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**Effects of gonadal hormone deficiency  
on bone mineral density. Can physical  
activity increase bone mineral density  
in women?**

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To my mother  
and in memory  
of my father.

## **ABSTRACT**

Osteoporosis with its associated fragility fractures is a global health care problem. The incidence of fragility fractures has increased dramatically the last 50 years. This has been suggested to at least in part be due to the sedentary lifestyle in the modern society. The prevalence of osteoporosis increases with increasing age. In the decade following menopause, most women experience more rapid bone loss than that caused by aging alone. This is mainly due to the decreased ovarian estrogen secretion. Bone mineral density (BMD) decrease can be prevented by estrogen therapy.

One of the aims of these studies was to investigate the effects of decreased levels of gonadal hormones on bone mineral density (BMD) in men and women. Men with prostate cancer were subjected to medical or surgical castration. This led to decreased testosterone levels and decreased bone mineral density. The decrease in bone mass was larger in the surgically castrated group. Treatment of fertile women with GnRH analogues for endometriosis for 6 months and hereby decreased estrogen levels led to a decrease in bone mineral density. Perimenopausal women with fluctuating estradiol levels and occasional ovulations were followed for 18 months. There was a significant decrease in BMD over an 18 months period.

The main aim of this thesis was to study if moderate physical training could prevent the loss of bone mass or even increase BMD in women with low circulating estradiol levels. Therefore young women with endometriosis treated with GnRH analogues for 6 months were randomized to physical training for 12 months or no intervention. The subjects trained during six months of GnRH treatment and during six months following cessation of therapy. Perimenopausal women with fluctuating estradiol levels and postmenopausal women with a forearm fracture and low bone mineral density were randomized to training or to controls for 18 and 12 months respectively. The results indicate a moderately positive effect of physical training in all three studies. The groups were small and no direct comparison was made. The most pronounced positive effect of training on BMD was found in the young women during six months following cessation of GnRH therapy. The least pronounced effect was found in the postmenopausal women with low stable estradiol levels. We concluded that moderate physical activity can prevent perimenopausal decrease in BMD, increase BMD in postmenopausal women with low bone mass and increase the speed of recovery of bone mass after GnRH therapy in women of fertile age with endometriosis.

Key words: Physical activity, bone mineral density, postmenopause, perimenopause, GnRH treatment, endometriosis, prostate cancer.

## LIST OF PUBLICATIONS

This thesis is based upon the following four papers, which will be referred to by their roman numerals.

- I. Bergstrom I, Gustafsson H, Sjoberg K, Arver S. Changes in Bone Mineral Density Differ Between Gonadotrophin-releasing Hormone Analogue- and Surgically Castrated Men with Prostate Cancer. *Scand J Urol Nephrol*. 2004;38(2):148-52
- II. Bergström I, Freyschuss B, Jacobsson H, Landgren BM. The Effect of Physical Training on Bone Mineral Density in Women treated with GnRH analogues: a pilot study. *Acta Obstet Gynecol Scand*. 2005 Apr;84(4):380-3.
- III. Bergstrom I, Freyschuss B, Landgren BM. Physical Training and Hormone Replacement Therapy Reduce the Decrease in Bone Mineral Density in Perimenopausal women: a pilot study. *Osteoporos Int*. 2005 Jul;16(7):823-8
- IV. Bergstrom I, Landgren BM, Brinck J, Freyschuss B. The Effect of Physical Training on Bone Mineral Density in Postmenopausal women with low bone mineral density and a forearm fracture: a randomized controlled study. Submitted June 2006 *Osteoporos Int*.

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## LIST OF ABBREVIATIONS

BMD	Bone mineral density
BMC	Bone mineral content
BMI	Bone mass index
BMP	Bone morphogenetic proteins
BMU	Bone metabolic unit
BUA	Broad ultrasound attenuation
CTX	C terminal propeptid
CNS	Central nervous system
dB/MHZ	Decibel/Megahertz
DEXA	Dual energy X-ray absorptiometry
DPA	Dual-photon absorptiometry
<i>E</i>	Young Modulus
ER	Estrogen receptor
EV	Estradiolvalerat
GH	Growth hormone
GnRH	Gonadotropine-releasing hormone
G6PD	Glucose-6-phosphate dehydrogenase
HT	Hormone therapy
ICTP	Carboxyterminal telopeptide of type 1 collagen
IGF	Insulin-like growth factors
MPA	Medroxyprogesteronacetat
NO	Nitric oxide
NS,ns	Not significant
NTX	N terminal propeptid
OPG	Osteoprotegrin
QCT	Quantitative computed tomography
PICP	Carboxyterminal propeptide of type 1 collagen
QUS	Quantitative ultrasound
RANK	Receptor activator of NF $\beta$ B
RANKL	Receptor activator of NF $\beta$ B ligand
SD	Standard deviation
SE	Standard error
SBU	The Swedish Council on Technology
SOS	Speed of sound
TGF	Transforming growth factors
WHO	World Health Organisation



# **1 INTRODUCTION**

## **1.1 BONE STRUCTURE**

The bone matrix has organic (35%) and inorganic (65%) components. The inorganic components consist primarily of calcium and phosphate, deposited as hydroxyapatite (1). The organic part consists of mainly (90%) collagen fibres type 1 and the rest of non collagenous proteins. Most of these proteins are synthesised by the osteoblasts, i.e. the bone forming cells (2). Bone is categorized into two types, trabecular or cancellous bone and cortical bone. The cortical bone has a higher true volume density than does trabecular bone, whereas trabecular bone has a larger surface area per unit volume (1). Bone is a metabolically active organ. A continuous process, called bone remodelling, involves continuous resorption and formation (3). Most of the bone tissue turnover occurs on the bone surface. Because of its larger surface area, the bone remodelling frequency is about five to tenfold greater in the trabecular bone compared to the compact bone (1).

A complex relationship between systemic hormones (parathyroid hormone, thyroid hormones, growth hormone, glucocorticoids, 1.25- Dihydroxy vitamin D and gonadal steroids) cytokines, growth factors, prostaglandins and mechanical stress is responsible for the control of bone metabolism (1).

## **1.2 BONE CELLS**

### **1.2.1 The osteoblast**

The osteoblasts are metabolically active mesenchymal-derived cells. They serve two main functions: They form the structural component of bone (matrix and mineral) and they produce regulatory factors that influence bone formation and resorption.

Osteoblasts synthesise the extracellular matrix of bone, both the collagenous matrix (predominantly type 1 collagen) and the noncollagenous matrix proteins (where osteocalcin is the predominant one). The bone-specific alkaline phosphatase correlates with bone mineralization and is localized in the plasma membrane of the osteoblast. The mineralization of the collagen substructure is a function of the osteoblast. The process of mineral precipitation is still unknown.

Many bone regulatory factors are produced by osteoblasts; bone morphogenetic proteins (BMPs), the beta transforming growth factors (TGF) the insulin-like growth factors (IGF) among others.

The osteoblast has the key role in the initiation of bone resorption. These cells have receptors for the regulation by molecules that act as bone resorbing stimulatory factors, such as parathyroid hormone, vitamin D, prostaglandins, interleukins, estrogens and TGF- $\beta$  and translate hormonal signals to control both bone formation and bone resorption (4). The cytokine RANKL is produced by the stromacell/-osteoblast (5).

### 1.2.2 The osteoclast

The osteoclasts are the bone resorbing cells. They arise from haematopoietic stem cells in the bone marrow. For differentiation and activation these cells require the presence of the cytokine RANKL, produced by stroma cells/osteoblast. RANKL binds to its receptor RANK on the osteoclast precursor cell (5,6). The process can however be inhibited by the false receptor osteoprotegerin, OPG, a competitive receptor that binds to the RANKL. OPG, exerts negative effect of the osteoclast differentiation through RANK/OPG ratio (5).

The osteoclast is a giant multinucleated cell. The most prominent area of the osteoclast is the deep foldings of the plasma membrane facing the bone matrix. The attachment of the cell to the matrix is performed through integrin receptors. Lysosomal enzymes are synthesized by the osteoclasts and secreted into the extra cellular compartment. First the hydroxyapatite crystals are solubilized by the acidic pH in the resorption lacunae. The collagen fibres are then digested by proteolytic enzymes as cathepsin K secreted by the osteoclast.

Recent evidence suggests that the osteoclast undergoes apoptosis after a cycle of resorption, a process enhanced by estrogens. This may be the explanation for the increased bone resorption after menopause (2).

### 1.2.3 The osteocyte

The cellular response to mechanical loading is initiated by the osteocytes (5). The osteocytes are the most common cells in bone (5). When the osteoblasts have produced enough matrix and have become encased in the mineralized matrix, they differentiate further into osteocytes. The transition from osteoblast to osteocyte takes approximately 3-5 days in humans. Osteocytes are embedded in bone matrix occupying spaces (lacunae) in the interior bone and are connected to adjacent cells by long cytoplasmic projections, enriched with microfilaments that lie within channels (canaliculi) through the mineralized matrix (7). These processes are organized during the formation of the matrix before its calcification; they form a network of thin canaliculi permeating the entire bone matrix and to the osteoblasts on the bone surface (2). This network may monitor the local strain and initiate organized net cell work in response to changes in strain (8). Fig 1.

Between the osteocyte plasma membrane and the bone matrix itself is the pericyte space. This space exists both in the lacunae and in the canaliculi, and is filled with extracellular fluid. The total bone surface area of the canaliculi and lacunae is 1000-5000m<sup>2</sup> in an adult (compared with a surface area of 140 m<sup>2</sup> for lung capillaries) (2).

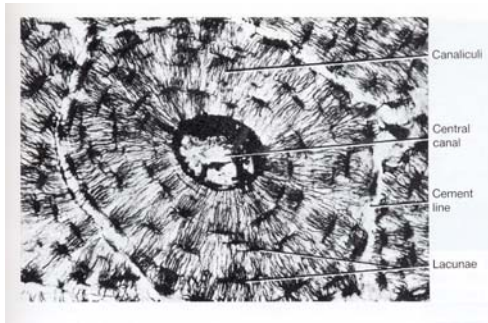
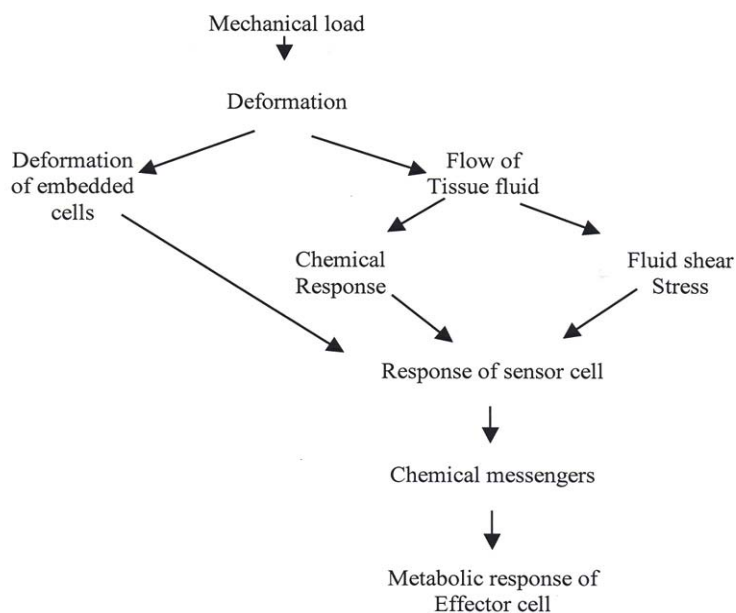


Fig 1: The canicular organisation of osteocyte. Reprinted from: Jee WSS 1983 The skeletal Tissue. In: Weiss L (ed.) Histology, Cell and Tissue Biology. Elsevier Biomedical, New York, NY, USA pp 200-205.

Osteocytes are metabolically and electrically coupled through gap junction's protein complexes. Gap junctions are essential for osteocyte maturation, activity and survival. The flow of extracellular fluid in response to mechanical forces throughout the canaliculi induces a spectrum of cellular response in osteocytes (8). Fig 2. The current concept of mechanotransduction in bone is thought to involve direct strain and fluid flow (9).



**Fig 2:** Lecture notes from seminars

“ The University of Edinburgh Scottish Mechanotransduction Consortium and the European Calcified Tissue Society”. Bone and Mechanical Loading 3-4sept 2003.

Studies have demonstrated that osteocytes produce significantly higher levels of the signalling molecules prostaglandin E2 and prostacyclin than osteoblasts do in response to pulsating fluid flow. It has been suggested that pulsating fluid flow transduces mechanical events into cellular signals by raising intracellular  $\text{Ca}^{2+}$  through ion channels and induce  $\text{Ca}^{2+}$  release from intracellular stores (10). Calcium influx precedes the rapid increase in G6PD activity, preceded by prostaglandin release and NO increase. The increased G6PD activity is then followed by increased Growth factor synthesis (10).

The osteoblasts on the bone surface are in direct chemical contact with the osteocytes within the mineralized bone. Strain-generating could be perceived by osteocytes and their regulatory information can be passed on to the osteoblasts (11). A recently identified signalling pathway activated by loading is the glutamatergic signalling system, (12) earlier only identified outside in the CNS but now known to function in many different tissues. It has been shown that the osteoblasts and osteocytes possess the necessary molecular apparatus to use a process with very close similarities to synaptic transmission between neurones (13).

It has also been suggested that mechanical loading may be an initiator of bone remodelling by modulating the balance of RANKL and OPG expression. (14).

Some data indicate that cellular response to loading are abolished in the absence of functional estrogen receptors and increased with overexpression of estrogen receptor (9).

Osteocytes will be phagocytised and digested together with other components during the osteoclastic bone resorption. Osteocytes may play a role as mechanosensors in the local activation of bone turnover (2). Increased numbers of empty lacunae and apoptotic cells are observed during bone turnover in aged human bone, in glucocorticoid treated mice and after estrogen withdrawal (8). Mechanical stimulation has been reported to protect osteocytes against apoptosis (15).

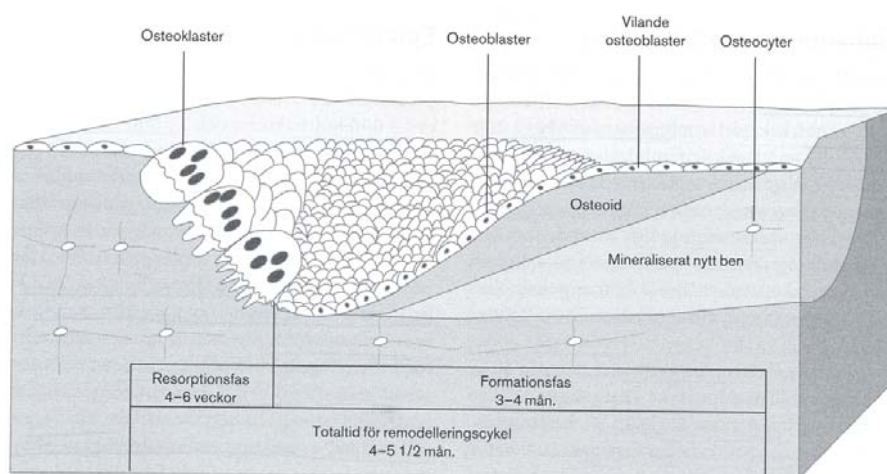
### **1.3 BONE REMODELLING: A PROCESS INVOLVED IN BONE GROWTH AND TURNOVER**

Bone remodels throughout life and adapts its material properties to the mechanical demands placed upon it (11). The bone remodelling is a surface phenomena and occurs in the periosteal, endosteal, haversian canal and trabecular surface (11). The remodelling in bone occurs in focal and discrete packets throughout the skeleton called bone metabolic units, BMUs. The remodelling that occurs in each packet is geographically and chronologically separated from other packets of remodelling and normally 90 % of these are dormant (11). There are 1-2 million BMUs in the skeleton and they are more abundant in the trabecular bone than in the cortical bone (5). The BMU of the cortical bone is the osteon or the haversian system, a cylinder running parallel to the long axis of the bone. The osteon forms approximately two thirds of the bone volume. In trabecular bone the BMU follows the same shape as the trabecular surface, most of which is concave towards the marrow (11). In the normal adult skeleton, the bone formation occurs predominantly in locations of previous bone resorption.

In the cortical bone osteoclasts dig out a tunnel creating a “cutting cone” and subsequently new bone is formed in the area of the “closing cone” leading to the creation of a new bone structural unit (1).

Osteoclast activation is the initial step in the remodelling sequence. Osteoclasts are activated in specific focal sites by mechanisms that are still not understood and the

mechanism for the initiation of bone remodelling is thus unknown (6). The osteocyte is probably a participant in this process. The osteocytes sense bone deformation, which is an indicator of the need for adaptive remodelling in bone size, shape, and distribution to accommodate prevailing loads. The death of osteocytes by apoptosis due to estrogen deficiency, in old age, after bone damage and during corticosteroid therapy may initiate the remodelling (16). Diminishing mechanical forces are also of importance and eliminate signals that maintain the osteocyte viability thereby leading to cell death (17). The number of osteocytes that undergo apoptosis may provide the topographic information needed to target removal of damage by osteoclasts (16). The resorptive phase of the remodelling process is followed by repair of the defect by a group of osteoblasts that are attracted to the site of the resorption defect and then presumably proceed to make new bone. This takes approximately 3 months (6) and the whole process lasts for approximately 200 days. Fig 3.



**Fig 3:** Bone Remodelling. Reprinted from: Törring O, Ljunggren Ö, Johnell O, Werner S 2004. Kalcium och Skelettm metabola sjukdomar. In: Werner S (eds.) 2004 Endokrinologi Liber AB Stockholm Bild 9.5 pp 208.

The cellular and humoral mechanisms responsible for mediating the coupling of bone formation to bone resorption are still not clear. Coupling may be mediated through osteoblast stimulating factors, such as IGF-1, IGF-2 or TGF- $\beta$ . These factors are released from the bone matrix during the process of osteoclastic bone resorption (6). At menopause an increase in bone resorption occurs. Estrogen deficit results in an increased activation frequency of bone remodelling units and increased resorption depths on bone lacunae (1). Many estrogen-dependent growth factors and cytokines are involved in bone remodelling. Estrogen modulates the production of bone-resorbing cytokines such as interleukin 1 and 6 and bone stimulating factors such as insulin like growth factor 1 and 2 and transforming growth factor B. Estrogen increases vitamin D receptors in osteoblasts (3). Estrogen plays a role in one of the key positions in the RANK-osteoprotegrin system described in the section “primary osteoporoses” below.

The volume of the cortical bone is regulated by the formation of the periosteal bone, by remodelling in the Haversian system and endosteal bone resorption. The periosteal bone formation continues to increase the diameter of the cortical bone throughout life (6).

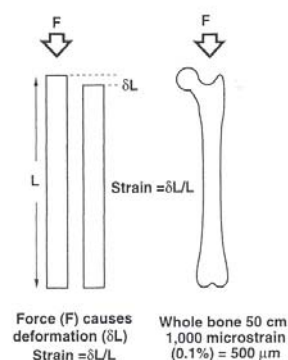
## 1.4 MECHANICAL LOADING

The sensitivity of bone to physical and environmental stimuli is readily evident in animal and clinically based studies that show the skeleton's response to exercise (9,18). A decrease in mechanical load due to immobilisation or weightlessness causes a reduction in bone mass. This is a result of an initial increase in bone resorption followed by a decrease in bone formation (19).

The thought that bone responds to its mechanical environment is ancient, but its origin in modern times is generally attributed to Wolf 1892 cited by Erlich and Lanyon (10). He said that mechanical forces give mechanical feedback and adaption of the bone. Wolf's law suggests that responsiveness to increased loads leads to stronger bone, whereas a reduction in loading or usage leads to bone loss. The crucial point of this "law" is that bone strives toward a structure optimized for the individual's levels of activities. This adaption of the skeleton has been demonstrated in clinical studies, for example tennis players whose racket holding arm has higher radius BMD than the other arm (18). The feedback system reduces bone with decreased load to a new optimal level of habitual activity (9).

Mechanic transduction is an active research field. Between 1970 and 1980 160 papers were published that included in title or abstract the words loading and bone, in the 1980s there were 860 papers published, in the 1990s there were 2300 and during the first years of this decade over 2300 have been published, (source PubMed) (9).

Load applied to the skeleton is generally described in terms of stress and strain. Stress is the force applied per unit area to a subject. Strain is a measure of deformation in response to the application of stress. Strain generates the adaptive response of loading. Strain is a measure of deformation and is calculated by dividing the change in an object's length by its original length (20). Strain is a dimensionless ratio, however it is commonly measured in microstrain (strain  $\times 10^{-6}$ ) 0.1% deformation of bone gives in humans 0,001 strain or 1000 mikrostrain (21). Fig 4.



**Fig 4:** Strain is the ratio of deformation divided by original length, so as a ratio has no units. Typical long bone strains are ranged of 1000-3000  $10^{-6}$ . Reprinted from Skerry TM 1998 The Regulation of gene expression in Bone by Mechanical Loading. In: Russel RGG, Skerry TM, Kollenkirchen U (eds) Novel Approches to treatment of Osteoporosis Springer Berlin pp 179-198(22).

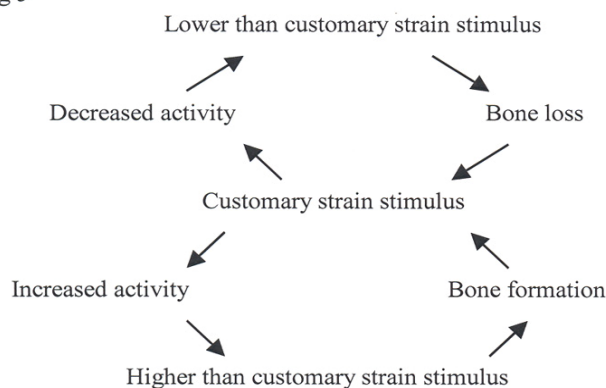
One theory is that dynamic loading creates fluid movements in bone's lacunar-canalicular network, which in turn generates shear stresses on the plasma membranes of osteocytes. When the Fluid Flow stimulates the cell wall processes, the cell deforms and creates subsequent metabolic activity via integrins in the cytoskeleton. This initiates a cascade of cellular events (10).

Animal experiments give us the information that bone cells prefer to respond to strains that are high and changing at fast rates and are presented in unusual distributions (10). Additionally numbers of cycles of loading, duration of loading, frequency of repetition and hold-or rest-time during an individual cycle appear to have effects on the osteogenesis (9).

According to the mechanostat theory by Frost 1987 the formation of bone is regulated. New bone is formed if the load induces strain that is higher than usual. However that same load when repeated and adapted to that particular remodelled bone will then induce a lower strain and then bone formation will not occur.

Frost suggested that when bone is loaded above 2500 mikrostrain, modelling is induced (20). The breaking strain for all bones occurs when the load causes a deformation of 8000 microstrain or above (9). However when applied loads cause 200 microstrain or less, modelling is inhibited. Substantial reduction in chronicle unloading (such as immobilisation or in low gravity) is associated with increased bone porosity, expansion of marrow cavity, thinning of bone cortex and ultimately bone that is less resistant to strain (20). Fig 5

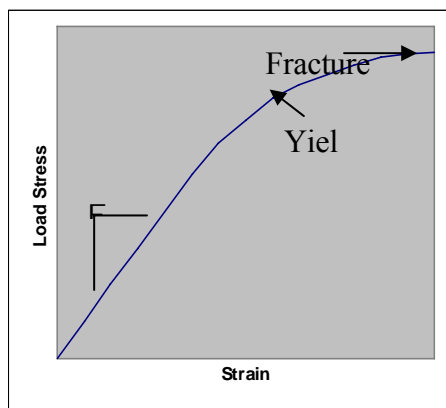
Fig 5



**Fig 5:** Strain feedback regulates net bone formation and resorption to optimize bone mass/architecture to function."Low habitual strains are responsible for maintenance of our current bone mass, and increased strains above the normal peak experienced less frequently during a day initiate new bone formation. In contrast, reduction of habitual strains is associated with bone loss, so that in each case after the end of the formative or resorptive process, strains are returned to habitual levels under new exercise/disuse regimen". Reprinted from Skerry TM, Suva LJ. Investigation of the regulation of bone mass by mechanical loading: from quantitative cytochemistry to gene array. *Cell Biochem Funct* 2003;21:224 fig1(12).

One obvious goal of strain mediated form/function formula is to avoid fracture. Bone loading and architecture must be coordinated to avoid tissues yield strain (18). The materials can be characterized as weak or strong, ductile or brittle, stiff or compliant (21).

Stiffness is a way materials answer to stress. A stiff material shows little strain for a large stress. The usual measure of materials stiffness is Young Modulus  $E$ . Bone in general has Young Modulus 20 and tendon has Young Modulus 1.5 (16b). Fig 6. Before failure the specimen deforms (strain). The slope of the linear region of the stress-strain curve is the Young's modulus  $E$  of the material. This represents the material stiffness. The greater the slope, the stiffer is the material. The point at which the stress-strain curve begins to bend is the yield point; yield strength. The area under the stress-strain curve is the amount of energy the tissue can withstand before failure and is called toughness. A bone that can sustain little strain after yield is considered brittle. The mineralization affects the material (21). Fig 6



**Fig 6:** Reproduced Burr D, Turner C 2003 Biomechanics on bone In: Favus (ed.) The Primer on the Metabolic Bone Disease and Disorders of Mineral Metabolism, 5<sup>th</sup> ed. American Society for bone and mineral Research, Washington DC, USA, pp 58-64 with permission from the American Society of bone and mineral research.

Activity produce different strain in different parts of the skeleton (22). Table 1.

Table 1

Activity	Skull	Tibia
Chewing	80	
Smiling	100	
Walking	50	720
Heading ball	200	840
Jump 0,45m	170	880
Jump 1,3m		2060

Table 1: Reprinted from Skerry TM 1998 The Regulation of gene expression in Bone by Mechanical Loading. In Russel RGG, Skerry TM, Kollenkirchen U(eds.) Novel Approches to treatment of Osteoporosis Springer Berlin pp 179-198.

Even very low loads can induce bone formation if applied at sufficient high frequencies (20). There is also a strong correlation between BMD and muscle strength at all skeletal sites (18). Low levels of high frequency strains arrive directly from muscle dynamics. These persistent low magnitude strains have been shown to be strongly osteogenetic and may represent a strong stimulus in defining the morphology of the skeleton. It has been suggested that ongoing activity of the postural muscles may be the dominant force of controlling bone mass (18).



## **1.5 OSTEOPOROSIS**

Osteoporosis is defined as a state of decreased bone mass accompanied by micro architectural changes. Osteoporosis results in a decrease in bone strength and an increased risk of low energy fractures (3, 23).

Osteoporosis with its associated fragility fractures is a global health care problem. The incidence of fragility fractures has increased dramatically the last 50 years (24). Epidemiologists conclude that one of the reasons to the increasing incidence of fragility fractures may be the sedentary lifestyle in the modern society (25).

Bone loss occurs during the normal aging process. The term “primary osteoporosis” refers to osteoporosis that is due to the involutional losses that come with aging, and in women, the physiological losses related to the menopause (26).

### **1.5.1 Primary Osteoporosis type 1**

The prevalence of osteoporosis increases with increasing age. However, in the decade following menopause, most women experience more rapid bone loss than that caused by aging alone. The first 5-10 years after menopause bone loss is accelerated and may be up to 10-fold higher than the premenopausal bone loss (26). This bone loss is due to estrogen deficiency caused by ovarian failure (26). The cessation of ovarian function and the following decrease in circulating oestrogen levels is associated with an increase in bone turnover. An increase in the number of osteoclasts in trabecular bone is observed in the estrogen-deficient state. Estrogens appear to shorten the life span of osteoclasts, the active agents in bone resorption, by stimulating early apoptosis. It has been proposed that interleukin-6 production from the marrow stromal and osteoblastic cell lines is inhibited by estrogen. Thus estrogen deficiency leads to an increase in interleukin 6 and a subsequent increase in the rate of osteoclastogenesis and bone resorption (3, 26).

The bone resorption factors stimulate the osteoblast to produce RANKL on the surface. On the preosteoclast there are RANKL-receptors. The activation starts proliferation and differentiation to matured osteoclasts. However a false receptor called osteoprotegrin, OPG, a cytokine derived from immunocompetent cells, becomes a competitive receptor by binding to the RANKL on the osteoblast surface and thereby interferes with the binding to RANK on the preosteoclast. This osteoprotegrin production is stimulated by estrogen. Adequate oestrogen levels maintain OPG levels thereby preventing the osteoclasts to mature. Low oestrogen levels thus lead to failure to inhibit osteoclast maturation (27).

The immediate postmenopausal bone loss is thought to be about 2 % per year. Later, 7-10 years after the menopause the rate of loss decreases. An increased number of anovulatory cycles reflected by irregular menstrual patterns are seen during the perimenopause. Some studies have described BMD decrease during transition to menopause. A loss of 1-2 % per year has been reported (28,29). It is well known that hormone therapy (HT) prevents bone loss after menopause (26).

### **1.5.2 Primary osteoporosis type 2**

Fundamental differences exist between the patterns of bone loss caused by aging compared to that caused by estrogen-deficiency. The bone loss caused by aging is to a greater extent related to a progressive decline in the number of osteoblasts available compared to the number needed (27). Several independent factors influence the rate of bone loss during aging including mal-nutrition, immobilization and decreased levels of gonadal hormones, growth hormone (GH) and insulin-like growth factor 1 (IGF-1)(30).

Low calcium intake and vitamin D deficiency are common in the elderly and are caused by a number of factors, including dietary habits, lack of exposure to sunlight, malabsorption and mal-nutrition. This may lead to a persistent secondary hyperparathyroidism, which in turn, leads to increased bone resorption and a significant decrease in bone mass. (30)

### **1.5.3 Male osteoporosis**

Thirteen to nineteen percent of the male population has decreased bone mineral density when defined according to the osteoporosis WHO criteria for women (31). Testosterone levels may decrease due to disease, but also with age in healthy men. Men with low testosterone levels have a decreased BMD and a higher fracture risk. Approximately half of the men with femoral fractures have been reported to have low testosterone levels (32). The trabecular bone loss that occurs during mid-life and accelerates later is slightly less than the changes found in women. The increase in periosteal bone formation that occurs in men may be greater than in women and has been postulated to contribute to the lower fracture risk observed in older men (33). Osteoporosis fractures are more common in females, but thirty percent of all hip fractures occur in men (34). Furthermore, men have a higher mortality rate than women after any type of fracture (35,36).

### **1.5.4 Secondary osteoporosis**

Several publications have stated that about 50% of men and 35 % of women with symptomatic vertebral fractures have osteoporosis secondary to disease or medication.

There are numerous causes of secondary bone loss, including adverse effects of drug therapy, hypogonadism and other endocrine disorders, eating disorders, immobilisation, bone marrow-related disorders, diseases of the gastrointestinal or biliary tract, renal failure, transplantation, genetic disorders, rheumatic diseases and some forms of cancer (33,37).

Glucocorticoids, anticoagulants, anticonvulsants, thyroxine and chemotherapy are examples of drugs that may interfere with bone metabolism (37).

Castration is known to increase bone-loss in adult men at all ages and thus increase the risk for development of osteoporosis and fragility fractures (38). In young men

castration causes a yearly loss of 7% in BMD in the spine during the first two years (39). Some studies report changes in BMD in men with prostate cancer treated with androgen deprivation (gonadotropine-releasing hormone - GnRH analogues) (40-42).

Women with endometriosis and myomas can be treated with GnRH analogues. They cause a suppression of circulating gonadotropins and sex hormones inducing menopausal estrogen levels and thus an increase in bone turnover resulting in a significant bone loss (43-50).

## **1.6 DUAL-ENERGY X-RAY ABSORPTIOMETRY**

DEXA, is the golden standard for bone mass measurement. It has been used since the late eighties. DEXA has an x-ray generator using two levels of energy obtained by a K-edge filter. The technique makes it possible to eliminate the effect of surrounding soft tissues on the bone density. The radiation dose is low, about 10% of the radiation acquired at a pulmonary x-ray. The DEXA scan presents with an image of the spine, hip, radius or total body. The scan time for hip or spine is 10-30 seconds to several minutes depending on the technique (23,51).

The pencil beam technique requires 4-10 minutes for imaging the hip or spine with multiple scans while the fan beam technique, which requires only a few sweeps over the region, takes about 10-30 seconds (52).

The bone mineral content is measured in a given area and we get the so-called bone mineral density BMD by dividing the content with the area ( $\text{BMD, g/cm}^2$ ).

BMD is therefore a projected areal density, not a true volumetric density.

The accuracy is about 8 % and the time precision is about 1 % (23,51).

A strong association has been found between the BMD estimated by DEXA and the fracture risk. A hip measurement is the best site for the prediction of hip fracture, and the BMD of the spine is the best predictor of vertebral fracture (53).

One disadvantage of the DEXA is that the results are affected by bone size. Since it does not take into account the three-dimensional aspects, larger bones appear denser than smaller ones (51). DEXA of the spine in the elderly frequently gives falsely high BMD due to spondylosis or vertebral compression (23,54).

## **1.7 QUANTITATIVE ULTRASOUND**

Quantitative ultrasound measures the bone density in peripheral sights most commonly the heel. It is suggested from in vitro studies that the mechanical properties of trabecular bone can be predicted with quantitative ultrasound measurements of the calcaneus (QUS) (55).

The quantitative ultrasound parameters are: SOS measures the speed of sound (m/sec) and BUA, the broad-band attenuation of the ultrasound beam in the heel (dB/MHz) (56).

Using these parameters a stiffness index can be calculated  $(0,67 \times \text{BUA}) + (0,28 \times \text{SOS}) - 420$  (57).

Researchers are still discussing which mechanical and/or structural parameters of the bone are being measured by the QUS. It may be related to the trabecular size and trabecular spacing, and parameters of bone mineralization, such as crystal size and orientation. The ultrasound parameters may reflect qualitative properties as the elasticity, structure, micro architecture that is strictly related to bone strength. (55,56). Results from the Epidos study in Europe showed that a combination of DEXA and QUS has a higher accuracy for prediction of a future fracture than either method alone (58). QUS is used with both water and gel. It takes about 2 minutes to perform and the patient must be able to remain in a sitting position during the procedure. The accuracy is about 20% and the time precision is about 2,5 % (23,51).

New techniques like Peripheral Quantitative Computed Tomography (pQCT), High resolution computed tomography (HRCT) and Magnetic resonance imaging (MRI) are under development. These methods may create the possibility to measure the bone area, the bone architecture (cortical and cancellous), the trabecular architecture, number of trabeculae, trabecular thickness and trabecular separation and other three-dimensional aspects of bone structure (59,60).

## **1.8 BONEMARKERS**

The makers of bone formation include total and bone alkaline phosphatase, osteocalcin (or bone gla-protein) and procollagen peptide (3). Bone specific alkaline phosphatase is a membrane bound enzyme that is produced in the osteoblasts and probably has a role in the mineralization process. Osteocalcin is also produced by osteoblasts and is the most prominent non-collagen protein in the matrix and is a valid marker of bone formation (4). The procollagens are also synthesized by the osteoblasts (4) and during the formation of type I collagen there is a split of a carboxyterminal PICP that can be measured in serum. (61).

Type I collagen is linked by pyridinoline crosslinks and deoxypyridinoline. During degradation of bone these are released and excreted in the urine and are thus bone markers for resorption. So are also the C terminal propeptid CTX and the N terminal propeptid NTX from the terminal regions of the type I collagen. CTX can also be measured in serum like ICTP, the carboxy-terminal across-linked telopeptid (62). All these markers show substantial individual variability. Marker measurements correlate poorly with bone mineral density (3).

However serum-osteocalcin, urinary pyridinoline crosslinks, and other markers of bone turnover are significantly increased after the menopause due to the menopausal dramatic increase of bone turnover. The values of the bonemarkers have been shown to return to premenopausal levels within a few months of hormone replacement therapy (61).

## **1.9 STUDIES ON THE EFFECT OF PHYSICAL ACTIVITY ON BMD IN POSTMENOPAUSAL WOMEN.**

Osteoporosis has become a significant health problem. Because inactive lifestyles are associated with increased risk of osteoporotic fractures the effects of increased regular exercise has been investigated in many prospective studies (63). Some of these studies have identified regular physical activity as one of the determinants of the maximum bone mineral density a person reach as an adult, the peak bone mass (64). The dramatic increase in hip fractures over the last 40 years is probably not only explained by an ageing population but may thus be associated with a sedentary lifestyle (65). During menopause the hormonal changes will accelerate bone density loss, a physical reduced activity under the menopausal period may then lead to further bone loss (66). Therefore a lot of prospective studies have focused on if physical exercise can reduce the decrease in bone mineral density caused by estrogen deficiency in postmenopausal women (66). In this thesis we have focused on studies of the effect of physical training in postmenopausal women and some are listed in table 2 and commented below.

### 1.9.1 Table 2

Author Year (Reference)	Number of patients included	Duration Months	Intervention	Measure- ment	Com- pliance	Drop Outs & Ex- cluded	Results neck	Results spine	Random & Influences	Ca +D
Bassey 1995 (63)	63  Age 54-55	12	50 heel drops daily  Cont	DEXA Spine Neck	84%	30%	NS	NS Subgroup with last menstruation>6 years Cont - 0.035 g/cm <sup>2</sup> ♦	Random not described.	Ca
Bravo 1996 (64)	124 with osteopenia.  Age 60	12	Walking, dancing, stepping exercise Muscular training 60 min 3 times /week at 60-70% of max heart rate. Cont	DEXA Spine Neck	nr	13%	Ex 0.002g/cm <sup>2</sup> Cont -0.004g/cm <sup>2</sup>	Ex 0.005 g/cm <sup>2</sup> Cont -0.012g/cm <sup>2</sup> ♦	Random described  Some used HT or bisfosfonate	a few
Ebrahim 1997 (65)	165 with fracture upper limb  Age 67	24	Brisk walking 40 minutes 3 times /week Cont did exercise for upper limb	DEXA Spine Neck	100%	41%	Difference between groups 0.019 g/cm <sup>2</sup> p = 0.056	+ 0.017 g/cm <sup>2</sup> both groups.	Random described Some used HT or bisfosfonate	-
Grove 1991 (66)	15  Age 56	12	Low impact High impact 1 hour 3 times /week Cont	DPA Spine	80%	6%	-	Low i vs. Cont ♦ High i vs. Cont ♦ Low + 000 g/cm <sup>2</sup> High + 0.02 g/cm <sup>2</sup> Cont -0.07 g/cm <sup>2</sup> ♦	Random described	+

Author Year (Reference)	Number of patients included	Duration Months	Intervention	Measure- ment	Com- pliance	Drop Outs & Ex- cluded	Results neck	Results spine	Random & Influences	Ca +D
Going 2003 (67)	ExHT 86 HT 73 Ex 91 Cont 70  Age 56	12	HT Ex=Weight- bearing, weight- lifting (free weights and machines) 3 days/week increasing Cont	DEXA Spine Neck Troch	72%	17%	ExHT Troch + 2.1% ♦ Neck + 1.5% ♦ Ex Troch +1.2% ♦ Cont Neck - 0.4% ♦	ExHT 0.8% ♦ HT 1 % ♦	Random not described   <i>Intention to treat</i>	Ca
Hatori 1992 (68)	EX 23 Cont 12  Age 45-67	7	Moderate ex 90% of maximal heart rate High ex 110 % of maximal heart rate at anabolic threshold Walking and stretching 3 times /week Cont	DEXA Spine	nr	n=2	-	Mod ex -1 %  High ex +1.1% Cont - 1.7 %  Cont vs. High ♦	Random not described	-

Author Year (Reference)	Number of patients included	Duration Months	Intervention	Measure- ment	Com- pliance	Drop Outs & Ex- cluded	Results neck	Results spine	Random & Influences	Ca +D
Heinonen 1998 (69)	101 Perimeno Endur 34 Call 36 Cont 35  Age 52	18	Endurance: 55-75% of VO <sup>2</sup> max, jogging, cycling, climbing Callisthenic: stretching with wrist and ankle band 1,2 kg Cont: light stretching 1 hour 3 times/week	DEXA Spine Neck	72%	25%	Endurance +0.013 g/cm <sup>2</sup> Cont -0.006 g/cm <sup>2</sup> Callisthenic no change  Endurance vs. Cont ◆	NS	Random described	Ca
Heikkinen 1997 (70)	78 two groups with HT treatment and one placebo  Age 53	24	The three groups were randomized to training with loading 1 hour three times /week	DEXA neck Spine	nr	12%	Significant increase in the HT groups  All groups Ex vs no Ex ◆	Significant increase in the HT groups.	Random described	
Iwamoto 1998 (71)	68 with osteoporosis  Age 65	12	Exercise daily brisk walk and gymnastics two sets a day 5days/week Cont	DEXA spine	nr	n=33	-	Ex + 4.48% Cont + 1 %  Ex vs. Cont ◆	Random not described	+



Author Year (Reference)	Number of patients included	Duration Months	Intervention	Measure- ment	Com- pliance	Drop Outs & Ex- cluded	Results neck	Results spine	Random & Influences	Ca +D
Iwamoto 2001 (72)	35 with osteoporosis  Age 64	24	Exercise daily brisk walk and gymnastics two sets a day 5days/week Ex only year one Ex two years Cont	DEXA spine	nr	nr	-	Ex 2 years + 4.29 % ♦  Cont + 0.96%  Ex vs. Cont ♦	Random not described	+
Kerr 1996 (73)	56  Age 40-70	12	One side of the body was trained 3 hour/week with resistance (high load) or endurance (low load) training more frequent Both increased level Cont side	DEXA Neck Troch Intertro	82%	n=10	Increase in high strength group vs. cont side In Troch 1.7% vs. -0.6% ♦ In Ward 2.3% vs. + 0.8 ♦	-	Random to left or right side	-
Kerr 2001 (74)	126  Age 60	24	1 Strength training that increased 2 Fitness training some strength & Bicycle 1 hour three times/week 3 Cont	DEXA Spine Hip	74%	30%	Strength group Total hip + 0.9% ♦ Intertroc + 1.1% ♦ Strength vs.Cont Intertroc ♦	NS	Random described	Ca
Kemmler 2003 (75)	137 with osteopenia  Age 55	14	Endurance, strength training 2 sessions/week+ home training increasing Cont	DEXA Spine Total hip	75%	15%	Cont -0,8% ♦	Ex group + 1.3% ♦ Cont - 1.2% ♦	Agreed to participate Non-random controlled	+

Author Year (Reference)	Number of patients included	Duration Months	Intervention	Measure- ment	Com- pliance	Drop Outs & Ex- cluded	Results neck	Results spine	Random & Influences	Ca +D
Kemmler 2005 (76)	137 with osteopenia  Age 55	38	Endurance, strength training 2 sessions/week+ home training increasing Cont	DEXA Spine Neck	2,4 session /week	Ex 21% Cont 29%	Ex vs. Cont 0.7 vs. -2.6% ♦	Ex vs. Cont 0.7% vs. -3% ♦	Random not described	+
Korpelaie * 2005 (77)	160 with osteopeni  Age 72	30	Weight bearing exercise 1 hour/week (6 month yearly) and a 20 min daily home program during 30 month. Mean 3 times a week. Cont	DEXA Hip	73%	Ex 19% Cont 14%	Ex no change  Cont Neck - 1.6% ♦ Troch - 1.1 % ♦	-	Computer randomized  Some used HT  <i>Intention to treat</i>	-
Martin D 1993 (78)	76  Age 57	12	Treadmill on 70- 85% of max heart rate 30 or 45 min three times/week Cont	DPA Spine	C:a 80%	n=21	-	NS Women<6years of last menstruation Ex -1.67% Cont -3.36% Ex vs. Cont ♦	Random not described	+

Author Year (Reference)	Number of patients included	Duration Months	Intervention	Measure- ment	Com- pliance	Drop Outs & Ex- cluded	Results neck	Results spine	Random & Influences	Ca +D
Miliken 2003 (79)	94 Ex 26 Cont 30 HTEx 17 HT 21  Age 40-65	12	Weight bearing exercise 75 min 3 times/week	DEXA Spine Neck		n=4	HT vs. no HT ♦  Wards Ex vs. Cont ♦  Trook ExHT vs. HT ♦	HT vs. no HT ♦	Random not described	ca
Nelson 1994 (80)	40  Age 59	12	Strength training 2 times/week  Cont	DEXA Spine Neck	87%	n= 1	Ex 0.9% Cont -2.5% Ex vs. Cont ♦	Ex 1% Cont -1.8% Ex vs. Cont ♦	Random not described	+
Prince 1995 (81)	168  Age 63	24	1 2 hour walks/week and weight bearing exercise 4 hours/week + Calcium  2 Calcium 3 Placebo	DEXA Spine Hip	39%	nr	Ca -0.18%/year Placebo - 0.67%/year  ExCa +0.28%/year ExCa vs. Ca ♦	NS	Random described	Ca or not Ca
Pruitt 1992 (82)	27  Age 55	9	Weight training 1 hour 3 times/week Cont	DPA Spine Neck	83%	n=1	Ex -2.7% Cont -0.8 %	Ex +1.6% Cont - 3.6% Ex vs. Cont ♦	Non random controlled	-

Author Year (Reference)	Number of patients included	Duration Months	Intervention	Measure- ment	Com- pliance	Drop Outs & Ex- cluded	Results neck	Results spine	Random & Influences	Ca +D
Revel 1993 (83)	78  Age 57	12	Psoas training two sessions 60 flexions daily compared with deltoid training	TBMD QCT	60%	n= 11	-	Psoas trained group +0.14 g/cm <sup>2</sup> vs deltoid -8.87 g/cm <sup>2</sup> ♦	Random not described  <i>Intention to treat</i>	-
Snow 2000 (84)	18  Age 64	60	Lower body resistance and jumping ex 3 days/week Cont	DEXA Neck	84%	0	Cont - 4.43% Ex + 1.54% Ex vs. Cont ♦	-	Non random controlled Some used HT	+

Text to table 2

The studies listed are randomized controlled studies or case control studies published from the beginning of 1990, in postmenopausal women up to 67 years of age. The intervention lasted at least 7 months. The women were healthy postmenopausal if not indicated. All studies are per protocol except when indicated. Significant changes from baseline and between groups are indicated ♦. Ca=Calcium, Cont=Controls, DPA=Dual-Photon Absorptiometry, Ex=Exercise, HT=Hormone therapy, n=number, nr=non reported, NS=non significant, vs.=versus, results noted in table without♦are non significant (\* 70 years, an intention to treat study).

### 1.9.2 Comments to table

The disadvantages of the studies are the high drop out frequency, low compliance and the small number of participants. In six of the studies the participants were known to have decreased bone mineral density at baseline (64,71,72,75,76,77). Fracture in the upper limb was an inclusion criteria in a study not listed by Krolner 1983 (86) and in the study of Ebrahim (65). Krolner found a significant change between training and controls over time and Ebrahim found a tendency. In some of the studies HT treatment or bisfosfonate treatment was not an exclusion criteria (64,65,77,84). Some studies also had HT as an intervention in combination with exercise (67,70,79). A positive impact of training (with the HT studies excluded) was found on the hip BMD in 8 studies (65,69,73,74,76,80,81,84) and on the spine BMD in 10 studies.

(66,68,71,72,75,76,78,80,82,83). All except two are per protocol studies.

The mechanism of action of exercise on the skeleton is through gravitational force (weight bearing, endurance training, high impact, low impact) or muscle pull, producing strains within the skeleton (strength training, non impact). The interventions in these studies differ. In some, the intervention was described in detail, and even the way of increasing the training was clearly defined (75,76). In other studies the intervention was monitored from % of the maximal heart rate (64,68,78). In several papers the purpose was to determine the type of exercise that most markedly influenced the skeletal status. In this résumé six studies with only impact training had positive influence on the BMD (65,66,68,69,77,81). In the hip region: 65,69,77,81 and in the spine: 66, 68. Positive impact with only strength training on the BMD was found in four studies (74,80,82,83). Nelson (80) found a positive impact in both sites. Kerr found a positive impact in the hip (74). Pruitt found a positive significant impact only in spine (82) and also Rewel who however only measured the spine (83). In several investigations there were a mix of impact and nonimpact training with positive effect on BMD (64,71,72,75,76,84) with a positive effect in the hip found in study: 75, 76 and 84. A positive impact of the mixed training was found in the spine in study 64,71,72,75,76.

Korpelainen gave the overall conclusion that the lack of reporting on the exercise characteristics (type, intensity, frequency, duration and mode) and interventions in postmenopausal women limits the conclusions that could be drawn about the effect of exercise (77). However Layon and Skerry found that many carefully conducted studies designed to show the effects of exercise on bone mass were wasted because the exercise regimen used was measured in terms of a variable which may have little relevance to strain in bone, such as cardiopulmonary performance (85).

## **2 AIMS OF THE STUDY**

### **2.1 GENERAL AIM OF THE STUDY**

To investigate the effects of gonadal hormones on bone mass in men and of physical activity and gonadal hormones on bone in women.

### **2.2 THE FOLLOWING WERE THE SPECIFIC AIMS AND ISSUES**

1. To assess the effect of surgical and medical castration in men with prostate cancer on bone mineral density.
2. To evaluate the effects of weight-bearing physical activity on bone mineral density in women with endometriosis treated with GnRH- analogues.
3. To investigate changes in bone mineral density during the perimenopausal period and compare the effect of hormone therapy and weight bearing physical activity on bone mineral density.
4. To evaluate the effect of weight-bearing physical activity on bone mineral density in postmenopausal women with osteopenia and osteoporosis and a wrist fracture.

### **3 MATERIALS AND METHODS**

#### **3.1 CLINICAL MATERIALS**

33 elderly men; 12 with prostate cancer treated with bilateral orchidectomi age 78.6 years (range 72-88 years), 10 with prostate cancer treated with GnRH analogues age 72.5 years (range 64-81 years) and 10 healthy men age 76.2 years (range 60-80 years). They were all attending the urology outpatient clinic at Danderyds Hospital (Paper 1).

19 women in reproductive age with endometriosis treated with GnRH analogues during 6 months. The age in the intervention group (no 8) was 24.4 years (range 23-35 years) and in the control group (no 11) was 31.3 years (range 23-38 years). They were attending the obstetrics and gynaecology department of Danderyd and Karolinska hospitals and they all had symptomatic endometriosis confirmed laparoscopically. They had not previously been treated with GnRH analogues (Paper 2).

60 apparently healthy perimenopausal women with irregular menstruation and/or sweating and flushes. 20 with age 47.3 years (range 44-51 years), 20 with age 48.4 years (range 45-55 years) and 20 with age 47 years (range 41-51). The women were recruited through advertisement in a daily newspaper (Paper 3).

113 apparently healthy postmenopausal women with at least one year after menstruation (range 1 –21 years), age 59.6 years (range 50-65 years) with decreased bone mineral density and wrist fracture were included. The women were recruited through advertisement in a daily newspaper (Paper 4).

The ethics committee of Karolinska Hospital, Huddinge Hospital and Karolinska Institutet South approved the studies.

Study 1: Dnr 95-170. Study 2: Dnr 96-197. Study 3: Dnr 381/98. Study 4: Dnr 27/02

#### **3.2 STUDY DESIGN**

##### **Study 1**

A prospective, controlled parallel-group study with a comparison every third month of the effect on bone mineral density of surgical and medical castration in men with prostate cancer compared with a control group during one year.

##### **Study 2**

Women in reproductive age with endometriosis aimed for 6 months treatment with GnRH analogues were randomised to physical training or to controls. Bone mineral density was studied at base line and after 6 months and one year.

### Study 3

Perimenopausal women were randomised to physical training or hormone therapy or to a control group. Bone mineral density was measured at start and after six and eighteen months of intervention.

### Study 4

Postmenopausal women with a wrist fracture and osteopenia or osteoporosis were randomised to physical training or to a control group. Bone mineral density was analysed at start and after one year.

## **3.3 INTERVENTION**

### Physical training

The physical training consisted of three fast thirty-minute walks plus one or two sessions of one-hour training per week. The aerobic training consisted of - 5 minutes warming up, -25 minutes strengthening exercise of arms, legs, back and stomach, - 25 minutes of aerobic exercise and 5 minutes of stretching. The individuals chose their own level of training. The patients filled in a protocol for each training episode. A study nurse questioned each subject for compliance every third months.

The training period lasted for one year. Paper 2 and paper 4.

The training period lasted for 18 months. Paper 3.

### Hormone therapy (HT)

The HT group received 2 mg estradiolvalerat (EV) daily for 9 days, 2 mg EV and 10 mg medroxyprogesteronacetat (MPA) for 12 days followed by 2 mg EV for 7 days, (Divina plus Orion Pharma Finland®).

## **3.4 METHODS**

### **3.4.1 Dual energy X-ray absorptiometry**

Paper 1: Bone mineral density was performed in the hip at base line and at 3,6 and 12 months (Lunar DPX-L; Lunar Corporation, Danderyd hospital).

Paper 2: Bone mineral density was performed in the spine and hip at baseline and after 6 and 12 months (Hologic model QDR 4500 ACCLAIM unit, Karolinska University hospital Solna)

Paper 3: Bone mineral density was performed in the hip and spine at baseline and after 6 and 18 months (Lunar DPX-L 7263 Karolinska University Hospital Huddinge)

Paper 4: Bone mineral density was performed in the hip and in the spine at baseline and after one year (Lunar Prodigy 10631 GE Medical Systems Karolinska University Hospital Huddinge).

### **3.4.2 Ultrasound**

Ultrasound of the heel was performed at baseline and after 3,6 and 12 months (Achilles ultrasound device; Lunar Corporation Danderyd Hospital). Paper 1.



### **3.4.3 Bone markers**

Osteocalcin was analysed in blood with a non competitive Immuno Radio Metric assay (ELSA-Osteo) from CIS Bio International, Gif-Sur-Yvette, France, and Deoxypyridinoline in urine was analysed with a automated competitive Enzyme Immuno Assay (EIA) (Pyrilinks-D) from DPC, Los Angeles, CA, USA using an Immulite 2000. All samples were run in the same batch and the total coefficient of variation (CV%) was well below 10% for both methods.

The bone makers were analysed at base line and after 6 and 18 months. Paper 3.

### **3.4.4 Hormones**

17 beta -estradiol and testosterone analyses were performed at baseline and after 3,6 and 12 months using commercial kits obtained from Diagnostic Product Corporation (Los Angeles, CA). Paper 1

### **3.4.5 Lower extremity muscle strength**

Paper 3. The muscle strength of the legs was measured at base line and after 6, 12 and 18 months. This was performed using a vertical jump technique with an arm swing in the start. The height of the jump was measured in centimetres, using a measuring tape fastened to the waist of the women. At each time point the subjects jumped at least twice and the highest height was recorded.

Paper 4. The timed-stands test is a performance-based measure that records the time needed to stand 10 times from a standard chair in seconds. A shorter time indicates better performance. Physical Rehabilitation Outcome Measures II (PROM II) Book Measure Review Template. Csuka M, McCarty J. Wisconsin USA 1985.

## **3.5 STATISTICAL METHODS**

Paper 1: Bone mineral assessments were analysed using two-way ANOVA with repeated measures on one factor. The factors were, GROUP with three levels and TIME with time-points 0, 3, 6 and 12 months. The 36 months evaluation was omitted due to too few subjects. Differences between levels of the time factor were evaluated by post-hoc contrasts.

In case of significance interaction between group and time, simple effects were examined, i.e. effects of one or more factors holding other factors fixed.

Endocrine assessments were analysed with the MannWhitney- U test.

Paper 2: The data contain missing values, supposed to be at random. The data were analyzed using procedure Mixed in SAS ®. The model was set up as a repeated measures design with the between factor GROUP (treatment/control) and the within factor TIME (0, 6 and 12 months). As the homogeneity of variances assumption was violated, a model with separate variances for each group was performed. Differences

between levels of the time factor were evaluated by post-hoc contrasts. In case of significant interaction between group and time, simple effects were examined, i.e. effects of one more factors holding other factors fixed.

Paper 3: The data contain missing values, supposed to be at random. The data were analyzed using procedure Mixed in SAS ®. The model was set up as a repeated measures design with the between factor GROUP (HRT, training and control) and the within factor TIME (0, 6 and 18 months). In case of significant interaction between group and time, simple effects were examined, i.e. effects of one factor holding the other factor fixed).

Paper 4: Statistical methods hip BMD and muscle strength.

The data were analysed using repeated measurement ANOVA method Statistica 7.0 software. The two groups (control and training) were the between-factor and the time (0 and 12 months) was the within-factor. The proportional differences over time in each group were calculated and compared. Missing values in the data set were supposed to be at random.

Statistical methods L2-L4 subgroup

A two-way ANOVA with repeated measures on one factor was used to analyse the BMD data. The between factor was Group (training and control) and the within factor was Time (baseline and after 12 months). According to our hypothesis, the years since last menstruation would be expected to influence the mean loss of BMD during the study period. Thus a three-way ANOVA with repeated measures on one factor was performed. The between factors were Group (training and control) and Years since last menstruation ( $\leq 6$  years and  $>6$  years) and the within factor was Time (baseline and after 12 months). The three-way interaction Group\*Time\*Years since last menstruation was of special interest. If this interaction is significant, then the two-way interaction Group\*Time is modified by Years since last menstruation, which means that the differences between control- and training patients, regarding the changes over time, will show different pattern within these two subgroups.  $P < 0.05$  is considered statistically significant.

## 4 RESULTS

Table 3. Summary of results.

Paper	Average age (range)	BMD neck	BMD spine	Ultra- sound heel stiff
<b>1</b> Healthy men n=10	76.2 (60-80)	+ 0.017 g/cm <sup>2</sup> + 1.26 %		+ 1.26 %
<b>1</b> Men with prostate cancer and ablation testis n=12	78.6 (72-88)	- 0.037 g/cm <sup>2</sup> - 4.53 % ♦ c vs ablatio ♦		-9.04 % ♦
<b>1</b> Men with prostate cancer, GnRH – treated n=10	72.5 (64-81)	- 0.027 g/cm <sup>2</sup> - 3.18 %		- 3.58 %
<b>2</b> Women with endometriosis GnRH treated n= 11	24.4 (23-38)	- 0.028 g/cm <sup>2</sup> - 3.6 % ♦	- 0.054 g/cm <sup>2</sup> - 5.1 %	
<b>2</b> Women with endometriosis GnRH treated FYSS n=8	24.3 (23-35)	- 0.0048 g/cm <sup>2</sup> - 0.6 % c vs FYSS ♦	- 0.028 g/cm <sup>2</sup> - 2.6 %	
<b>3</b> Peri- menopausal women n= 20 1,5 years	47 (41-51)	-0.013 g/cm <sup>2</sup> -1.4 %	- 0.03 g/cm <sup>2</sup> - 2.7 % ♦	
<b>3</b> Peri- menopausal women HT n= 20 1,5 years	48.4 (43-55)	+0.007 g/cm <sup>2</sup> + 0.7 %	+ 0.008 g/cm <sup>2</sup> + 0.7 % c vs HT ♦	

<b>3</b> Peri-menopausal women FYSS n= 20 <i>1,5 years</i>	47.2 (44-51)	-0.012 g/cm <sup>2</sup> -1.2 %	- 0.007 g/cm <sup>2</sup> -0.5 % c vs FYSS	
<b>4</b> Post-menopausal women with osteopenie and wrist fracture n= 39	59.6 (51-64)	- 0.003 g/cm <sup>2</sup> -0.4%	- 0.004 g/cm <sup>2</sup> -0.4%	
<b>4</b> Post-menopausal women with osteopenia and wrist fracture and FYSS n=37	59.7 (50-65)	+0.007 g/cm <sup>2</sup> +0.8 %   ♦ c vs FYSS ♦	+ 0.006 g/cm <sup>2</sup> +0.65%	
<b>4</b> Post-menopausal women with oteopenia and wrist fracture and menopause < 6years n= 9	55,3 (52-59)	-	- 0.019 g/cm <sup>2</sup> -1.7%	
<b>4</b> Post-menopausal women with osteopenia and wrist fracture and menopause < 6years and FYSS n= 9	55,9 (52-61)	-	+0.014 g/cm <sup>2</sup> +1.4% c vs FYSS ♦	

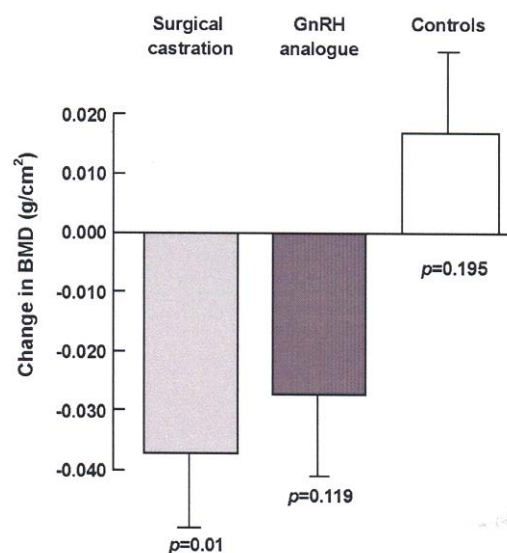
Text to table 3. The results from each study are summarized in table 2. Change from base line to 1 year respectively 1½ year in BMD, in different periods of life, (age) medical conditions and treatments. ♦ = Significance. FYSS= physical training group. c=controls.

## 4.1 PAPER 1

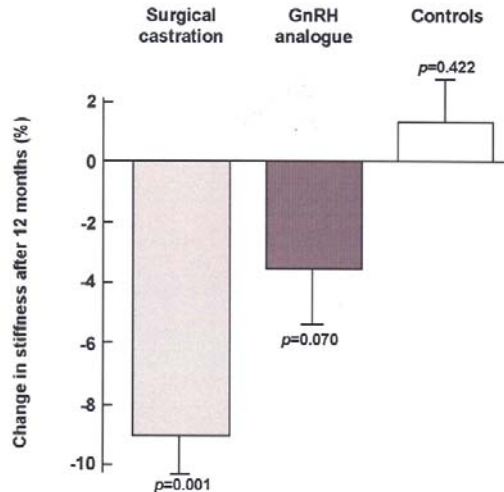
Changes in bone mineral density differs between GnRH-analogue and surgically castrated men with prostate cancer – a prospective controlled parallel group study.

The main question in this paper was whether medical castration induces a smaller rate of bone loss than surgical castration. Therefore twenty eight men presenting with prostatic cancer who had been selected to undergo medical or surgical castration and ten healthy men with benign urological disorders were followed from baseline observations and assessed for BMD at 3, 6, 12 and 36 months. Serum hormone levels were also assessed. Orchidectomy and treatment with GnRH analogues caused an expected rapid decrease in serum testosterone levels with no difference between these two groups. The mean BMD in the femoral neck measured by DEXA in surgically castrated men and the GnRH treated men was 0.037g/square centimetre (SE 0.013),  $p=0.010$  (4.53 %) and 0.027 g/square centimetre (SE 0.014),  $p=0.119$  (3.18 %) respectively, at 12 months while the controls gained 0.017 g/squared centimetre (SE 0.013),  $p=0.195$  (1.26%) Fig 7. Stiffness in the heel measured with ultrasound surgically castrated men lost 9.04% ( $p<0.001$ ), the GnRH treated lost 3.58% (ns) vs. and the controls gained 1.26% (ns). Fig 8.

Conclusion: The current study suggested a higher rate of bone loss in men with metastatic prostate cancer who were treated with surgical castration than those that were treated with GnRH analogues.



**Fig 7.** Changes in BMD in the femoral neck assessed with DEXA after 12 months treatment with surgical or medical castration compared to a controlled group. Mean and SEM are shown.



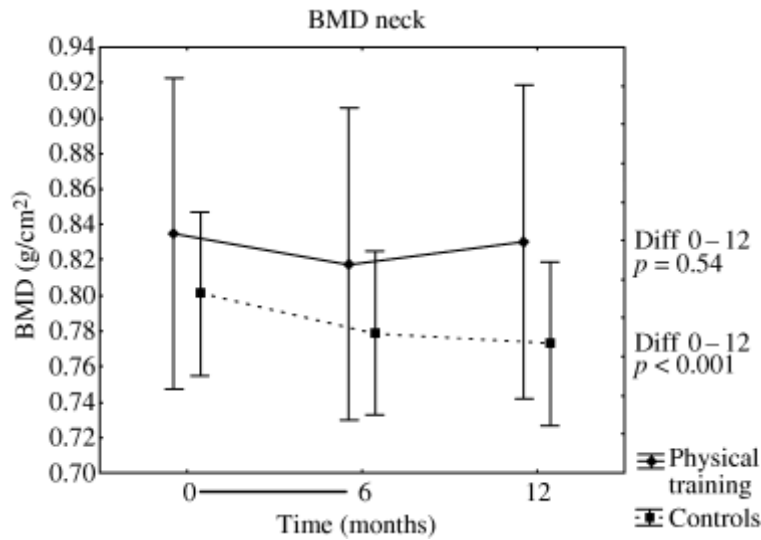
**Fig 8.** Change in heel stiffness assessed with ultrasound after 12 months treatment with surgical or medical castration compared to a control group. Mean and SEM are shown.

## 4.2 PAPER 2

The effect of Physical Training on Bone Mineral Density in Women with endometriosis treated with GnRH analogues-a pilot study.

GnRH treatment of endometriosis causes amenorrhea, loss of estrogen and bone loss. The main question was whether this bone loss could be prevented with physical training. Therefore nineteen Caucasian premenopausal women aged 23 to 38 years were included in the study. The subjects were all treated with 21.6 mg goserelin during 6 months. The patients were randomized for physical training n=8 or to a control group n=11. The total period of training was 12 months, whereas GnRH treatment was terminated after 6 months. Bone mineral density was measured in the femoral neck area and the lumbar spine using Dual energy X Ray Absorptiometry. This was performed just before treatment, after 6 and 12 months. Seven women fulfilled the training during 12 months of observation. In the control group ten women were followed up for 12 months.

BMD in femoral neck: After 6 months the women in the physical training group were 2.1% below baseline. 6 months later they had gained BMD in the femoral neck and were 0.6 % below baseline. The control group lost 2.8 % after 6 months and was 3.6 % below baseline after 12 months. The difference in loss of BMD after 12 months between the groups was significant ( $p=0.0288$ ). Fig 9. In the spine there was no significant difference between the two groups. In the present study physical training was shown to regained bone mineral density after treatment with GnRH analogues when compared to a control group. This effect could be demonstrated 6 months after cessation of GnRH treatment.



**Fig.9** Bone mineral density in the femoral neck at baseline, 6 and 12 months in 19 premenopausal women with endometriosis undergoing physical training or control (mean and 95% confidence interval). The black bar indicates GnRH therapy

### 4.3 PAPER 3

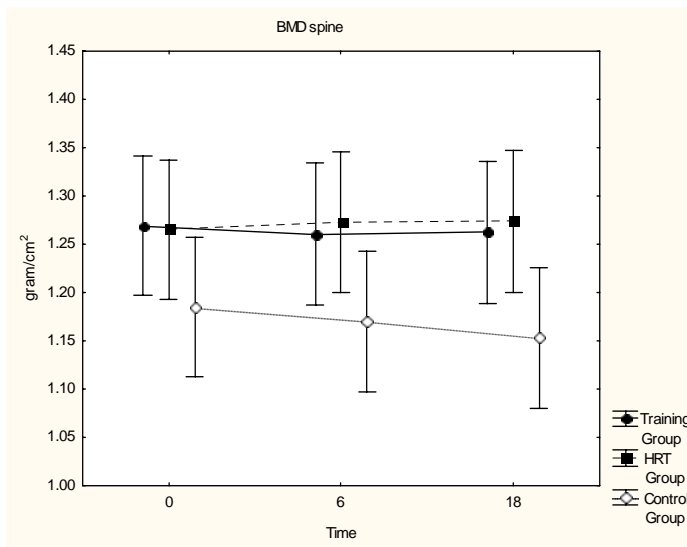
Physical Training and hormone replacement therapy reduce the decrease in bone mineral density in perimenopausal women - a pilot study.

Transition to menopause characterized by an increased number of anovulatory cycles reflected by irregular menstrual patterns. A few studies have described BMD decreases during perimenopause. The main question was whether this period induces bone loss and to study the effects of HT and physical activity on BMD during the perimenopause. Therefore sixty perimenopausal women were included. The subjects were randomised to either physical training (n=20), HT (n=20) or to a control group (n=20).

After 18 months the BMD in the spine had not decreased in either the training group or in the HT group. In the control group the spine BMD had significantly decreased ( $p = 0.014$ ). Fig 10.

U-deoxypyridinoline and osteocalcin was increased significantly in the control group ( $p = 0.0198$ ,  $p = 0.0295$ ). No significant changes in bone marker levels were found in the training group or the HT group.

We found that both HT and physical training can prevent a decrease in spine BMD in perimenopausal women over a period of 18 months.



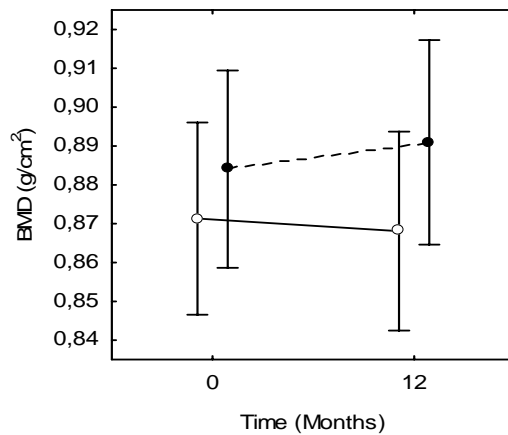
**Fig 10** Sixty perimenopausal women were randomized to three different treatments: control, physical training, and HRT. All received calcium. DXA of the lumbar spine (L2–L4) was performed at baseline and after 6 and 18 months of treatment. The results are presented as mean and 95% confidence interval ;  $p=0.0014^*$

#### 4.4 PAPER 4

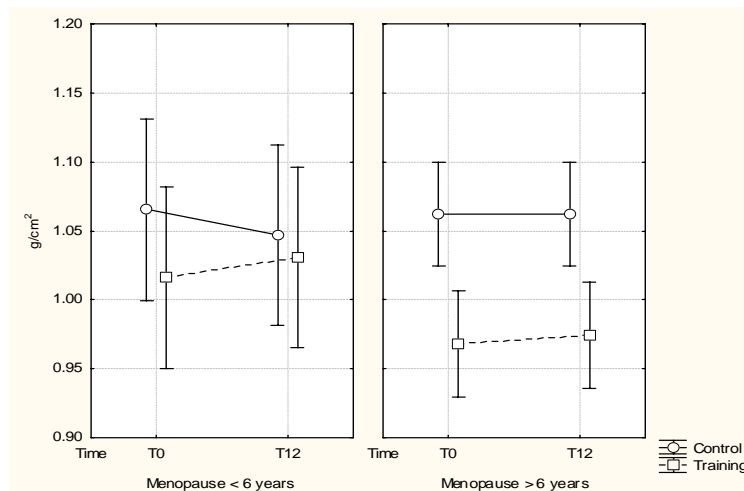
The effects of Physical Training on Bone Mineral Density in postmenopausal women with low bone mineral density and a forearm fracture - a randomized controlled study. The aim of the study was to investigate if moderate physical activity can prevent bone loss in postmenopausal women 45 to 65 years of age with a forearm fracture and low bone mass. One hundred and twenty one women with a BMD T-score in the interval  $-1$  to  $-3.0$  were contacted for inclusion, and 112 were randomized. The physical training consisted of three fast thirty-minute walks plus one or two sessions of one-hour training per week in a training centre outside the hospital. The Bone Density was measured in the hip and the lumbar spine at baseline and after one year. Only data from the patients who had been compliant with the intervention was included, thus a per protocol analysis. After exclusions and drop outs there were 37 in the training group and 39 in the control group. In total hip BMD the interaction for the group x time variables was significant ( $p=0.029$ ). The increment in BMD during 12 months in the training group was  $+0.0069 \text{ g/cm}^2$  ( $\pm 0.019$ ) ( $p=0.037$ ) while the control group decreased their mean BMD with  $-0.0032 \text{ mg/cm}^2$  ( $\pm 0.019$ ) ( $p=0.31$ ). The mean proportional change in BMD over time was  $-0.39 \%$  for the control group and  $+0.80 \%$  for the training group. Fig 11. No significant change in spine BMD was observed. However the difference between control- and training patients regarding the mean loss in BMD in L2-L4 in spine after 12 months was analyzed within the subgroups, menopause  $\leq 6$  years (no 18) and menopause  $> 6$  years. Within the subgroup menopause  $\leq 6$  years, the Group\*Time interaction was significant,  $F(1.67)=5.3$ ,  $p=0.02$ , which means that the mean loss in BMD during the study period differed between the control- and the training women. Further analyses,



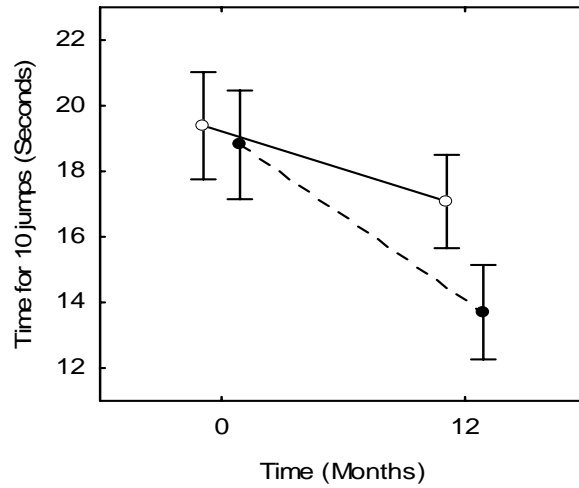
within this subgroup, revealed that BMD decreased  $0.019 \text{ g/cm}^2$  (-1.7%) after one year in the control group,  $p=0.07$ , and increased  $0.014 \text{ g/cm}^2$  (+1.4%) in the training group,  $p=0.16$ . Fig 12. At base line both the control and the training group had equal leg strength. After 12 months the measurement was repeated and showed that the training group had increased their strength compared with the control group. Fig 13. Our results indicate a weak but positive effect of training on hip BMD in postmenopausal women with low bone mass.



**Fig 11.** Figure Comparison of Bone Mineral Density (BMD) in the hip (total content) at baseline and after 12 months in the control and training group in postmenopausal women with decreased bone mineral density and a forearm fracture. The patients' total bone mineral content of the hip was measured at baseline and at 12 months. There was a significant increase in BMD in the training group compared to the control group after 12 months when calculated with repeated measurement ANOVA,  $p=0.029$ . Legends. Open circles, Control group; Filled Circles, Training group. Vertical bars denote 95% Confidence interval.



**Fig 12.** Figure Comparison of Bone Mineral Density (BMD) in L2-L4 at baseline and after 12 months in the control and training group in the sub group with last menstruation < 6 years and > 6 years. group. (Postmenopausal women with decreased bone mineral density and a forearm fracture). The patients' total bone mineral content of L2-L4 in the spine was measured at baseline and at 12 months as described in material and methods. In the subgroup with last menstruation < 6 years there was an indication of difference in BMD in the training group compared to the control group after 12 months when calculated with repeated measurement ANOVA. Vertical bars denote 95% Confidence interval



**Fig 13:** Figure Comparison of muscle strength in legs at baseline and after 12 months in the control and training group in postmenopausal women with decreased bone mineral density and a forearm fracture. The patients' muscle strength in the legs was measured at baseline and at 12 months as described in material and methods. There was a significant increase in leg strength in the training group (less seconds) compared to the control group after 12 months when calculated with repeated measurement ANOVA,  $p=0.003$ . Legends. Open circles, Control group; Filled Circles, Training group. Vertical bars denote 95% Confidence interval.

## 5 GENERAL DISCUSSION

### 5.1 THE EFFECT ON BONE MINERAL DENSITY OF WITHDRAWAL OF HORMONES

Bone mineral density begins to decrease in men and women in their mid 40s. The initial loss is slow, about 4% per decade (31). The results of longitudinal studies suggest that bone loss in elderly men is approximately 5–10% each decade, with bone loss accelerating after 75 years of age (33). One of the causes of decreased BMD in men is decreasing testosterone levels (31).

When testosterone levels are rapidly decreased in elderly men, as they are when men with prostate cancer are treated by means of castration, their bone mineral density decreases (40-42). In our study this was verified when looking at men one year after surgical castration or one year after ongoing GnRH therapy. Both the castrated groups showed that testosterone levels rapidly decreased to the same extent. However, we found more pronounced bone loss in the femoral neck when the men had been subjected to surgical castration – 4.6% compared with medical castration – 3.2% (NS). The explanation for this might be that estradiol levels were more decreased in the surgically castrated group than in the medically castrated group ( $p=0.058$ ). It is now well accepted that even estradiol is of importance for the male skeleton. Testosterone is converted to estradiol by the enzyme aromatase. Aromatase deficiency is rare and the few men identified with this condition have had low BMD (87). A recently published study has shown that free estradiol in men is an independent predictive factor of BMD at all bone sites studied (88).

In a recent publication it was suggested that high circulating concentrations of follicle-stimulating hormone (FSH) cause hypogonadal bone loss (89). Treatment by means of surgical castration will elevate the circulating concentration of FSH, whereas treatment with GnRH analogues will decrease it (43). This may be one other possible explanation for the greater loss of BMD in the group subjected to surgical castration.

In medically castrated patients the testes are left intact. The testes produce many biologically active substances that reach peripheral tissues, but their possible relevance in the regulation of BMD is unknown.

Women of reproductive age with endometriosis are treated with GnRH analogues in order to suppress ovulation and decrease circulating estrogens to postmenopausal levels, thereby inducing atrophy of the endometric lesions. Bone loss is a well-known negative side-effect of low serum estradiol levels. Therefore, the duration of treatment is restricted to 6 months. We found that the rapid withdrawal of estrogens during 6 months' treatment with GnRH resulted in significant BMD loss in the femoral neck (3.6%) and in the spine (5.1%; NS) (study 2) one year after the start of the GnRH treatment. These results confirm previously reported data (90).

A few studies have shown a decrease in BMD during the transition to menopause. A loss of 1–2% per year has been reported (29, 91). An increased number of anovulatory cycles with fluctuating estradiol levels, reflected by irregular bleeding, are seen during the perimenopausal period. The hormonal environment is probably permissive as regards bone loss (29). In study 3 the perimenopausal women lost BMD in the spine over a period of 18 months (2.7%), and there was also a loss in the femoral neck (1.4%; NS). The women were treated with calcium.

After menopause circulating estrogen levels decrease as a result of cessation of ovarian function. Postmenopausal bone loss is estimated to be 0.5–2% per year. It has been noted that approximately half of the bone loss observed during the first ten years after menopause occurs within the first three years. Later, the rate of loss decreases (27,91). In study 4 no change in total hip or in spine BMD was observed over a period of one year in healthy postmenopausal women with decreased bone mineral density. This may have been a result of the fact that all subjects received calcium and vitamin D during the study and this treatment has been shown to increase BMD slightly in postmenopausal women (92). However, in a subgroup of early postmenopausal women a decrease in spine BMD (-1.7%,  $p=0.07$ ) was observed.

In the light of the results of these studies we might conclude that a rapid withdrawal of gonadal hormones (brought about by surgical or medical intervention) results in substantially decreased bone mineral density. A slower decline in BMD is found in the perimenopausal period, when estradiol levels are fluctuating with occasional ovulation, and in early menopause when the levels of estradiol decrease (91,93)

## **5.2 CAN PHYSICAL TRAINING PREVENT BONE**

### **LOSS WHEN OESTROGEN LEVELS ARE DECREASED?**

In women with endometriosis treated with GnRH for six months, physical training during the GnRH therapy and for the following 6 months led to restitution of BMD in the femoral neck, whereas the control group did not show normalised BMD (paper 2). In the perimenopausal period, training for 18 months preserved spine BMD, whereas a decrease was observed in the control group (paper 3). In postmenopausal women with low BMD and a Colles' fracture, training for one year increased BMD. Compared with the control group the training group gained 1.2% in total hip BMD (paper 4). No significant changes were seen in spine BMD when the whole sample of women was included. In the subgroup of early postmenopausal women, the one-year difference in percentage change in bone mineral density between the training and control groups was 3.1% in the spine. However, subgroup analyses should always be regarded with scepticism. This was the case here, as the subgroup in our analysis had not been predetermined and it was not large enough to ensure precision.

Our data imply that weight-bearing exercise maintains BMD during withdrawal of oestrogen. To our knowledge no previous study has involved investigation of the effects of physical training on women treated with GnRH analogues or on women

during the perimenopausal period. Physical training can restore bone density in women with GnRH-induced bone loss, according to the results of our pilot study. Our data suggest that physical training can prevent bone loss during the perimenopausal period.

A small effect on BMD (measured by DEXA) is observed using physical training as secondary prevention in women with osteopenia and Colles' fracture. Only two other studies can be found that have involved investigation of the effects of physical activity on BMD in postmenopausal women with a fracture occurring as a result of osteoporosis, and they have shown a significant positive effect (86) or an indication of a positive effect (65). The results of randomised prospective studies among postmenopausal women with or without osteopenia, assessing the effect on BMD of weight-bearing or muscle-strengthening training over one year show an overall gain of about 1–3% (measured by DXA) in the spine or hip (65,66,68,69,71-75,80,82,84). In studies that have been ongoing for more than one year (24 to 38 months) the training group has shown a 3.3% overall gain compared with controls (72,76). In summary, the overall impression is that physical training has a moderately positive effect on BMD.

### **5.3 POSSIBLE PRE-CLINICAL EXPLANATION**

A main finding in our studies was that participation in physical activity may preserve or even increase bone mass during a period of decreased estrogen levels. Our data and others suggest that the younger the skeleton is, the higher the possibility to react to physical training. Several mechanisms are probably involved in the decreased response to mechanical loading with age. One possible reason may be the lack of estrogen, which seems to decrease the effectiveness of the adaptive response related to mechanical load (85). In both clinical and laboratory studies, removal of estrogen reduces bone mass and increases the frequency of remodelling events (1, 85). According to the mechanostat theory, estrogen lowers the set point for mechanical adaptation. This means that bone formation normally starts at lower strains than during estrogen deficit (75). Studies suggest that the presence of estrogen receptors (ERs) is necessary for an adaptive response to load. The expression of ERs in osteocytes appears to be diminished in the absence of estrogen (85). In fracture callus from humans, in which there was clear evidence of osteogenesis, biopsies were analysed for estrogen receptor expression in bone cells (94). The expression of ERs tended to be decreased in osteoblasts and osteocytes in women over 40 years of age compared with younger women.

In study 2, when the GnRH-treated women with endometriosis had stopped the GnRH treatment, there was a rapid positive effect of physical training on BMD. This was probably a result of a return of ovulation immediately after cessation of the GnRH treatment. In study 3 a more pronounced effect of physical training on BMD was observed in the perimenopausal women compared with the postmenopausal

women in study 4. One possible explanation is that perimenopausal women have higher circulating estradiol levels and some ovulatory cycles and thus more ER expression in the osteoblasts and osteocytes than postmenopausal women. The fact that the subgroup of women with menopause within the previous 6 years appeared to respond better to physical activity as regards spine BMD than those with menopause more than 6 years previously (in study 4) might be a result of lower numbers of ERs in the osteocytes and osteoblasts later after menopause. This has not yet been investigated. As aging is a complex process, several other mechanisms of equal or more importance are likely to be involved.

#### **5.4 TRAINING PROGRAMME**

Strain is a measure of deformation of bone and monitoring the events would thus be invasive. Unfortunately, in human studies strains cannot be measured (20). The mechanostat theory states that the same training programme (same load, intensity and frequencies) will result in different individual responses. If the bone is already adapted to strain stimulus as a result of a training programme, bone formation will not occur at that same level of training. However, in a non-adapted bone the same programme will stimulate bone formation (fig. 5). Therefore, training programmes must be individualized if the goal is to increase bone mass. In our studies the subjects trained at a level that suited them and they probably increased their activity levels automatically when a steady state was reached. We have to make an assumption that the more well trained you become, the levels of strain can be increased (but not measured, however), thereby further inducing bone formation (fig. 5). In our studies the training programmes were not therefore decided beforehand, or described in detail. The women were encouraged to increase the level and intensity of training if possible. The time spent training each week was the only directive the participants had to follow. Brisk walking has been shown to have a positive effect in a few studies. The best effect of brisk walking is probably when it is combined with another weight-bearing exercise (95). Our studies included three fast thirty-minute walks and one or two sessions of one-hour aerobic training per week in a training centre outside the hospital. The fact that the training sessions were at different levels and took place several times a day, every day of the week, gave the subjects good accessibility to training. The training consisted of 5 minutes of warming up, 25 minutes of arm, leg, back and stomach-strengthening exercise, 25 minutes of aerobic exercise and 5 minutes of stretching. The most osteogenic exercise would involve jumping. However, this kind of strain-generating exercise is not suitable for this age group as it provokes incontinence.

The women recorded each training episode. The study nurses assessed attendance after 3, 6 and 12 months (papers 2, 4) and after 18 months for each subject (paper 3). The intervention was moderate and easy to join and did not extensively interfere with daily life.

## 5.5 PREVENTION AND TREATMENT

Several drugs have been shown to be effective in fracture prevention in high-risk individuals (23) and should be used according to their specific treatment indications. In individuals with lower fracture risk, however, these drugs are not cost-effective and use of less expensive treatment options with fewer side-effects may be a more sensible alternative. The standard treatment of symptomatic endometriosis involves GnRH analogues, and there are well-documented benefits. Reduction of BMD is, however, a negative side-effect. Many studies of different medical agents have been performed in regard to primary prevention of expected bone-loss. However, the effect of these drugs is not well documented in these young populations as the studies are small and of short duration (48,49,50). Add-back therapy with estrogens may increase the symptoms of endometriosis (47). The fracture risk is small in the short term in this relatively young population. The bone loss observed during GnRH treatment may, however, increase fracture risk in the longer term and minimization of bone loss is therefore of importance for prevention of future fractures. As medical therapy suppresses but does not cure endometriosis the need for re-treatment is common; thus a cumulative effect of several treatments on bone mass might become a problem. Our results suggest potential beneficial effects of physical activity on preservation of bone mineral density in women with endometriosis receiving GnRH analogue therapy. During the first 6 months of training during concomitant GnRH treatment the intervention did not eliminate bone loss. However, physical training increased the rate of bone recovery in the femoral neck after cessation of GnRH therapy, compared with the control group. This was only a pilot study.

It has earlier been suggested that BMD decreases in the perimenopausal period (91). Our findings indicate that both HT and physical training can prevent bone loss in the spine over a period of 18 months in perimenopausal women. Hormone therapy remains a cornerstone in the treatment of vasomotor symptoms and as prophylaxis against osteoporosis. Today it is well known and accepted that HT should only be used for a limited period of time ( $< 5$  years) owing to the increased risk of breast cancer (23). Our pilot study highlights the beneficial effects of physical activity and its potential as an alternative to HT for preservation of BMD in perimenopausal women. However, larger prospective studies are required.

The lifetime risk for a Swedish woman aged 50 years is 46% as regards any osteoporotic fracture and 22% for a wrist fracture (23). The results of retrospective studies indicate that women with a forearm fracture have a 1.9-fold increased risk of a hip fracture later in life (96). According to the new recommendations from NICE (National Institute for Clinical Excellence, UK) antiresorptive treatment is recommended for secondary prevention of osteoporotic fragility fractures in postmenopausal women below 65 years of age with a T-score  $\leq -3$ , or if they have a T-score  $\leq -2.5$  plus one additional clinical risk factor (97).

In our study healthy postmenopausal women up to 65 years of age, with a wrist fracture and T-scores of -1.5 to -3 were included. In a Swedish study involving 122 women of 50–75 years of age with a wrist fracture, 33.6% had osteoporosis and 51% had osteopenia (98). In our cohort of 167 apparently healthy postmenopausal women of 45–65 years of age with wrist fracture, 23% had normal BMD, 59% had osteopenia and 18% had osteoporosis (99). In the present study a positive effect on total hip BMD after one year of physical training was observed in women with decreased BMD and a wrist fracture. While an overall treatment effect of over 1.2 % /year appears small for an individual, this might, however, have a significant impact on the number of osteoporotic fractures in a large population (100). Other prospective studies in postmenopausal women have shown more pronounced overall treatment effects: Hatori et al. found 2.8 % (68), Iwamoto et al. 3.5 % (71), Kemmler et al. 2.5 % (75) and Nelson et al. 2.6 % (80) after 1 year of training. These results are comparable to those observed after one year of treatment with raloxifene, an antiresorptive drug (101) with proven vertebral anti-fracture efficacy (101). A BMD 1% lower than average increases the risk of hip fracture by 5% (102). Low bone mass in the hip is a risk factor for fractures (103) and interventions that prevent bone loss in the hip may be of importance for fracture prevention. Our results indicate that physical activity might be positive in secondary prevention and might be suitable for postmenopausal women with a wrist fracture and osteopenia, which would include about 50% of postmenopausal women with wrist fracture. However, to confirm this, larger prospective long-term studies are needed. Our assumptions on fracture prevention are solely based on increases in BMD, as fracture data are lacking.

## **5.6 MUSCULAR TRAINING**

Physical training not only affects BMD, but also other parameters that may be of importance as regards fracture, such as balance and muscle strength (104). Increases in physical activity are beneficial for both the skeleton and for neuromuscular function. The positive influence on balance and muscle strength plays a role in decreasing the risk of falls (105). It has often been emphasized that the positive effect of training in the elderly is primarily the increase in muscle strength that per se prevents osteoporotic fractures.

In the present work the effect of training on muscle strength was tested in perimenopausal and postmenopausal women. We found no changes over time in perimenopausal women (paper 3) but a remarkably positive effect was seen in the postmenopausal women (paper 4). After one year of training their muscular strength was equal to that of women ten years younger, according to the results of the Timed-Stands Test.



## **5.7 MONITORING THE EFFECT OF PHYSICAL TRAINING ON BONE**

The effects of physical training on bone, measured using DEXA, are small compared with those seen during treatment with bisphosphonates such as alendronate. However, the changes measured using DEXA do not fully reflect true changes in bone strength, nor are they fully correlated to a reduction in the risk of fracture. This is also true for the fracture reduction observed during treatment with antiosteoporotic drugs. Treatment with fluoride increases BMD as measured with DEXA even more than alendronate, but it may actually increase the risk of fracture (23). Raloxifene brings about small changes in spine BMD, but it still leads to a significant reduction in vertebral fractures (101). Thus, other changes in bone, often referred to as changes in bone quality, are important, but cannot be measured using DEXA. Physical activity may actually have a larger impact on bone strength than is reflected by changes in BMD.

Mineral content is not the only factor important for bone strength and its resistance to fractures. Size and structural characteristics also have to be incorporated in the evaluation (106). Bone is three-dimensional, and has a geometric structure and a micro-architecture. It consists of inorganic and organic components. The relative proportions and geometric organisation of the organic and inorganic components of bone affect strength. The quantity of inorganic substances (bone mineral content [BMC] or BMD) is often used as a surrogate measure of bone strength. However, absorptiometry does not take into account micro-architecture or bone quality (106).

## **5.8 ANIMAL MODELS OF THE EFFECTS OF TRAINING ON BONE, AND FRACTURE RATE**

Skerry and Lanyon (85) rightly emphasize that evaluating the effect of physical training on bone mineral density may be an inappropriate method of measuring the adaptive changes of bone architecture resulting from bone loading. Studies indicating the lack of effect of exercise as regards increased BMD do not necessarily provide evidence against the beneficial influence of exercise on bone architecture (85). Bone quality is probably as important as bone quantity. If we want to evaluate fracture prevention regimes we will probably have to use other tools in the future. Some evidence from animal studies points in this direction. A physical training study involving hens, of 5 weeks duration, showed no changes in BMC. However, the ultimate breaking strength was 12–14% greater in the exercised hens than in the non-exercising hens (106). In another study, the right ulnae in female rats were subjected to 360 loading cycles daily (106). The loaded bone had greater resistance to fracture (64%–87% in ultimate force) but only a 5–10% increase in BMC. The results of these two animal studies suggest that physical activity induces strength in bone by way of internal architecture changes first, and then by way of an increase in BMC (106).

## 5.9 CONCLUSIONS AND FUTURE ASPECTS

There is much evidence that shows the beneficial effects of physical activity on BMD, bone strength and fracture rate and the fact that physical activity is an important determining factor of bone quality and metabolism. This has been described in studies carried out *in vitro* (10, 18) and in animal studies (106). The beneficial effects are supported by immobilization and bed rest studies, showing a rapid decrease in BMD of up to 30% before a new steady state is reached. It is a well-known fact that astronauts lose bone during space flights as a result of weightlessness (107). Many observational cross-sectional studies indicate beneficial effects of physical training on bone. The results of observational studies on exercising individuals show that they have higher BMD than non-exercising individuals (20) and that they have lower fracture risk (106). Small effects of physical training have been observed in prospective studies when measuring BMD with DEXA. Thus, we have to evaluate all these results with caution in order to avoid being over-positive or over-negative. The positive results from the observational studies may be biased. On the other hand we may lack the proper tools for evaluating the effects of training on bone prospectively.

Physical training is important during adolescence to maintain a strong skeleton into maturity, and probably, in later life, to minimize bone loss after the age of 50 (106). The ideal primary intervention against bone loss would be one that incorporates all aspects of normal bone turnover, not one that annihilates any given part of it (18). Physical activity is therefore an optimal primary intervention, with target site-specific regimens for the inhibition and/or reversal of bone loss, and this is achieved without interrupting the delicate interplay between the cells responsible for bone remodelling. It is also evident that physical activity is beneficial as regards hypertension, hyperlipidaemia, obesity, diabetes type II and impaired glucose tolerance (105). It is of importance for public health to create a positive attitude around physical activity, an attitude that to a large extent can be encouraged by academia. Professor Åstrand, Department of Physiology and Pharmacology, Karolinska Institute, states that studies of normal people provide an important baseline for the study of diseases. Exercise physiology provides a unique opportunity to study how different functions are regulated and integrated and it should therefore be included in medical education (105). This could involve the science of mechanical loading and bone. The idea that mechanical loading plays a role in bone metabolism by way of osteocyte mediation could be emphasised. The physician would thereby be well educated as regards the possibility of physical activity as a means of primary and secondary prevention of a number of diseases, including moderate osteoporosis. This scientific field would probably be more successful if there were closer connections and more collaboration between preclinical and clinical researchers.

Does physical training prevent bone loss and fractures? In order to answer this question prospective randomised studies with a larger number of participants followed for longer periods of time are needed, as well as new tools to evaluate bone

quality. In the absence of this type of evidence, we still have to address the question. I do believe that physical training is beneficial for the bone and that an increase of physical activity in the population would lead to a decrease in fracture incidence. I have to admit that my position rests on low-grade scientific evidence, i.e. observational and animal studies. On the other hand we know for sure that immobilization is harmful to the skeleton. Although several scientists in the bone field have concluded that “physical training has little effect on postmenopausal bone”, we must not forget that lack of evidence is not proof of absence of an effect. A lot of research is still to be performed before we can truly make that statement and it would probably be more beneficial for public health if we were more careful in stating such conclusions.

According to earlier and recent findings, patients undergoing withdrawal of oestrogen may receive the following information: Modest training without cessation may preserve bone mass. It is my strong belief that we have to go on moving to stay healthy and this goes for the skeleton too.

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