6, 7]. An aging population contributes to the increased incidence, as the risk of osteoporotic fracture increases with age [8]. However, aging can only partially explain the increasing incidence as the age-specific incidence has also increased [2, 9-11]. In Sweden, the lifetime risk of sustaining an osteoporotic fracture is as high as 50% for women and 25% for men [4, 12].

Osteoporotic fractures are associated with high mortality and morbidity [1, 13, 14], increasing healthcare costs, and place a heavy burden on the healthcare system. In Sweden, the number of osteoporotic fractures is about 70,000 per year [4]; the number of bed days associated with these fractures exceeds 500,000, which is greater than the number of bed days for breast cancer and prostate cancer combined, and is even higher than the bed days for myocardial infarction [15]. The

direct annual costs associated with osteoporotic fractures in Sweden is staggering, totaling about 5.6 billion Swedish crowns or 0.6 billion euros according to the latest calculations [16, 17]. If the total societal burden of osteoporosis in Sweden is calculated, including quality adjusted life years lost, the cost is in excess of 15 billion Swedish crowns [17].

Osteoporotic fractures

In osteoporosis, the bone mineral density is reduced and bone structure deteriorates; this leads to reduced bone strength and increased fracture susceptibility (Figure 2). This can potentially increase fracture risk at any site, but fractures occur primarily at sites with a high proportion of trabecular bone.



Normal bone structure Osteoporotic bone

Figure 2. Bone structure deteriorates in osteoporosis. Note the absence of a normal internal trabecular framework in osteoporotic bone, leading to decreased bone strength.

The most common fracture types are wrist fractures, hip fractures, vertebral compression fractures, and proximal humeral fractures (Table 1).

Common osteoporotic fractures in Sweden

Fracture type	Annual number
Wrist	25 000
Hip	18 000
Vertebral compression	15 000
Proximal humeral	10 000

Table 1. The incidence of osteoporotic fractures in Sweden [18].

The treatment of osteoporotic fractures is challenging. Because the bone quality is poor, osteosynthesis can be difficult and post-operative care is often complicated by co-existing morbidities, general frailty, and dementia. The mortality rate is elevated after osteoporotic fracture, especially for vertebral and hip fractures, and about 25% percent of hip fracture patients are dead within a year of the fracture event [14].

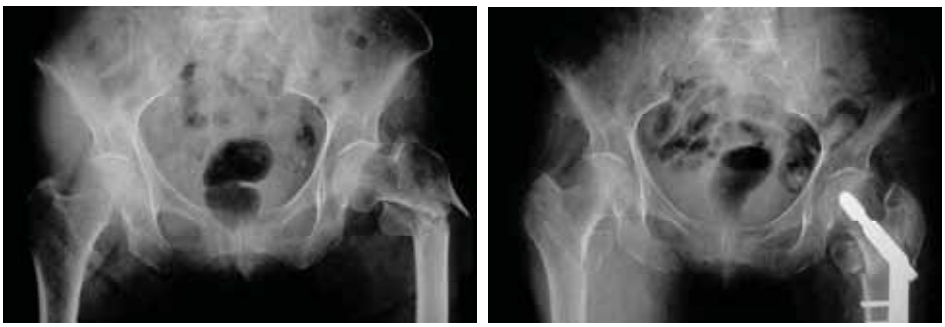


Figure 3. Fracture of the left hip, before and after surgery.

Diagnosis of osteoporosis

Osteoporosis is an insidious disease. Severe osteoporosis can develop in an individual without any symptoms. In fact, a sudden fracture after minimal trauma is often the first manifestation of the disease. The treacherous nature of the disease makes it difficult to diagnose, and it can be difficult to motivate patients to take preventive measures, including medication, even after they sustain a fracture. The condition is most prevalent in the old and frail, a group of patients with little ability or energy to act forcefully for better prevention, diagnosis, and treatment. Osteoporosis awareness by both the medical community and society is still insufficient.

Several clinical risk factors for osteoporosis have been identified (Table 2). These factors can help identify patients at risk for osteoporotic fractures and can thus aid in the selection of patients for diagnostic work-up.

Risk factors that cannot be influenced	Risk factors that may be influenced
Age	Physical inactivity/immobilisation
Previous fracture	Low bodyweight/anorexia nervosa
Female sex	Glucocorticoid medication
Premature menopause	Cigarette smoking
Family history of fractures	Poor vision
Ethnicity	Alcohol abuse
Height	Vitamin D deficiency
Neuromuscular disorders	Low bone mineral density
	Calcium deficiency
	Hypogonadism
	Hyperthyroidism
	Hyperparathyroidism
	Malabsorption
	Renal disorders
	Certain pharmaceuticals
	Propensity for falling

Table 2. Risk factors for osteoporosis and osteoporotic fractures, adapted from John Kanis and SBU [4, 19]

The diagnosis of osteoporosis is primarily based on the assessment of bone mineral density (BMD). This is usually done with dual energy X-ray absorptiometry (DXA or DEXA), preferably by measuring the hip and lumbar spine. Other less costly techniques measure peripheral sites, often the calcaneus, using peripheral DXA (pDXA), peripheral quantitative computed tomography (pQCT), or quantitative ultrasound (QUS). DXA is generally accepted as the gold standard because the measurements are precise, the radiation exposure is minimal, the most clinically relevant regions can be evaluated, and most research and treatment regimens are based on DXA measurements (see "Bone Mass Measurements" section).

The quantitative thresholds for the diagnosis of osteoporosis have been determined by WHO and modified by the International Osteoporosis Foundation [19-21]. Bone mineral density has a normal distribution, and the WHO thresholds are expressed as standard deviations ("T-score") of a reference population of young adults in the same population (Table 3). These criteria were developed for women, but research has shown that the same absolute BMD values in the hip convey about the same fracture risk to both men and women [19, 20].

WHO definitions of osteoporosis

Normal: Bone density is within 1 SD (+1 or -1) of the young adult mean.

Osteopenia: Bone density is 1 to 2.5 SD below the young adult mean (-1 to -2.5 SD).

Osteoporosis: Bone density is 2.5 SD or more below the young adult mean (≤ -2.5 SD).

Established osteoporosis: Bone density is more than 2.5 SD below the young adult mean, and the individual has had one or more osteoporotic fractures.

Table 3. The WHO definitions of osteoporosis[19-21].

BMD is an important predictor of fracture risk. However, other factors also play significant roles in the assessment of fracture risk. Age is a very important factor as the risk of hip fracture increases exponentially with age [8]. Medications can increase fracture susceptibility through a direct effect on skeletal metabolism; examples include anticonvulsants, anticoagulants, antipsychotics [22], antidepressants, high dosages of thyroid hormones, and cortisone treatment [23]. Some medications can also increase the risk of falling; examples include diuretics, neuroleptics, and benzodiazepines [24].

Bone structure

Mature human bone contains bone mineral, organic bone matrix, and several types of bone cells [4, 25, 26].

Bone mineral

Bone mineral consists mainly of calcium and phosphorous organized into hydroxyapatite crystals, $Ca_{10}(PO_4)_6(OH)_2$, but also contains trace elements such as magnesium, strontium, and fluoride [4, 26]. The bone mineral provides rigidity, resistance to compressive forces, and hardness. Bone mineral is important for calcium homeostasis since 99%, or about 1300 grams, of the body's calcium is stored in the adult skeleton [26].

Bone matrix

The bone matrix constitutes more than 25% of bones [26]. The chief component of bone matrix is collagen, which constitutes about 65% of the organic content of the bone. Several types of collagen exist in the bone, but fibrillar type I collagen is predominant [26]. The collagen fibers are made up of three long polypetide chains that form a triple helix. Crosslinking of the three polypeptide chains further enhances collagen fiber strength (Figure 4). The collagen in mature bone is mineralized and interwoven into the bone's hydroxyapatite structure. The collagen fibers are organized into complex patterns that reinforce the mineralized bone.

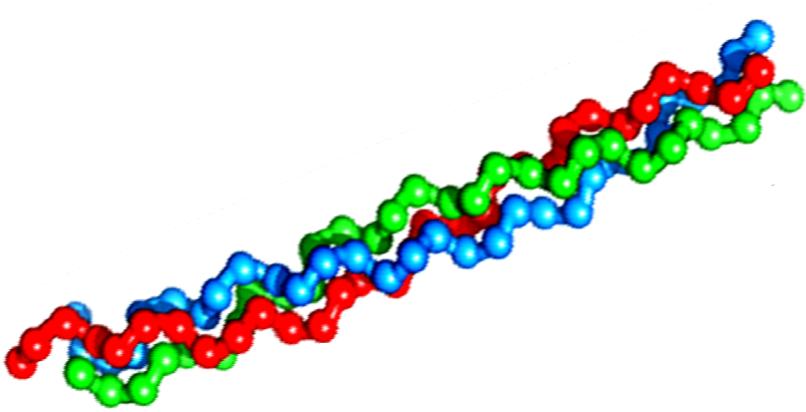


Figure 4. Illustration of the collagen triple helix. Collagen fibers reinforce the skeleton.

Another important organic matrix component is osteocalcin, which constitutes up to 20% of the bone's organic matrix. Osteocalcin is secreted by the bone-building cells, osteoblasts, and has a high affinity for the bone mineral hydroxyapatite [27]. It is believed to play an important role in bone mineralization [27], and the maturation of osteocalcin is dependent on adequate levels of vitamin K [26].

Proteoglycans, glycoproteins, and osteonectin are complex molecules that are also part of the organic bone matrix. They may be involved in maintaining the integrity of the bone matrix and in binding the hydroxyapatite to the matrix [26]. Several other types of proteins and compounds have been detected in bone tissue; these components may stimulate or inhibit mineralization [26].

Bone cells

There are several types of bone cells in the skeleton that contribute to the health and integrity of the bones [26]. Osteoblasts are bone-forming cells that produce the bone matrix, which is subsequently mineralized. The action of osteoblasts is balanced by osteoclasts, which are multinuclear cells related to macrophages that break down bone. The osteoclasts are very effective, and can move on the skeletal surfaces between different sites.

The entire bone surface is covered by cells; most are inactive osteoblast-derived cells that are called bone lining cells. There are similar inactive cells within the bone in lacunae; these cells are called osteocytes, and they are derived from osteoblasts (Figure 5). Osteocytes are thought to be mechanosensors and are interconnected to each other through canaliculi within the bone [28].

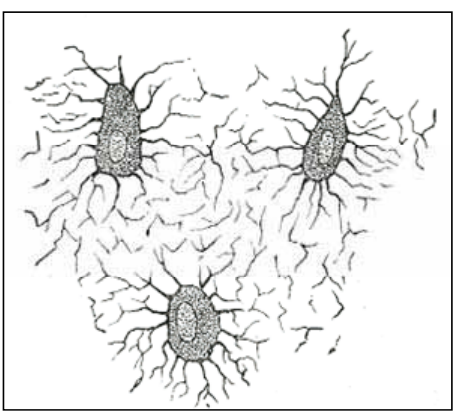


Figure 5. Osteocytes are embedded within the bone and are connected by canaliculi.

Remodeling of the skeleton

In the adult skeleton, even after the cessation of growth, there is still continuous metabolic activity. The skeleton is constantly remodeled in order to prevent and repair microdamage, and to adapt the skeleton to changes in mechanical loading. This continuous process takes place on the skeletal surfaces at bone multicellular units (BMUs), which are small areas where osteoclasts and osteoblasts work in unison to replace old bone [26] (Figure 6). The multinucleate osteoclasts adhere to the bone surface and secrete demineralizing and proteolytic substances to create a pit on the bone surface of for example trabeculi, or a canal into cortical bone. The newly exposed bone surface is then covered by osteoblasts that produce osteoid; this protein mixture makes up the bone matrix, which is subsequently mineralized. The process of remodeling is tightly regulated, and there is a close coupling between resorption and formation. Remodeling ensures that good quality bone tissue is maintained, however various factors can affect the remodeling process. In aging and in osteoporosis, for example, uncoupling of resorption and formation occurs, leading to progressive bone loss. Nutritional and hormonal factors, inflammatory processes, pharmacological agents, and skeletal loading can also influence remodeling.

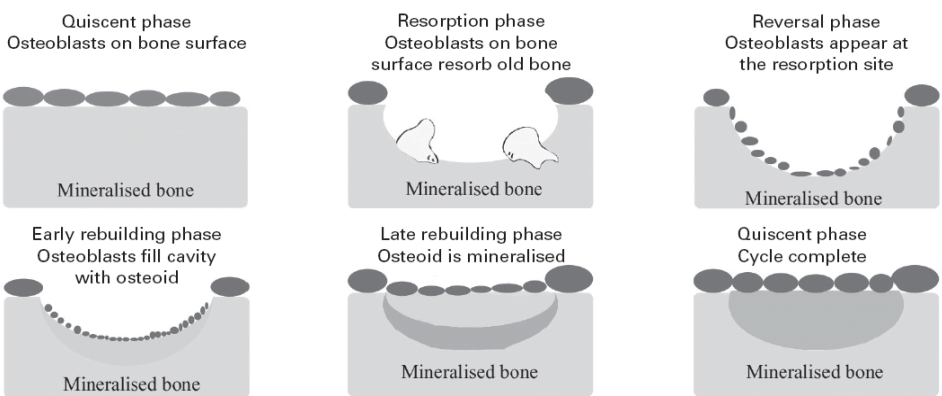


Figure 6. Bone remodeling at a bone multicellular unit (BMU).

Bone development

Growth and development in infancy

The newborn infant's skeleton is very small and only contains about 30 g of calcium [29]. Gestational age is a major variable in affecting the bone mass at birth, since the majority of bone accumulates during the last trimester. An infant's size is determined more by maternal nutrition and placental and intrauterine factors than by genetic factors [29, 30]. During the next two decades, the skeleton increases in size and in bone mineral content (BMC). In fact, the calcium content increases 40-fold to about 1200 g (Figure 7), and the phosphorous content increases from 17 g to 700 g [26, 31]. This growth takes place at the epiphyseal growth plates at the end of the long bones, and the process, called endochondral ossification, is responsible for increasing the length of the bones [32, 33]. As bone length increases, the bone must also increase in diameter. This increase in width by appositional growth occurs when osteoblasts form compact bone around the external bone surface. Meanwhile, cancellous bone in the center is gradually resorbed around the medullary cavity in a process called endosteal resorbtion [34]. Simultaneously, but not necessarily at the same pace, the BMC increases. During infancy, BMD of the total body increases by 157%, and whole body BMC increases by 389%. The strongest predictor of BMD and BMC during this period is body weight [35].

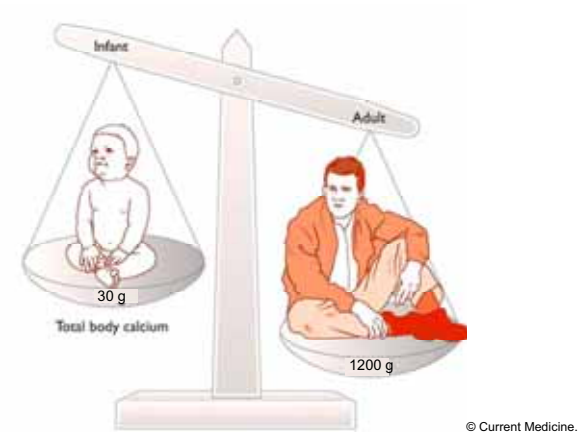


Figure 7. The calcium content of the skeleton increases 40-fold from infancy to adulthood.

In the first 6 months after birth, the volumetric bone density decreases by about 30% [36-38]. This is mainly due to endocortical resorption that leads to an increase in the size of the marrow cavities [36, 37, 39]. During this time, the bone starts to grow rapidly in length; bone strength is maintained by simultaneous periosteal apposition of bone on the bone surface where the effect on stability is the highest [40, 41]. The result is a redistribution of bone from the endocortical to the periosteal surface (Figure 8) [30, 42].

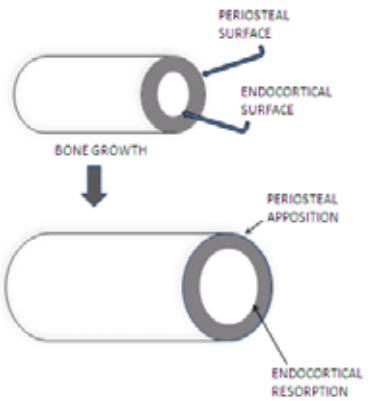


Figure 8. Bone remodeling during growth by periosteal apposition and endocortical resorption.

Growth and development in childhood: 2-12 years

During childhood, the bone is subjected to greater forces than in infancy: The longitudinal growth increases lever arms and bending moments, and it has been suggested that the added muscle force increases bone formation during muscle contractions [40, 43]. Growth during the childhood period is strongly influenced by growth hormone (GH) and thyroid hormone [30]. GH is essential for the proliferation of cartilage cells at the epiphyseal plate, and thus stimulates linear growth; furthermore, GH stimulates protein synthesis and inhibits the formation of fat and carbohydrates. Thyroid hormone is also essential for growth during childhood, and jointly with GH promotes cartilage and bone formation [30, 44]. Childhood is characterized by relatively steady growth: Generally, a child grows 5-6 cm per year and gains about 2.5 kg/year [30].

Growth and development in adolescence

Adolescence is the period when a child matures into an adult. It starts with puberty, when secondary sex characteristics develop in both boys and girls. During the adolescent growth spurt, the skeleton grows rapidly and the bones increase in size, followed slightly later by a rapid increase in BMC. This is a particularly important time in skeletal development, and largely determines the adult skeleton's size and BMC (and thus bone density). Hormonal increases mark the onset of puberty and occur at approximately 11 years of age in girls and 13 years in boys [45](Figure 9). The pubertal period begins when gonadotropin-releasing hormone (GnRH) pulses trigger an increase in the amplitude of follicle stimulating hormone (FSH) and luteinizing hormone (LH) pulses. The result is an increase in gonadal sex steroid output that increases GH and IGF1 production [30, 46]. Both the sex steroids and the GH-IGF1 axis play important roles in skeletal maturation, pubertal growth, and bone and muscle mass accrual, and they probably work in concert [30, 47, 48]. The increases occur in the transition between Tanner stages 1 and 2, with peak levels occurring around Tanner stages 4 and 5 followed by a rapid decline. Moreover, sex hormones stimulate renal 1,25-dihydroxyvitamin D production, which contributes to bone accumulation during puberty [42, 46]. 1,25-dihydroxyvitamin D levels increase gradually in Tanner 1 and 2, peaking in mid-puberty. The increase in 1,25-dihydroxyvitamin D is associated with increases in total body BMC or BMD and radial BMD [42]. Adequate calcium intake is important during puberty: The daily requirement is higher as calcium is needed for rapid bone growth during this period.

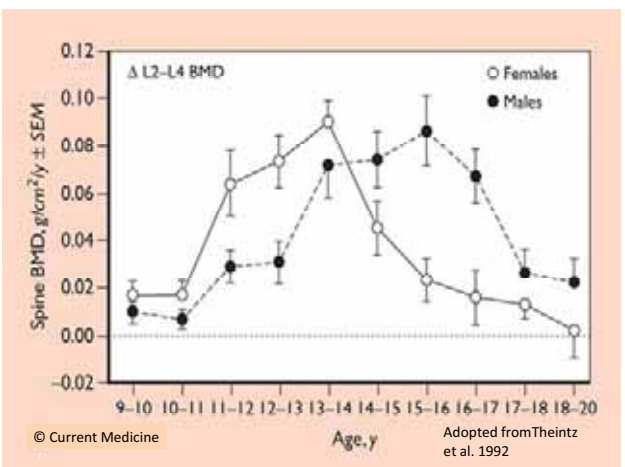


Figure 9. Adolescent bone accrual [49].

Adolescence is a period of rapid skeletal growth, during which the skeletal mass approximately doubles [50]. The result of puberty is a rapid increase in skeletal mass, which is partly due to increases in longitudinal growth and partly due to increases in cortical thickness. The increase in cortical thickness is thought to be mediated through both periosteal envelope expansion and reduction in marrow width, similar to that which occurs during childhood growth (Figure 8) [48]. High levels of bone formation and resorption markers are observed in early- and mid-puberty [51, 52] as indicators of increased bone remodeling. Linear growth precedes bone mineral accrual (Figure 10). Peak bone mineral accrual occurs approximately 0.7 year after the time of peak height velocity (PHV) [53]. Here at about 11.6 years of age for girls and 13.5 for boys, the adolescents have reached 90% of adult height but only about 60% of adult BMC values. The discrepancy between bone size and mineral content during this growth spurt can lead to transient bone weakness, possibly contributing to the high incidence of fractures at this age [54].

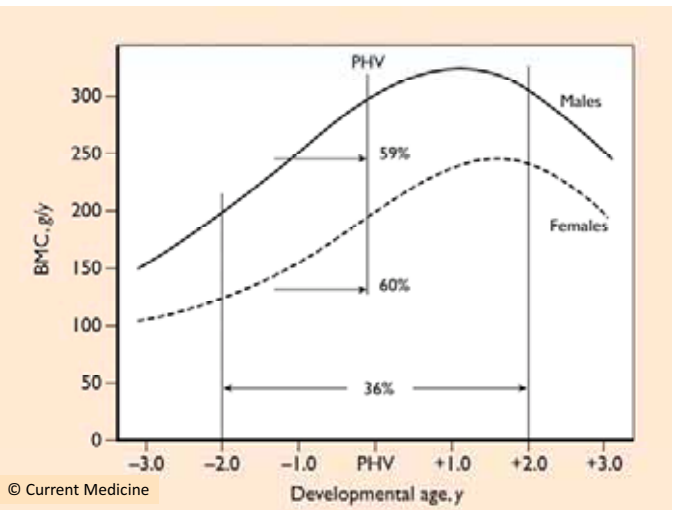


Figure 10. Peak height velocity precedes peak bone mineral accrual. This may lead to transient bone weakness in adolescence [53, 54].

The pattern of growth differs in boys and girls, partly due to the two-year delay in the start of puberty in boys compared to girls, and partly due to the fact that the pubertal growth spurts in boys last almost 1 year longer than in girls[49, 55-58]. Estrogen is responsible for the ossification of the growth plate, which causes cessation of growth in late puberty [59]. These differences may help explain the up to 10% greater height and 25% greater peak bone mass in boys [60].

Peak bone mass

Peak bone mass is a very important concept in skeletal physiology. It refers to the point in time at which a person's bone mass reaches its peak level. However, the timing of peak bone mass is not entirely uniform: Lumbar spine, femoral midshaft, and neck are estimated to obtain peak bone mass at 16-18 years of age [49, 57, 61], whereas the radius, skull, and whole body are estimated to reach peak bone mass as late as 35 years of age [61, 62]. After the attainment of peak bone mass there is a progressive loss of bone, but the peak bone mass accounts for more than half of the variation in bone mass until at least 65 years of age [63, 64].

The period of bone growth around the time of attainment of peak bone mass is therefore of great interest, as even small increases in peak bone mass may have a great influence on osteoporosis and fracture risk [64, 65] (Figure 11).

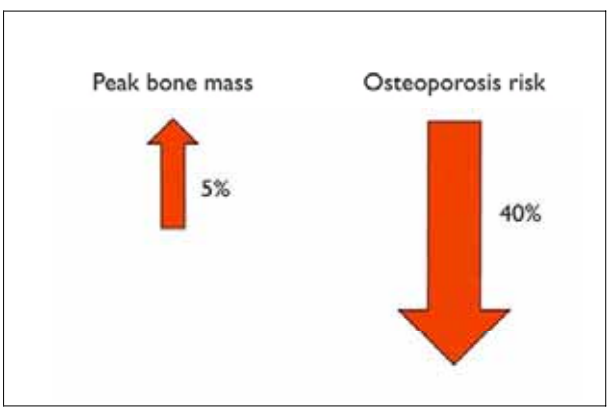


Figure 11. Small increases in peak bone mass may greatly influence osteoporosis risk [65].

Biochemical markers of bone turnover

The skeleton is metabolically active, and bone formation and bone resorption continually rebuild the skeleton. Normally bone formation and resorption are closely coupled, but in osteoporosis these processes can be un-coupled so that bone resorption exceeds bone formation, leading to net bone loss.

If bone loss could be detected early in the disease process, early diagnostic and preventive measures could be initiated. Loss of bone tissue can be monitored by bone densitometric techniques such as DXA. However, the rate of bone loss is usually slow and gradual, and the precision of even the best densitometric techniques do not allow detection of short-term bone losses. While the short-term precision of DXA can be high, around 1%, the long-term precision is about 2-3% [66-68]. Because average postmenopausal bone loss is on the order of 1% per year, it can thus take several years to detect changes, making it difficult to estimate the rate of bone loss by DXA [69-71]. It is also difficult to monitor osteoporosis preventive measures or the efficacy of osteoporosis medications as the bone density response is slow. Bone research is similarly complicated, as small changes in bone structure and density are difficult to detect by current techniques; this makes bone research time consuming and tedious.

It is desirable to detect bone loss at a much earlier stage and to develop more sensitive methods for monitoring bone changes. This would allow earlier diagnosis of osteoporosis and better monitoring of osteoporosis interventions. Pharmacological treatments can be costly, be prescribed for many years, and have undesirable side effects. Efficient monitoring of bone biology could potentially identify the treatment that is most suitable for an individual patient. Research efforts have focused on developing bone analysis methods using biochemical markers [72], and many different biochemical bone markers have been evaluated. Over the years, many studies have demonstrated that the levels of several different bone markers correlate with bone density, bone loss, and fracture risk [73-77], and can be used to evaluate aspects of drug treatment and drug compliance [78-81]. Biochemical bone marker analysis is fraught with many difficulties, limiting its clinical usefulness. Information on frequently utilized bone biochemical markers is summarized in the next section.

Markers of bone formation

The most frequently used markers of bone formation are alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BAP), osteocalcin (OC), and the procollagen extension peptides, aminoterminal propeptide (PINP) and carboxyterminal propeptide (PICP). These markers can all be analyzed in serum samples [4].

Alkaline phosphatase

ALP is a cell membrane enzyme in liver, bone, kidney, and placenta; the primary sources of serum ALP are liver and bone, and it is produced by osteoblasts and osteoblast precursors [82]. Studies have demonstrated that although it is not bone-specific, ALP is related to fracture risk, current bone density, and bone loss after menopause [73, 74]. BAP can now be separated from liver-specific ALP using monoclonal antibodies [82]; however, BAP levels are believed to be related to excess spent enzyme from several types of bone cells (osteoblasts, pre-osteoblasts, lining cells, and osteocytes), and therefore may not provide very specific or reliable information about bone metabolism [82]. Similar to ALP levels, BAP levels correlate with bone loss in postmenopausal women [71].

Osteocalcin

OC, also called bone Gla-protein, is secreted by osteoblasts as they deposit new bone matrix. OC is a part of the organic bone matrix and is thought to play an important role in bone mineralization [72, 82-86]. It is a bone-specific marker [85, 86]. OC levels are related to fracture risk, current bone density, and bone loss after menopause [71, 73, 74, 87]. OC is dependent on vitamin K for gamma carboxylation, and a vitamin K deficiency can lead to an increasing proportion of OC becoming undercarboxylated, resulting in impaired bone mineralization. Undercarboxylated osteocalcin (ucOC) has therefore also been used as a bone marker. Correlations between ucOC and hip fractures and hip BMD have been reported [88-91].

Procollagen 1 extension peptides

PINP and PICP are cleaved from procollagen type 1 prior to fibril formation and are considered measures of newly-formed collagen type 1 [85, 86]. They are not entirely bone-specific as they are also secreted from other tissues, but almost all circulating PINP/PICP originates from bone. Intra- and inter-assay variation is 3-7% [72, 85, 92].

Markers of bone resorption

Collagen type 1 is the predominant bone matrix protein, and its degradation products are frequently used as bone resorption markers since their levels increase as bone is broken down. Several different assays for these markers are available.

Collagen pyridinium crosslinks

Immunoassays also exist for the collagen crosslinks pyridinoline (PYR) and deoxypyridinoline (DPYR), which bind collagen fibrils together [72, 86]. These crosslinks are released when bone matrix resorption occurs; they enter the circulation and are excreted in urine [86]. DPYR is more bone-specific as PYR also exists in articular cartilage [86]. PYR and DPYR levels correlate with bone loss in postmenopausal women [71].

CTX/NTX

Among the most frequently used collagenous resorption markers are C-terminal crosslinks (CTX), a fragment from collagen type 1 crosslinked C-telopeptide, and N-terminal crosslinks (NTX), a fragment from collagen type 1 crosslinked N-telopeptide [86]. Both of these fragments can be analyzed by immunoassays using serum or urine [72, 86]. CTX and NTX are regarded as sensitive and specific indices of bone resorption, and have been successfully used to monitor and predict the response to osteoporosis treatments; in fact, they may be the most specific and responsive markers for osteoclast activity [80, 81, 87, 93-95]. NTX levels generally correlate with BMD measurements [76], although not all studies have found significant correlations [96]. CTX is an independent predictor of hip fracture risk [97]. In a study of elderly women, Garnero et al. found that a combination of bone density measurements (dual energy X-ray absorptiometry or calcaneal ultrasound broadband attenuation) plus CTX analysis increased the sensitivity in predicting hip fractures [97, 98]. Furthermore, it was found that combining CTX measurements with fracture history predicted hip fractures as well as hip DXA.

Utility of bone markers

As outlined above, several studies have demonstrated that common bone markers correlate with current bone density, rate of bone loss after menopause, and fracture risk. While the use of bone markers may potentially increase the sensitivity of fracture prediction when used in addition to BMD, no studies on pharmacological treatment on the basis of bone marker measurements have been performed; therefore, the clinical usefulness for treatment selection remains unclear [4, 98, 99]. Furthermore, the results of bone marker studies may not be sufficiently uniform to allow prediction of BMD or fracture risk for individual patients. Biochemical markers are not recommended for diagnosing osteoporosis, nor is it clear whether or how these markers can be used clinically for fracture prediction [4, 99-101]. A study of urinary NTX in a clinical setting found that the test rarely influenced osteoporosis management by clinicians [102]. Further, a study of postmenopausal women on HRT or placebo concluded that neither BAP, OC, DPYR/PYR, nor CTX/NTX offered useful information for predicting BMD or BMD changes [101].

Bone mass measurements

The diagnosis of osteoporosis is primarily based on bone density measurements. Over the last several decades, different techniques for bone density measurements have been developed. Each of these techniques has its advantages and drawbacks: Some are well investigated and proven, while others are fairly new and have yet to prove their worth in research and clinical practice. The multitude of available methods is both a blessing and a curse. The field of clinical osteoporosis diagnosis and treatment is still fairly new, and the different available methods that use different reference values and measuring sites adds to the difficulty for clinicians entering this field. For hospital management, it can be difficult to decide what equipment is the most appropriate and most needed. A brief outline of current methods is therefore in order.

DXA

Dual Energy X-ray Absorptiometry (DXA, sometimes called DEXA) is regarded as the "gold standard" in osteoporosis research and practice (Figure 12) [103-106]. The WHO osteoporosis criteria (see section Osteoporosis) and most treatment guidelines are based on DXA results. Most bone and osteoporosis research employing BMD measurements has also been performed using DXA.

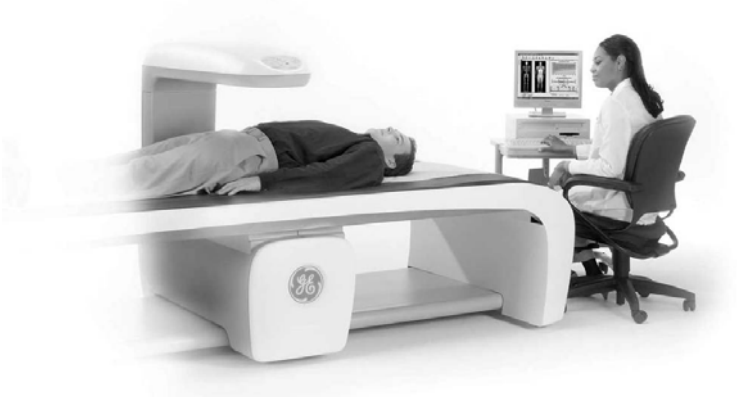


Figure 12. DXA equipment: the GE Lunar iDXA.

DXA is a radiation-based technique that has replaced other less accurate radioabsorptiometric methods. DXA employs two different energy levels of radiation to separate calcium from soft tissue by means of computer analysis, thus eliminating the need to manually correct for soft tissue thickness. Simultaneously, DXA enables accurate and precise body composition analysis (Figure 13), providing information about tissue fat content and fat-free or lean content (the latter roughly corresponds to muscle mass) [107].

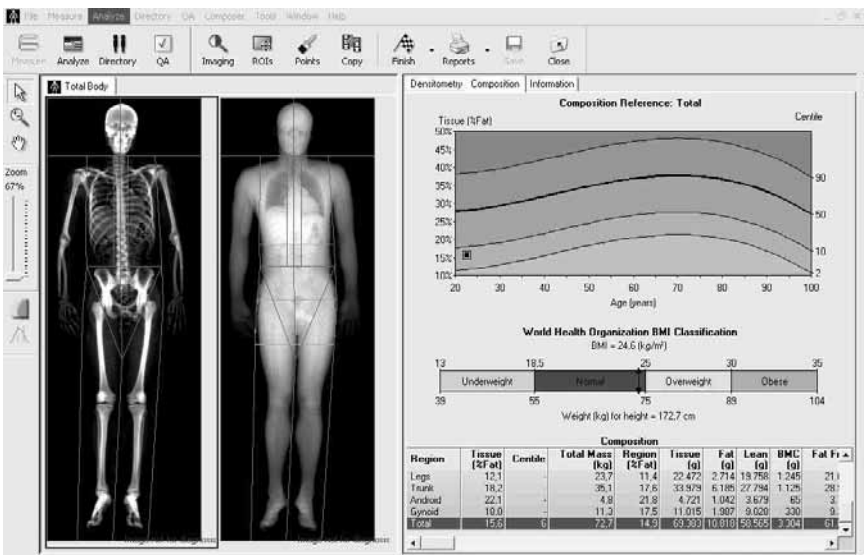


Figure 13. DXA measures not only bone parameters, but also help estimate body composition parameters such as fat mass and muscle content, which can be very useful for both research and clinical purposes.

First generation DXA equipment employs a narrow pencil beam of radiation; while the scans are time consuming, they have no magnification error and the radiation dose is very low—about 2 microSievert (μSv) per scan. In contrast, natural background radiation is about 2400 μSv per year [108]. This means that no radiation shielding is needed for patients or technicians during DXA testing. Newer DXA systems often employ a "fan-beam," resulting in much faster scanning time and higher resolution. The newest computer software can even detect vertebral compression fractures from the scan. Drawbacks of the fan-beam DXA equipment include problems with magnification errors, which do not affect BMD, but do affect BMC, bone area, and soft tissue measurements. These errors can, however, be corrected [109-112]. Another issue is radiation exposure. Although fan-beam DXA systems use higher levels of radiation than do pencil-beam systems, the patient doses are still very low; however, technician doses could be a concern [113-115].

As noted above, one advantage of DXA is very low radiation exposure, making it safe for both patients and hospital staff. This also means that DXA can be used in regular hospital rooms, since no special radiation shielding is needed. Another advantage of DXA is that the measurements are very precise. The coefficient of variation is often reported to be <1% between scans; accuracy remains a larger problem, but is usually within 10% [66-68, 70]. Current fan-beam DXA scans take just a few minutes, making it possible to scan many patients per day at a relatively low cost. DXA can measure BMC and BMD of any desired skeletal site, albeit with varying precision (Figure 14).

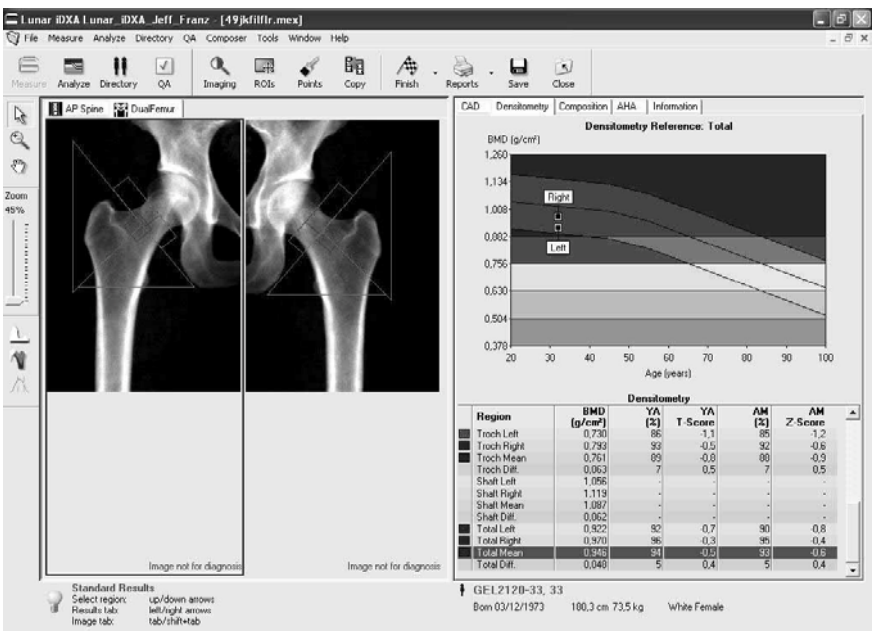


Figure 14. DXA equipment can measure the BMD of any skeletal region, including the most clinically relevant sites such as the hip.

DXA can thus measure the BMD of the most clinically relevant sites, such as hips and vertebrae. It has been shown that site-specific measurements correspond better to bone strength at the site than do non-site-specific measurements [106, 116]. DXA can also predict fracture risk very well, even better than blood pressure readings predict stroke, and better than serum cholesterol predicts cardiovascular disease [20, 117]. Metaanalyses indicate that fracture risk roughly doubles for each standard deviation reduction in BMD [4, 117]. Especially proximal femur measurements may

predict future fractures well: A decrease in bone density of 1 standard deviation at the femoral neck corresponds to a 2.6-fold greater risk for hip fracture, a similar decrease at the spine corresponds to a 2.3-fold greater risk for vertebral fracture (Figure 15) [4, 117, 118].

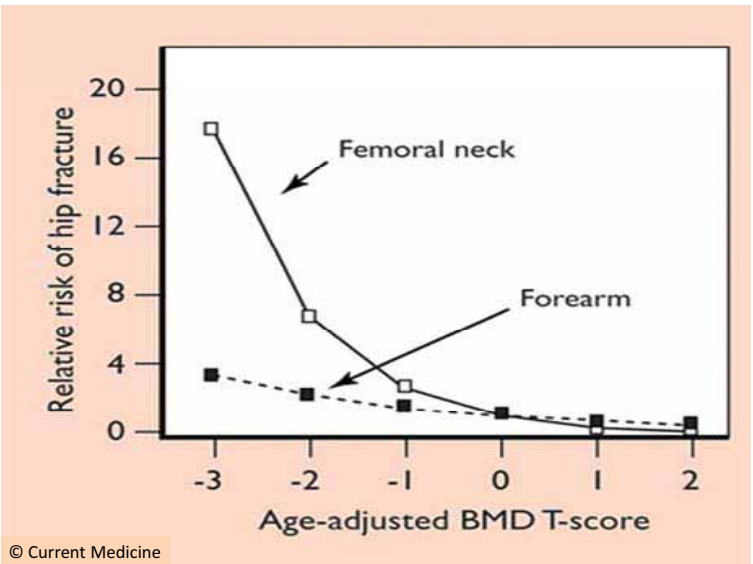


Figure 15. The relationship between DXA BMD measurements and fracture risk [118].

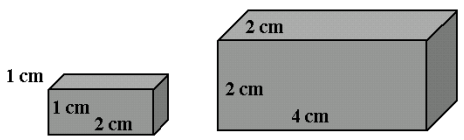
DXA measures areal bone mineral density (aBMD) as opposed to true volumetric density (vBMD). That is, the bone mineral per square centimeter of bone area viewed by the scanner is recorded and expressed as g/cm^2 instead of being expressed as g/cm^3. This means that bone size affects the results of a measurement: A large bone will have a higher aBMD than a small bone, even if the vBMD is the same (Figure 16). This can lead to some difficulty in interpreting aBMD results; however, this might be compensated by the fact that larger bones are suggested to be stronger [119, 120]. So far, attempts to switch to vBMD measurements have not been successful in terms of clinical relevance [119-121].

A disadvantage of DXA is that interpretation of lumbar spine BMD measurements in older subjects is difficult as compression fractures or degenerative changes can give falsely elevated values; thus, most current guidelines suggest that the BMD of

the femoral neck should be used for diagnosing osteoporosis [19, 70]. It should also be noted that different DXA machines may give different results [109, 122-124]. The reasons for this include different dual energy methods, different calibration, different detectors, different edge detection software, different regions of interest, and different reference populations. Therefore follow-up measurements should be made on the same DXA equipment. In multicenter studies, cross-calibrations of the DXA machines are required.

Effect of bone size on bone mineral measurements

(two bones with equal volumetric bone mineral density)



Projected area, cm^2	2	8
Volume, cm^3	2	16
vBMD, volumetric bone density, g/cm^3	1	1
aBMD, areal bone density, g/cm^2	1	2
BMC, bone mineral content, g	2	16

Figure 16. Bone size influences DXA measurements.

Standard whole body DXA scanners are large and expensive, costing around 1 million Swedish crowns (~100,000 euros), and require specially trained technicians. Facilities with limited patient volume and limited funding may hesitate when considering the cost of the equipment. Therefore a market for small, inexpensive, and portable peripheral scanners has emerged. Heel DXA scanners, which measure calcaneal BMD, have been developed to meet this demand. There is currently a lack of reference data, as well as limited knowledge of how the peripheral data relate to more central measurements, to the WHO osteoporosis criteria, and to treatment cut-off values. As previously discussed, there is better correlation for fracture risk if site-specific DXA measurements of hip and spine are performed [117], and site-specific measurements correlate better to bone strength [106, 116].

Quantitative ultrasound

Quantitative ultrasound (QUS) bone density scanners have also been developed to meet the demand for inexpensive, radiation-free portable devices (Figure 17). The "broadband ultrasound attenuation" (BUA) and "speed of sound" (SOS) techniques, or modifications of these techniques, are typically used [104]. BUA measures loss of energy in the ultrasound passing through the bone, and SOS measures the speed of sound, since the speed of sound increases in cortical bone (and, to a lesser extent, in trabecular bone) [104]. It has been suggested that these techniques may provide additional information about bone quality properties, but this is not yet well-established [125-128]. Studies combining densitometric techniques with bone strength testing indicate that QUS is inferior to DXA in predicting femoral and radius bone strength [106, 116]. However, there is an association between low QUS values (both by BUA and SOS) and elevated peripheral fracture risk. The predictive value is similar to DXA, with relative risk increasing >1.5-fold for every standard deviation decrease [129, 130]. The precision of QUS is good, with coefficients of variation of less than 4% for BUA and less than 0.5% for SOS [103, 131, 132]. A drawback of QUS is that measurements are limited to peripheral bone sites such as the calcaneus; as discussed previously for heel DXA, this measurement has yet to be related to current values used to diagnose osteoporosis.

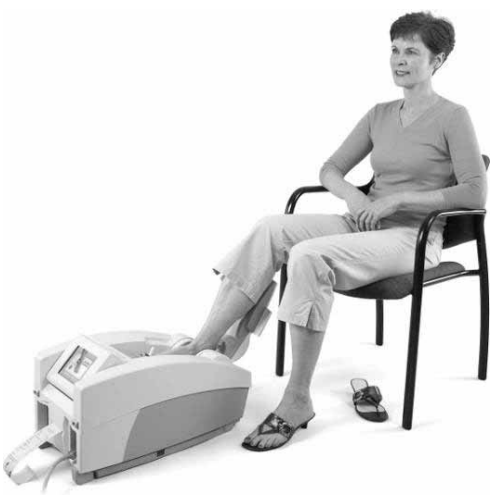


Figure 17. A QUS scanner is relatively inexpensive, but can only measure peripheral skeletal sites such as the calcaneus.

There are furthermore great technical differences between equipment from different manufacturers. QUS scanners may employ different type of transducers, different ultrasound frequencies, analyze data in different ways, and measure different skeletal sites or different regions of a site [104]. Therefore it is not possible to talk about QUS as one specific method as it is a multitude of related technologies [127]. This also makes it difficult to compare the results of different scanners. A big problem is that T-scores measured with QUS cannot be compared to DXA T-scores. QUS does not measure bone mineral, and different reference databases are used [104]. It is not known whether patients with a low QUS T-score will benefit from traditional osteoporosis medication as very few studies have looked into the subject [127].

Quantitative computerized tomography and peripheral quantitative computerized tomography

Quantitative computerized tomography (QCT) is a radiological technique that can measure volumetric bone density. It can potentially discriminate between cortical and trabecular bone density, and further refinement may allow high resolution mapping of trabecular bone structure. QCT is very precise, with variation as low as <1% [133, 134]. Peripheral QCT (pQCT) is another newly-developed technology that measures peripheral sites. Whether QCT and pQCT techniques offer clinical benefits compared to DXA is unclear, as studies comparing these techniques and their relation to bone strength have not found advantages compared to DXA [106, 116]. A drawback of QCT is the high radiation exposure compared to DXA.

Magnetic resonance imaging

Progress in high resolution magnetic resonance imaging (MRI) techniques enables detailed analysis of trabecular bone structure; in addition, MRI-based bone densitometry is under development [135-137]. MRI can be used to investigate the microstructure of the axial skeleton, as well as the peripheral skeleton, without the high radiation exposure associated with QCT. MRI techniques can potentially provide very detailed bone structure analysis that can be used to assess bone mechanical strength and fracture risk [135, 137]; these techniques have yet to prove their clinical usefulness.

Summary: Bone mineral density measurements

In conclusion, central DXA will remain the gold standard for bone densitometry in the near future. Peripheral techniques such as pDXA and QUS may be useful for screening in facilities in which central DXA is not available. QCT technology is promising, but is currently not a viable alternative for routine scans due to its use of higher levels of radiation. MRI bone analysis and densitometry are also very promising, but are still in the early stages of development. This is a rapidly developing field, and further research and new technologies are likely to impact clinical practice in the coming years.

Physical activity and bone

In 1892, the German anatomist Julius Wolff stated that bone tissue adapts to the loads it is subjected to. This is known as "Wolff's law". If a bone is subjected to heavy loading, it will remodel itself to become stronger; conversely, the bone will lose strength if loading is decreased [138]. He specifically proposed that bone trabeculi become aligned with the predominant loads, a principle that has since been confirmed in animal experiments [139, 140]. More recently, H. M. Frost has presented a very comprehensive "mechanostat" theory on how bone modeling and remodeling allow bones to adapt to changing loads [141, 142]. In both animal and human studies, researchers have found ample support for the principles originally outlined by Wolf.

Effects of skeletal unloading in animal studies

Animal studies clearly demonstrate that skeletal unloading is followed by bone demineralization. Immobilization of rats' hind limbs by either sciatic neurotomy or tenotomy results in rapid bone demineralization and loss of trabecular and cortical bone volume [143, 144]. In a study of hind limb-suspended rats in which one group was allowed to exercise and the other group was not, the exercising group preserved femoral BMD while the passive rats lost both femoral BMD and muscle mass [145]. Simulated weightlessness in mice, created by suspending the mice by their tails, led to rapid osteocyte apoptosis, increased osteoclast numbers, loss of trabecular and cortical volume, and reduced BMD and bone strength [146].

Effects of unloading/immobilization on bone in humans

The effects of unloading on the human skeleton have been thoroughly studied in experimental settings. For example, significant bone loss is observed in astronauts, who experience unloading for long periods of time (Figure 18) [147]. The same results were obtained during prolonged experimental bed rest [148]. In both cases, bone loss was followed by at least partial recovery after skeletal loading resumed [147, 148].

Bone loss following unloading has also been observed in clinical settings. Limb disuse or immobilization resulting from painful joint disease, trauma, or surgery results in bone loss, predominantly in the affected limb[149-152]. In stroke (patients with hemiparesis, there is rapid bone loss on the affected side that is subjected to less loading; in contrast, there is increased bone density on the non-affected side, possibly due to increased loading on the healthy side [153, 154]. This phenomenon has also been observed in patients with spinal cord injuries in which rapid bone loss

occurred in paralyzed areas: while quadriplegics suffer bone loss in both arms and legs, paraplegics lose bone primarily in the lower limbs while maintaining BMD better in the arms [155-157] .

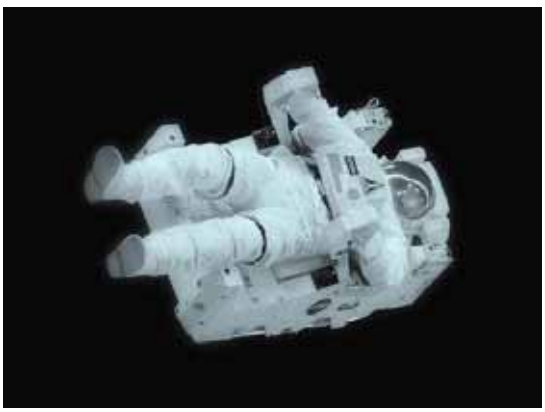


Figure 18. Unloading of the skeleton during spaceflight can result in bone loss.

Effects of skeletal loading in animal studies

Studies in animals have revealed that the best osteogenic response is achieved by intense and regular dynamic skeletal loading; only a low number of loading cycles per day is necessary, and increasing the number of loading cycles does not confer additional benefits [158-161]. In response to loading, trabecular orientation is altered, new bone is formed, and increases in bone weight, cortical area, and breaking strength can been observed [139, 158, 162].

Effects of physical activity on bone in children

The effects of physical activity on bone in children have been studied extensively. Physical activity seems to have a beneficial effect on the growing skeleton in both boys and girls. In cross-sectional studies, non-randomized intervention trials, and randomized controlled exercise intervention trials in children, both girls [163-169] and boys have better bone mineral accrual, higher BMD gains, higher BMD values, and in some studies, greater bone size compared to controls who exercise less(Figure 19), the effects tend to be site-specific, so that, for example, lower extremity loading primarily leads to lower limb improvements [163, 165-167, 170-172]. Overall, there is ample and convincing evidence that physical activity has a positive effect on bone development in children of both sexes.

Figure 19. Physical activity in childhood is associated with BMD gains.

Effects of physical activity on bone in adolescents

In adolescents, the relationship between physical activity and bone development is not as clear as in pre-pubertal children. Cross-sectional studies usually demonstrate higher BMD levels in athletes compared to controls, and these BMD increases are seen primarily in loaded regions of the skeleton [173-176]. There may be critical periods in adolescence during which physical activity has a greater impact on skeletal growth, while in other periods exercise may have less of an impact. This seems to be the case for girls in particular.

Thus, Morris et al. studied the effect of a physical activity intervention program in pre-menarcheal 9- to 10-year old girls. The intervention group had significantly greater gains in total body, proximal femur, and femoral neck BMD compared to the control group [177]. In a randomized controlled intervention study of girls' physical activity, McKay et al. found that physical activity correlated with improved bone accrual in femoral neck and spine in early pubertal girls, but not in pubertal girls [164]. Similar results were noted in a controlled intervention study by Heinonen et al. in which high-impact step aerobic training in pre-menarcheal girls was associated with BMD gains, whereas identical training in post-menarcheal girls was not associated with BMD gains [178]. In pubertal girls, physical activity seems to have less of an effect on bone. In a study of 14- to 18-year-old girls by Blimkie et al., strength training was associated with high strength gains in arms and legs but not with BMD gains [179]. In a intervention study by Sundberg et al., the effects of increased physical education in school was studied in 40 boys and 40 girls from age 12 to 16 and compared to control groups [180]. In boys, increased physical education

correlated with increased BMD and BMC in weight-loaded regions of the skeleton, but no such effects were seen in the girls. Male weightlifters 15-20 years old have significantly higher BMC compared to matched controls [181, 182]. Young adolescent male athletes followed from age 16 to 19 years demonstrated significant gains in total body, humerus, and femoral neck BMD compared to controls [183].

In summary, the timing of physical activity intervention may be of great importance. Bailey reported that the peak bone accrual velocity in the femoral neck occurs about 2 years earlier in girls than in boys [53], and Theintz et al. reported similar results [49](Figure 20). It seems plausible that training interventions that coincide with the period of maximum bone growth would result in better bone mineral gains than interventions that take place after this period. This may explain the data from studies comparing training interventions in pre-menarcheal and post-menarcheal girls [164, 177, 178] (Figure 20).

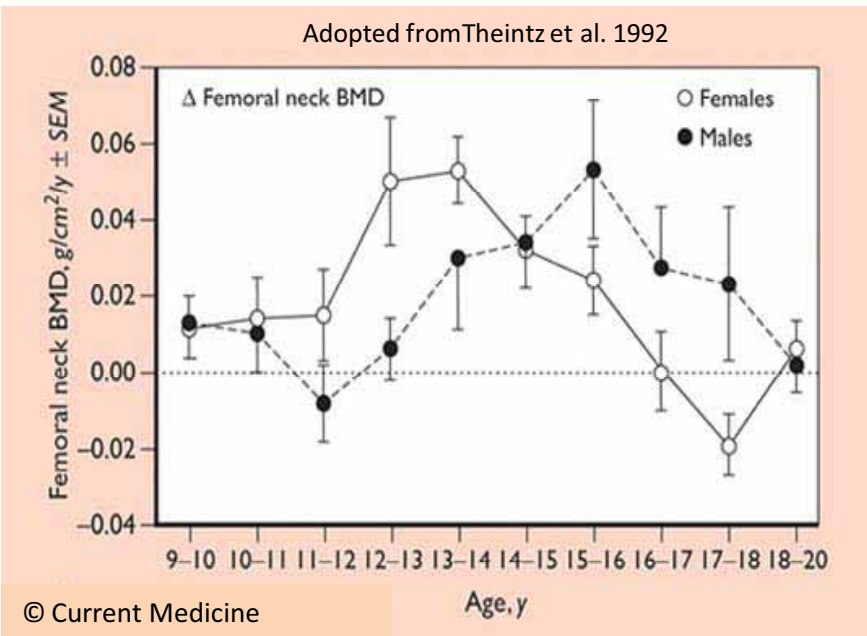


Figure 20. Peak bone accrual occurs about 2 years earlier in girls than in boys[49, 53].

Effects of physical activity on the skeleton in premenopausal women

Cross-sectional studies in premenopausal women usually demonstrate a positive association between physical activity and BMD levels, but these studies are difficult to interpret as they may also reflect previous physical activity [184-189]. Intervention studies in adult premenopausal women have yielded varying results. In a randomized controlled intervention trial, Heinonen et al. found that high-impact exercises such as aerobics and step aerobics correlated positively with BMD gains in the proximal femur, but not at non-weight bearing sites. Aerobic and muscular performance improved simultaneously [190]. Another randomized controlled high-impact study by Bassey et al. found that proximal femur BMD improved significantly compared to controls after jumping exercises, but lumbar spine BMD did not improve [191]. This suggests that high-impact exercises may have a positive but site-specific effect on the skeleton. Studies involving resistance training have produced less favorable results: While muscular strength improves, little or no effect on the skeleton is observed [192, 193].

Effects of physical activity on the skeleton in postmenopausal women

In postmenopausal women, randomized controlled intervention trials studying the effect of physical activity on bone have produced varying results. Several randomized controlled studies have failed to show any positive effects on BMD from physical activity. A study of 50- to 60-year-old postmenopausal women participating in a program involving heel-drops found that this activity did not result in BMD differences compared to a control group training only with low-impact activities [194]. In an 18-month study involving high-impact exercise, Bassey et al. found no difference between postmenopausal exercisers and controls [191].

Other studies have shown a modest effect of exercise on skeletal health. In a 12-month Canadian study, subjects performing impact physical activity maintained spine BMD, while the control group lost spinal BMD; however, the training program had no effect on femoral BMD [195]. In a 2-year study comparing calcium supplementation with and without physical activity intervention, smaller losses in femoral neck BMD were observed in the physically active group [196]. In a brisk walking intervention program, increases were seen in ultrasound-determined calcaneal BMD compared to controls, although there were no significant differences in femoral neck and spine BMD [197]. Vincent et al. found that resistance training had a positive effect on femoral neck BMD in elderly subjects [198].

In summary, the effect of physical activity interventions on BMD in postmenopausal women is unclear, and the gains are modest at best.

Effects of physical activity on the skeleton in men

Cross-sectional studies in men have reported higher BMD levels in athletes participating in weight-bearing sports compared to controls, and the effects have been sport-specific with the greatest BMD differences at skeletal sites subjected to high loads [187, 199-202]. Low-impact sports such as swimming do not convey BMD benefits in men [202].

In a 4-year randomized controlled study of regular low-impact, aerobic physical activity in males ages 53-62, there was no beneficial effect on BMD parameters compared to non-active controls. Cardio respiratory performance, however, improved by 13% [203]. In a randomized controlled study of heavy resistance training in elderly men (and women), significant BMD gains were seen in the femoral neck [198].

In adult men, there is ample and convincing cross-sectional data supporting a positive effect of physical activity on BMD. There is little data from randomized controlled prospective studies that support these findings.

Long-term effects of physical activity on osteoporosis and fracture risk

Taken together, the studies discussed in the previous sections generally suggest that physical activity favorably influences BMD, especially in males. In females the effects of physical activity on bone are less clear and seem to be related to maturity and hormonal changes. The optimal timing, duration, and intensity of the physical activity is not completely clear. It would be helpful to determine whether the beneficial effects on bone observed in athletes and in some intervention studies can be maintained into old age and whether these beneficial effects decrease the risk of osteoporosis and fracture. Several studies in both men and women have demonstrated the long-term BMD benefits of prior physical activity [204-207]. In the elderly population, the effects of previous physical activity may be attenuated, with other lifestyle factors having a larger influence [206]. Even decades later, prior physical activity seems to correlate with a decrease in fracture risk, at least in men [208, 209].

Vitamin D and bone

During the last decade, vitamin D and its effect on the skeleton and on fracture risk have received a lot of interest and generated considerable debate. Vitamin D is a fat-soluble vitamin with hormone-like properties [210](Figure 23). Two main forms exist, vitamin D_2 and D_3, along with several precursors and metabolites. For humans, the most important source of vitamin D (80-100%) is endogenous production in the skin. Vitamin D_3 is synthesized from the cholesterol derivative 7-dehydrocholesterol under the influence of sunlight or UV light [210, 211] (Figure 21).

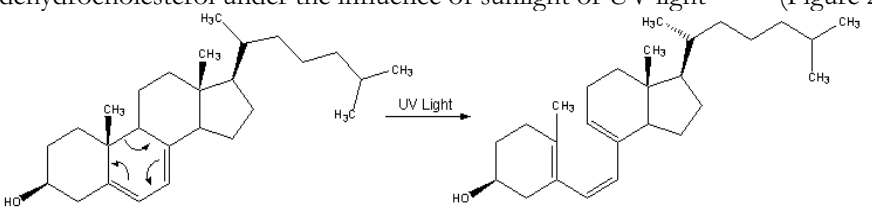


Figure 21. In the skin, 7-dehydrocholesterol is converted to cholecalciferol (Vitamin D_3) by UV radiation.

Adequate sunlight is needed for this process. In northern latitudes such as Scandinavia, vitamin D_3 can only be synthesized in the skin during the summer months; the stored vitamin D is then gradually depleted during the fall, winter, and spring [212-214]. The skin's ability to synthesize vitamin D is reduced in the elderly. Clothing that blocks sunlight can also reduce vitamin D synthesis in the skin, and Muslim women that use a veil are at particularly high risk for vitamin D deficiency [215].

Standard diets contain low and inadequate amounts of vitamin D [210, 216-218]. Small amounts of vitamin D_2 are available from plants; limited amounts of vitamin D_3 can be found in animal-based foods such as meat, fish, and eggs, and fish liver oil is a good source of vitamin D_3 [210, 216](Figure 22)

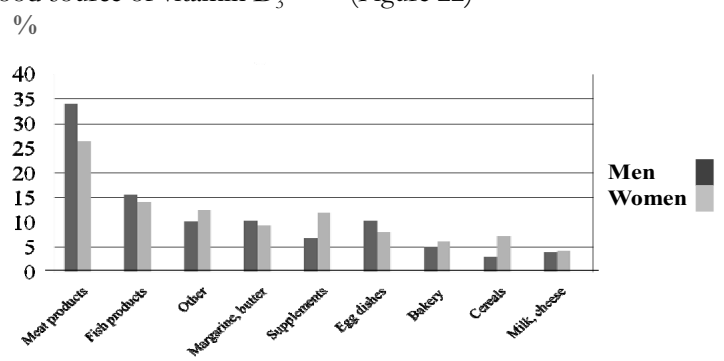


Figure 22. Relative contributions (%) of various foods to total vitamin D intake in Ireland [216].

Simplified overview of vitamin D metabolism

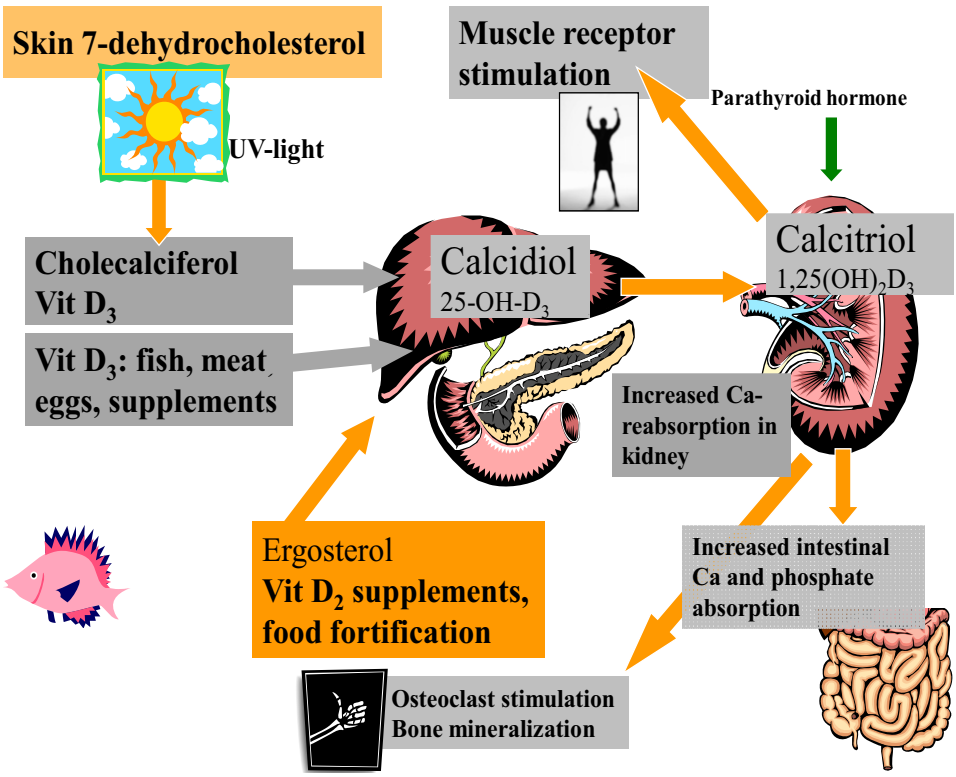


Figure 23. **Simplified overview of vitamin D metabolism and actions** [210]. Vitamin D is primarily synthesized in the skin upon exposure to UV light. It is also available in the diet, but to a lesser extent. Skin-synthesized and dietary vitamin D, which is predominantly vitamin D_3, is transported to the liver where it is 25-hydroxylated. The liver, together with fat tissue, serves as a storage depot for 25-OH-vitamin D_3. In the kidney, 25-OH-D_3 is converted into its active form, 1,25(OH)2-D_3 (calcitriol). This step is tightly regulated. The parathyroid gland senses plasma calcium levels and secretes parathyroid hormone (PTH) in response to decreased calcium levels. PTH then stimulates calcitriol production in the kidney. Calcitriol enhances intestinal calcium resorption and renal calcium resorption, and can also stimulate osteoclast activation. Recent research has identified vitamin D receptors in muscle tissue [219, 220].

Vitamin D deficiency is very prevalent worldwide. If a serum level of 75 nmol/l is chosen as the cut-off level, as is currently recommended, 87% of the white British population (45 years of age) are affected by vitamin D deficiency during the winter and spring [221]. A high prevalence of hypovitaminosis D has been found in other populations as well [222-225]. A study by Välimäki et al. reported rather severe hypovitaminosis D in healthy Finnish military recruits, with as many as 39% of the recruits having less than 20 nmol/l during the wintertime [226]. However, even in areas that receive a lot of sunlight, vitamin D insufficiency is prevalent [227]. In hospitalized patients, the prevalence of hypovitaminosis D is very high, almost universal [228-230].

It has been known for a long time that vitamin D protects the skeleton against rickets. For over a hundred years, vitamin D-containing cod liver oil has been used to prevent that disease. In a landmark study, Chapuy et al. found that vitamin D_3 supplementation significantly and substantially reduced fracture incidence in elderly institutionalized women [231]. The fracture-reducing efficacy of vitamin D supplements in the elderly has since been confirmed in other studies. Three metaanalyses concluded that vitamin D supplementation can reduce the fracture incidence in older people; however, a daily dose of about 800 IU is required [223, 232-234]. These results, combined with the known beneficial effects of vitamin D on calcium metabolism, have resulted in universal adoption of vitamin D for the prevention and treatment of osteoporosis. This practice has been called into question after two large British studies of predominantly elderly females found no evidence that vitamin D supplementation reduced fractures [235, 236]. Thus there is now considerable debate about the benefits of supplementation. Critics assert that these two studies had low compliance and inadequate vitamin D level testing [224]. Recent reports also indicate that higher dosages of vitamin D than are currently recommended would be needed to achieve optimal anti-fracture effects. One reason for this may be the observation that optimal suppression of the osteoclast-stimulating parathyroid hormone is not achieved until serum levels reach about 75 nmol/l; previously, much lower levels were deemed acceptable [222, 223, 237, 238].

Vitamin D influences physiological processes in addition to its effect on skeletal health. Vitamin D receptors have been identified in human muscle tissue [219, 220], and vitamin D levels positively correlate with muscle strength, walking ability, and balance, all of which are factors that could influence fracture risk [239-241]. Furthermore, supplementation with vitamin D has been found to reduce the incidence of falls [242, 243].

To summarize, vitamin D supplementation seems a sensible choice, especially for the elderly. It affects not only bone and calcium metabolism, but may also favorably affect muscular function and reduce fracture risk. The present dosage regimens may need to be reevaluated as higher dosages than are currently recommended seem to confer greater anti-fracture efficiency.

Vitamin A (retinol) and bone

Vitamin A, also called retinol, is of great importance for human health. Vitamin A is a fat-soluble vitamin belonging to a family of chemical compounds known as retinoids (Figure 24). Important food sources of vitamin A include fish oils, liver, eggs, and dairy products. It is also available in the provitamin carotenoid form in carrots and in green leafy and yellow vegetables [244]. Retinol is stored as retinyl esters in the liver and is important for eyesight as retinol is a key component of retinal pigments. Retinol deficiency can cause decreased dark adaptation and night blindness. More serious deficiency can cause corneal drying, which can progress into corneal degeneration and blindness (so-called xerophtalmia). This is a major cause of blindness, and worldwide more than 5 million children are afflicted each year [244, 245]. Vitamin A is also important for epithelial cell function: Deficiency causes hyperkeratosis, vulnerability and dry skin, and may also affect the epithelium of the respiratory tract mucosa and the gastrointestinal and urinary tracts. This increases the incidence of infections in these systems, which can sometimes lead to death [244, 245]. Vitamin A malnutrition is prevalent in developing countries, and in addition to the severe consequences noted above can also cause growth retardation in children.

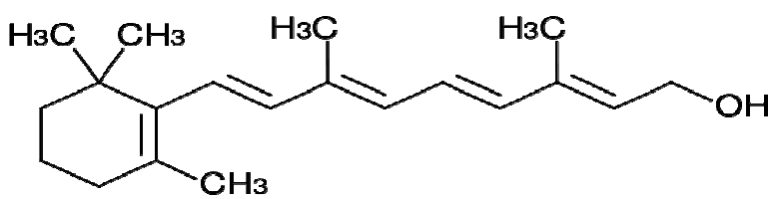


Figure 24. Retinol.

Acute vitamin A toxicity is rare, but can result from the ingestion of large doses of vitamin A supplements or from eating polar bear or seal liver that contains a very high concentration of retinol. Symptoms include drowsiness, headaches, irritability, vomiting, and skin peeling [244].

In developed nations, vitamin A deficiency is rare. In spite of this, vitamin A supplementation and food fortification is widespread. In Sweden and other countries, milk is often fortified with vitamin A. Vitamin A-containing supplements are prescribed to children. The increasing availability of vitamin A in fortified food and supplements may frequently lead to intake above the recommended dietary allowance [246, 247].

Retinol analysis is difficult and expensive, as it requires costly laboratory equipment [248-250]. Retinol-binding protein 4 (RBP-4) is a transport protein for retinol that is released into the circulation from the liver. RBP-4 can reliably be used as a sensitive and specific marker for retinol status, and levels of RBP-4 are highly correlated to plasma retinol status and can be measured by simple and inexpensive methods [248-250]. Acute phase response, protein malnutrition, liver disease, and renal failure can to varying degrees affect RBP-4's potential as a surrogate marker for retinol [248, 251-253].

Vitamin A influences bone health by several different mechanisms. It has been known for decades that toxic doses of vitamin A cause fractures in rats. Animal studies have demonstrated that even moderately elevated concentrations of retinol inhibit collagen synthesis, induce bone resorption, cause bone fragility, and antagonize the bone-sparing effects of vitamin D [254-257]. In humans, vitamin A intake seems to decrease intestinal calcium absorption and antagonize the action of vitamin D on calcium [258], although the mechanism by which retinol antagonizes vitamin D has not been fully elucidated [255, 258]. Treatment of rats with retinol further intensified ovariectomy-induced bone loss and attenuated the bone-sparing effects of bisphosphonates [259, 260]. Furthermore, retinol is metabolized into several biologically active substances, some of which affect bone tissue. The retinol metabolite retinoic acid has been reported to suppress interleukin 6 production in human osteoblast cells, while simultaneously decreasing cell differentiation and alkaline phosphatase and osteocalcin production in these cells [261]. Jacobson et al. demonstrated that retinoic acid causes an increase in the RANK-L/osteoprotegerin ratio in human osteoblasts, which could be a mechanism of bone resorption [262]. Another retinol metabolite, the retinoid isotretinoin, has proved to be highly detrimental to the human skeleton , isotretinoin occurs naturally in the body, but is also used as a pharmacological agent, especially in dermatology [263-266].

High dietary intake of vitamin A or high serum levels of retinol may correlate with increased fracture risk in both men and women [246, 267-269]. Studies have also demonstrated an association between high vitamin A intake and reduced BMD [267, 270, 271]. It has even been suggested that high vitamin A intake could be a factor contributing to the high incidence of osteoporotic fractures in Scandinavia [267, 272].

Other studies have yielded that sub-toxic retinol levels may be detrimental to bone health and result in an increased fracture rate, and supplemental vitamin A may be harmful even at dosages below the upper limit of the normal dietary allowance [246, 267, 268, 270]. However, several studies have failed to establish any relationship between retinol levels or vitamin A supplementation and bone density [273-277] or fracture rate [275, 278]. A possible rationale for the discrepancies are findings suggesting that there

is an inverse U-shaped relationship between retinol levels and hip fracture risk, indicating that both low and high levels of retinol may be harmful [269]. Furthermore retinol intake in both the low and in the high range have been associated with reduced BMD [270]. This would imply that there is a narrow optimal dosage range for retinol.

In view of these equivocal findings, the current practice of vitamin A supplementation and food fortification is a matter of debate [246, 267, 268, 272, 279, 280].

Fatty acids and bone

Recent research suggests that fatty acids play an important role in bone metabolism [281]. Fatty acids are important for human nutrition. They are an important source of energy, and can be stored in the body's adipose tissues as triglycerides in which three fatty acid chains are connected to a glycerol molecule [282](Figure 25). Fatty acids are also building blocks for other lipids such as phosphoglycerides, sphingolipids, and cholesteryl esters [282]. Fatty acids are important for cell membrane function and for nerve and brain function. Most fatty acids can be synthesized in the human body from dietary carbohydrates and proteins; exceptions are the essential fatty acids α-linolenic acid and linoleic acid, and their derivatives, which must be provided by the diet [282]. Subcutaneous adipose tissue provides insulation for the body, conserving body temperature and energy in addition to being an energy storage depot. Visceral fat also provides support and padding for internal organs. Fat-soluble vitamins (A, D, E, K) and essential fatty acids are ingested with dietary fats. Dietary fat is usually available as triglycerides, and common sources include meat and fish, animal fats, and vegetable oils such as olive, linseed, corn, and coconut oils [282].

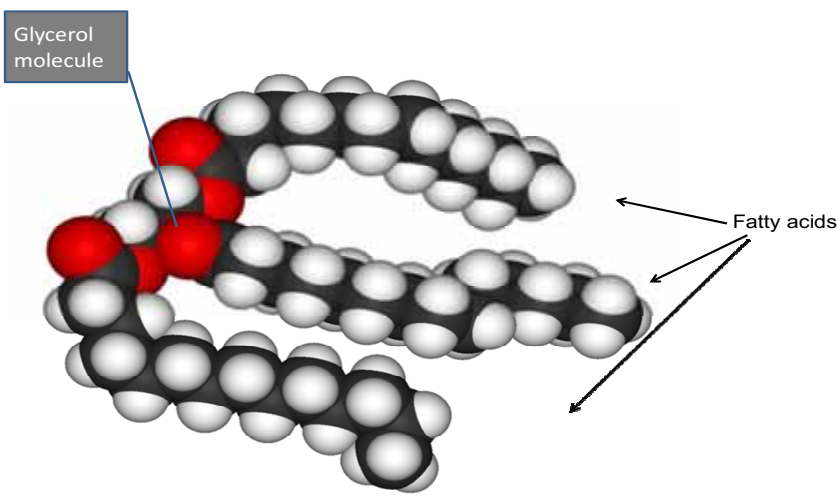


Figure 25. A triglyceride molecule is formed from three fatty acid chains joined to a glycerol molecule.

Fatty acids are described by the formula R-COOH, with R representing an alkyl chain made up of carbon and hydrogen atoms. The chain length is variable, and the terms "short-chain" and "long-chain" fatty acids are sometimes used [282]. There are different families of fatty acids: saturated fatty acids, monounsaturated fatty acids, and two families of polyunsaturated fatty acids (PUFAs) [282]. The principal difference is the degree of saturation in the alkyl chains (Figure 26). In saturated fatty acids, there are no double bonds between the carbon atoms—instead, hydrogen molecules occupy these bonds. In monounsaturated fatty acids, there is one double bond in the alkyl chain. In PUFAs, there are two or more double bonds.

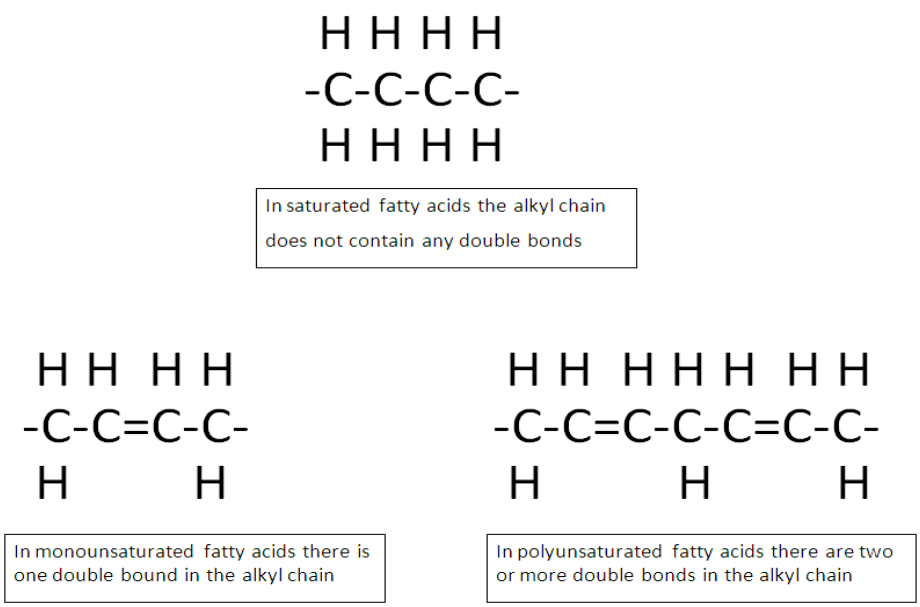


Figure 26. Saturated, monounsaturated, and polyunsaturated fatty acids have different alkyl chains.

Saturated fats are more solid at room temperature than unsaturated fats. Butter, cream, cheese, pork, tallow, lard, and coconut oil all contain high levels of saturated fatty acids. Various health risks have been attributed to saturated fatty acids, and saturated fat intake is associated with negative health effects including hypertension [283], coronary heart disease [284], and negative effects on plasma lipid levels [285].

The two PUFA families are n-6 fatty acids, also known as omega-6 fatty acids, and n-3 fatty acids, also known as omega-3 fatty acids. They are essential fatty acids that cannot be produced by the body as humans lack the necessary enzymes. The two essential fatty acids families start with 2 short chain polyunsaturated fatty acids. α-linolenic acid is the base for the n-3/omega-3 family of PUFAs, and linoleic acid is the base for the n-6/omega-6 family. To some extent in humans, these short-chain PUFAs can then be gradually lengthened into longer n-3 and n-6 PUFAs. The n-3 and n-6 PUFAs differ in the position of the first double bond, with the n number indicating the position of the double bond counted from the omega-end (last carbon) of the fatty acid as illustrated below (Figure 27).

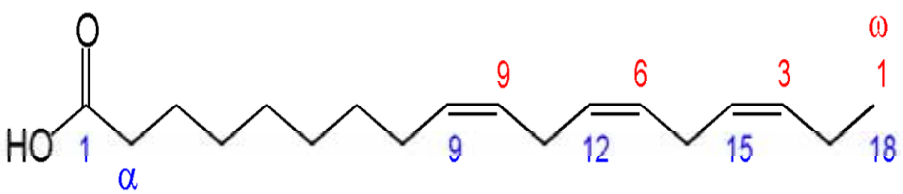


Figure 27. The chemical structure of alpha-linolenic acid (ALA), an n-3 fatty acid. Note that counting from the omega end, the first double bond appears at the third carbon-carbon bond (double line segment).

PUFAs in general, and n-3 fatty acids in particular, have evoked a lot of interest in recent years. Many health benefits have been attributed to n-3 fatty acids, including incidence reduction or disease modification of coronary heart disease, Alzheimer's disease, macular degeneration, asthma, type 1 diabetes, multiple sclerosis, cancer, inflammatory bowel disease, rheumatoid arthritis and psoriasis [286-288].

It has been suggested that poly-unsaturated fatty acids may positively influence human bone accrual in young individuals [289], and supplementation with PUFA may decrease bone turnover and improve BMD in elderly patients [290]. The ratio of n-6:n-3 PUFAs may be important, and a low n-6:n-3 ratio may be preferable. In a study in which the ratio of n-6:n-3 PUFAs was estimated in dietary intake, a higher n-6:n-3 ratio was associated with lower hip BMD in elderly men and women [291].

For decades it has been known that dietary fat composition can influence bone in animals, and that PUFA deficiency can lead to decreased collagen synthesis, bone demineralization, replacement of bone by adipose tissue, and bone weakness [292]. Animals studies have shown that n-3 PUFAs can inhibit bone loss after ovariectomy and reduce osteoclast activity [293-295]. Also in animals, a low n-6:n-3 PUFA ratio is associated with improved bone formation [296].

The mechanisms behind the beneficial effects of n-3 PUFAs on bone metabolism are not entirely clear, but several different modes of action have been proposed. Fatty acids may alter bone metabolism by influencing prostaglandin and cytokine levels, and n-3 fatty acids may reduce inflammatory prostaglandins and cytokines. In contrast, n-6 PUFAs increase production of pro-inflammatory substances such as PGE_2, TNF-α, and IL-6, which may promote bone resorption [281, 286, 297]. Another proposed mechanism is that n-6 fatty acids may activate the nuclear receptor PPARγ, which modulates genes that regulate metabolic functions and promotes the differentiation of pluripotent stem cells into adipocytes rather than osteoblasts [286, 298]. Studies also suggest that n-3 fatty acids increase intestinal calcium absorption and reduce renal calcium excretion [292, 298, 299].

In the light of their beneficial effects on bone, it has been suggested that n-3 PUFA supplementation could be used as a safe adjunct to other osteoporosis therapies or as a treatment in its own right [286, 290]. Further research is needed to explore these possibilities.

Aims and hypotheses of the thesis

The overall aim of the thesis was to study the influence of physical activity, vitamins and fatty acids on bone mass and bone accrual in young men. The following were the specific aims:

1. To investigate prospectively whether two sports with different physical loading patterns have different effect on bone mineral accrual in young men. We also aimed to evaluate the influence of heritable factors on bone mass by investigating also the young men's fathers and mothers.

Our hypothesis was that sports with different loading patterns have different effects on bone mineral accrual.

2. To investigate the relationship between current high-, medium-, and low impact physical activity and bone mass in male and female medical students.

Our hypothesis was that high impact physical activity in particular would correlate with physical activity.

3. To investigate the association between vitamin D_2, vitamin D_3 and peak bone mineral density.

Our hypothesis was that vitamin D levels, both D_2 and D_3, would correlate positively with bone mineral density.

4. To investigate the relationship between retinol and its carrier protein, RBP-4, and peak bone mineral density, fat mass, and markers of bone metabolism.

Our hypothesis was that levels of vitamin A and RBP-4 would show a negative association with bone mass and markers of bone metabolism, and a positive association with fat mass.

5. To investigate the relationship between fatty acids and peak bone mineral density.

Our hypothesis was that concentrations of n-3 fatty acids, but not n-6 fatty acids, would be positively associated with bone mineral density.

Materials and methods

Subjects

In this thesis, 3 different cohorts were studied. The cohort for study I was a group of young males along with their parents; for study II, a cohort of male and female medical students was used; and for studies III, IV, and V, a third young male cohort was used.

Study I subjects

This study included 117 healthy Caucasian males, about 17 years old at baseline. The subjects were volunteers and were not randomly selected. Sixty-five were ice hockey players, 22 were badminton players, and 30 men were recruited as a control group(Figure 28), they did not participate in any organized training. Four years later, a follow-up was conducted. Of the original 117, 110 participants were contacted and agreed to participate. Of these, 14 ice hockey and 3 badminton players were excluded as they had ended their active sports career before the follow-up; 2 subjects were excluded due to illness.

Figure 28. The subjects in study I consisted of male ice hockey players, badminton players, and control subjects.

In study I, we also wanted to study the BMD and BMC of the parents of the 3 different groups to evaluate the influence of heritable factors. Eighty percent of the

fathers and 80% of the mothers were contacted and agreed to participate. Participation rate was 86% for ice hockey parents, 79% for badminton parents, and 65% for the control parents.

Study II subjects

Study II involved medical students at the University of Umeå who were recruited at the Sports Medicine Unit. Participants included 198 student volunteers (Figure 29). Thirty-three male and 41 female medical students were excluded. Exclusion criteria were recent or multiple fractures, medication interfering with bone metabolism, irregular menstrual cycles, substantial weight loss, eating disorders, current or prior smoking habits, and chronic disease. Twelve of the medical students were excluded due to disease, 11 due to smoking, 3 due to multiple fractures, 6 because of ethnicity, and one due to leg amputation; a further 41 were excluded for not returning questionnaires. Thus, 124 students remained for the study: 62 men and 62 women. The mean age of the male medical students was 28 years, and the female mean age was slightly lower, 25 years.

Figure 29. The subjects in study II were male and female medical students.

Study III, IV, and V subjects

Studies III, IV, and V all involved the same cohort of young men. The participants were recruited through advertisements and information given at two high schools and local athletic clubs. A flowchart is provided to illustrate how the young men participated in the different studies (Figure 30).

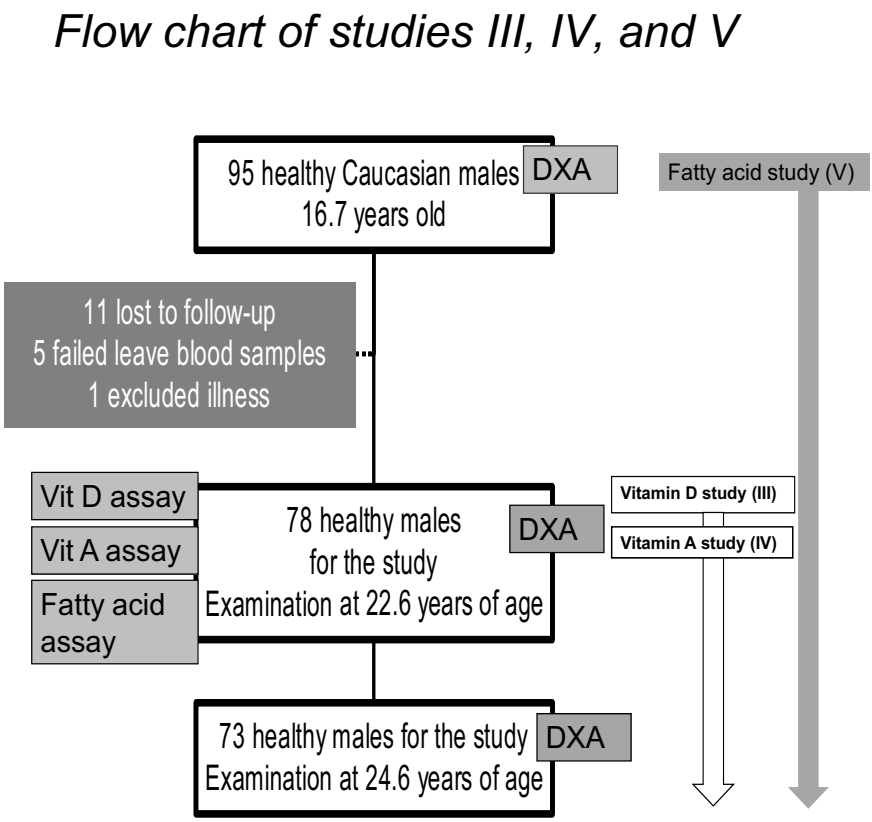


Figure 30. A flow chart of studies III, IV, and V. Note that the fatty acid study (study V) was longer than the other studies.

DXA measurements of bone mass and body composition

In study I, the BMD (g/cm^2) of the total body and the BMD and BMC (g) of the lumbar spine and right proximal femur were measured in the study cohort at baseline and at follow-up. These parameters were also measured in the parents using the same Lunar DPX-L (GE-Lunar, GE Healthcare) dual-energy X-ray absorptiometer, software version 4.6e (Figure 31). The bone areas of the total hip and femoral neck and the bone area and height of the lumbar spine (L2-L4) were also measured using the same equipment. The BMD, BMC, and bone area of the dominant humerus in the young men were derived from the total body scan, using the scaling option to maximize precision. The same investigator performed 90% of the scans.

Figure 31. DXA scan at the Sports Medicine Unit using the Lunar DPX-L.

In study II, BMD (g/cm^2) of the total body, femoral neck, and lumbar spine (L2-L4) was measured using the same Lunar DPX-L (software version 1.3). DXA (Figure 31). Body weight, lean body mass, and fat mass were obtained from the total body scan. The BMC, the area of the femoral neck and lumbar spine, and the height of the lumbar spine were obtained using the lumbar spine and femoral neck software. From these measurements, the volumetric bone density (vBMD, mg/cm^3) of the femoral neck and lumbar spine was estimated using the assumption that these two sites are cylindrical in size. The vBMD was then obtained by

dividing the total volume of this cylinder by the BMC of the same site. The same investigator performed all of the scans.

Studies III, IV, and V also used the Lunar DPX-L, software version 4.6e. The BMD (g/cm^2) of the total body and right hip was measured. The BMD of the spine and the lean body mass were derived from the total body scan, and abdominal fat mass was derived from the total body scan using the region-of-interest program. One investigator performed all the analyses. To maximize precision, the scaling option was used and set to 200.

The coefficient of variation (CV, SD/mean) was determined by scanning one person seven times on the same day, with repositioning between each scan. The CV values were 0.7% for the total body scan, ~1% for the BMD of the femoral neck/total hip scan, 0.6% for the lumbar spine BMD, 2.3% for the humeral BMD, 0.7-1.5% for the different measured bone areas, 0.7% for lean body mass, and 2% for abdominal and total fat mass. To evaluate the region-of-interest program, two different persons were scanned. The first person was scanned seven times on the same day, with repositioning between each scan. The CV value was then calculated to be 1.1% for spine BMD. The second person was scanned a total of 10 times on different days. The CV value then increased to 2.5%. The equipment was calibrated each day using a standardized phantom to detect drifts in the measurements and to test machine functions. The equipment was also evaluated regularly during the studies using a spine phantom. No drifts in BMD were detected.

Laboratory analyses

Determination of vitamin D analog concentration

In study III, blood samples were obtained from the 78 subjects at 22 years of age. Serum (150 μL) was diluted with 450 μL 2-propanol containing hexadeuterio-25OHD3 as an internal standard and butylated hydroxytoluene (BHT) as an antioxidant. After thorough mixing for 15 minutes followed by centrifugation for 10 minutes (4,000 x g at 10°C), a 100 μL aliquot from the supernatant was injected into the high-performance liquid chromatography (HPLC) system. HPLC was performed with an HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA), interfaced by atmospheric pressure electrospray ionization to a Hewlett-Packard (Palo Alto, CA) mass spectrometer operated in single ion monitoring mode. Vitamin D analogues were separated on a 2.1 x 50 mm reversed phase column (column temperature: 40°C). A one-point calibration curve was constructed from analysis of an albumin solution enriched with a known vitamin D concentration. Recovery was >95%, the method was linear from at least 5-400 nmol/L, and the limit of detection was 1-4 nmol/L. The CVs for the standards (SD/mean) were 5.8% (29.4 nmol/L) and 5.2% (73.6 nmol/L).

Determination of retinol and RPB-4 concentration

In study IV, serum was obtained under non-fasting conditions from 78 men at baseline (i.e., at 22 years of age), and stored in a dark freezer at -80° Celsius until analyzed. Eighty microliters of plasma was diluted with 300 μL 2-propanol containing the internal standard, retinol acetate, and the antioxidant, butylhydroxytoluene. After thorough mixing for 15 minutes and centrifugation for 10 minutes (4000 x g at 10°C), a 7 μL aliquot of the supernatant was injected into the HPLC system. HPLC was performed with an HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA) with a single wavelength UV detector operating at 325 nm. Retinol was separated from the matrix and internal standard on a 4.6 x 25 mm reversed phase column. A 2-point calibration curve was constructed from plasma calibrators with known concentrations of retinol. The column temperature was 40°C. Recovery was >95%, the method was linear from 0.1 to ≥10 μM, and the limit of detection was 0.01 μM. The relative standard deviations were 4.9% (1.2 μM) and 5.8% (1.7 μM). RBP-4 plasma concentration was determined with a radial immunodiffusion technique (Human RBP-4 NanoRID Kit, Binding Site Ltd., Birmingham, UK). The CV% for the method was 10.2% (3.1 μM). This method measures total RBP-4, i.e. apo-RBP, free holo-RBP, apo-RBP-TTR, and holo-RBP-TTR.

Measurement of bone metabolism markers

Bone metabolism markers were analyzed in studies III and IV. Parathyroid hormone (PTH), osteocalcin, and the carboxy-terminal telopeptide of type 1 collagen (CTX, Beta CrossLaps) were measured using a direct chemoluminescence technique based on the sandwich technique in which proteins in the patient sample and specific antibodies form a complex during the first incubation. During the second incubation, streptavidin-marked microparticles are added, and the protein is separated magnetically. The chemoluminescence process is initiated and subsequently measured by a photo multiplicator. The CV is 1.7- 5.2%, depending on the concentration and on the particular molecule being measured.

Fatty acid profile of serum phospholipids

In study V, serum was obtained under non-fasting conditions from the 78 subjects at 22 years of age. Total plasma lipids were extracted according to Folch et al. [300] and the phospholipids were isolated on 400 mg aminopropyl solid-phase extraction columns according to Helland et al. [301]. Phospholipids were eluted from the columns with methanol, evaporated with hot nitrogen, and transmethylated with fresh sodium methoxide. Fatty acid methyl esters were extracted into hexane containing 20 mg/L butylated hydroxyl toluene (BHT) as an antioxidant and separated on a 100 m x 0.25 mm (internal diameter) capillary gas chromatography column (SP-22566, Supelco, Bellefonte, PA, U.S.A.), with hydrogen as the carrier gas and flame ionization detection. The results were expressed as grams fatty acids per 100 grams of serum phospholipids.

Statistical analyses

The SPSS software for personal computers (versions 11.5, 12.0 and 14.0, SPSS Inc. Chicago, IL) was used for statistical analysis in all studies.

Study I statistics

Differences among the three groups were determined using analysis of variance and Bonferroni's post hoc test, and analysis of covariance was conducted with body weight as a covariate. The independent contributions of the badminton group, the ice hockey group, and the control group to the changes in bone variables during the 4-year follow-up period were investigated using linear regression. The three different groups (badminton, ice hockey, and control) were first transformed into two dummy variables, the first of which tested whether the badminton group was significantly different from the ice hockey group and the second of which tested whether the badminton group was significantly different from the control group. These dummy variables were then used in the linear regression as independent variables, together with changes in weight, height, and physical activity during the 4-year study period. Bivariate correlations were investigated using Pearson's coefficient of correlation. SPPS for PC (version 12.0) was used for statistical analyses. A p-value less than 0.05 was considered significant.

Study II statistics

Bivariate correlations were calculated between the bone variables, body composition parameters, and the physical activities with different impacts using Pearson's coefficient of correlation. Multiple linear regression analyses were then conducted to identify the variation explained at each site by all these variables. The different amounts of high-, medium-, and low-impact activity were then analyzed together to evaluate independent associations with BMD. A p-value less than 0.05 was considered significant.

Study III and IV statistics

All data were presented as means ± standard deviations. Bivariate correlations were calculated using Pearson's coefficient of correlation. The independent contributions of vitamin D analogs 25OHD3, 25OHD2, and total 25OHD to BMD as well as the independent contributions of retinol and RBP-4 to BMD were investigated using linear regression. In study IV, to allow for a U-shaped relation, a second-degree polynomial was used. A p-value less than 0.05 was considered significant.

Study V statistics

Again, all data were reported as means ± standard deviations. Bivariate correlations were calculated using Pearson's coefficient of correlation. The independent contributions of fatty acids to the different BMD sites were investigated using linear regression, including weight, height, and physical activity as independent variables. Differences between 3 groups were investigated by analysis of variance (ANOVA) with Bonferroni's test for post hoc comparisons. Again, a p-value less than 0.05 was considered significant.

Ethics

Informed consent was given by all participants and the study protocols were approved by the Ethics Committee of the Medical Faculty, Umeå University for all the studies (I-V).

Summary of results

Study I

Effects of Different Types of Weight-Bearing Loading on Bone Mass and Size in Young Males: A Longitudinal Study

Whether different types of weight bearing loading have different effects on bone mineral accrual in young adults is not well investigated. We measured BMD, BMC, and bone area (cm^2) at different sites, in 46 ice hockey players, 18 badminton players and 27 controls, all 17 years of age at baseline. A follow up was conducted four years later. The gains in BMD and BMC of the femoral neck and in BMC of the humerus were significantly higher ($p < 0.05$) in badminton players compared with controls during the follow-up time. The badminton players also gained more hip BMC and area compared with the ice hockey players ($p < 0.05$). At the follow-up, the badminton players had higher BMD and BMC at all sites compared with controls ($p < 0.05$). After adjustment for body weight, badminton players had higher hip BMD and BMC, femoral neck BMC, and humeral BMC compared with ice hockey players ($p < 0.05$) at the follow-up. To investigate the influence of heritable factors on bone mass, the fathers of the young athletes and controls were investigated. After adjustment for differences in age, there were no differences in BMC or BMD among fathers of badminton players, ice hockey players, or controls. This may suggest an absence of selection bias concerning the differences found in bone mass.

Study II

Current Physical Activity is Related to Bone Mineral Density in Males but not in Females

The aim of the present study was to investigate the association between high-, medium-, and low-impact physical activity in males and females at the time of peak bone mineral density in young adulthood. The cohort studied consisted of 62 male medical students (mean age 28.1 $\pm$ 3.9) and 62 female medical students (mean age 25.1 $\pm$ 3.9). The BMD of the total body, femoral neck, and lumbar spine, and the BMC and area (cm^2) of the femoral neck and lumbar spine was measured using dual energy X-ray absorptiometry. Volumetric BMD (vBMD, mg/cm^3) of the femoral neck and lumbar spine was estimated. The amount of physical activity per week, was divided into high-impact, medium-impact, and low-impact activity. In

the male cohort, estimated hours of high-impact physical activity per week was associated with BMD and BMC of all sites ($r = 0.27$-0.53, $p < 0.05$) and bone area of the femoral neck ($r = 0.38$, $p < 0.01$). Total amount of physical activity per week was associated with BMD of the total body and femoral neck, BMC of femoral neck and lumbar spine, femoral neck vBMD, and the lumbar spine area ($p < 0.05$ for all). Using linear regression, high-impact physical activity was independently associated with BMD (beta = 0.27, $p < 0.05$) and BMC (beta = 0.34, $p < 0.01$) of the femoral neck. In the female cohort there was no association between amount or type of physical activity to BMD, BMC, vBMD, or the bone area of any site. Instead body weight, lean body mass, or fat mass were significantly related to BMD and all BMC sites in this group.

Study III

Relationship between Vitamin D Metabolites to Bone Mineral Density in Young Males: A Cross-Sectional and Longitudinal Study

The aim of this study was to investigate the association between vitamin D analogs and peak bone mineral density in young men. The cohort studied consisted of 78 males with a mean age of 22.6 years at baseline. BMD of the total body, hip, and spine and lean body mass were measured at baseline and at a follow-up 2 years later. Blood samples were assayed for 25-hydroxyvitamin D_2 ($25OHD_2$), 25-hydroxyvitamin D_3 ($25OHD_3$), and total 25-hydroxyvitamin D (25OHD) at baseline, using high-performance liquid chromatography. Levels of $25OHD_3$ was significantly correlated with BMD at all sites and to lean body mass ($r = 0.23$-0.35, $P < 0.05$), at baseline. In contrast, levels of $25OHD_2$ significantly negatively correlated with BMD of the total body ($r = -0.28$, $P = 0.01$) and spine ($r = -0.27$, $P = 0.02$). After adjustment for the influence of age, body weight, body height, and physical activity (hours/week), levels of $25OHD_3$ were independently associated of BMD of the total body (beta = 0.24, $P = 0.03$) and spine (beta = 0.25, $P = 0.03$). Levels of 25OHD2 were an independent negative predictor of the same sites (beta = -0.23 for both, $P = 0.03$). There was a negative association between levels of $25OHD_3$ and levels of $25OHD_2$ ($r = -0.31$, $P = 0.006$).

Study IV

Retinol, Retinol-Binding-Protein-4, Abdominal Fat Mass, Peak Bone Mineral Density and Markers of Bone Metabolism in Men: The NO_2-Study

The influence of retinol on BMD in males after puberty in men is not well investigated. The purpose of the present study was to investigate the association between retinol, RBP-4 and BMD, abdominal fat mass, and markers of bone metabolism in young men. The cohort studied consisted of 78 healthy males with a mean age of 22.6±0.7 yr at baseline. A follow up was conducted in 73 of the participants 2.0±0.4 yrs later. Associations between serum concentrations of retinol and RBP-4, BMD, and serum concentrations of osteocalcin, and carboxyterminal telopeptide of type 1 collagen (CTX) were investigated. Both retinol and RBP-4 showed an inverse relationship with that of osteocalcin ($r = -0.23$ to -0.25, $P < 0.05$). Levels of RBP-4 ($r=0.26$, $p=0.02$) and osteocalcin ($r=-0.23$, $p=0.04$) were also related to abdominal fat mass. Neither retinol nor RBP-4 concentrations were associated with BMD at any site or CTX as baseline, or changes in BMD during the two year follow-up period. Levels of RBP-4 showed a strong association with levels of retinol ($r=0.61$, $p<0.001$).

Study V

n-3 Fatty Acids are Positively Associated with Peak Bone Mineral Density and Bone Accrual in Healthy Men: the NO_2 Study

The knowledge concerning the influence of polyunsaturated fatty acids on bone mass in humans is limited. The objective of the present study was to investigate the role of fatty acids in bone accumulation and the attainment of peak bone mass in young men. The cohort studied consisted of 78 healthy young men with a mean age of 17 years at baseline. BMD of total body, hip, and spine was measured at baseline and at 22 and 24 y of age. Fatty acid concentrations were measured in the phospholipid fraction in serum at 22 y of age. Concentrations of n-3 fatty acids were positively associated with total BMD (r=0.27, P=0.02) and spine BMD (r=0.25,P =0.02) at 22 y of age. A positive association between concentrations of n-3 fatty acid and the changes in BMD at the spine (r=0.26, P=0.02) between 16 and 22 years of age was found. Concentrations of docosahexaenoic acid (DHA, 22:6n-3) were positively associated with total BMD (r =0.32, P=0.004) and BMD at the spine (r =0.30, P=0.008) at 22 y of age.

General discussion

Osteoporosis is a major public health problem. The lifetime risk for osteoporotic fracture is about 50% in women and 25% in men [4, 12]. Osteoporosis is also a very costly disease that puts a heavy burden on public healthcare systems. Genetic factors play a very significant role for the risk of developing osteoporosis and for sustaining a fracture [302, 303], and several other factors also play a role. Physical activity and nutrition are among the major factors that can be influenced and may reduce the future risk of osteoporosis. This may be especially important in adolescence and in young adulthood, when about 50% of the total bone mass is accumulated and peak bone mass is achieved [46, 304]. Peak bone mass is believed to influence the future risk of developing osteoporosis and fractures [209, 305].

Although osteoporosis and osteoporotic fractures are more prevalent in women than in men, osteoporosis is a problem in the male population as well. Osteoporotic fractures may have increased at a faster rate in males than in females [306, 307]. Given the impact of the disease, male osteoporosis has garnered little attention, and it is an under-diagnosed and under-treated condition [308-310].

In the present thesis, we investigated the influence of physical activity on the BMD of men and women, as well as the influence of different nutritional factors on bone parameters in men around the time of peak bone mass attainment.

The influence of physical activity on the skeleton was investigated in studies I and II. Specifically, in study I we examined the effects of playing badminton and ice hockey on bone mass in young men, and we compared the results with a control group after 4 years of follow-up. The study started when the subjects were 17 years old. After adjustments for body weight, badminton players initially had higher BMD and BMC at all skeletal sites compared to the control group. The same was true for ice hockey players, except for humeral BMC.

At follow-up 4 years later, the effect of badminton play on the skeleton was even more evident. During the study, the badminton players had made significant gains in femoral neck and humeral BMD compared with controls. The hip BMC of the badminton players also increased more than the BMC of the ice hockey players. At the end of the study, after adjustment for differences in body weight, the badminton and ice hockey players had significantly higher BMD and BMC at all measured skeletal sites compared to controls. The badminton players also had significantly higher hip BMD and BMC, and femoral neck and humeral BMC compared to the ice hockey players.

Study I also found that bone size could be affected by physical training. After 4 years, the badminton players had a greater increase in hip area than did the ice hockey players; after adjustment for body weight, the badminton players also had greater dominant humeral areas. As an increase in bone size increases the moment of inertia and thus the bone strength, this could be of great importance with regard to breaking strength [119, 201, 311] (Figure 32).

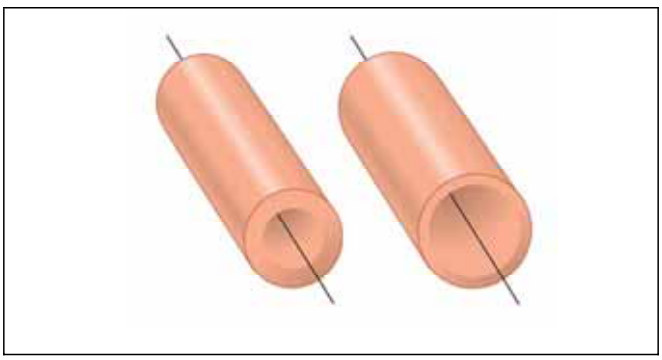


Figure 32. An increase in bone size increases the cross-sectional moment of inertia even if the bone mineral mass is the same, leading to better bending resistance and decreased fracture risk [119, 311].

Badminton is a sport that involves a lot of jumping and quick changes in direction. This puts a lot of strain on the skeleton, especially in the lower extremities, and can explain why badminton (Figure 33) seemingly induces a more powerful osteogenic response than does ice hockey [175]. Another racquet sport, tennis, has also been associated with high BMC or BMD values compared to controls, especially in the lower extremities and in the dominant arm [173, 187, 312-315]. Many previous investigators have found that skeletal response to training seems to be site specific, e.g. humeral loading is related to higher humeral bone measures and soccer increase lower limb bone measures [189, 201, 202, 311, 316].

We do not know of any previous study in which different weight-bearing sport (badminton vs. ice hockey) have shown different effects on both bone mass and bone size. Our results confirm that weight-bearing loading in men is important for bone accrual after puberty and into young adulthood. Badminton in particular seems to induce a good osteogenic response in the male skeleton, at least up to the time of peak bone mass.

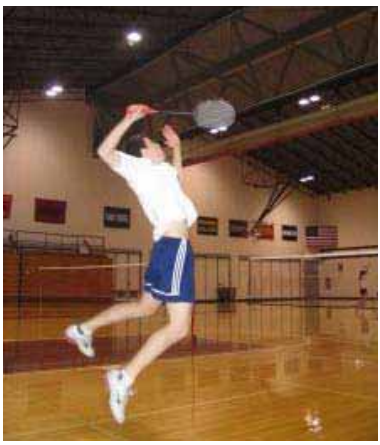


Figure 33. Playing badminton induces a strong osteogenic response in young males.

As heritable factors might influence both BMD and also an individual's propensity for a certain sport, we also checked the bone parameters of both the mothers and fathers of the participants. No BMD or BMC differences were identified when comparing parents of athletes with parents of controls. This suggests that the observed skeletal benefits stemmed from physical activity rather than from heredity.

In study II we examined the relationship between current physical activity and BMD in both young men and young women. This was a cross-sectional study involving 62 men and 62 women, all medical students. We recorded the amount and type of physical activity the students engage in; the activities were classified as low-, medium-, or high-impact activities. In the men we found that high-impact activities were significantly associated with BMD and BMC at all measured sites as well as with femoral neck area. Interestingly, these results closely mirror the results of study I. In the female cohort, the results were different—we could find no relationship between any type of weight bearing activity and BMD, in spite of the fact that women had similar training hours and similar amount of high-impact activity as the men. We found that body weight, lean body mass, and fat mass were significantly related to BMD and BMC at all sites in women.

Our study indicates that men and women respond differently to skeletal loading. Two previous studies have also noted that women do not have the same beneficial skeletal response to training as men [180, 317]. Possible explanations include the high prevalence of exercise-induced menstrual and hormonal disorders in training women, though these were not detected in our study [318-320]. A few studies in younger pre-pubertal girls have demonstrated positive bone mass gain as a result of

exercise [164, 177]. Therefore, it may be that the optimal time for exercise-induced bone gain is in pre-puberty in women, and that later training efforts have little effect on the female skeleton. However, there are other studies of young adult women that found a significant association between physical exercise and BMD gains [176, 321, 322].

Adequate nutrition is a prerequisite for any type of growth. In studies III-V we examined some of the nutritional factors that could be important for skeletal growth after puberty.

Study III investigated the influence of vitamin D on peak bone mass. Vitamin D has long been used in osteoporosis prevention and treatment, usually in combination with calcium. Most treatment protocols employ vitamin D and calcium supplementation as first-line treatment. Nearly all studies of other pharmacological treatments use vitamin D in both the active treatment and placebo groups. In studies of the elderly, Vitamin D supplementation has significantly reduced fracture incidence [231]. Metaanalyses have also indicated that vitamin D supplementation can reduce fracture incidence in older persons; however, a daily dose of about 800 IU is required [223, 232-234]. These results, combined with the known beneficial effects of vitamin D on calcium metabolism, have resulted in universal adoption of vitamin D for the prevention and treatment of osteoporosis.

In our study 78 young males with a mean age of 23 years at baseline were followed for two years. Serum levels of vitamin D_3 and D_2 were recorded at baseline. Weight, height, and BMD were measured at baseline and at follow-up at 25 years of age. We found that vitamin D_3 showed a positive association with BMD at all measured sites. Surprisingly, vitamin D_2, which is often used in supplements, was a negative predictor of BMD. There was a negative association between the two analogues. This is the first study of vitamin D_3 and D_2 and their respective association with BMD. Previous research has studied the total amount of vitamin D rather than vitamin D_3 and D_2 separately. A study by Armas et al. found that vitamin D_2 was much less effective than D_3 in maintaining 25OHD serum concentrations and furthermore that $25OHD_3$ levels actually declined with vitamin D_2 supplementation [323]. There was a strong association between vitamin D_3 levels and the month the sample was obtained, making it likely that sunlight exposure rather than nutritive factors had the greatest impact on vitamin D_3 levels.

The relationship between vitamin D levels and measures of bone in puberty and young adults has been studied by others. In a study of adolescent girls by Lehtonen-Veromaa et al., correlations between vitamin D levels and lumbar spine and femoral neck BMD gains were found [324]. Välimäki et al. found significant positive correlations between serum 25OHD and BMC at lumbar spine, femoral

neck, trochanter, and total hip sites in male subjects near the time of peak bone mass [226].

In summary, the results of study III might indicate that vitamin D_3 is of significant importance for bone mineral accrual in young adults near the time of peak bone mass. Vitamin D_2, on the other hand, may have a negative association to the skeleton and to D_3 levels. This is important as many nutritional supplements contain vitamin D_2, a practice that should definitely be reevaluated.

In study IV, we investigated vitamin A (retinol), its transport protein RBP-4, and their association with BMD, abdominal fat mass, and markers of bone metabolism in young men. The rationale behind this study included the results of previous experimental studies in which vitamin A hypervitaminosis caused decreased bone formation in rats and the retinol metabolite retinoic acid negatively affected human osteoblasts cells [254, 261]. Several clinical studies have also found an association between high retinol levels or high retinol intake and fracture risk [267-269]. Furthermore, associations between high vitamin A intake and reduced BMD have been observed [267, 270]. Thus, it is possible that even moderately elevated vitamin A levels or intake could contribute to the development of osteoporosis [267, 268, 280, 325].
In study IV, we used the same participants as in the vitamin D study (study III). The participants' mean age was 22.6 years at the start of the study when the DXA scan was performed and blood samples were obtained. Two years later, repeat BMD measurements were performed.

A negative association between retinol, RBP-4 and osteocalcin was observed. Osteocalcin is as outlined earlier a bone-specific protein secreted by osteoblasts. The association found may suggest a decreased bone formation in subjects with high retinol levels. However, levels of retinol were not related to BMD at any site or changes in BMD during the follow up period of two years. Both levels of RBP-4 and osteocalcin were related to abdominal fat mass. These association are supported by recent experimental and clinical research, where both osteocalcin and RBP-4 have been found to interact with fat mass and insulin sensitivity [234, 326, 327]. In our study, the relationship between RBP-4 and osteocalcin disappeared when adjusting for abdominal fat mass. Thus, from the results of the present study, we can draw no certain conclusion about the relationship found between RBP-4 and osteocalcin. In this rather small cohort, we found no evidence for an influence of levels of retinol on peak BMD in men.

In study V we investigated the role of fatty acids in bone accrual and in the attainment of peak bone mass in young men. The influence of fatty acids on human bone growth is not very well characterized. Animal studies have

demonstrated correlations between n-3 polyunsaturated fatty acid (PUFA) intake, reduced bone resorption, and increased bone formation [293, 294, 328-330]. In children, ingested PUFAs and saturated fatty acids may influence bone growth [289, 331]. In a study of elderly women, lumbar spine BMD was preserved and femoral neck BMD increased compared to control in a group taking combined evening primrose oil and fish oil supplements [290]. As the supplement mixture contains several different types of fatty acids, it is hard to draw any firm conclusions.

The cohort for study V consisted of the same 78 young men as in the previous studies that investigated the associations of vitamin A and vitamin D with bone mass (studies III and IV). In young men who were 22.6 years of age, significant positive correlation was found for serum levels of n-3 fatty acids and total body and spine BMD. The n-3 fatty acids also correlated positively with spine BMD accrual. In contrast, a significant negative association was found between monounsaturated fatty acids and total body BMD. Thus, our main findings from study V were the positive association between n-3 fatty acids and total body and spine BMD as well as the positive association between n-3 fatty acids and spine BMD accrual.

Summary and conclusion

Badminton training seems to induce a favorable osteogenic response in the skeleton of young males after puberty compared to ice hockey training. The skeletal response was site-specific.

High impact physical activity similarly was associated with higher bone mass in a cohort of young men but not in a cohort of young women. In the women, body weight, lean body mass, and fat mass showed positive associations with the bone parameters.

Vitamin D_3 levels showed a positive association with BMD at all measured sites in a young male cohort. Conversely vitamin D_2 levels were negatively correlated with total body and spine BMD. There was a negative association between the two vitamin D analogs.

Retinol and RBP-4 levels were not associated with BMD in a young male cohort. Negative correlations between retinol, RBP-4 and the bone formation marker osteocalcin were observed. However, these associations were also influenced by abdominal fat mass.

In a young male cohort, n-3 PUFAs correlated positively with total body BMD, spine BMD, and finally spine BMD accrual. Monounsaturated fatty acids conversely correlated negatively with total body BMD.

In conclusion, the results of the present thesis may suggest that several modifiable life style factors could be influenced in young males to increase peak bone mass.

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Sammanfattning på svenska

Benskörhetsfrakturer drabbar ungefär varannan kvinna och var fjärde man. Då det inte finns någon effektiv bot mot benskörhet är prevention av största betydelse. Även om ärftliga faktorer har stor betydelse för risken att drabbas av frakturer finns det även vissa omgivningsfaktorer som går att påverka. Diagnosen benskörhet ställs genom att mäta skelettets innehåll av benmineraler, den så kallade bentätheten. Den maximala bentätheten nås strax efter 20 års ålder och genom att maximera den kan man sannolikt minska risken att drabbas av frakturer längre fram i livet. I denna avhandlings har vi undersökt betydelsen av fysisk aktivitet, nivåer av vitamin D, vitamin A samt fettsyror för bentätheten hos unga män omkring den tidpunkt då maximal bentäthet uppnås.

Betydelsen av fysisk aktivitet undersöktes prospektivt under fyra år hos 46 ishockeyspelare, 18 badminton spelare och 27 kontroller som ej deltog i idrottsträning. Alla grupper bestod av unga män som var 17 år vid första undersökningstillfället. Under uppföljningstiden ökade badmintonspelarna mer i benmassa i överarmen jämfört med kontrollgruppen och ökade mer i benmassa i höften än både kontrollgruppen och ishockeyspelarna. Dessutom ökade badmintonspelarna mer i benstorlek i höften jämfört med ishockeyspelarna. Vi bedömer att skillnaderna i benmassa inte beror på ärftliga faktorer, då det inte fanns någon skillnad i benmassa mellan föräldrar till grupperna av idrottsmän och föräldrar till kontroller. Relationen mellan fysisk aktivitet och bentäthet undersöktes även i en annan studie där materialet bestod av 62 manliga och 62 kvinnliga medicine studerande. Graden av fysisk aktivitet delades upp i aktiviteter med hög stötbelastning (high impact), medelhög och låg stötbelastning. Hos männen var mängden hög stötbelastande fysisk aktivitet relaterad till bentätheten både i höft och rygg och även benstorleken i höften. Hos kvinnorna däremot var graden av fysisk aktivitet inte relaterat till bentätheten utan den var istället relaterad till kroppskonstitution.

Betydelsen av vitamin D, vitamin A och fettsyror för utvecklingen av den maximala benmassan undersöktes hos 78 unga män. Vitamin D_3-nivåer var positivt signifikant relaterade till bentäthet både i ryggen samt i höften medan nivåer av vitamin D_2 visade en signifikant negativ relation till bentätheten hos denna kohort. Det fanns även en signifikant negativ relation mellan nivåer av vitamin D_3 och D_2. Nivåerna av retinol var inte relaterade till bentäthet, men både retinol och retinolbindande protein 4 visade en signifikant negativ relation till osteocalcin, som

är en markör för benformation. Slutligen var koncentrationen av omega 3 fettsyror i serum hos denna kohort relaterade till bentätheten i både rygg och hela kroppen.

Dessa studier visar sammanfattningsvis starka relationer mellan påverkbara kostfaktorer och fysisk aktivitet till utvecklingen av den maximala bentätheten hos män. Graden av fysisk aktivitet visade inte samma starka relation till maximala bentätheten hos kvinnor som hos män.

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