ILARI PAJAMÄKI

Mechanosensitivity of Bone

■

ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the small auditorium of Building K,
Medical School of the University of Tampere,
Teiskontie 35, Tampere, on January 12th, 2007, at 12 o'clock.

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION
University of Tampere, Medical School
Tampere University Hospital, Department of Surgery
UKK Institute
National Graduate School for Musculoskeletal Diseases
Finland

Supervised by
Docent Teppo Järvinen
University of Tampere
Docent Harri Sievänen
University of Tampere

Reviewed by
Professor Petri Lehenkari
University of Oulu
Professor Tim Skerry
University of Sheffield, U.K.

Distribution
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 3 3551 6055
Fax +358 3 3551 7685
taju@uta.fi
www.uta.fi/taju
http://granum.uta.fi

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 1202
ISBN 978-951-44-6815-5
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 585
ISBN 978-951-44-6816-2
ISSN 1456-954X
http://acta.uta.fi

Tampereen Yliopistopaino Oy – Juvenes Print
Tampere 2007

To Elias and Siiri

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based upon the following original publications, referred to in the text by their roman numerals (I-IV)

I Järvinen TLN, Kannus P, Pajamäki I, Vuohelainen T, Tuukkanen J, Järvinen M and Sievänen H (2003): Estrogen deposits extra mineral into bones of female rats in puberty, but simultaneously seems to suppress the responsiveness of female skeleton to mechanical loading. Bone 32:642-651.

Seeman E and Zebaze RMD (2004): On Jarvinen et al. Letter to the editor. Bone 34:231-232.

Pajamäki I, Sievänen H, Kannus P and Järvinen TLN (2004): Response to Seeman and Zebaze. Reply to letter to the editor. Bone 34:233-235.

II Pajamäki I, Kannus P, Vuohelainen T, Sievänen H, Tuukkanen J, Järvinen M and Järvinen TLN (2003): The bone gain induced by exercise is not preserved through a virtually life-long deconditioning: A randomized controlled experimental study in male rats. J Bone Miner Res 18:544-552.

III Järvinen TLN, Pajamäki I, Sievänen H, Vuohelainen T, Tuukkanen J, Järvinen M and Kannus P (2003): Femoral neck response to exercise and subsequent deconditioning in young and adult rats. J Bone Miner Res 18:1292-1299.

IV Pajamäki I, Sievänen H, Kannus P, Jokihaara J, Vuohelainen T and Järvinen TLN (2006): Anabolic skeletal effects of estrogen and loading are structurally distinct. (Submitted).

ABBREVIATIONS

aBMD	Areal bone mineral density, g/cm^2
ANOVA	Analysis of variance
AP	Anteroposterior direction
BMC	Bone mineral content, g or mg
BMU	Bone multicellular unit
BV	Bone volume, mm^3
BV/TV	Bone volume fraction, %
cCSA	Cortical cross-sectional area of bone, mm^2
CSMI	Cross-sectional moment of inertia
cvBMD	Cortical volumetric bone mineral density, g/cm^3 or mg/mm^3
CV_{rms}	Average-root-mean-square coefficient of variation
DC	Deconditioning
DXA	Dual energy x-ray absorptiometry
E^+	Estrogen-replete
E^-	Estrogen-deplete
ER	Estrogen receptor
ERα	Estrogen receptor – alpha
ERβ	Estrogen receptor – beta
ERT	Estrogen replacement therapy
EX	Exercise
Fmax	Fracture load of bone, N
L^+	Loading included
L^-	Immobilization
LBM	Lean body mass
MES	Minimal effective strain
MESm	Minimal effective strain for modeling
MESr	Minimal effective strain for remodeling
ML	Mediolateral direction
OVX	Ovariectomy
pQCT	Peripheral quantitative computed tomography
SD	Standard deviation
SEM	Standard error of mean
SHAM	sham operation
tBMC	Total bone mineral content, g or mg
tvBMD	Total volumetric bone mineral density, g/cm^3 or mg/mm^3
Tb.N	Trabecular number, 1/mm
Tb.Sp	Trabecular separation, mm
Tb.Th	Trabecular thickness, mm
tCSA	Total cross-sectional area of bone, mm^2

TV	Total volume of bone, mm^3
tvBMD	Total volumetric bone mineral density, g/cm^3 or mg/mm^3
μCT	Microcomputed tomography
vBMD	Volumetric bone mineral density, g/cm^3 or mg/mm^3

ABSTRACT

The objectives of this thesis study were to investigate the effect of gender, estrogen and age on the responsiveness of bone to mechanical loading (mechanosensitivity), and furthermore, to assess the ability of bone to maintain the exercise-induced bone benefits after the exercise is ceased. In addition, the independent and potentially interactive effects of estrogen and loading on the structural characteristics of bone were characterized. The mineral status and structure of bone were assessed using peripheral quantitative computed tomograghy (pQCT) and/or microcomputed tomography (μCT), and materials testing machine was used for the determination of the structural strength of bone. We observed the bones of female rats exhibiting a clearly lower responsiveness to exercise than male rats and the phenomenon was evident in both young and adult rats. Furthermore, the removal of estrogen secretion (via ovariectomy) resulted in enhanced mechanosensitivity of female bones to exercise. However, rather than contributing this phenomenon to the actions of estrogen per se, the effect appeared to result from the estrogen-induced deposition of mechanically excess mineral into bone consequently increasing the rigidity of bone and thus, indirectly resulting in lower mechanosensitivity. In a continuation of this study, it was shown that the exercise-induced bone benefits obtained during the period of rapid skeletal growth were eventually lost when the exercise was completely ceased. We found no quantitative differences in the responsiveness of bone to exercise between young and adult rats indicating that aging is not related to reduction in the mechanosensitivity of bone. However, an apparent trend for different mechanisms of adaptation to exercise was observed so that the young bones mainly adapted through geometrical changes (increase in bone size) whereas adult rats seemed to adapt mainly through increase in bone density. Likewise, the ability of bone to preserve the exercise-induced bone benefits did not seem to be related to age, since the loss of bone in the young and adult rats was identical after the cessation of exercise. By separately or simultaneously removing the effect of mechanical loading (cast immobilization) and/ or estrogen, it was shown that mechanical loading is the principal determinant of bone geometry and strength. The loading effect was shown to be direction-specific as loading was found to have a significant stimulatory effect on the bone surfaces in the primary loading direction. Estrogen, in turn, was shown not to have its primary effect on the structural particulars of bone but rather, on accrual of bone mass. Furthermore, the skeletal actions of mechanical loading and estrogen were shown to be completely independent and also very distinct within the structure of trabecular bone compartment.

YHTEENVETO

Tämän väitöskirjan tarkoituksena oli selvittää sukupuolen, estrogeenin ja iän merkitystä luun kuormitusvasteen säätelyssä sekä fyysisellä kuormituksella aikaansaadun luulisän pysyvyyttä kuormituksen lopettamisen jälkeen. Lisäksi selvitimme mekaanisen kuormituksen ja estrogeenin rooleja luun rakenteen ja lujuuden säätelijöinä. Luun mineraalimassan, -tiheyden ja rakenteen määritimme perifeerisellä kvantitatiivisella tietokonetomografialla (pQCT) ja/tai mikro-tietokonetomografialla (μCT). Luun mekaaninen lujuus määritettiin mekaanisella koestuslaitteella. Tutkimuksessamme osoitimme, että urosten luun vasteen fyysiselle kuormitukselle oli merkitsevästi suurempi naaraisiin verrattuna sekä nuorilla että aikuisilla rotilla. Lisäksi osoitimme estrogeenin vaikutuksen poistamisen (munasarjojen poisto) naarailla lisäävän luun kuormitusvastetta. Tämä estrogeenin luun kuormitusvastetta vähentävä vaikutus ei kuitenkaan näyttäisi olevan suora, sillä havaitisimme estrogeenin ”pakkaavan” luuhun ylimäärin mineraalia johtaen luun lujuuden kasvuun, samalla epäsuorasti aiheuttaen luun kuormitusvasteen heikkenemisen. Jatkotutkimuksessa havaitsimme kasvuiässä fyysisen kuormituksen avulla aikaansaatujen luumuutosten pysyvän jonkin aikaa liikunnan lopettamisen jälkeen, mutta seurannassa nämä positiiviset luumuutokset kuitenkin lopulta hävisivät. Kasvavien ja täysikasvuisten rottien luita analysoimalla osoitimme, että ikääntymisellä ei ole kvantitatiivisesti (massa, lujuus) arvioiden vaikutusta luun kuormitusvasteeseen. Sen sijaan kvalitatiivisesti arvioiden näyttäisi, että kasvuikäinen luu reagoi liikuntakuormitukselle pääasiallisesti geometrisia ominaisuuksia (luun koko) muuttamalla ja täysikasvuinen luu puolestaan luun mineraalitiheyttä lisäämällä. Lisäanalyysina tarkastelimme vielä, onko luilla iän suhteen eroa niiden kyvyssä säilyttää liikunnan avulla aikaansaatuja muutoksia, mutta mitään viitteitä tällaisestakaan emme pystyneet osoittamaan. Poistamalla mekaanisen kuormituksen (kipsi-immobilisaatio) ja/ tai estrogeenin vaikutuksen osoitimme, että mekaaninen kuormitus on pääasiallinen luun rakennetta ja lujuutta säätelevä tekijä. Kuormituksen aikaansaama luulisä kertyi suunta-spesifisesti eli luupinnoille, joihin kohdistuu suurimmat voimat luuta kuormitettaessa. Estrogeenilla ei aiemmasta tiedosta poiketen näyttäisi olevan itsenäistä luun kuormitusvastetta tai kokoa säätelevää vaikutusta vaan nämä vaikutukset tulevat esille epäsuorasti estrogeenin luun mineraalipitoisuutta säätelevän vaikutuksen kautta. Täten estrogeenin luustovaikutukset näyttäisivät kohdistuvan vain luun mineraalipitoisuden säätelyyn. Lisäksi osoitimme sekä kuormituksella että estrogeenilla olevan itsenäinen, mutta eri mekanismeilla toimiva, hohkaluun rakennetta säätelevä vaikutus.

INTRODUCTION

The modern human skeleton represents an end point of million years of ongoing adaptation since our separation into an independent evolutionary lineage. The skeleton integrates several vital non-mechanical functions (mineral homeostasis, hematopoiesis) in conjuction with its primary locomotive purpose into a single organ. Although it is nowadays well established that the primary function of the skeleton is locomotion (Burr 1997, Frost 1997, Parfitt 1998), the non-mechanical functions of the skeleton have been the main attraction of skeletal researchers. For example, most osteoporosis experts still attribute osteoporoses to disorders in the regulation of the "effector cells" (osteoclasts or osteoblasts), so causes and cures of diseases should be sought in those cells and/or in their regulation by nonmechanical factors. This view has led to dramatic growth of cell- and molecular-biologic research on effector cells and their roles in skeletal problems, simultaneously leading to the ignorance of skeletal adaptations to mechanical needs.

All body movements are produced by co-ordinated contractions of skeletal muscles, while the concomitant dynamic muscle work provides the fundamental source of mechanical loading to the skeleton. Thus, bones must be able to gradually adapt themselves to the prevailing loading environment in order to produce each bone with mechanically-appropriate material and geometric properties (Einhorn 1992, van der Meulen et al. 2001) which ultimately determine the whole-bone strength, the bottom line (Einhorn 1992, Järvinen et al. 2005). In order for bones to adapt to prevailing loading environment, bones are equipped with a mechanosensory feedback system that senses the loading-induced deformations within the bones and copes with the locomotive challenges through modifications in bone size and shape – i.e. through geometric, structural and architectural adaptation (Frost 2003). New bone is laid on regions which are subject to loading that exceeds clearly the customary loading range, while bone is removed from regions which experience reduced loading well below the customary loading range.

Several nonmechanical factors such as age, hormonal status and gender, are believed to exert an influence to the bone's mechanosensing pathway, thus altering the adaptive response of skeleton to mechanical loading. However, as a result of the current improved research methodology available for the evaluation and characterization of the skeleton, the conclusions have been attained virtually exclusively from the studies focusing on the cellular- and molecular level actions of these factors simultaneously resulting in ignorance of the possible effect on the ultimate phenotype of the skeleton, i.e. the structural rigidity and strength (Einhorn 1992, Järvinen et al. 2005). Thus, it seems at times that the methodological surge has occurred at the cost of studies becoming method-

driven, instead of being hypothesis-driven - seeking the true biological mechanisms and relationships.

The purpose of this series of experiments was to use a structurally oriented approach to evaluate the potential role of gender, estrogen and age in modulating the mechanosensitivity of bone. The reason in focusing on these particular factors is that according to the previous studies, these factors are among the primary factors exerting an effect on the adaptive response of bone to mechanical loading. We also explored the ability of the bone to maintain the exercise-induced bone benefits after the exercise is ceased. Finally, our goal was to assess the skeletal effects of estrogen, as it is considered to possess a *direct* effect on the bone structure and strength in addition to its proposed effect on the mechanosensing pathway of bone.

REVIEW OF THE LITERATURE

1. Bone biology

1.1. Bone composition and structure

Bone is a specialized form of connective tissue that, like the other connective tissues, consists of cells and extracellular matrix. The feature that distinguishes bone from other connective tissue is the mineralization of the matrix. This produces a hard and strong type of tissue capable of providing mechanical integrity for efficient body motion and protection for the internal organs. By weight, approximately 70% of the bone tissue is mineral or inorganic matter, water comprises 5 to 8%, and the organic or extracellular matrix makes up the remainder. Approximately 95% of the mineral phase is composed of a spesific crystalline hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_3$], whereas 98% of the organic phase is composed of Type I collagen and a variety of noncollagenous proteins; cells accounting for the remaining 2% of the organic phase. (For review, see Buckwalter et al. 1995, Einhorn 1996).

On the basis of shape, bones can be classified into four groups, long bones (e.g. the tibia and the metacarpals), short bones (e.g. carpal bones of the hand), flat bones (e.g. the bones of the calvarium and the sternum), and irregular bones (e.g. vertebra). Long bones have a shaft called the diaphysis and two expanded ends, each called an epiphysis**.** The flared portion of the bone between the diaphysis and the epiphysis is called the metaphysis (Figure 1). It extends from the diaphysis to the epiphyseal line. A large cavity filled with bone marrow, called the marrow or medullary cavity, forms the inner portion of the bone that is supported or surrounded by bone tissue and periosteum (Ross et al. 1995).

The bone tissue is classified as either cortical (compact or dense) or trabecular (spongy or cancellous) (Figure 1). Cortical bone forms the outside of the bone as a solid structure with low surface-to-volume ratio. Cortical bone has two surfaces, a fibrous connective tissue capsule covering the outer surface of bone, known as the periosteum, and the other on its inner surface lining the marrow cavity, known as the endosteum (endocortex). Trabecular bone consists of a lattice of rods, plates and arches forming a meshwork in the interior of the bone.

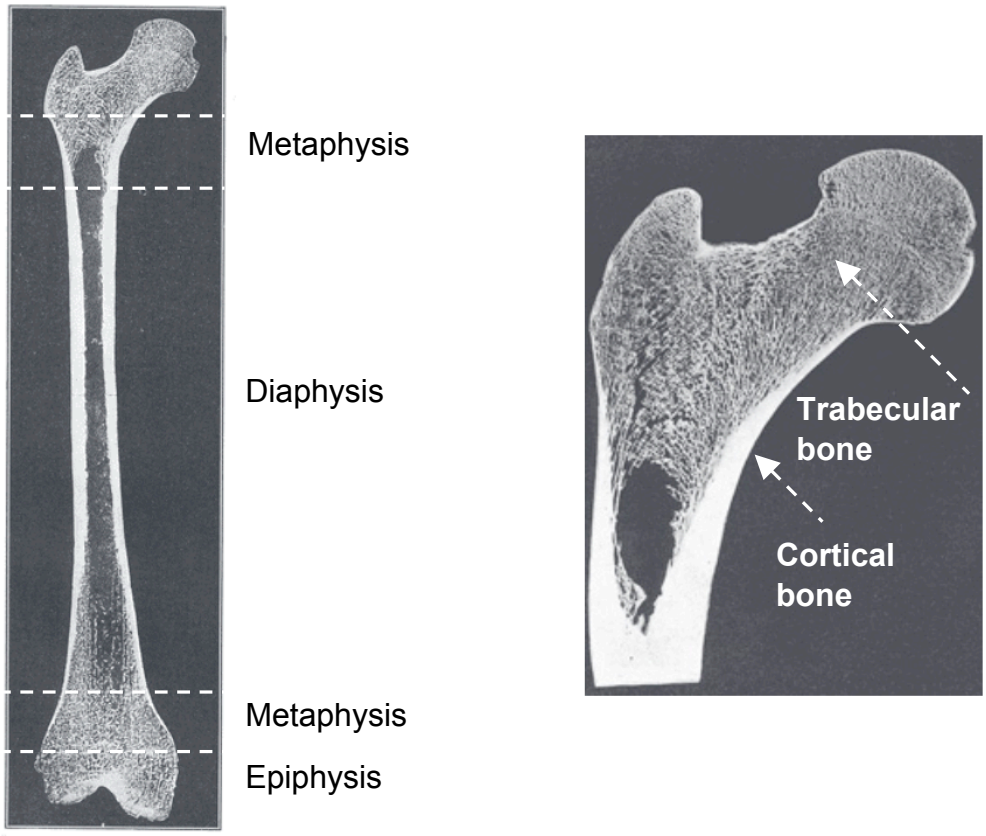

Figure 1. Structure of a typical long bone (femur).

The spaces of the meshwork are continuous and are occupied by marrow and blood vessels. Cortical and trabecular bone tissue are located in specific parts of bones. In the long bones, the diaphysis is primarily cortical in structure, whereas the epiphysis and metaphysis are mainly filled with trabecular bone with a thin layer of cortical bone on the outside. Cortical bone comprises 80% and trabecular bone 20% of the skeletal mass (For review, see Ross et al 1995, Einhorn 1996).

There are four cell types found in bone (mesenchymal stem cells, osteoblasts, osteoclasts, and osteocytes). Mesenchymal stem cells are multipotent cells that have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma (Pittenger et al. 1999). With the exception of osteoclast, mesenchymal stem cells (presented in adult bone marrow) are considered as progenitor cells for other bone cells. In contrast, osteoclast has its origin in a different cell line arising from the hematopoietic precursors (monocyte family). The osteoblast is the differentiated bone-forming cell that secretes both the collagen and the ground substance that constitutes the initial unmineralized bone or osteoid. The osteoblast is also responsible for the calcification of the matrix. The osteocyte is a differentiated osteoblast, also considered the mature bone cell. It is enclosed by bone matrix that it previously secreted as an osteoblast. Each osteocyte occupies a space or lacuna that conforms to the lenticular shape of the cell. The osteocytes extend cytoplasmic processes through the fine tunnels or canaliculi in the matrix to contact, by means of gap junctions, processes of neighboring cells. The osteoclast is a large

multinucleated cell whose function is to resorb bone (For review, see Ross et al. 1995, Einhorn 1996).

1.2. Bone turnover

The modeling is defined as the simultaneous removal and formation of bone at different sites by two mediator mechanisms called resorption and formation drifts. Osteoblasts in formation drifts add new bone, and osteoclasts remove bone in resorption drifts over broad surface regions during the modeling process. Modeling is generally defined in two forms (Frost 1990a). Macromodeling refers to alterations in the gross shape, size and strength of bones (Frost 1990a, Kimmel 1993), whereas micromodeling is responsible for the alignment of trabeculae within cancellous bone regions to accommodate patterns of customary usage (Frost 1990a, Kimmel 1993).

Bone remodeling refers to the renewal process whereby small pockets of old bone, dispersed throughout the skeleton and separated from others spatio-temporally, are replaced by new bone throughout adult life. It has been estimated that in humans, as much as 25% of trabecular bone and 3% of cortical bone is resorbed and replaced each year (Parfitt 1984). A remodeling site is initiated by the appearence of osteoclasts (and precursors) following any of several humoral or local stimuli to resorption. The osteoclasts proceed to resorb an amount of bone which produces a small resorption pit. The bone-resorbing activity of osteoclasts is regulated by extracellular calcium (Ca^{2+}) concentrations (Li et al. 2006). During the subsequent formative phase, actively synthesizing osteoblasts appear and begin to deposit uncalcified matrix which is later mineralized. Resorption and formation always occur successively in the same location and always in the same order. This sequence of resorption and formation has been referred to as a basic multicellular unit of bone turnover (BMU), and the process of bone resorption followed by an equal amount of formation has been termed coupling (Parfitt 1984, Frost 1987a, 1987b and 1990b). BMU-based remodeling occurs on all four skeletal "envelopes" – the periosteal, haversian, cortical-endosteal and trabecular surfaces – and it does so throughout life being responsible for much of the bone turnover after the completion of skeletal growth, whereas (macro)modeling drifts only affect cortical bone and primarily during growth. Bone remodeling is controlled by several circulating hormones and locally produced factors, and intercellular communication among the diffrent bone cells is an integral part of these mechanisms.

1.3. Non-mechanical function of bone

Bone is an important reservoir of mineral ions, and the skeleton contains 99% and 88% of the body's calcium and phosphate, respectively. Calcium is an essential ion for many physiological processes and thus the maintenance of normal blood calcium levels is critical to health and life. Calcium may be removed from the bone matrix to the blood if the circulating levels of calcium

fall. Conversely, excess blood calcium may be removed from the blood and stored in the bone. In mammals, these physiological processes are regulated by a negative feedback mechanism that involves the alimentary tract, the kidneys, and bone. The so-called ”calciotropic” hormones- parathyroid hormone (PTH), vitamin D, and to a lesser extent, calcitonin- maintain the equilibrium of calcium pool (For review, see Ross et al 1995, Einhorn 1996).

The skeleton also serves as the primary site for the formation of blood cells (hematopoiesis) after birth. Under appropriate stimuli, the pluripotent hematopoietic stem cells residing close to the endosteal surfaces of bone marrow (trabecular bone regions) differentiate into blood cells (Taichman 2005). Osteoblasts and hematopoietic stem cells are closely associated with each other in the bone marrow and current data suggest that osteoblasts play a central role in hematopoiesis as osteoblasts have been shown to produce many factors essential for the survival, renewal, and maturation of hematopoietic stem cells (Taichman 2005). Osteoblasts have also been shown to induce the expansion and maturation of osteoclasts from hematopoietic precursors and activate osteoclastic bone resorption (Taichman 2005). Thus, the more rapid response of trabecular bone sites (metaphysis) to various stimuli (e.g. immobilization, hormones) compared to the bone sites composed mainly of cortical bone (diaphysis) is attributable to the location of the osteoclastic precursors on the endosteal bone surfaces.

2. Bone biomechanics

The biomechanical properties of bone can be described at two levels: 1) the material properties are defined by the tissue-level qualities of bone, 2) the structural properties describe the bone as a whole anatomical unit (Einhorn 1992, Turner and Burr 1993). The material properties of bone tissue are typically determined by testing uniform, prepared bone specimens subjected to simple, well-defined loads. During mechanical testing, the bone sample generates an internal force, *stress*, to resist the testing load. Stress is defined as a force per unit area at the failure location of the sample. The original shape of the bone sample is also deformed, described as *strain*, when the bone is subjected to an applied force. Strain is defined as the percentage change in length during the sample load by a force direction, or deformation (Einhorn 1992, Turner and Burr 1993, Currey 2001).

The relationship between stress generated by applied loads to a structure and strain in response to the load is called a *stress-strain curve*. The stress-strain curve can be divided into two regions: the *elastic strain region* and the *plastic strain region*. The elastic strain region and the plastic strain region of the stress-strain curve are divided by the yield point. Within the elastic or preyield region, the strain increases linearly with increasing stress, and after the load is released the bone will return to its original shape. The slope of the elastic region of the stress-strain curve is called the elastic or *Young’s modulus* and is a measure of

the *intrinsic stiffness* or *rigidity* of the material. Within the plastic or postyield region, stresses cause permanent damage to the bone structure, and if the load is increased further, the specimen will eventually fail or fracture at the ultimate stress and strain point (Turner and Burr 1993, Currey 2001). The area under the stress-strain curve is a measure of the amount of energy needed to cause a fracture. This property of a material is called energy absorption, or toughness. In testing an entire bone or a functional bone structure, the relationship between applied load and deformation is defined by a *load-deformation curve* (Figure 2).

Inorganic matrix (mineral mass) mainly determines bone's stiffness as a material whereas the organic component of the tissue (collagen fibers and fibrils) is responsible for the elasticity of the material, allowing the transient deformation of the bone under the applied loads. In entire bones, the structural strength and stiffness depend primarily on the size, shape, distribution of bone mass in space and internal architecture (Currey 1984 and 2001). Bone's microarchitectural and material properties vary relatively little with age, sex, species, bones, and disease, thus contributing less to the structural strength of bones (Currey 1984).

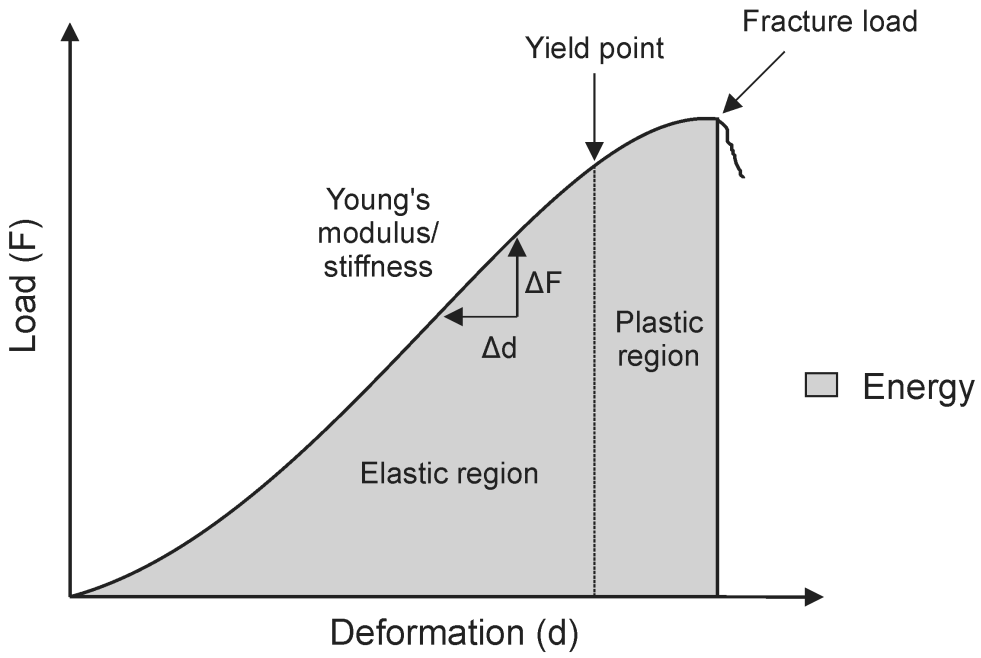

Figure 2. Typical load-deformation curve for a bone loaded until fracture.

Stresses in bone can be classified into three principal components: compressive, tensile and shear. These basic stress types, either alone or in combination, can result in a variety of complex loading configurations experienced by bones in nature: tension, compression, bending (combination of tensile and compressive forces), and torque (shear stresses along the entire length of bone) (Einhorn 1992, Turner and Burr 1993). The highest stresses in the bone diaphysis during normal activities are caused by bending and torsional loading, and thus resistance to these loads is the most relevant for these bone sites. Assuming the cross-sectional shape of diaphysis as circular hollow cylinder, the

most efficient design for resisting bending and torsional loads involves distributing the bone mass as far as possible from the neutral axis of bone, quantitatively described by the cross-sectional moment of inertia [CSMI = $\pi/64(r^4-r_i^4)$] (Figure 3A). Thus, only small additions of bone at the periosteal surface increase the CSMI considerably since the CSMI is proportional to the fourth power of radius. The redistribution of bone material displays a major role in maintaining skeletal integrity during aging when bone is lost at endosteal surfaces with simultaneous additions occurring on periosteal surfaces (Ruff and Hayes 1982). Although the cortical thickness decreases with age, the strength of bones is efficiently preserved by relocating the bone to a position where it has a maximum positive impact on the CSMI, i.e. at periosteal surface (Kimmel 1993).

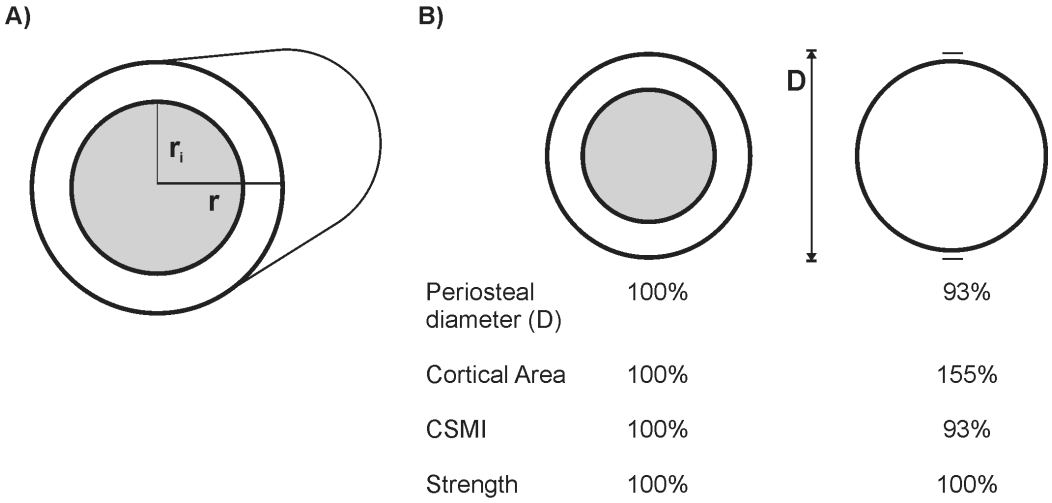

Figure 3. (A) Cross-sectional moment of inertia (CSMI), where r is the outer radius (from the neutral axis to periosteum) and r_i is the inner radius (from the neutral axis to endosteal surface) of the hollow cylinder. **(B)** The influence of cross-sectional geometry on the structural strength. The solid cylinder has an equal torsional strength to a hollow cylinder with only 7% lower periosteal diameter but 55% higher cortical area. Adapted from van der Meulen et al. (2001).

3. Bone functional adaptation

> "Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws" *(Wolff 1892)*

Although the "form follows function" relationship of bone, known as the Wolff's law, was proposed over a century ago, for a long time skeletal scientists believed that bone architecture, health, and disease depended mainly on nonmechanical cell- and molecular-biologic features. According to this view, nonmechanical factors influenced bone's effector cells (osteoblasts and osteoclasts). Osteoblasts added bone, osteoclasts removed it, they functioned independently of each other, and mechanical influences had little effect on bone strength and mass

(Weinmann and Sicher 1955, Snapper 1957, McLean and Urist 1961). However, the primary function of the bones is to bear the muscle contraction- and gravity-induced mechanical forces exerted on them without breaking, and consequently, to enable the efficient locomotion of the body (Burr 1997, Frost 1997). During growth and development, the skeleton optimizes its architecture by subtle adaptations to these mechanical loads.

3.1. Mechanotransduction

Mechanotransduction – conversion of a biophysical force into a cellular response- is a multistep process comprising of four distinct phases: 1) mechanocoupling, the transduction of mechanical force applied to the bone into a local mechanical signal perceived by a sensor cell; 2) biochemical coupling, the transduction of a local mechanical signal into a biochemical signal and, ultimately, gene expression or protein activation; 3) transmission of signal from the sensor cell to the effector cell, i.e. the cell that will actually form or remove bone; and 4) the effector cell response, the appropriate tissue-level response (Turner and Pavalko 1998) (Figure 4).

Mechanical forces cause deformation of the bone tissue and cells, and generate pressure gradients that drive extracellular fluid flow through the canalicular spaces in bone (Weinbaum et al. 1993, Turner and Pavalko 1998). This fluid flow further causes electric fields in bone, called streaming potentials (Chakkalakal 1989). Each of these tissue-level effects of mechanical loading probably plays some role in mechanotransduction, as bone cells in culture have been shown to respond to mechanical strain (Somjen et al. 1980), fluid flow (Reich et al. 1990), and electric fields (Korenstein et al. 1984). However, it is generally believed that the fluid flow is the most important mediator of the mechanical signal to strain sensing cells (Reich et al. 1990, Turner et al. 1994, Han et al. 2004).

The ideal location, interconnection with each other through functional gap junctions and their sensitivity to fluid flow make osteocytes and bone lining cells (osteocyte-bone lining cell complex) the best candidates for mechanosensory cells in bone tissue (Cowin et al. 1991, Lanyon 1993, Turner et al. 1994, Klein-Nulend et al. 1995, Mullender and Huiskes 1997). Since neither bone lining cells nor osteocytes can actively form or resorb bone, they produce several intermediaries, such as prostaglandins (PGs) and nitric oxide (NO), for cell-to-cell communication with the effector cells within minutes of being exposed to mechanical loading (Rawlinson et al. 1991, Johnson et al. 1996, Zaman et al. 1997).

The effector response involves both nonproliferating and proliferating osteoprogenitor cell populations. Mechanical loading induces an early bone formation response within 48h that involves osteoblasts recruited from bone lining cells or nondividing osteoprogenitor cells. Proliferating osteoprogenitor cells are also stimulated by mechanical loading and they differentiate into osteoblasts 72-96 hours after a mechanical stimulus (Boppart et al. 1998, Turner and Pavalko et al. 1998).

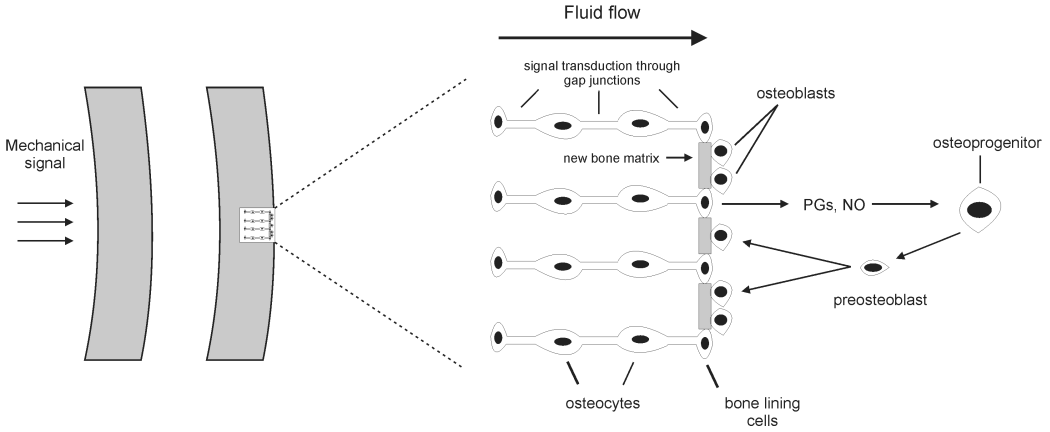

Figure 4. The mechanotransduction pathway in bone. The mechanical signal induces a fluid flow through canalicular channels which is detected by mechanosensory cells (osteocytes and bone lining cells). The intermediaries (PGs and NO) are released stimulating the recruitment of osteoblasts and differentiation of osteoblasts from osteoprogenitor cells. Adapted from Turner and Pavalko (1998).

3.2. Bone's mechanostat and the concept of mechanosensitivity

At birth, many features of skeletal architecture and the biologic mechanism that can change it already exist as "baseline conditions" and "baseline activities". After birth, these activities adapt the skeleton to its mechanical loads and strains, and the bone cells as osteoblasts and osteoclasts render it possible for the skeleton to do it.

Mechanostat theory - a theory proposed by an orthopaedic surgeon Harold Frost - suggests the existence of mechano-biologic negative feedback mechanisms that would work under the control of a subject's mechanical usage, adjusting skeletal architecture in ways that tend to prevent that mechanical usage from causing structural failures of skeletal tissues and organs (Frost 1987a, 1987b and 2003). Mechanostat works homologous to a thermostat controlling the temperature in a house, sensing and perceiving the incident loading-induced strain distribution within the bone and subsequently removing bone tissue from sites where the concomitant stresses are marginal while forming new bone tissue at sites subjected to increased stress. In order for the mechanostat to work, in vivo strain studies (Lanyon and Smith 1970, Lanyon 1973 and 1984, Lanyon et al. 1975, Bouvier 1985) suggest the existence of certain threshold levels (minimally effective strains, MES) of those strain-dependent signals for switching the two bone mass and strength controlling functions, i.e. modeling and remodeling, ON and OFF (Figure 5). The bone's modeling threshold range (MESm) designates the strain region where mechanically-controlled modeling begins and bone mass is increased, while the bone's BMU-based remodeling threshold range (MESr) designates the strain region in and below which maximal "disuse-mode" remodeling activity occurs and bone is removed next to marrow. Above the MESr, bone resorption and formation by completed BMUs tend to be

in balance and existing bone mass and strength is maintained; that is the "conservation mode". The threshold ranges are believed to be genetically determined, modeling threshold strain range centered near 1000 microstrain and remodeling threshold centered near 50-100 microstrain. For comparison, loads that fracture a healthy bone cause strains centered near 25,000 microstrain in young adults (Frost 2003). However, it should be noted that this suggestion of certain strain magnitudes to induce modeling and remodeling are the same in all bones and different regions of a single bone is most likely an oversimplification of mechanostat and may only apply e.g. in the midshaft of a long bone (Skerry 2006). It has been shown that not only magnitude, but also rate, frequency, rest periods, and to some extent duration or number of cycles of loading and their timing of application all appear to have effects on the osteogenic nature of bone (O'Connor and Lanyon 1982, Rubin and Lanyon 1984, Rubin and McLeod 1994, Robling et al. 2002).

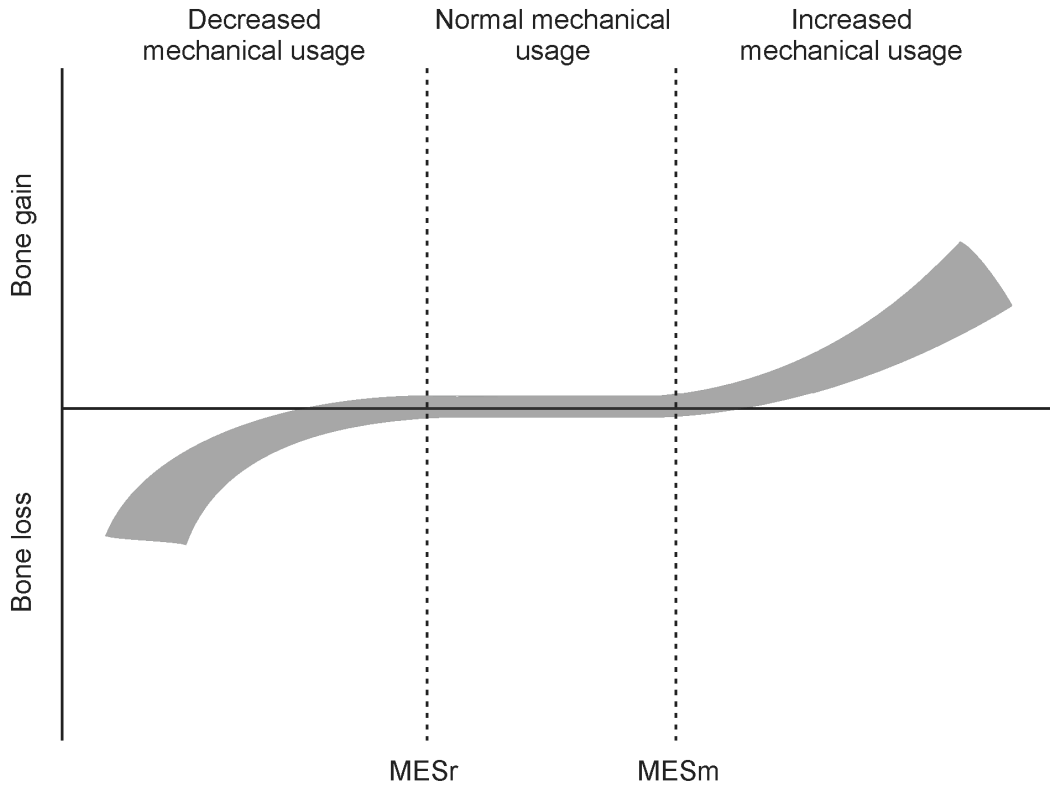

Figure 5. The mechanostat theory. The bone's modeling threshold range (MESm) designates the strain region where mechanically-controlled modeling begins and bone mass is increased, while the bone's BMU-based remodeling threshold range (MESr) designates the strain region in and below which maximal "disuse-mode" remodeling activity occurs and bone is lost. Above the MESr, bone resorption and formation by completed BMUs tend to equalize and existing bone mass and strength is maintained. Adapted from Frost (2003).

This mechanosensory feedback system maintains the skeletal rigidity under the typical voluntary loads exerted by muscle contractions. However, bones can withstand temporary loads far greater than the maximum loads caused by typical voluntary loads without breaking. Thus, bones do have a certain *strength safety factor*, which equals to bone's maximum strength divided by its modeling threshold (Frost 2003). It has been estimated that healthy young-adult mammalian bones can withstand loads about 6 times greater than loads generated by typical voluntary loads without breaking (Frost 2003). The existence of safety factor is essential, since the entire adaptational process (from mechanosensing until altered structure and strength) of bone to mechanical loading takes time to proceed, also known as "adaptational lag". In other words, bones have adapted to the prevailing loading environment and do not "foresee" the possible alterations in loading conditions, so they must be equipped with a certain safety factor. However, occasionally forces generated, for example by severe trauma, clearly exceed the safety-factor and traumatic fractures are produced (Frost 2003).

Mechanosensitivity - the ability of bone tissue to detect mechanical loads - is believed to be directly modulated by systemic (hormones such as estrogen and growth hormone) (Halloran et al. 1995, Cheng et al. 1996 and 1997, Jagger et al. 1996, Westerlind et al. 1997, Turner 1999, Joldersma et al. 2001, Lanyon and Skerry 2001) and local (growth factors such as insulin-like growth factor 1 and 2) (Bikle et al. 1994, Kostenuik et al. 1999) factors. It is also believed that the degree to which an individual responds to a mechanical stimulus depends partly on age (Rubin et al.1992, Buhl et al. 2001, Klein-Nulend 2002) and genetics (Robling and Turner 2002). According to one theory (Frost 1987b), a possible modulator of the bone's mechanosensory apparatus could "sensitize" or "desensitize" bones to mechanical loading by altering the modeling (MESm) and remodeling (MESr) thresholds of bone, which would tend to increase, decrease or conserve bone mass (and consequently strength), by making smaller or larger strains than before turn modeling and conservation- or disuse-mode remodeling ON or OFF.

3.4. Aging and mechanosensitivity

Age is speculated to modulate the skeletal sensitivity to mechanical loading, as exercise interventions have been shown to induce significant bone gains in young (growing) individuals, but hardly result in any increases in mature skeleton, and seem to only preserve, at best, the existing bone stock in the elderly (Dalsky et al. 1988, Kannus et al. 1995, Heinonen et al. 1996, Berard et al. 1997, Ernst 1998, Haapasalo et al. 1998, Seeman 2002). Human studies thus quite uniformly suggest that adolescence (puberty) provides a particularly opportune time to intervene with loading exercise (Kannus et al. 1995, Khan et al. 1996 and 2000, Haapasalo et al.1998, MacKelvie et al. 2002).

The existing *in vivo* experimental data on the age-dependence of the skeletal responsiveness to external loading is far more controversial, as studies have shown the responsiveness of the aged skeleton to be increased (Buhl et al. 2001), reduced (Rubin et al. 1992, Turner et al. 1995), or unaffected (Raab et al. 1990,

Umemura et al. 1995). Raab et al. (1990) reported a comparable skeletal response to exercise in young and old (2.5 and 25 months old, respectively) rats, but used a different running velocity in the two age groups, thus somewhat hampering valid comparisons. Umemura et al. (1995) reached the same conclusion that the effects of exercise were not limited by age in their comprehensive comparison of rats of 3-, 6-, 12-, 20- and 27 months of age subjected to both jump training and running. In contrast, Rubin et al. (1992) showed, using their classic experimental model of externally loadable functionally isolated turkey ulna preparation, that a physical signal clearly osteogenic in the 1-year-old young adult skeleton was hardly acknowledged in the older (3-year-old) bone tissue. Similarly, Turner et al. (1995) observed that both the periosteal and endocortical surfaces of the tibiae of 19-month-old rats were significantly less responsive to mechanical loading than those of 9-month-old rat tibiae. However, the use of historical controls and inappropriate statistical comparisons diminish the strength of this latter study. In agreement with these two studies, Dehority et al. (1999) used a model completely opposite to the one used in all the studies noted above, that is the skeletal unloading by hindlimb suspension, to demonstrate that the effects of non-weight bearing are prolonged and have a greater relative effect on bone formation in the adult than in the young growing rats. To add yet another dimension to an already confused situation, Buhl et al. (2001) recently reported that 22-month-old male rats had a greater sensitivity to squatlike-exercise than their younger counterparts (4- and 12-month-old male rats).

The reduced capacity of the aged skeleton to respond to changes in the loading environment has been attributed to decreased sensitivity of the mechanosensory cells of the bones to mechanical loading-induced stimuli (Rubin et al. 1992, Pearson and Lieberman 2004), but a recent study on human bone cells found no evidence for the loss of mechanosensitivity with donor age (Klein-Nulend et al. 2002). It has also been proposed that the osteogenic responsiveness to mechanical loading may differ *qualitatively* between different age groups as follows: during the longitudinal growth period and particularly during puberty, increased loading can produce actual structural changes in bone through periosteal expansion (altered bone geometry), whereas additional bone acquired after skeletal maturity is probably deposited along the existing bone structures (Forwood and Burr 1993, Kannus et al. 1995).

3.4. Maintenance of the exercise-induced bone gain

Although the effects of increased mechanical loading on skeleton are obvious, the skeleton's ability to preserve the exercise-induced bone gain after the cessation of exercise is not clear (Seeman 2002). Current view is that if physical activity is started after the rapid period of skeletal growth (in adulthood), the beneficial effects of exercise may become lost (Dalsky et al. 1988, Yeh and Aloia 1990, Vuori et al. 1994, Seeman 2002). However, controversy still exists whether the exercise-induced additional bone mass gained during the rapid growth period can be maintained into adulthood and old age, because it has been

speculated that increased mechanical loading during growth could induce such changes in bone structure and size that may persist despite the cessation or reduction of loading (Kontulainen et al. 1999 and 2001). If these exercise-induced benefits are preserved into old age, physical activity during growing years could provide an effective prevention strategy against age-related bone loss and consequent fractures.

Retrospective cross-sectional studies on former athletes and their controls have given preliminary evidence that at least part of the exercise-induced bone benefits obtained during growing years may persist despite decreased physical activity (Karlsson et al. 1995 and 1996, Bass et al. 1998) and contribute to the lower incidence of fragility fractures in former athletes later in life (Karlsson et al. 2000, Nordström et al. 2005). However, the results of retrospective cross-sectional clinical studies are prone to many confounding factors such as selection bias of the subjects, the evaluation of quantity and quality of physical activity of former athletes during the active training years or other bone-affecting living habits, and furthermore, the amount of exercise-induced bone gains prior to cessation of exercise is unknown. There are only limited number of clinical longitudinal studies examining the maintenance of the exercise-induced bone benefits obtained during growing years after the physical activity is decreased or ceased (Kontulainen et al. 1999 and 2001, Gustavsson et al. 2003, Nordström et al. 2005). Kontulainen et al. (1999 and 2001) showed that positive side-to side BMC difference between the playing and nonplaying upper extremity remained during 4- and 5-year follow-up period in male and female raquet sports players. However, in these studies the study subjects continued the playing activity, although at significantly decreased rate, which may influence the results.

Some animal studies support the above noted contention that bones can preserve the exercise-induced benefits if the exercise occurs during the period of the rapid skeletal growth (Silbermann et al. 1991, Kiuchi et al. 1998, Singh et al. 2002) whereas opposite findings showing the loss of the exercise-induced bone gain after cessation of exercise have also been reported (Yeh and Aloia 1990, Iwamoto et al. 2000). The lack of consistency in these experimental deconditioning studies may arise from differences in the study design, exercise protocols, gender of the animals, and duration of the experiments.

4. Skeletal function of estrogen

Sex steroids play an important role in skeletal homeostasis (Compston 2001). Especially estrogen, the predominant female sex hormone, has drawn the attention of skeletal researchers since Fuller Albright, a clinician working in the 1930s, introduced a classic concept on postmenopausal osteoporosis (Albright et al. 1940 and 1941). Although estrogen has traditionally been regarded as a female sex hormone, estrogen is the major biologically active bone steroid in males, too (Emans et al. 1990, Smith et al. 1994, Morishima et al. 1995, Carani et al. 1997, Bilezikian et al. 1998, Khosla et al. 2002). The actions of estrogen have also been linked to the mechanical control of bone homeostasis (Westerlind

et al. 1997, Turner 1999, Lanyon and Skerry 2001). However, the precise mechanism of action of estrogen on the skeleton is still not entirely clear.

4.1. Estrogen and bone metabolism

Estrogen is considered a bone mass conserving hormone (Riggs et al. 2002). The current view is that the principal skeletal effect of estrogen at tissue level is suppression of bone turnover, maintaining balanced rates of bone formation and bone resorption (Riggs et al. 2002). Consequently, the loss of estrogen function at menopause is associated with marked increase in bone remodeling caused by simultaneous increase in bone formation and bone resorption in each BMU. However, the rates of increase of bone resorption and formation are disproportionate, with resorption clearly exceeding formation, resulting in net loss of bone.

At the cellular level, estrogen inhibits the outflow of osteoclastic precursors from hematopoietic lineage cells and early osteoblastic precursors from mesenchymal lineage cells in the marrow (Jilka et al. 1992 and 1998). In addition to reducing outflow of osteoblast and osteoclast precursors from lineage cells, estrogen has been shown to affect osteoclast development, activity, and apoptosis (Hughes et al. 1996, Manolagas et al. 2002). The antiresorptive effect of estrogens on bone is largely attributed to its inhibition of osteoclasts to produce number of bone resorbing cytokines, such as interleukin-1 and –6 and tumor necrosis factor-α (Pacifici 1998).

The skeletal effects of estrogen might result from either direct, receptor-mediated actions or from indirect actions on systemic hormones (Compston 2001). However, the indentification of estrogen receptors (ERs) in normal osteoblast-like cells in 1988 (Eriksen et al. 1988, Komm et al. 1988) suggest for the bone actions of estrogens being direct receptor mediated rather than indirect via secondary effects on other systemic hormones such as calcitonin and parathyroid hormone (Lindsay 1987, Schot and Schuurs 1990, DeCherney 1993). In addition to osteoblasts, osteoclasts (Oursler et al. 1991 and 1994) and osteocytes (Tomkinson et al. 1998, Braidman et al. 2000) have been suqsequently discovered to contain functional ERs. Today, two forms of estrogen receptors have been identified, estrogen receptor-α (ERα) and estrogen receptor-β (ERβ). The human ERα was originally cloned in 1986 by P. Chambon and his colleagues (Green et al. 1986). ERα was found to have homology between species and until recently was thought to be the primary mediator of estrogen action. However, findings of residual estrogen activity in ERα knockout mice led to the discovery of a second receptor, ERβ (Kuiper et al. 1996). Bone cells contain both subtypes of ERs, although their distributions within bone differ. Their significance for estrogen effects on bone has yet to be demonstrated, since residual effects of estrogens in double ERα/ ERβ knockout mice suggest existence of third ER or alternatively the potential for signaling via membrane-bound ERs may mediate some residual estrogen activity (Sims et al. 2002 and 2003).

4.2. Estrogen effect on skeletal maturation

During skeletal growth and maturation, sex steroids have, in combination with other hormones, several major effects and are presumed to be responsible for the sexual dimorphism of the skeleton. During skeletal growth, sex steroids affect the size, shape, and peak mass of the human skeleton (Garn et al. 1966).

4.2.1. Estrogen and longitudinal bone growth

Longitudinal bone growth results from expansion of the growth plate cartilage by regional chondrocyte proliferation, hypertrophy, and secretion of extracellular matrix (Ross et al. 1995). During the prepubertal years, the rate of skeletal growth is comparable in both sexes. The initiation of pubertal growth spurt by sharp rise in sex steroid production, in concert with other hormones, begins at about age 11 years in females and about age 13 to 14 years in males and lasts about 2 years in both sexes. The average of a 2 years longer prepubertal period of growth in combination with greater pubertal growth velocity in males than in females is responsible for the greater ultimate length of long bones in males compared to females (Cameron et al. 1982). Some other vertebrates, for example rats (Hansson et al. 1972), exhibit similar gender difference in length of long bones.

There is clinical and experimental evidence that estrogen plays a pivotal role in determining longitudinal bone growth in both genders (Wronski et al. 1988 and 1989, Smith et al. 1994, Morishima et al. 1995, Carani et al. 1997). Ovariectomy has been shown to result in transient increase in longitudinal bone growth (Wronski and Yen 1991) which is inhibited by estrogen treatment. Furthermore, the longitudinal bone growth rate is cyclic during the estrous cycle in the normal adolescent female rat, with the slow phase of bone growth occurring when estrogen levels are maximal and the fast phase occurring when estrogen levels are minimal (Whitson et al. 1978). These findings suggest that estrogen is the principal ovarian hormone active on growth plate cartilage. However, the mechanism of action of estrogen on growth plate cartilage is poorly understood.

4.2.2. Development of bone mass in relation to estrogen status

The rapid skeletal growth at puberty is associated with generalized accelerated increase in bone mineral mass in both genders (Figure 6.) according to plain radiographic studies (Garn 1970), the more recent dual energy X-ray absorptiometric (DXA) studies (Zanchetta et al. 1995, Bass et al. 1999) and most recently, studies employing peripheral computed tomography (pQCT)-measurements (Neu et al. 2001a and 2001b, Schöenau et al. 2001). Since its introduction in 1987, dual-energy X-ray absorptiometry (DXA) has been considered the method-of-choice for assessing bone mineral status [bone mineral content (BMC) and areal bone mineral density (aBMD)] and is currently the cornerstone in osteoporosis diagnosis (Faulkner et al. 1991, Johnston et al. 1991,

NIH 2001). The aBMD measured by DXA is the BMC per unit projected bone area and expressed in g/cm^2, an unrecognized unit in standard SI nomenclature. This planar nature of DXA makes the accurate assessment of the geometry, true composition and mechanical competence of bones impossible, and only rather crude approximations of bone's structural properties can be attained (Sievänen 2000). In contrast, pQCT allows for the determination of the actual or "true" (volumetric) BMD [a function of the BMC per volume of bone, that is volumetric BMD (vBMD) in terms of g/cm^3] and structural properties of bone, e.g. characterization of trabecular and cortical compartments, and estimations of bone strength (Sievänen et al. 1998).

The growth patterns of DXA-derived aBMD exhibit similar trends with the whole body (or site-specific) BMC (Lu et al. 1994, Zanchetta et al. 1995) and significantly higher postpubertal values in males compared to females have been reported (Zanchetta et al. 1995, Lu et al. 1996). However, as the bone size increases during skeletal growth period (and more so in males compared to females), the inability of DXA to take into account the bone size gives erroneous impression that tremendous increase in bone density occurs, e.g. increase of 70% in females and 105% in males in aBMD of distal radius between 6-20 years of age (Zanchetta et al. 1995), and males exhibit denser bones than females (Seeman 2001). Indeed, more recent pQCT-derived studies have suggested that high proportion of this increase in aBMD is due to increases in bone size (also explaining the gender difference in aBMD due to longer growth period in males), not in "true" bone density (Neu et al. 2001a, Schöenau et al. 2002) (Figure 6). The pQCT measurements between different bone sites and regions within a bone have also provided evidence for the existence of site- and gender-specificity in the accumulation of bone mineral during skeletal growth (Neu et al. 2001a and 2001b, Schöenau et al. 2002, Wang et al. 2005).

Current view on the effects of sex steroids on the regulation of bone mass accrual during growing years is that estrogen is the major biologically active bone steroid in females as well as in males (Emans et al. 1990, Smith et al. 1994, Morishima et al. 1995, Carani et al. 1997, Bilezikian et al. 1998, Khosla et al. 2002). Studies on female adolescents with estrogen deficiency (Emans et al. 1990) and males with congenital defect in estrogen receptor sensitivity (Smith et al. 1994) or estrogen synthesis (Morishima et al. 1995) have shown that in the absence of estrogen, skeleton displays reduced BMC and aBMD, and administration of estrogen is accompanied by reversal of the bone mineral loss, i.e. increase in bone mass and areal density is observed (Emans et al. 1990, Carani et al. 1997, Bilezikian et al. 1998).

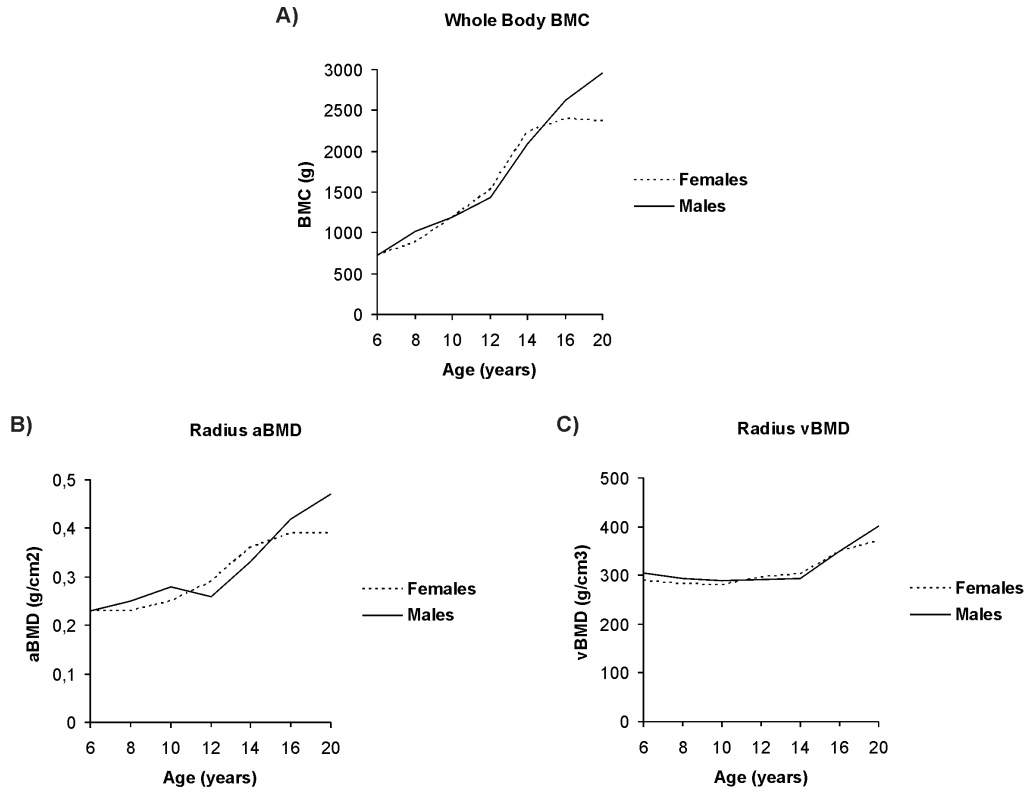

Figure 6. The development of **(A)** whole body BMC, **(B)** aBMD of distal radius and **(C)** vBMD of distal radius between 6-20 years of age in both genders. Adapted from Zanchetta et al. (1995) and Neu et al. (2001a).

To add another dimension to the discussion on the bone mass development during skeletal growth period, an interesting observation was introduced by Schiessl et al. in 1998 (Schiessl et al. 1998). By re-analyzing the data of whole body bone mineral content and body composition in Argentine boys and girls from 2 to 20 years old (Zanchetta et al. 1995), it was shown that the increase in bone mass in both sexes seems to closely accompany the increases in lean body (muscle) mass until just prior to menarche, i.e. the onset of cyclic estrogen secretion. Thereafter, this uniform pattern in the development of male and female skeletons suddenly dissociates, as the female skeletal mass starts to increase rapidly and disproportionately to the concurrent increase in lean body mass (Figure 7). Based on this finding, it was hypothesized that increasing estrogen secretion in females at puberty "sensitizes" bones to mechanical loading-induced stimuli via lowering the remodelling threshold and makes bone mass relative to muscle mass (the principal regulator of bone mass) increase faster than before making the bones heavier/stronger relative to muscle mass than in males. These authors also speculated, although did not directly couple the phenomenon to estrogen, that this extra packing of bone mineral into female skeleton (condensation of bones) could be most likely an evolutionary *safety measure* against the anticipated bone loss caused by pregnancy and lactation.

The experimental studies in both humans and animals have supported the above mentioned sex differences in bone-muscle mass relationship during growth (DeMoss and Wright 1998, Schöenau et al. 2000, 2001 and 2002, Wang

et al. 2003), and furthermore, recent pQCT-based studies have actually shown higher vBMD values in postpubertal females compared to males (Haapasalo et al. 2000, Kontulainen et al. 2002, Schöenau 2002, Riggs et al. 2004). Although the higher estrogen level in females than in males after the onset of puberty has been speculated to be responsible for this gender-specific development of bone-muscle mass relationship and "condensation" of female skeleton, it has not been proved to be the exact cause.

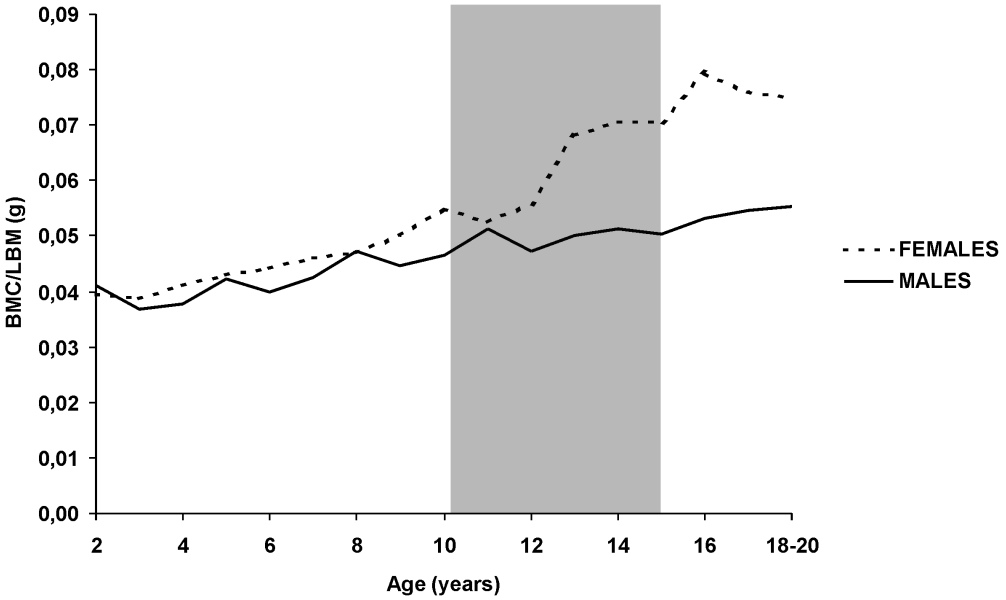

Figure 7. A graph showing the development of whole body BMC relative to lean body mass (LBM) in females and males between 2-20 years of age. Around 10-12 years of age, bone mass begins to increase faster in females than in males relative to lean body mass (muscle mass). Adapted from Zanchetta et al. (1995).

4.2.3. Estrogen and cross-sectional structure of bone during skeletal growth

In addition to the effects of estrogen on longitudinal bone growth and bone mass accrual, estrogen actions have been considered to influence skeletal architecture by modifying the radial growth of bones. The absolute and relative movements of the periosteal and endosteal surfaces of cortical bone determine the diameter of the long bone, the cortical bone mass, cortical thickness, and the distance the cortical bone mass is placed from neutral axis of bone (Seeman 2001).

There is little difference between sexes in the relative increase in cortical bone mass and periosteal and endocortical growth until puberty (Garn 1970, Neu et al. 2001b, Schöenau et al. 2001). At puberty the sexual dimorphism of the skeleton appears. The rapid skeletal growth during puberty is associated with accelerated periosteal apposition and fairly constant expansion of the medullary cavity in males, whereas in females the periosteal apposition occurs at considerably lower extent compared to males (Garn 1970, Zhang 1999, Neu et al. 2001b, Kontulainen et al. 2005). However, controversy exists about the behaviour of the endocortical surface and medullary cavity in females during

pubertal years (Garn 1970, Bass et al. 1999, Zhang et al. 1999, Neu et al. 2001b Högler et al. 2003, Kontulainen et al. 2005, Wang et al. 2005). Observations at the metacarpal determined from plain radiographs (Garn 1970) (Figure 8) or DXA-derived estimations at the femoral shaft (Bass et al. 1999) suggest that cortical wall thickness increases as a result of endosteal apposition in females. In contrast, recent comparisons of cortical bone structure by more sophisticated measurement techniques pQCT (Neu et al. 2001b, Kontulainen et al. 2005) and MRI (Högler et al. 2003) have provided evidence that the size of the medullary cavity remains relatively constant or even shows minor increase in females during skeletal growth period. Endocortical contraction may also be region-specific, and related to whether bone is weight-bearing (femur, lumbar spine) or not (radius, metacarpal) (Bass et al. 1999).

These gender-specific differences in the behaviour of periosteal and endosteal surfaces have been linked to direct effects of sex hormones, and predominantly estrogen, on number and activities of cells within bone. Periosteal apposition is considered to be inhibited in fertile-aged women by direct inhibitory effect of estrogen on periosteal bone cells, as estrogen deficiency has been shown to result in increased medullary area (Turner et al. 1987a and 1987b), periosteal bone formation (Turner et al. 1987a, 1987b, 1989 and 1992), osteoblast number and size (Turner et al.1992) and the mRNA levels for bone matrix proteins (Turner et al. 1990 and 1992), whereas the estrogen treatment has been shown to reverse the above mentioned estrogen deficiency induced changes on periosteal cells.

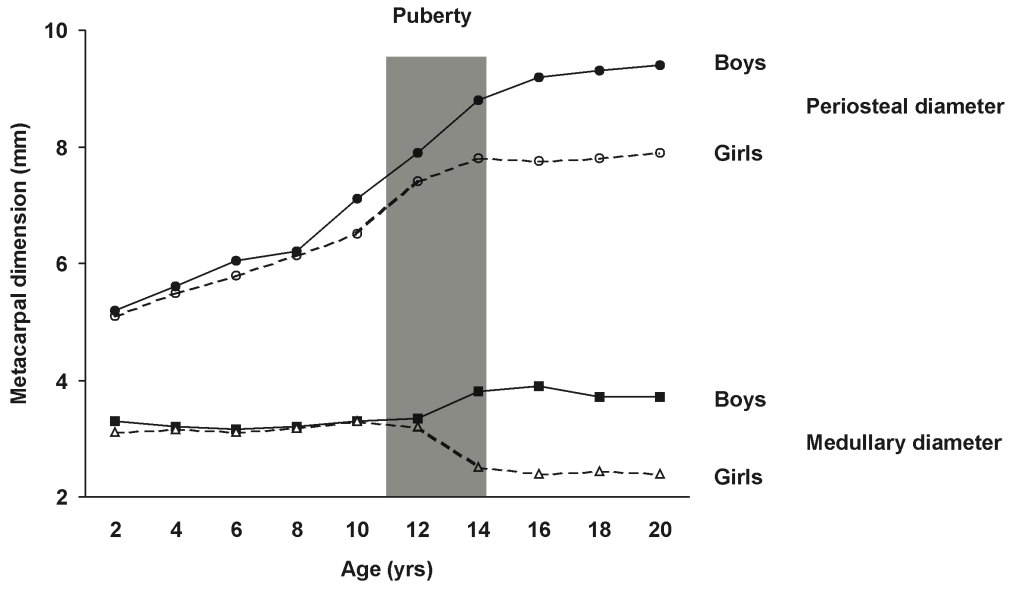

Figure 8. The original observation by Garn (1970) showing no gender-specific difference in periosteal or medullary diameter of the metacarpal bones before puberty. During puberty the periosteal diameter expands in boys and ceases to expand in girls whereas medullary diameter remains fairly constant in boys throughout growth but contracts in girls. Adapted from Garn (1970).

4.3. Estrogen related skeletal changes during aging

4.3.1. Estrogen and postmenopausal bone loss

Fuller Albright was the first to highlight the adverse effects of sex steroid deficiency on bone in a series of clinical descriptions in 1940s (Albright et al. 1940 and 1941). In his original observations, Albright noted the prevalence of ovariectomy among osteoporotic women being higher than expected, and almost invariably on these women the surgery had been performed at an age younger than the average age of natural menopause. Furthermore, Albright was the first to show that the negative calcium balance characteristic of osteoporotic post-menopausal women was reversed by estrogen administration. Based on these clinical findings, he postulated that the main skeletal action of estrogen was stimulation of osteoblast function (Albright et al. 1940).

Since Albright's original proposal - the hypo-osteoblastic hypothesis with decreased bone formation - alternative explanations for the origin of the postmenopausal osteoporosis have included disturbance in osteoclasia with increased bone resorption (Heaney and Whedon 1958, Frost 1961, Nordin 1964), negative calcium balance (Nordin 1960, 1961 and 1964), disturbance in calcium homeostatic control mechanisms (Jasani et al. 1965, Heaney 1965), increased skeletal sensitivity to parathyroid hormone (Heaney 1969 and 1974), deficiency of calcitonin (Stevenson et al. 1981, Tiegs et al. 1985) and calcitriol (Riggs and Melton 1983), altered activities of growth factors and cytokines (Canalis 1983, Pacifici et al. 1987 and 1991, Pacifici 1993, Mundy 1993, Manolagas 1994, Manolagas and Jilka 1995, Hustmyer et al. 1993), alterations in the local regulation of osteoclastogenesis (Walker 1975, Baron et al. 1986, Mundy and Rodman 1987, Kalu 1990, Jilka et al. 1992), changes in mechanical usage set-points (Frost 1992), failure in bone's adaptation to mechanical loading (Lanyon and Skerry 2001, Lee et al. 2003 and 2004), and estrogen deficiency-induced inhibition of osteoclast apoptosis (Hughes et al. 1996), as well as derangement in the birth and death of osteoblasts and osteoclasts (Manolagas 2000).

The unitary model of involutional osteoporosis that identified estrogen deficiency as the major cause of bone loss in postmenopausal women and as contributing cause of bone loss in elderly men was first presented by Riggs and Melton in 1986 (Riggs and Melton 1986) and subsequently refined in 1998 (Riggs et al. 1998). According to this model, the postmenopausal bone loss in females is divided into two separate phases: 1) type I osteoporosis (transient, accelerated bone loss) occurring essentially in the first decade after menopause and accounting for 20-30% of cancellous bone loss and 5-10% of cortical bone loss in females, and subsequent 2) type II osteoporosis (gradual, continuous bone loss) accounting for 20-30% of cancellous and cortical bone losses in both genders (Figure 9). There is nowadays mounting evidence that the accelerated bone loss associated with loss of estrogen secretion at menopause can be efficiently prevented by estrogen replacement therapy (ERT) (For review, see Riggs et al. 2002).

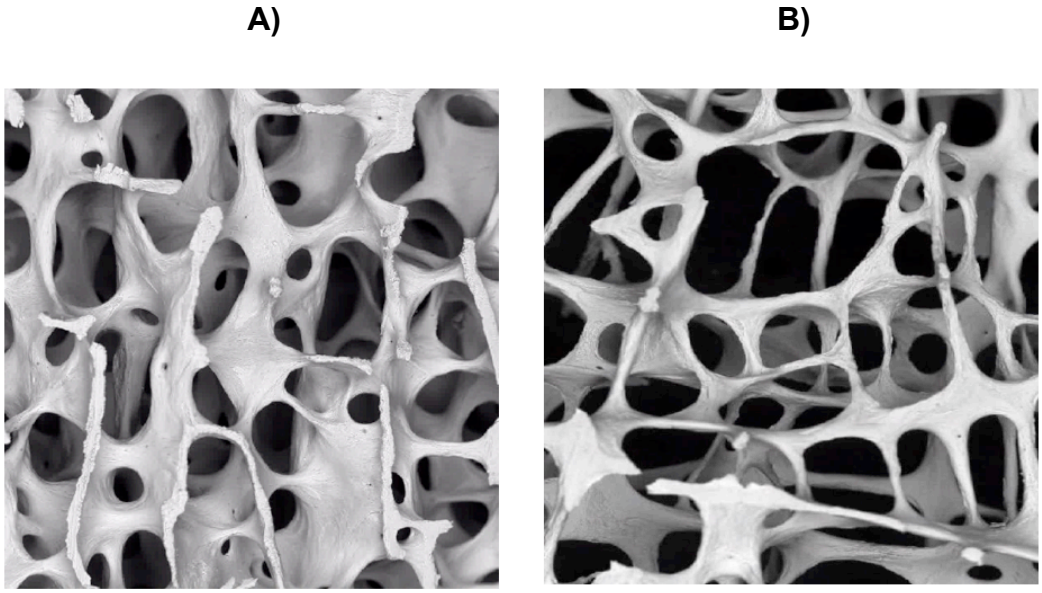

Figure 9. (A) Normal bone architecture in the third lumbar vertebra of a 30-year-old woman and, **(B)** osteoporotic architecture in the fourth lumbar vertebra of an 89-year-old woman.

4.3.2. Estrogen and periosteal and endosteal surfaces during aging

There is nowadays ample evidence that bone diameters increase with age (Smith and Walker 1964, Ruff and Hayes 1982 and 1988, Mosekilde and Mosekilde 1990, Heaney et al. 1997, Stein et al. 1998, Beck et al. 2000, Feik et al. 2000, Ahlborg et al. 2003). The age-related periosteal apposition is believed to occur in men (Ruff and Hayes 1988, Mosekilde and Mosekilde 1990) and, to a lesser extent, in women (Ruff and Hayes 1982 and 1988, Bouxsein et al. 1994, Heaney et al 1997). Smith and Walker (1964) were the first to demostrate expansion of the femoral cross-sectional diameter in aging women. In their original study, anteroposterior plain radiographs of femurs were obtained from 2030 women aged 45 to 90 years, and cortical thickness, periosteal and endosteal diameters of femoral midshafts were measured. They observed a simultaneous gain in periosteal diameter and expansion of endosteal diameter, the endosteal expansion exceeding periosteal enlargement resulting in thinning of the cortical wall.

A unique study by Ruff and Hayes (1982) using archeological samples of femora and tibiae from a large late prehistoric and protohistoric site in New Mexico provided further evidence supporting a model of general subperiosteal expansion of long bones with aging. Furthermore, a sex-specific difference in pattern of cortical expansion with aging was introduced: although the aging related increases in total subperiosteal area were fairly similar in both sexes (averaging 7 percent in males and 11 percent in females between 20 and 60 years of age), the percentage increases in medullary area were much greater in females (39 percent compared to 19 percent in males).

The data supporting the age-related expansion of bone diameters were derived primarily from cross-sectional studies until Heaney and colleagues (1997) reported the results of prospective study consisting of 170 women

followed for a mean period of 21 years from the age of 40 years. In their study, the femur shaft and neck diameter (assessed from standardized X-ray films) showed mean increases of 0.23%/ year and 0.14%/ year, respectively. In addition, Ahlborg and colleagues (2003) recently reported the results of prospective study consisting of 108 women followed from the time of menopause for a mean period of 15 years. Although the bone mass and skeletal structure were evaluated by a nowadays outdated method (single-photon absorptiometry), the results indicated a medullary expansion and simultaneous periosteal apposition occurring after menopause. In their study, the periosteal apposition partly compensated the decreased bone strength (strength index of bone showed no significant decrease until 14 years after menopause) caused by the postmenopausal bone loss (Figure 10).

As the inhibition of periosteal enlargement at puberty in females is commonly considered to be caused by direct estrogen mediated inhibition of periosteal bone cells, the expansion of the periosteal envelope after menopause (estrogen deficient state) is analogously attributed to removal of this estrogen-induced constraint on periosteal apposition (Seeman 2003). Studies in postmenopausal women are in concept with this notion as the use of ERT is associated with inhibited periosteal expansion compared to nonusers of ERT (Heaney et al. 1997, Beck et al. 2001).

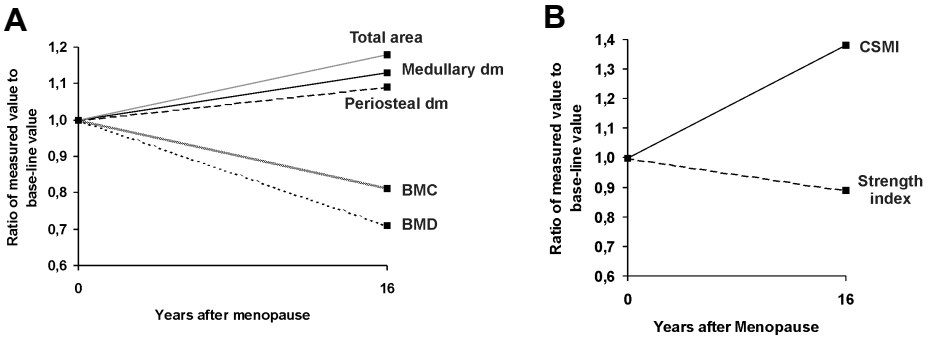

Figure 10. Relative changes in structural parameters, bone mass and strength index at the cortical site of the distal radius in women followed 16 years from menopause. Adapted from Ahlborg et al. (2003).

4.3.3. Rat as an animal model for postmenopausal osteoporosis

The ovariectomized (OVX) rat is the most commonly used animal model for postmenopausal bone loss and there is extensive literature studying the OVX rat including histomorphometric changes, biochemical markes, methodology for bone densitometry and evaluation of bone fragility (Wronski et al. 1985 and 1986, Wronski and Yen 1991, Frost and Jee 1992, Kalu 1991). The benefits of the OVX rat model include the exhibition of most of the characteristics of human postmenopausal osteoporosis in OVX rats, inexpensiveness as a model, easy housing, and the general acceptance of the public to the use of rodents in research (Turner 2001). However, the rat is considered a poor animal model to

study the effect of OVX on cortical bone because of the lack of Haversian (intracortical) systems, while another limitation is the absence of impaired osteoblast function during the late stages of estrogen deficiency (Wronski and Yen 1991). However, rats do show significant elevation of cortical porosity in response to immobilization (Sietsma 1995). The mechanism of increased intracortical porosity observed as a consequence of aging and certain diseases in human ribs and iliac crest biopsies (Wu et al. 1967, Brockstedt et al. 1993) is though to be an increased activation of Haversian remodelling systems accompanied by increased Haversian canal diameter. However, the relevance of porosity in rib or iliac crest biopsies to other bone sites (for example femoral neck) has not been fully investigated. More importantly, the relationship between cortical porosity and strength of a bone has not been adequately studied although some studies have shown increased cortical porosity in the femoral neck in patients suffering from the femoral neck fracture compared to age- and gender-matched controls (Bell et al. 2000).

4.4. Estrogen and mechanosensory system of bone

Estrogen is generally considered to increase the mechanosensitivity of bones (Cheng et al. 1996 and 1997, Jagger et al. 1996, Westerlind et al. 1997, Turner 1999, Joldersma et al. 2001, Lanyon and Skerry 2001). This permissive role of estrogen on the osteogenic effects of mechanical loading on bone is based solely on *in vitro* and early-stage loading-induced *in vivo* responses through the proposed involvement of the estrogen receptor(s) in the mechanosensing pathway of bone cell (Damien et al. 1998 and 2000, Jessop et al. 2001). It has even been suggested that the presence of functional ER-α and/or ER-β in bone cells is a prerequisite for the bones to respond to mechanical loading (Lee et al. 2003 and 2004). Prompted by these findings, it was recently suggested that postmenopausal osteoporosis *per se* would be attributable to the estrogen-withdrawal-induced de-sensitation of bones to loading-induced stimuli (Lanyon and Skerry 2001, Lee et al. 2003)

However, it has recently been increasingly acknowledged that bones, as primary locomotive organs, should be considered as structures with the mechanical strength and rigidity representing their ultimate phenotype (Seeman 1997 and 2002, van der Meulen et al. 2001, Boskey et al. 2003). It has even been argued that conclusions based solely on observations obtained either from cell culture experiments *in vitro* or from *in vivo* studies assessing bone mass or other surrogates of bone strength are insufficient and likely misleading (van der Meulen et al. 2001, Boskey et al. 2003, Järvinen et al. 2005). Regarding bones, if we do not know whether the bone as an organ has truly strengthened, we have no certainty of knowing whether a change in any of the intermediate or surrogate measures of bone strength denote only a transient phenomenon – like a "snap-shot" of a dynamic movement eventually fading away - or actually a strengthened bone structure as a response to the stimulus of interest (Järvinen et al. 2005).

Current literature provides only few experimental studies (Honda et al. 2001

and 2003) exploring the estrogen-loading interaction on bone characteristics that provide the direct measurement of bone strength and appropriate 2 x 2 factorial (estrogen and loading as factors) statistical analysis of the results. In those studies, estrogen and mechanical loading were found to have independent and additive or interactive effect on bone mass and/or histomorphometric properties, but no interaction was observed between estrogen and loading on bone strength. Also, clinical observations exploring the effect of estrogen (ERT) and exercise in postmenopausal women are sparse (Kohrt et al. 1995 and 1998, Heikkinen et al. 1997). In each of these studies, subjects with both ERT and exercise had the highest BMD compared with the other study groups after the completion of the intervention. However, the actual within group *responses* to exercise (change in BMD/BMC, controls versus exercised) between the estrogen-deplete and -replete groups were not carried out in any of these original studies.

AIMS OF THE STUDY

The main objective of this thesis was to evaluate the effect of gender, estrogen and age on the mechanosensitivity of bone, and furthermore, to assess the ability of the bone to maintain the exercise-induced bone benefits. In addition, the respective roles of estrogen and loading on the bone characteristics were investigated. More specifically, the aims of the individual studies were the following:

I. To assess whether there is any sex-related difference in the mechanosensitivity of bone to exercise, and if so, whether estrogen per se possesses a modulatory effect on the mechanosensitivity.

II. To evaluate whether the possible exercise-induced bone benefits attained during the period of fastest skeletal growth in a rat can be maintained into adulthood and old age after the exercise is ceased.

III. To determine whether the adaptive response of bone to exercise differs quantitatively and/or qualitatively between young and adult rats, and whether aging modulates the ability of the skeleton to maintain the exercise-induced skeletal changes.

IV. To explore the independent and potentially interactive effects of estrogen and loading on the structural characteristics of bone in a rat by separately or simultaneously removing their influence.

MATERIALS AND METHODS

1. Animals

A total of 270 rats (140 female and 130 male) of Sprague-Dawley strain were used in the experiments of this thesis (I-IV, Table 1.). Animals were housed five animals per cage at 20 ^{o}C with a light cycle of 12 hours, and fed standard laboratory chow (Ca^{2+} 0.9%, P 0.7%, and vitamin D 0.6 IU) and water ad libitum. In the experiments involving ovariectomized (OVX) rats (I, IV), each cage of OVX rats (two animals per cage) was pair-fed with a cage of control rats with access to food ad libitum in order to control the well-known gain of extra weight associated with OVX (Kalu 1984, Wronski et al. 1987). The pair-feeding was executed as follows: Each control cage was matched with an OVX cage, the food consumption of the control cage was followed (weighted) every other day and an identical amount of pellets was given to the OVX cage the next day. Otherwise the conditions were similar to all animals. All experiments were approved by the Ethics Committee for Animal Experiments of the University of Tampere and the animals maintained according to the guidelines of NIH standards established in the "Guidelines for the Care and use of Laboratory Animals".

2. Ovariectomy (I, IV)

At the beginning of the experiments (I, IV), 3-week-old female rats were randomly subjected to either bilateral sham (SHAM, E^{+}) or ovariectomy (OVX, E^{-}) surgery under fentanyl–midazolam anesthesia using a dorsal approach described in detail previously (Waynforth 1988). Both ovaries were exposed and removed in the OVX animals. In the SHAM animals, the ovaries were exposed and left intact.

3. Experimental loading models

3.1. Increased loading (exercise) (I-III)

During the first 1-2 weeks of the studies prior to assignment into actual control and exercise groups, all rats were run on a flatbed treadmill at a slow speed

(10–20 cm/s) for 3 min/day 3 days a week to acclimatize the animals to the treadmill running and to remove those animals refusing to run. After the acclimatization period, the rats were randomly assigned into control and exercise groups. The actual exercise was conducted on the treadmill once a day, 4–5 days a week for 14 (I-III) and 16 (E^+EX and E^-EX in study I) weeks. The inclination and speed of the treadmill and the duration of each session were increased progressively within the physical capacity of the animals (Table 2). The training regimen between corresponding study groups (Male-EX vs Female-EX, EX_1 vs. EX_2, and E^+EX vs. E^-EX) was identical.

Study	**Number and (age) of the animals**	**Study groups (gender and intervention)**
I (n=160)	50 (5-19wk)	males [14 wk exercise (**Male-EX_1**), controls (**Male-C_1**)] females [14 wk exercise (**Female-EX_1**), controls (**Female-C_1**)]
	50 (33-47wk)	males [14 wk exercise (**Male-EX_2**), controls (**Male-C_2**)] females [14 wk exercise (**Female-EX_2**), controls (**Female-C_2**)]
	60 (5-21wk)	sham-operated [16 wk exercise (**E^+EX**), controls (**E^+**) ovariectomized [16 wk exercise (**E^-EX**), controls (**E^-**)]
II (n=100)	100 (5-61 wk)	5-19 wk: 14 wk exercise (**EX**), controls (**C_{14}**) 5-33 wk: **EX**+ 14 wk deconditioning (**DC_{14}**), controls (**C_{28}**) 5-47 wk: **EX**+ 28 wk DC (**DC_{28}**), controls (**C_{42}**) 5-61 wk: **EX**+ 42 wk DC (**DC_{42}**), controls (**C_{56}**)
III (n=100)	50 (5-33wk)	5-19 wk: 14 wk exercise (**EX_1**), controls (**C_1**) 5-33 wk: **EX_1**+ 14 wk deconditioning (**DC**), controls (**C_2**)
	50 (33-61wk)	33-47 wk: 14 wk exercise (**EX_2**), controls (**C_3**) 47-61 wk: **EX_2**+ 14 wk deconditioning (**DC**), controls (**C_4**)
IV (n=30)	30 (3-11 wk)	sham-operated (**E^+**) right hindlimb normally loaded (**E^+L^+**) left hindlimb immobilized 8 wk (**E^+L^-**) ovariectomized (**E^-**) right hindlimb normally loaded (**E^-L^+**) left hindlimb immobilized 8 wk (**E^-L^-**)

Table 1. Animals and study groups.

STUDIES I-III					STUDY I (SHAM+EX, OVX+EX)				
Week	**Age** *(weeks)*	**Duration** *(min)*	**Speed** *(cm/s)*	**Inclination** *(deg)*	**Week**	**Age** *(weeks)*	**Duration** *(min)*	**Speed** *(cm/s)*	**Inclination** *(deg)*
1	5-/ 33-	5	20	5	1	4-	5	20	5
2	6-/ 34-	10	20	10	2	5-	5	25	5
3	7-/ 35-	10	20	15	3	6-	5	25	10
4	8-/ 36-	10	30	15	4	7-	5	30	15
5	9-/ 37-	10	30	20	5	8-	5	30	20
6	10-/ 38-	10	30	20	6	9-	5	30	25
7	11-/ 39-	10	30	25	7	10-	5	35	30
8	12-/ 40-	10	30	25	8	11-	5	35	30
9	13-/ 41-	10	30	30	9	12-	5	35	35
10	14-/ 42-	10	30	30	10	13-	5	40	35
11	15-/ 43-	10	30	30	11	14-	5	40	35
12	16-/ 44-	10	30	30	12	15-	5	40	35
13	17-/ 45-	10	30	30	13	16-	5	35	35
14	18-19/ 46-47	10	30	30	14-16	17-19	5	35	35

Table 2. The exercise regimen in the studies I-III.

3.2. Deconditioning (II, III)

In study II, the four exercise groups (EX, EX+ DC_{14}, EX+ DC_{28}, and EX+ DC_{42}) underwent an exercise program for 14 weeks (Table 2) between 5-19 weeks of age. After the exercise period of 14 weeks, the exercised animals in the group EX were sacrificed and specimen were collected. The remaining exercise groups (EX+ DC_{14}, EX+ DC_{28}, and EX+ DC_{42}) underwent a deconditioning period of different time lengths: after the exercise period of 14 weeks, the rats were allowed to move freely in the cage for 14 weeks, 28 weeks, and 42 weeks until the sacrifice in the groups EX+ DC_{14}, EX+ DC_{28}, and EX+ DC_{42}, respectively.

In study III, the young (EX_1, EX_1+DC) and adult exercise groups (EX_2, EX_2+DC) were subjected to exercise program for 14 weeks beginning at the age of 5 and 33 weeks, respectively (Table 2). After the exercise period of 14 weeks, the exercised animals in groups EX_1 and EX_2 were sacrificed and specimen were collected. The remaining exercise groups (EX_1+DC and EX_2+DC) underwent a deconditioning period of 14 weeks until the sacrifice at the age of 33 (EX_1+DC) and 61 (EX_2+DC) weeks of age.

3.3. Withdrawal of loading (IV)

After the OVX- or SHAM surgery under the same fentanyl–midazolam anesthesia, the left hind limb (L^-) of each study animal was immobilized for 8 weeks with a padded tape from the toes to 1 cm above the knee (stifle). The knee was fixed in 100° flexion and the ankle (hock) in 60° plantar flexion so that the calf muscles were relaxed. The fixation was checked daily and replaced or reinforced if necessary. The contralateral (right) limb (L^+) was kept free and served as non-immobilized control.

Study	Group	Age (weeks)	Animal weight ENTRY (g)	Animal weight FINAL (g)	Muscle weight (g)	Femur length (mm)
I	Male-C_1	5-19	147 ± 25	434 ± 39	3.06 ± 0.17	38.9 ± 0.55
	Male-EX_1	5-19	147 ± 23	394 ± 33	2.85 ± 0.28	38.9 ± 0.94
	Female-C_1	5-19	123 ± 20	261 ± 25	2.10 ± 0.13	34.2 ± 0.73
	Female-EX_1	5-19	120 ± 21	268 ± 23	2.13 ± 0.16	34.9 ± 0.76
	Male-C_2	33-47	515 ± 52	549 ± 60	3.64 ± 0.40	42.2 ± 0.73
	Male-EX_2	33-47	510 ± 50	478 ± 34	3.16 ± 0.29	41.6 ± 0.73
	Female-C_2	33-47	301 ± 17	313 ± 24	2.28 ± 0.19	36.5 ± 0.95
	Female-EX_2	33-47	296 ± 26	296 ± 24	2.14 ± 0.28	37.0 ± 1.22
	E^+	4-19	101 ± 12	271 ± 21	2.07 ± 0.19	34.6 ± 0.64
	E^+EX	4-19	106 ± 15	268 ± 18	2.04 ± 0.17	34.6 ± 0.75
	E^-	4-19	106 ± 13	303 ± 22	2.39 ± 0.23	35.5 ± 0.91
	E^-EX	4-19	100 ± 20	289 ± 17	2.25 ± 0.23	36.2 ± 0.59
II	C_{14}	5-19	147 ± 25	434 ± 39	3.06 ± 0.17	38.9 ± 0.6
	C_{28}	5-33	143 ± 25	525 ± 50	3.53 ± 0.32	41.4 ± 0.9
	C_{42}	5-47	145 ± 18	549 ± 60	3.64 ± 0.40	42.2 ± 0.7
	C_{56}	5-61	152 ± 12	576 ± 31	3.49 ± 0.23	42.0 ± 1.2
	EX	5-19	147 ± 23	394 ± 33	2.85 ± 0.28	38.9 ± 0.9
	EX+DC_{14}	5-33	146 ± 26	514 ± 37	3.56 ± 0.32	41.4 ± 0.9
	EX+DC_{28}	5-47	141 ± 21	544 ± 44	3.63 ± 0.22	42.2 ± 1.0
	EX+DC_{42}	5-61	142 ± 18	572 ± 78	3.51 ± 0.30	42.4 ± 1.0
III	C_1	5-19	147 ± 25	434 ± 39	3.06 ± 0.17	38.9 ± 0.6
	EX_1	5-19	147 ± 23	394 ± 33	2.85 ± 0.28	38.9 ± 0.9
	C_2	19-33	445 ± 40	525 ± 50	3.53 ± 0.32	41.4 ± 0.9
	EX_1+DC	19-33	396 ± 27	514 ± 37	3.56 ± 0.32	41.4 ± 0.9
	C_3	33-47	515 ± 52	549 ± 60	3.64 ± 0.40	42.2 ± 0.7
	EX_2	33-47	510 ± 50	478 ± 34	3.16 ± 0.29	41.6 ± 0.7
	C_4	47-61	536 ± 27	576 ± 31	3.49 ± 0.23	42.0 ± 1.2
	EX_2+DC	47-61	511 ± 45	583 ± 49	3.58 ± 0,43	41.7 ± 0,7
IV	E+L+	3-11	97 ± 14	195 ± 16	1.45 ± 0,12	30.8 ± 0,5
	E+L-	3-11	97 ± 14	195 ± 16	0.77 ± 0.11	30.2 ± 0.7
	E-L+	3-11	101 ± 13	217 ± 12	1.65 ± 0.10	31.1 ± 0.7
	E-L-	3-11	101 ± 13	217 ± 12	0.86 ± 0.10	31.3 ± 1.0

Table 3. The gross characteristics of the animals used in studies I-IV. The results are expressed as mean ± standard deviation (SD).

4. Samples

At sacrifice, the rats were killed with carbon dioxide inhalation and body weights were recorded (Table 3.). The right calf muscles (gastrocnemius, soleus, and tibialis plantaris) were carefully prepared and weighed, both femora were carefully excised and all surrounding tissues (skin, muscle, and soft tissue) removed. The right femora (both femora in study IV) were then wrapped in saline-soaked gauze bandages, and stored frozen at -20°C in small Ziploc freezer bags and the left femur was placed in 70% ethanol solution (I-III). In addition, in studies I and IV the success of ovariectomy was confirmed by clinically examining the absence of ovarian tissue and measuring uterine weight.

5. Bone measurements

At the day of measurements, the bones were slowly thawed at room temperature at least 12 h before actual mechanical testing and kept wrapped in the saline-soaked gauze except during measurements. For each rat, all measurements were performed successively in the same order.

5.1. Peripheral quantitative computed tomography (pQCT)

The cross-sections of the right femoral necks (both femoral necks in study IV) were scanned with a commercial pQCT system Stratec XCT 960A with software version 5.20 (Stratec Medizintechnik GmbH, Birkenfeld, Germany) (Figure 11). The scanner employs a 45 kV/0.3 mA X-ray source. The scan time is 2.5 min and the size of the image matrix is 128 x 128, with the voxel size being 0.092 x 0.092 x 1.25 mm^3 and the number of projections being 72. The bones were cut approximately at the midshaft and the proximal part of the femur was inserted, with the femoral neck in an axial direction, into a specially constructed plastic tube for the measurement. The scan line was adjusted to the midneck using the scout view of the pQCT software. Total cross-sectional area (tCSA), total bone mineral content (tBMC), and total volumetric bone mineral density (tvBMD) at the femoral midneck were recorded as given by the pQCT software. Each bone was measured twice with repositioning and the average of these measurements was taken as the outcome variable. In our laboratory, the reproducibility (average root-mean-square coefficient of variation, CV_{rms}) for repeated measurements in the femoral neck are 3.9% for the tCSA, 2.2% for the tBMC, and 2.1% for the tvBMD.

The midshafts of the right femora (both femora in study IV) were scanned with a Norland/Stratec XCT 3000 scanner (Stratec Medizintechnik GmbH) (Figure 11). The bones were inserted into a specially constructed plastic tube for the measurement and one cross-sectional slice from each bone was scanned at 50% of the measured length of the femur. The cross-sectional image of the femoral midshaft was scanned with a voxel size of 0.3 x 0.3 x 2.5 mm^3. tCSA,

cortical cross-sectional area (cCSA), and cortical volumetric BMD (cvBMD) were recorded as given by the pQCT software. Our CV_{rms} in the femoral midshaft are 0.9% for the tCSA, 1.5% for the cCSA, and 0.6% for the cvBMD.

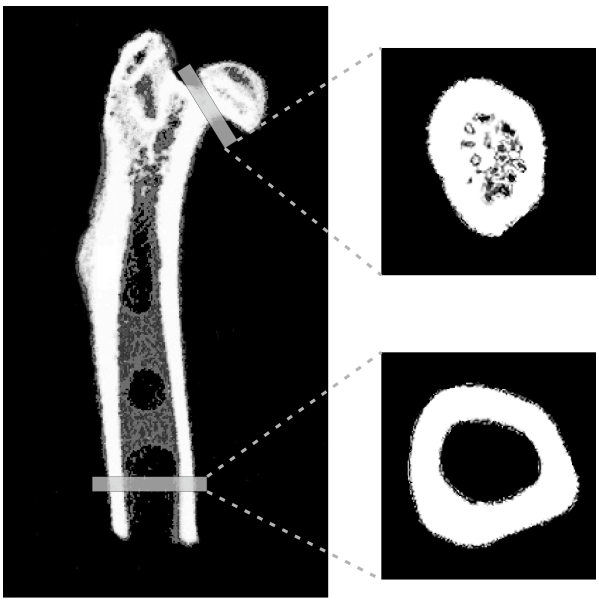

Figure 11. A pQCT derived longitudinal slice of femur and cross-sectional slices of femoral neck and midshaft used for analysis, the zone under the shaded region indicating the scanned region of interest.

5.2. Microcomputed tomography (μCT)

The distal metaphysis of femora were scanned using the SkyScan 1072 microtomograph (SkyScan, Antwerp, Belgium) (IV). An X-ray source of 61 kV/163μA was employed, yielding an image with matrix size 1024 x 1024 and pixel size of 14.5 μm (*x*) x 14.5 μm (*y*). The analyzed bone region (volume of interest, VOI) consisted a 4.5 mm longitudinal section (extending proximally from the mid-growth plate) of the trabecular bone of the distal femoral metaphysis (Figure 12). Approximately 300 slices were acquired per bone using a slice increment of 29 μm and the trabecular bone of each slice was separated from the cortical bone by manually drawing the contours. The entire trabecular bone region of the distal femoral metaphysis was evaluated, thereby minimizing sampling errors incurred by random deviations of a single section. A three-dimensional analysis of the VOI was performed using the software (CTAn version 1.03.2) provided by the manufacturer of the μCT scanner. A global threshold was used to distinguish bone and marrow (150 of maximal grey-scale value). The total bone marrow volume including the trabeculae (TV), trabecular bone volume (BV), and trabecular bone volume fraction (BV/TV) were determined for the entire VOI. Furthermore, mean trabecular thickness (Tb.Th), mean trabecular number (Tb.N), and mean trabecular separation (Tb.Sp) were determined using direct three-dimensional approach (that does not rely on any assumptions about whether the underlying structure is either plate- or rod-like) (Ulrich et al. 1999).

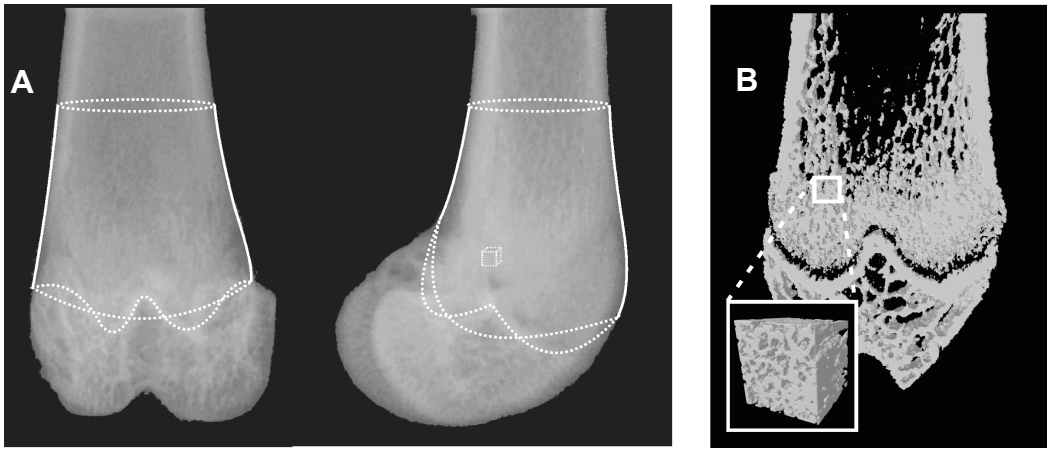

Figure 12. The micro CT analysis of the distal metaphysis of femur. **(A)** The volume of interest (VOI) consisted a 4.5mm longitudinal section extending proximally from the mid-growth plate. **(B)** 3D analysis of the trabecular bone region.

5.3. Geometrical measurements

A digimatic caliper (Mitutoyo 500, Andover, UK), providing a resolution of 0.01 mm, was used to measure the bone dimensions and geometry. The length of femur (L) (I-IV) was measured from the tip of the greater trochanter to the intercondylar notch. The width (W) and thickness (T) of the femoral shaft was measured in the mediolateral (ML) and anteroposterior (AP) directions, respectively (IV). Likewise, the inside width (w) and thickness (t) of the medullary canal of the femoral shaft were determined at the break line after three-point bending of the shaft (see subsequent text) (IV). Additionally, the femoral shaft was considered a hollow, elliptic-shaped structure, and the following geometric indices were determined according to common engineering principles: (1) cortical wall thickness in ML and AP directions, CWT_{ML} = (W-w)/2 and CWT_{AP} = (T-t)/2; (2) cross-sectional moment of inertia in the ML and AP directions, $CSMI_{ML} = \pi/64[(W^3T)-(w^3t)]$ and $CSMI_{AP} = \pi/64[(T^3W)-(t^3w)]$; and (4) section modulus in the ML and AP directions, $Z_{ML} = CSMI_{ML}/(W/2)$ and $Z_{AP} = CSMI_{AP}/(T/2)$ (IV). The CV_{rms} for these variables range from 0.2% to 4.0% in our laboratory (Järvinen et al. 1998a).

5.3. Biomechanical testing

After the pQCT analysis of the femoral shaft, the right femora (both in study IV) were subjected to mechanical testing. A Lloyd material testing machine (LR5K; J. J. Lloyd Instruments, Southampton, UK) was used for the anteroposterior three- point bending of the femoral shafts and compression of the femoral necks. For the three-point bending, the femora were placed on their posterior surface on the lower supports of the bending apparatus (Figure 13). For each bone, these supports were placed individually (just distal to the trochanter minor and the other just proximal to the condyles of the femur). After the adjustment of the supports, a small stabilizing preload was applied on the anterior surface of the

femur at a rate of 0.1 mm/sec using a brass crossbar. The bending load was then applied at a rate of 1.0 mm/sec to the femoral midshaft perpendicularly to the long axis of the bone until the failure of the specimen. The breaking load (Fmax) of the femoral shaft was determined. In our laboratory, the CV_{rms} of the Fmax for three-point bending is 5.0% (Järvinen et al. 1998a).

After the three-point bending of the femoral shaft, the proximal part of each specimen was collected and femoral neck subjected to pQCT measurement and subsequently, to compression test using the testing machine. For the compression test, the proximal half of each femur was mounted in a specially constructed fixation device (Figure 14). The specimen was then placed under the materials testing machine, and a vertical load was applied to the top of the femoral head using a brass crossbar. As in the three-point bending, a small preload was applied at a rate of 0.1 mm/sec. The bending load was then applied at a rate of 1.0 mm/sec until fracture of the femoral neck. The breaking load (Fmax) of the femoral neck was determined from the load-deformation curve. In our laboratory, the CV_{rms} of the Fmax for the femoral neck compression is 7.6% (Leppänen et al. 2006).

Figure 13. Three-point bending test of the femoral midshaft.

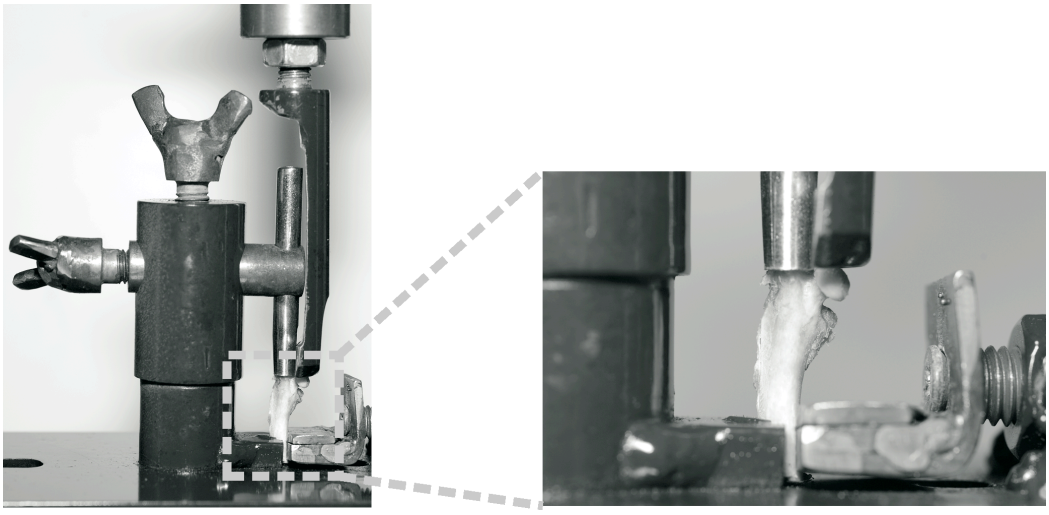

Figure 14. The compression test of the femoral neck.

6. Statistical analysis

All measurements were blinded to the group assignments. Results of each group of animals were expressed as the mean and standard deviation (II, III) or mean and standard error of mean (I, IV). Two-way factorial analysis of variance (ANOVA) (I-IV) was used to determine the effect of physical activity type (treadmill training/ immobilization) on the bone parameters and whether the response to mechanical loading differed between males and females (I), between young and adult (III), or between the E^+ and E^- rats (I, IV). The mechanical loading modality (exercise/ immobilization, control) and sex (male, female) (I), age (young, adult) (III) or estrogen status (E^+, E^-) (I, IV) were used as fixed factors. To eliminate the inherent bias arising from comparisons between experimental groups that differ in body weight and size (i.e., females vs. males, control vs. exercised, and E^+ vs. E^-) the weight of the calf-muscle (I-III) (representative of muscle mass which exert the principal mechanical loads on the skeleton) or femoral length and body weight (IV) were used as covariates. In all tests, a α level less than 5% ($p < 0.05$) was considered significant.

RESULTS

1. Age- and gender dependent development of bone mineral mass, size and strength

The development of tCSA, tvBMD, tBMC and Fmax, and also muscle mass adjusted values for tBMC and Fmax, of the femoral neck and midshaft in male and female rats between 19-61 weeks of age are presented in Figures 15 and 16, respectively. As readily apparent in Figures 15 and 16, the size, mineral mass and mechanical strength of femoral neck and midshaft increased steadily with aging in both genders. Interesting but false illusion of the declining tCSA of the femoral neck with age evident in males between 47-61 weeks of age and in females 33-47 weeks of age (Figure 15A) most likely resulted from the interaction of 1) actual age-related lengthening of the femoral neck accompanied with 2) limitations of the pQCT technology: In each study group, the scan line was adjusted anatomically to the midneck using the scout view of the pQCT software. The cross-sectional image of the femoral neck was then scanned with a voxel size of 0.092 x 0.092 x 1.25 mm^3, 1.25 mm being the thickness of the slice. In the younger study groups, the thickest portion of the neck was "captured" in the pQCT slice, but with age-related lengthening of the neck, the thicker portion was left out of the slice, and thus, the "illusion" of declining tCSA emerged. This also explains the similar pattern in the behavior of tBMC Figure 15C) with aging for example, at 33 weeks of age in females pQCT "sees" bigger bone naturally containing more mineral than at 47 weeks of age.

Furthermore, males exhibited significantly larger cross-sectional size and mineral mass resulting in mechanically stronger bone compared to female counterparts at each study point. However, the vBMDs of both bone sites (Figure 15B and 16C) remained rather constant during the entire study period in both genders. More interestingly, female rats exhibited ~ 20% higher total vBMD of the femoral neck compared to corresponding male rats. Furthermore, the femoral necks of female rats contained significantly more bone mineral (20-30%) (Figure 15D) and showed actually greater (~ 20%) mechanical competence (Figure 15F) in relation to muscle mass (represented here by calf-muscle weight). The findings were similar in the femoral midshaft (Figure 16F and G), although the difference in cortical BMC/ muscle mass did not reach statistical significance suggesting the existence of skeletal site-specificity.

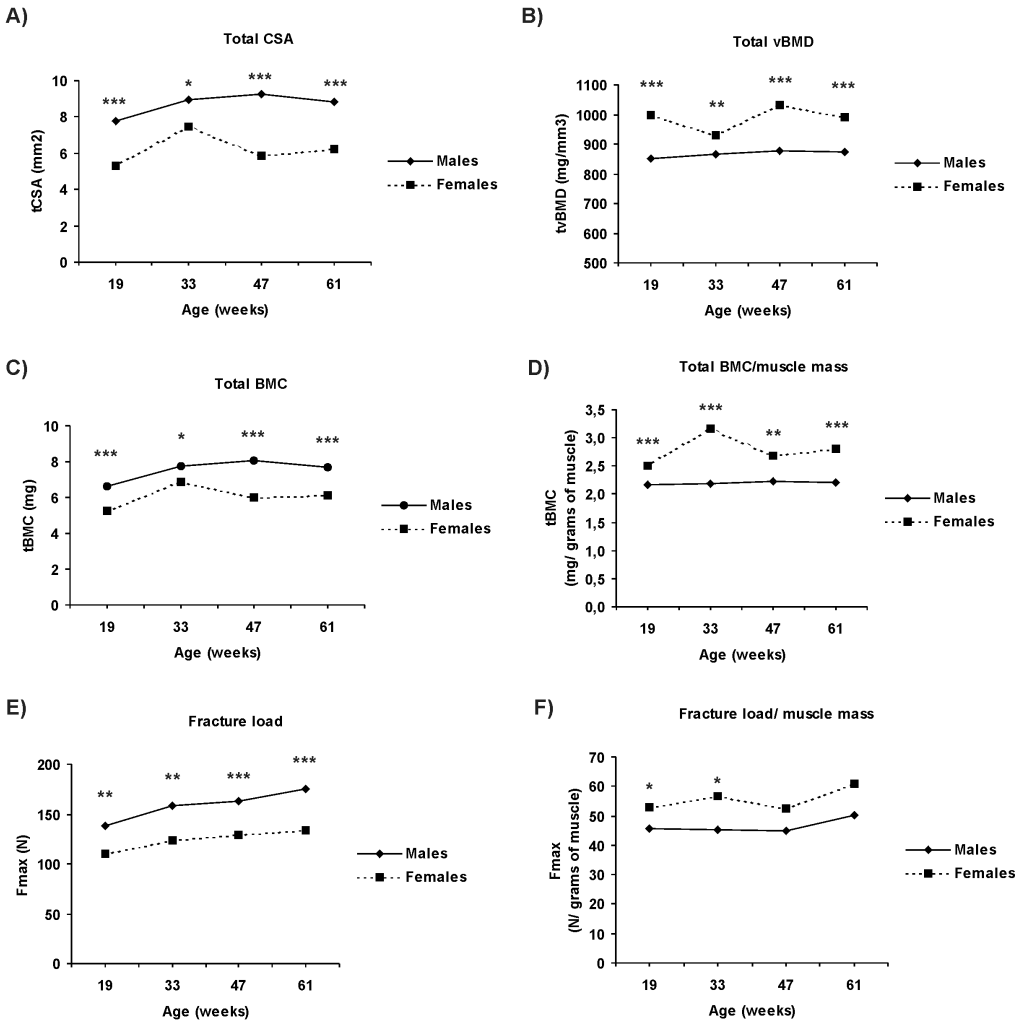

Figure 15. The development of **(A)** total cross-sectional area (tCSA), **(B)** total volumetric bone mineral density (tvBMD), **(C)** total bone mineral content (tBMC) and **(E)** fracture load (Fmax) of femoral neck in male and female rats between 19-61 weeks of age. Furthermore, the development of tBMC and Fmax in relation to muscle mass (calf-muscle weight) in both genders are presented in **(D)** and **(F)**, respectively. Statistically significant difference between females vs. males at each study point is indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

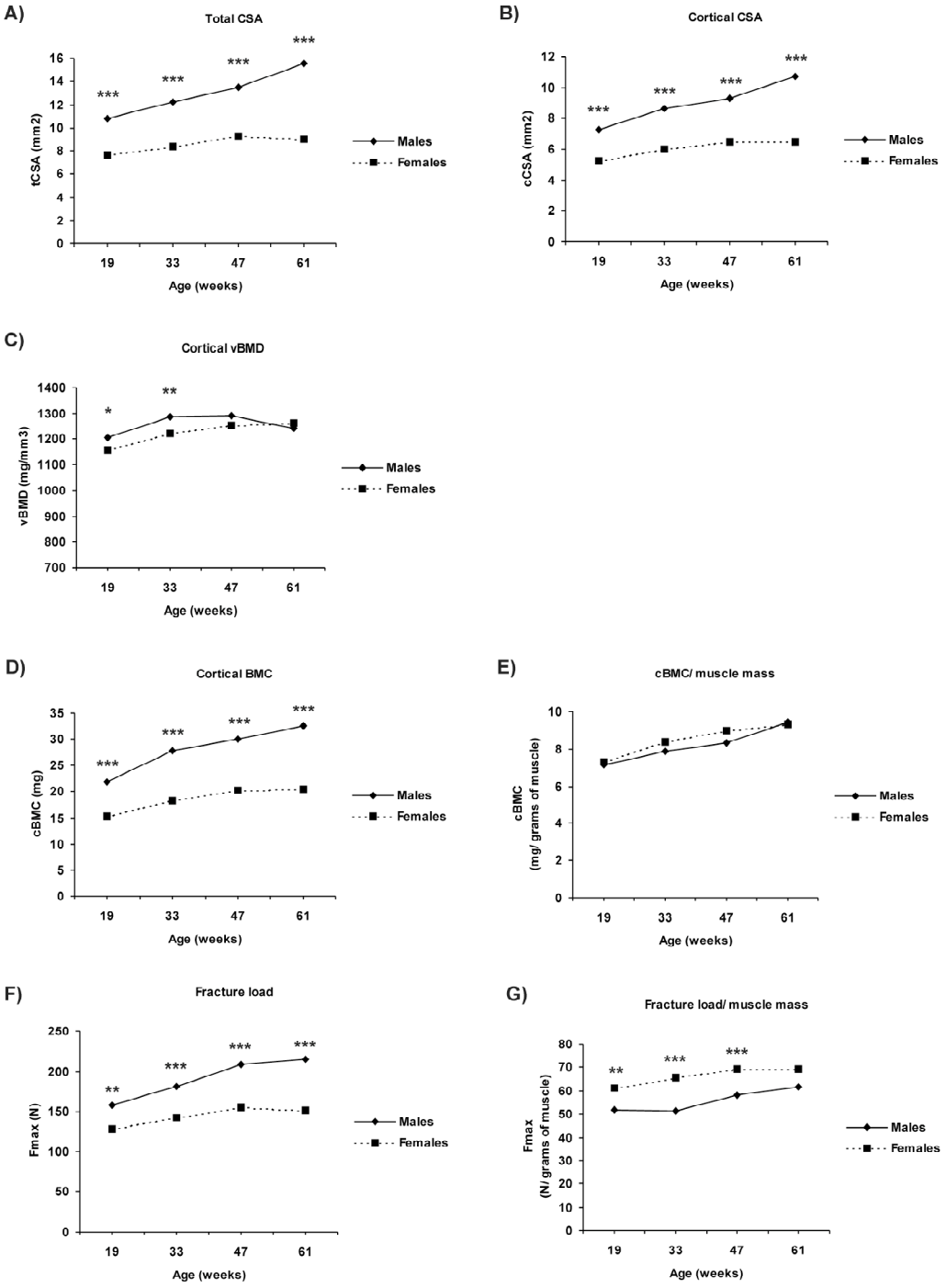

Figure 16. The development of **(A)** tCSA, **(B)** cCSA, **(C)** cBMD and **(D)** cBMC, **(E)** Fmax, and **(F)** cBMC and **(G)** Fmax in relation to muscle mass (calf-muscle weight) of femoral midshaft in male and female rats between 19-61 weeks of age. Statistically significant difference between females vs. males at each study point is indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

2. The effect of gender on mechanosensitivity

In our comprehensive analysis of the femoral neck characteristics, statistically significant exercise-induced benefits were observed in almost all measured femoral neck parameters in young males (5-19 weeks of age) after the 14-week period of treadmill training (Figure 17). In contrast, the identical exercise-regimen resulted in only minor/modest (and non-significant) effects in femoral neck characteristics of female rats. On the average, the exercise-induced bone benefits in males were 4-to 6-fold to those observed in young females after the 14 week period of treadmill training.

To ensure that the results observed in young rats (reduced mechanosensitivity in females compared to males) were not restricted only to the rapid growth period, the experiment was repeated using 33-47 wk-old adult rats. The results concerning the bones of adult rats were comparable to those observed in young rats, as the adult males showed significantly better mechanosensitivity to exercise compared to female counterparts (Figure 17).

In contrast to the data of the femoral neck, no exercise-induced benefits were observed in any of the measured femoral midshaft parameters in either gender.

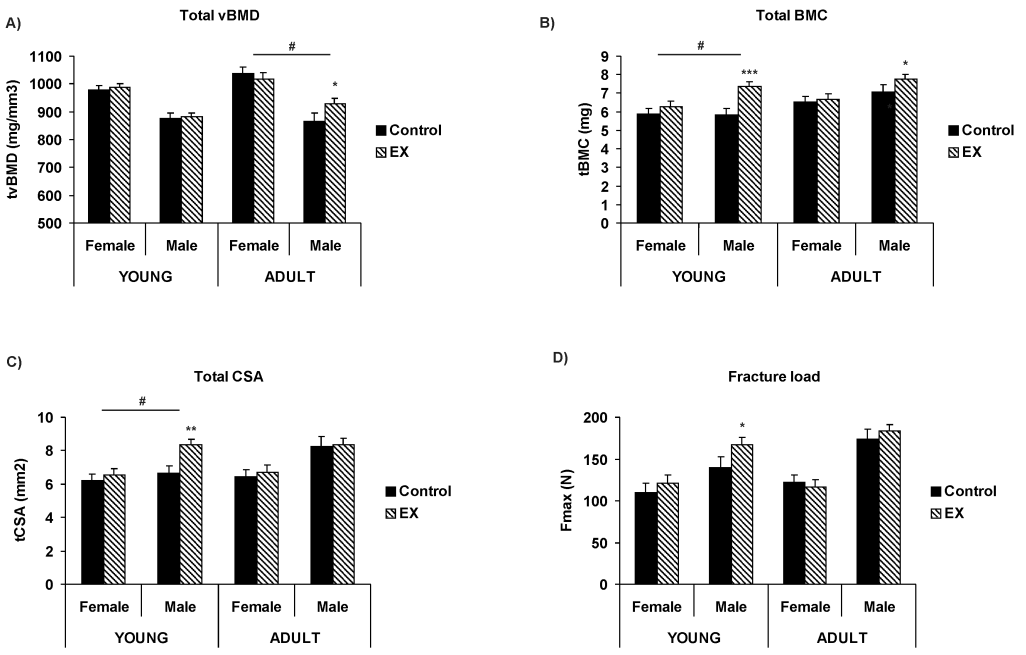

Figure 17. The effect of 14-week period of exercise on the femoral neck characteristics in young and adult female and male rats. Bars represent the mean ± standard error of mean (SEM). Significant differences are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (female/ male control vs. exercised within age group); # $p < 0.05$ sex-related difference in the response (within age group).

2. Estrogen and mechanosensitivity

The two experiments in young and adult rats (I) suggested that in comparison to males, the bones of female rats have, on one hand, a considerably higher bone mineral density, and higher bone mass and strength relative to the incident loading, but, on the other, a clearly reduced mechanosensitivity to increased loading conducted via exercise. Based on this, it was hypothesized that if estrogen did account for the observed deposition of extra stock of bone into female skeleton, then withdrawal of estrogen should not only result in a reduced bone density but also in an increased mechanosensitivity to exercise. The results in estrogen-repleted (E^+) and estrogen-depleted (E^-) female rats (I) confirmed that estrogen is actually responsible for the deposition of mechanically excess mineral into skeleton, as the removal of estrogen secretion was shown to result in decreased tvBMD, tBMC and Fmax (although statistically non-significant, $p = 0.123$) of the femoral neck in relation to muscle mass (Figure 18). More interestingly, the estrogen withdrawal in females resulted in highly comparable values to those observed in age-matched male rats. Furthermore, the E^- female rats exhibited enhanced mechanosensitivity of the femoral neck to exercise compared to E^+ counterparts (Figure 19).

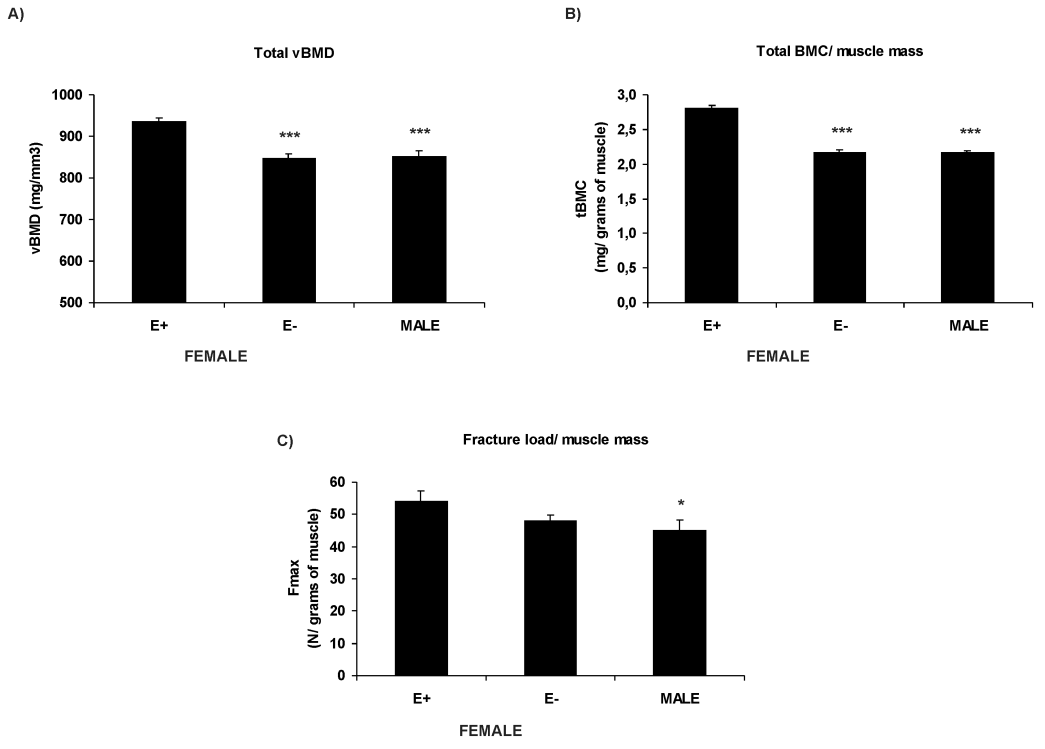

Figure 18. The total vBMD **(A)**, and muscle mass adjusted values for total BMC **(B)** and fracture load **(C)** of the femoral neck in estrogen-replete (E+), estrogen-deplete (E-) female and male rats. Bars represent the mean ± SEM. Significant differences between E+ and E-/male rats are indicated as follows: * $p < 0.05$, *** $p < 0.001$.

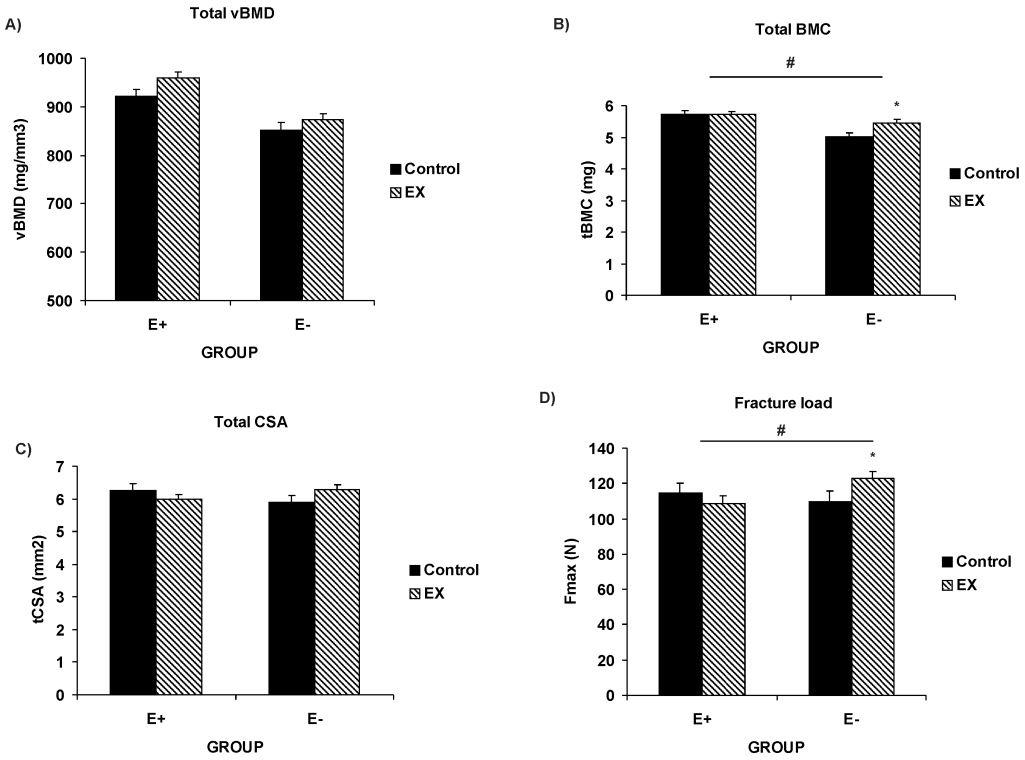

Figure 19. The effect of 16-week exercise period on the femoral neck characteristics in estrogen-repleted (E+) and estrogen-depleted (E-) female rats. Bars represent the mean ± SEM. Significant differences are indicated as follows: * $p < 0.05$ (control vs exercised within treatment group); # $p < 0.05$ (exercise effect between E+ and E-).

To further explore the possible modulatory effect of estrogen on the skeletal mechanosensitivity, 8-week period of hindlimb immobilization (removal of typical everyday loading) was executed in E^+ and E^- rats (IV). In concordance to study I, the magnitude of the loading-induced anabolic effect on the tCSA and Fmax of the femoral neck was virtually identical in the E^+ and E^- groups, indicating the absence of modulatory effect of estrogen on skeletal mechanosensitivity (Figure 20). On the contrary, while both loading and estrogen displayed significant main effects on the tBMC (again, the loading-effect being clearly more prominent than that of estrogen), a significant interaction was observed between the two factors on tBMC of the femoral neck, i.e. the loading-effect was clearly more pronounced under the influence of estrogen than without estrogen, However, considering that the estrogen-effect became significant only when the bone was loaded, not in the unloaded bone, our results actually indicated that loading is permissive to the skeletal influence of estrogen and not *vice versa* as commonly presumed today. To test the veracity of this unprecedented finding, we quantified the tBMC also in the femoral midshaft with pQCT, and perfectly in line with the findings at the femoral neck, the estrogen-effect on the bone mass of the femoral midshaft was evident only in the loaded but not in the unloaded bone.

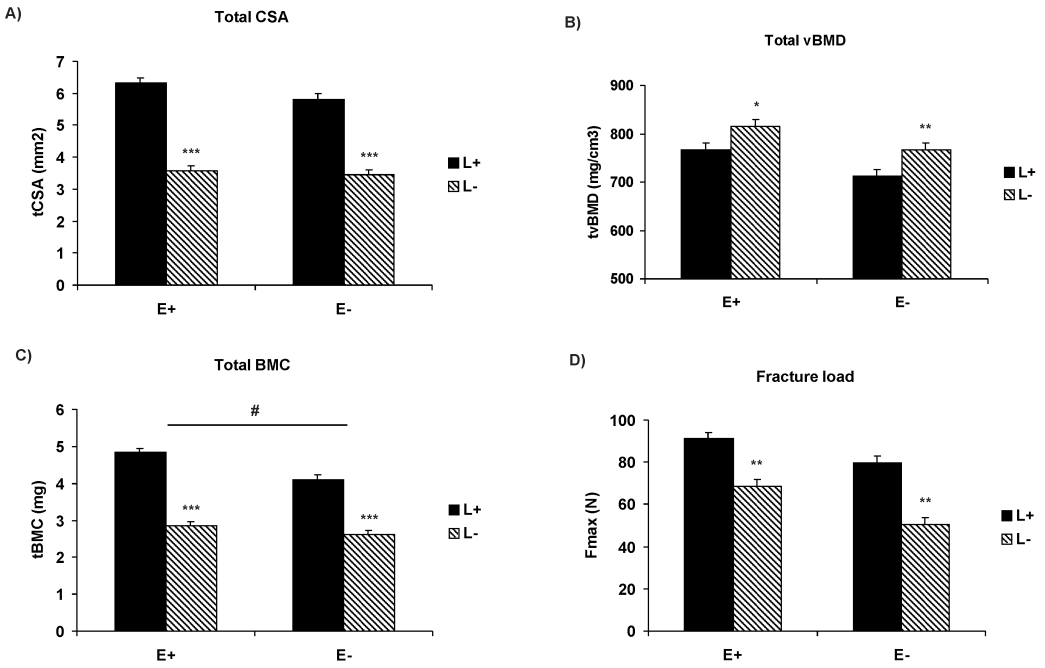

Figure 20. The effect of immobilization on the femoral neck characteristics in estrogen-replete (E+) and estrogen-deplete (E-) female rats. Bars represent the mean ± SEM. Significant differences are indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001 [unloaded (L-) vs. normally loaded (L+) limb]; # p < 0.05 (loading effect between E+ and E-).

3. Estrogen, loading and bone structure

Using the compression testing of the femoral neck, we could show that both estrogen and loading had highly significant main effects on Fmax, the magnitude of the estrogen-effect being approximately half that of loading (IV) (Figure 21). Furthermore, analysis by pQCT showed that loading had a highly significant main effect on the tCSA of femoral neck, whereas the corresponding effect of estrogen remained negligible (Figure 21).

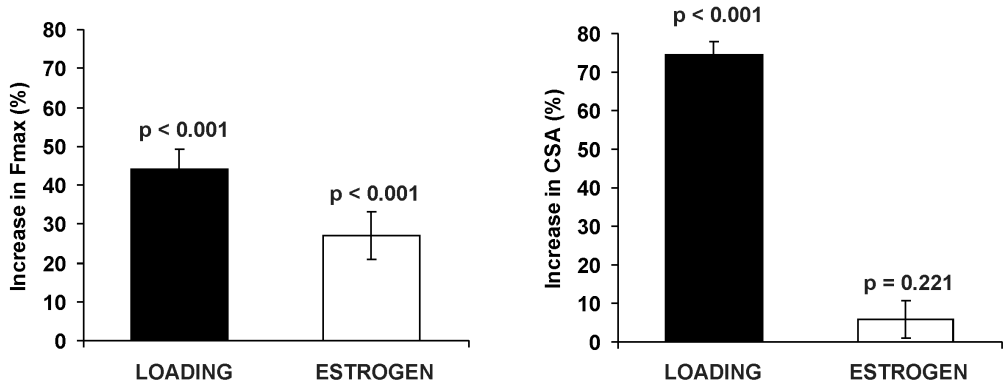

Figure 21. The independent effects of loading and estrogen on the fracture load and cross-sectional area of the femoral neck. Bars represent percent (%) difference ± SEM [L + vs. L- (black), and E+ vs. E- (white)].

Subsequent characterization of their respective effects on the structural characteristics of the femoral midshaft provided corroborative evidence that estrogen has no significant independent effect on bone geometry. However, the loading-effect was highly significant along both endosteal (resorption) and periosteal (apposition) surfaces regardless of estrogen status (Figure 22). Furthermore, the loading-effect was evident quite exclusively in the direction of principal loading direction, the mediolateral (ML) axis. Consequently, this redistribution of bone further from the neutral axis resulted in significantly increased section modulus (bending strength) of the midshaft (Z_{ML}+16%) in this primary loading direction. In the orthogonal anteroposterior (AP) direction, the loading-effects were similar, but not significant (Z_{AP} +11%). More interestingly, the estrogen-induced new bone formation (although non-significant) was found not only in the loaded bone but also explicitly in the ML axis (Figure 22). In addition, the respective influences of estrogen and loading were anatomically very distinct: the loading-induced addition of new bone was found on the periosteal surface with a concomitant removal of bone from the endocortical surface, while the estrogen-effect was manifest as an overall increase in cortical thickness.

Micro-CT-analysis of distal femoral metaphysis confirmed the previous finding that loading, but not estrogen, has a significant effect on the bone size (TV). However, both factors displayed a significant stimulatory effect on the trabecular BV and the trabecular BV/TV of the region. The mechanism of action of the two factors on the trabecular structure was shown to be very distinct: loading increased the Tb.Th and Tb.N without influencing Tb.Sp (explained by simultaneous loading-induced increase in TV), while the estrogen-effect was mediated through an increase in Tb.N without affecting Tb.Th, thus resulting in decreased Tb.Sp (as TV showed no estrogen related effect) (Figure 23). Altogether, our analysis of the distal femoral metaphysis showed that both loading and estrogen have a significant stimulatory effect on the trabecular bone, but due to their structurally distinct mechanisms of action, the effects were shown to be both completely independent (the p-value for interaction > 0.05 for all analysis) and additive in nature.

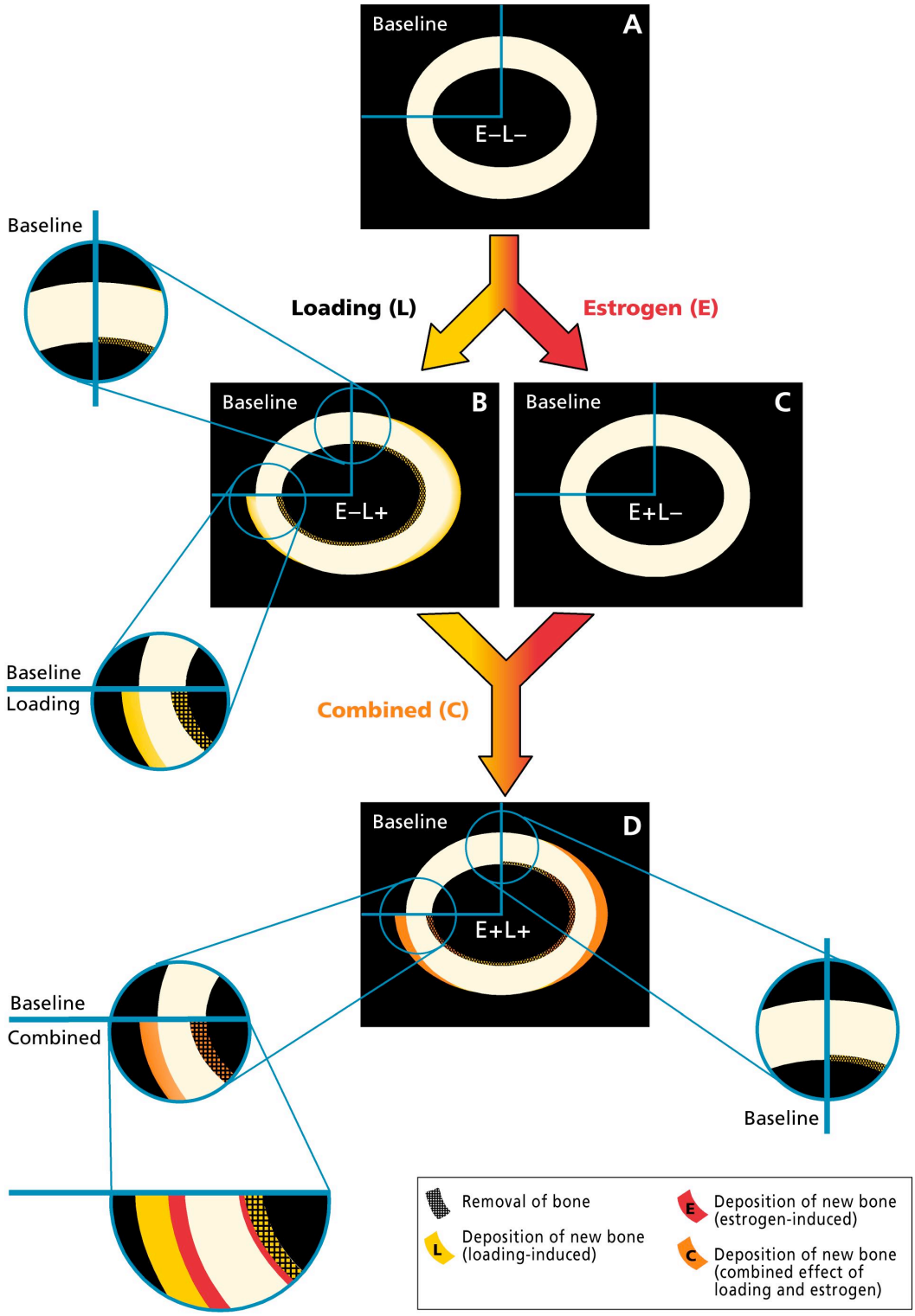

Figure 22. Schematic presentation (in scale) of the direction-specificity of the effects of mechanical loading and estrogen on the dimensions of the femoral midshaft. **(A)** The non-loaded, estrogen-deplete (E-L-) bone was used as the reference to illustrate the respective effects of loading (**B**, marked as yellow), estrogen (**C**, marked as red), and their combination (**D**, marked as orange) on the periosteal and endosteal surfaces of the femoral midshaft.

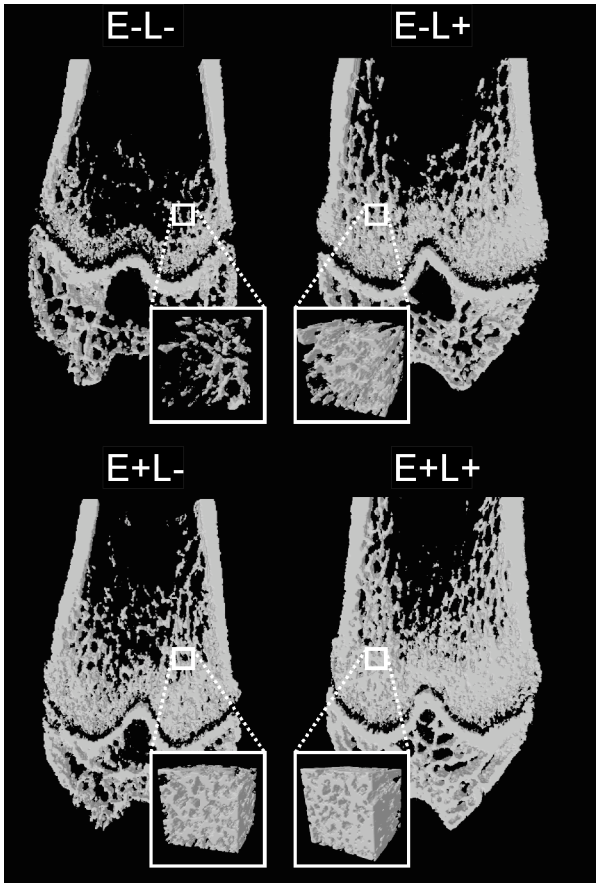

Figure 23. Effects of mechanical loading and estrogen on the trabecular bone texture in the distal femoral metaphysis.

4. Aging and mechanosensitivity

The identical 14-week period of exercise resulted in significant beneficial effects on the femoral neck characteristics in both the young and adult male rats (Figure 24). Although the responses between the two age groups were highly comparable quantitatively (tBMC and Fmax), an apparent trend for difference in mechanisms of adaptation (qualitatively) was observed: The young rats displayed striking exercise-induced increase in the tCSA while the tvBMD of the femoral neck showed only a minor increase, whereas a practically opposite response, i.e. increase in tvBMD and no change in tCSA, was observed in the adult rats. Furthermore, similarly to the unability to show any age-related difference in the skeletal mechanosensitivity to exercise, no age-related difference was seen in the ability of the bones to preserve the exercise-induced bone benefits, since the loss of bone in the young and adult rats was identical during the subsequent 14-week period of deconditioning (Figure 25).

No exercise (or deconditioning) -induced effects were observed in any of the femoral midshaft measurements in either age group.

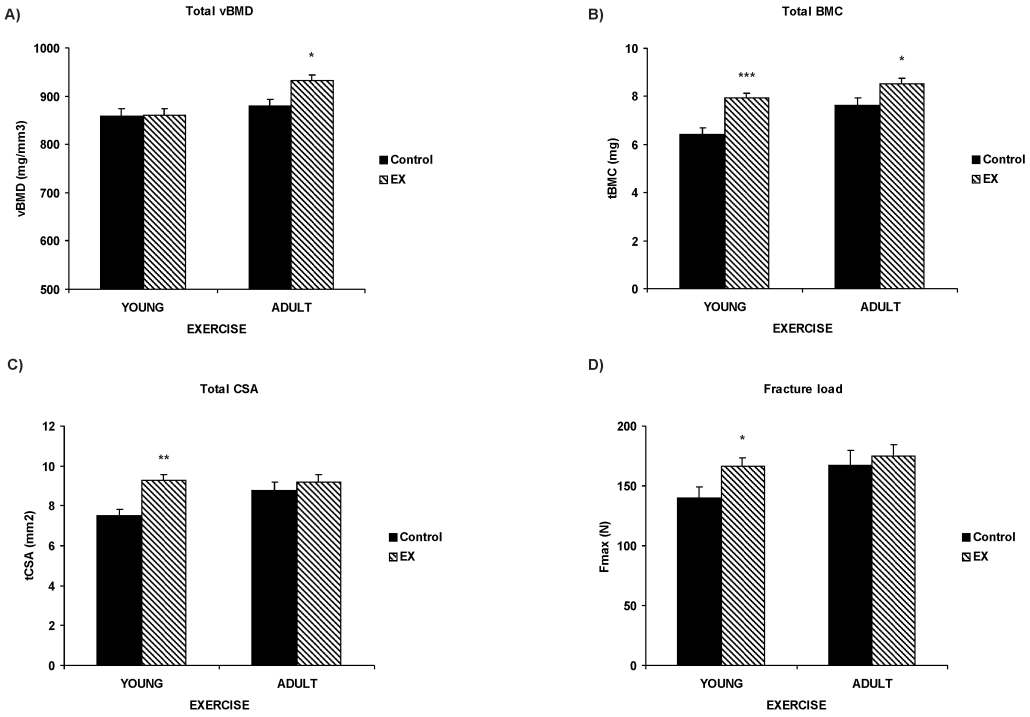

Figure 24. The effect of 14-week period of treadmill training on the femoral neck characteristics in young and adult male rats. Bars represent the mean ± SEM. Significant differences are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (control vs. exercised within age-group).

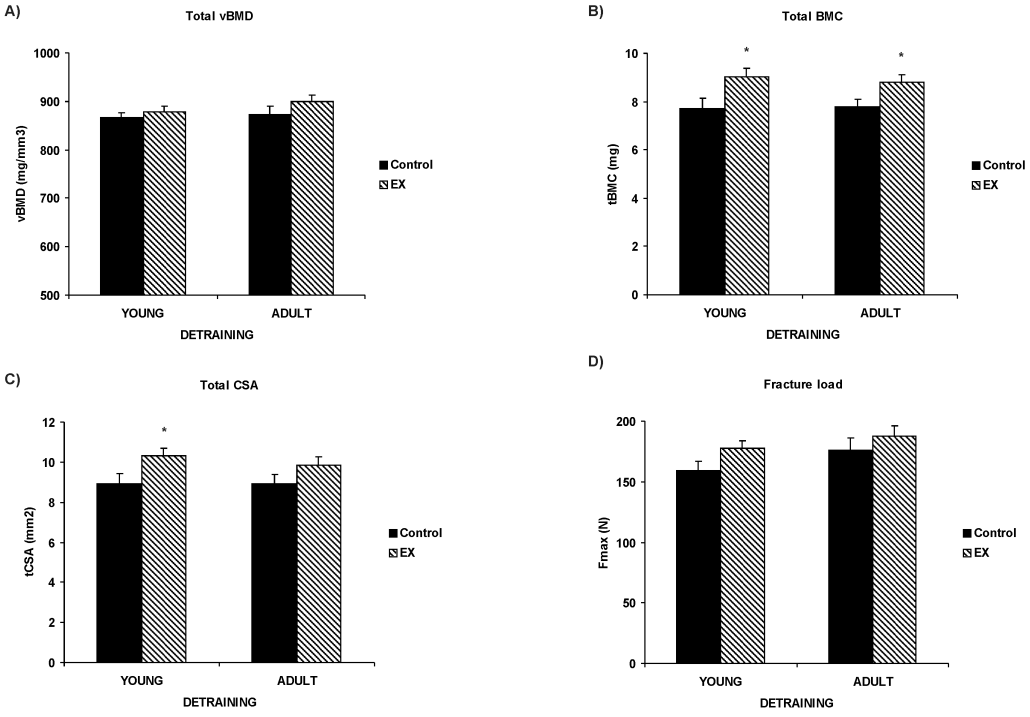

Figure 25. The 14-week deconditioning effect on the femoral neck characteristics in the previously exercised young and adult male rats. Bars represent the mean ± SEM. Significant differences are indicated as follows: * $p < 0.05$, ** $p < 0.01$ (control vs. previously exercised within age group).

5. Maintenance of the exercise-induced bone gain

The exercise-induced bone benefits obtained during the rapid growth period (5-19 weeks of age) in male rats were partially maintained during the subsequent 14-week deconditioning period: the tCSA and tBMC of the femoral necks of the previously exercised rats were still significantly higher than those in the control group. However, no significant differences were observed in any of the measured parameters of the femoral neck between the previously exercised and the control groups at either 28 or 42 weeks of deconditioning, indicating that the beneficial effects of exercise eventually disappeared (Figure 26).

Again, the femoral midshaft showed no exercise- (and consequenly deconditioning) induced effects in any of the measured parameters.

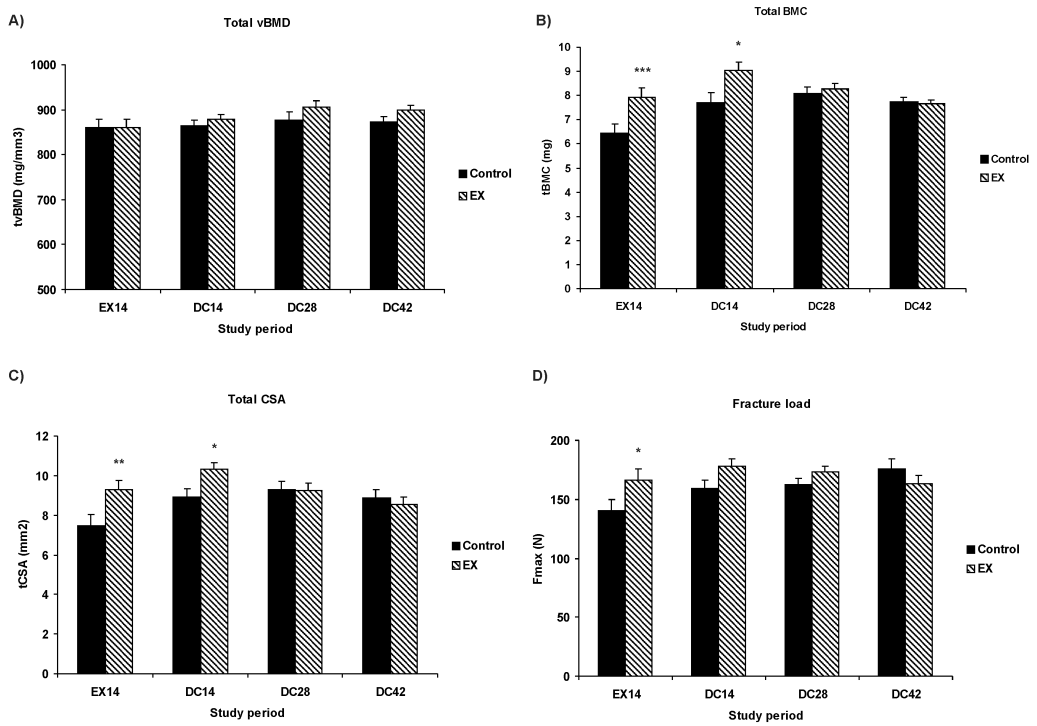

Figure 26. The maintenance of the exercise-induced bone benefits in male rats. Bars represent the mean ± SEM. Significant differences are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (control vs. exercised/ exercised + deconditioned).

DISCUSSION

The main objective of this thesis was to characterize the possible factors modulating the responsiveness of bone to changes in its loading environment (mechanosensitivity). Of the possible modulators of the mechanosensitivity of bone, estrogen has probably received the most attention, which is not surprising given its essential role as a primary origin of postmenopausal osteoporosis (Riggs et al. 1998). The current assumption is that estrogen directly enhances the mechanosensitivity of bone by lowering the modeling and remodeling thresholds of bone (Cheng et al. 1996 and 1997, Jagger et al. 1996, Westerlind et al. 1997, Turner 1999, Joldersma et al. 2001, Lanyon and Skerry 2001). However, from the evolutionary perspective, one could question, why would estrogen, a *reproductive* hormone, directly control the set-points of mechanosensory system of the skeleton? There hardly is a feedback loop that would inform any endocrine system about bone structure and its rigidity.

The results of this thesis clearly oppose the above mentioned assumption of estrogen having direct modulatory effect on the mechanosensory control system of bone (I, IV). In study I, we found the bones of female rats exhibiting a substantially lower responsiveness to increased loading (above those experienced during typical voluntary movement) conducted through exercise than male rats. Furthermore, this sex-dependent effect was shown to be independent of the age since the phenomenon was evident in both young and adult animals. In the subsequent corroborative experiment, we compared the mechanosensitivity of estrogen-deplete (E^-) and estrogen-replete (E^+) female rats and our data clearly demonstrated that the withdrawal of estrogen secretion improved the responsiveness of bone to exercise (I). Relative to body size and muscle weight (surrogates of incident loading), the bones of E^+ females were considerebly stronger and had higher bone mass than males and E^- counterparts (I). This was interpreted as a plausible explanation for the observed reduced mechanosensitivity in E^+ females compared to males and E^- females. Rather than attributing the observed reduced mechanosensitivity of estrogen-replete (E^+) animals to *direct* inhibitory effect of estrogen on the load-sensing mechanism, it seemed obvious that the effect was secondary to the estrogen-driven packing of mechanically excess mineral into female skeleton, and a consequent increase in the rigidity of bones. In other words, the already "overcondensed" female bones most likely need to respond to nothing but considerable increases in their incident loading, those clearly exceeding or differing from their customary loading (i.e. the extra stock of bone mineral damps the sensitivity of bone to adapt to changes in its loading environment).

The earliest reports supporting our finding of estrogen-driven condensation of female skeleton can be dated back some 80 years, when Sherman and

MacLeod (1925) showed that in female rats, the skeleton has significantly higher bone mass relative to the body and lean (muscle) mass than in males. These authors speculated, although did not directly couple the phenomenon to estrogen, that this extra packing of bone mineral into female skeleton was most likely an evolutionary *safety measure* against the anticipated bone loss caused by pregnancy and lactation. This finding of extra bone mineral relative to muscle mass in females has recently been corroborated by more sophisticated means in rats (DeMoss and Wright 1998, Bowman and Miller 1999, Wang et al. 2003), and finally in 1998 the seminal observations by Schiessl et al. (1998) incisively provided clinical evidence for the puberty-related faster increase of bone mass relative to muscle mass in females compared to males. On the other hand, these experimental and clinical findings suggests that if estrogen is responsible for deposition of extra stock of mineral into female skeleton at puberty, then withdrawal of estrogen secretion at menopause should result in unpacking of roughly the same amount of mineral. Indeed, the data from a study by Rico et al. (1994), in which the BMC and body composition were measured in a population of both sexes between 15 and 83 years of age, showed that the total body BMC relative to lean body-mass (muscle mass) remains higher in females compared to males the entire fertile period of human life-span. At menopause, the BMC/ lean body mass ratio begins to decline at a relatively rapid rate in females and eventually exhibits comparable value to males, thus providing a quite convincing corroborative clinical evidence for the above noted unpacking of this extra bone stock at menopause. Accordingly, the results of study I in this thesis provide a new etiological explanation for the accelerated phase of bone loss (type I osteoporosis) in women at menopause: once the female reproductive function ceases, the estrogen-driven packing of extra bone mineral to the female skeleton becomes useless, and accordingly, this mechanically excess mineral is shed from the bones. It is noteworthy that previous hypotheses concerning pathogenetic mechanism(s) of type I postmenopausal bone loss have generally presumed that female skeletal mass (bone stock) existing before menopause under normal secretion of estrogen represents an appropriate baseline (WHO 1994, Kanis and Gluer 2000). Thus, type I postmenopausal bone loss has been considered an inherent estrogen withdrawal-triggered *failure* in the delicate balance between the osteoblast and osteoclast activities that exists normally (e.g. before menopause). However, this view may overlook the potentially important role of estrogen in promoting the baseline level of mineral from which menopausal bone loss begins.

Although our results provide a rather simple evolution-based explanation for the accelerated phase of bone loss at menopause, it is acknowledged hereby that the postmenopausal osteoporosis is nowadays considered a complex event governed by multiple factors such as genetics, hormonal, nutritional and other environmental factors, and the interaction of them (Rizzoli et al. 2001, Liu et al. 2003). Especially, the genetics of osteoporosis represents one of the most active areas for research in bone biology. As most of the skeletal functions of estrogen are currently believed to be mediated by ERα, the role of ERα function and polymorphism of the ERα-gene in the development of postmenopausal osteoporosis has received wide attention in the field of bone research during the

past years. Bone biopsies from postmenopausal women have shown that the number of ERα-positive osteocytes is decreased (~50%) in estrogen-deplete women compared to estrogen-replete women (Hoyland et al. 1999). Furthermore, the results revealed cell-specific mechanisms by which ER expression is controlled in bone as osteoblasts and osteocytes showed different response in ERα number and ERα mRNA expression to estrogen withdrawal (Hoyland et al. 1999). In addition, polymorphisms of the ERα restriction enzymes *Xba*I and *Pvu*II has been suggested to exert an influence to the risk of osteoporosis and treatment response to estrogen in postmenopausal women (Ioannidis et al. 2004, Rapuri et al. 2006). The studies of ERα polymorphisms effect on BMD and fractures have so far provided inconclusive results, but in a recent meta-analysis involving over 18,000 individuals (Ioannidis et al. 2004), an association between ERα polymorphisms and fractures was observed so that in women homozygous for the absence of an *Xba*I recognition site, the adjusted odds of all fractures were reduced by 19%. More importantly, several studies have suggested that early responses of osteoblast cells to mechanical loading and estrogen share a common pathway, which involves ERα (Damien et al. 1998 and 2000, Jessop et al. 2001). Furthermore, Lee et al. (2003 and 2004) recently showed in transgenic mice that the adaptive response of bone to mechanical loading requires a functional ERα as the ulnae of mice lacking ERα showed a three-fold lower response to loading than wild-type mice with functional ERα. In addition, the ERα genotype has also been suggested to modulate the mechanosensitivity of bone to exercise (Suuriniemi et al. 2004). Thus, the current data suggest that at least part of the inter-individual differences in the risk of osteoporosis and osteoporotic fractures, response to estrogen treatment and to mechanical loading may be under genetic control.

As one may argue that the increased loading model (treadmill training) used in the experiment I does not represent a totally controlled change in the loading environment of the skeleton, we pursued on with a subsequent experiment to fully explore the proposed interaction between the skeletal effects of estrogen and locomotion (IV). By separately or simultaneously removing estrogen and mechanical loading, we confirmed the positive interaction between estrogen and mechanical loading on bone mineral accrual (BMC), a finding in accordance with the prevailing understanding on a direct enhancing effect of estrogen on the mechanosensitivity of bone. However, once extending our analysis beyond bone mass to bone geometry and particularly to the structural strength of bone - the ultimate property of bone relative to its primary locomotive function - the alleged modulatory effect of estrogen on the mechanosensitivity of bone vanished. Thus, in view of our data regarding the alleged *direct* modulatory effect of estrogen on the mechanosensitivity of bone, it was quite persuasively shown that the loading-induced anabolic effect on the bone characteristics was virtually identical regardless of the estrogen status of the animals.

This finding, the *indirect* effect of estrogen on the mechanosensitivity of bone (through the "mechanically excess" bone mineral), necessitates one to re-evaluate the whole concept of mechanosensitivity. The primary function of the mechanosensory control system of bone is to detect the alterations in its strain environment and then adjust the mechanical competence of bone through

modeling and remodeling until a new steady state is attained, i.e. the mechanical loading-induced bone adaptation returns peak strains within physiological thresholds or "customary mechanical usage range". Accordingly, the apparent goal of the mechanosensory control system is 1) to keep the mechanical competence of the bone in balance relative to the incident loads subjected on bone and 2) to keep the loading-induced deformations well below a specific safety margin in order to avoid failure of the structure (= fracture of bone).

Previous studies have generally presumed that the skeletal phenotype existing before the onset of an intervention represents an appropriate baseline, but it can be argued to be flawed. There is an apparent homology between the mechanosensory control system of a bone and thermostat controlling the temperature. To provide a clarifying example on the effect of baseline level to the observed *sensitivity* of the corresponding control system to adapt to changes, I will use an analog to thermostats controlling the temperature in two different swimming pools. At baseline, two identical thermostats (designated as A and B) are turned OFF so that temperatures of swimming pools A and B are controlled by the prevailing temperature of the surrounding environment; 10 C° and 25 C°, respectively (Figure 27A). When the desired temperature is then set to 30 C° in both swimming pools, the thermostats' heating systems are turned ON and they remain so until the desired temperature is achieved in both pools. Eventually, thermostat A increased the temperature in pool A by 20 C° and thermostat B 5 C° in pool B so that both swimming pools exhibit a same temperature of 30 C°. Although the *absolute* increase of temperature was greater in pool A (20 C° vs. 5 C° in pool B), it's not reasonable to consider thermostat A being more sensitive than thermostat B since both thermostats accommodated equally appropriately when the "target level" was adjusted to 30 C°. The key in this example is the difference in the "baseline" conditions under which the two identical thermostats worked prior to the adjustment of the new "target point" (temperature of 30 C°). In perfect agreement with this, the young and adult female rats exhibited considerably stronger bones relative to incident loading than corresponding males at baseline and thus, identical loading regimen resulted in significantly greater response in males compared to females (Figure 27B). However, all groups displayed identical bone strength as a result of the loading period, i.e. the bone adaptation to loading was equally succesful in each group.

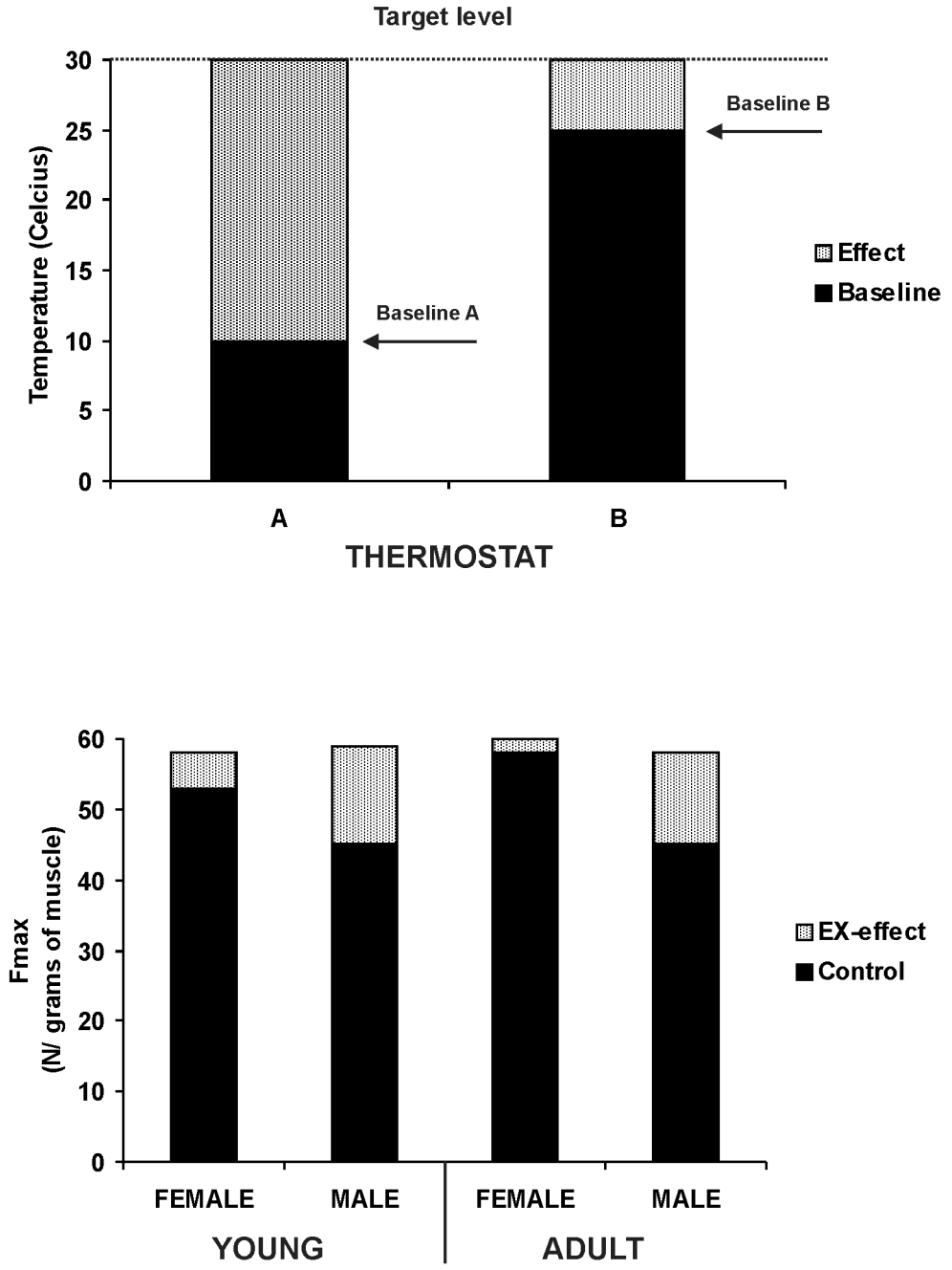

Figure 27. **(A)** An example of two thermostats controlling the temperature in swimming pools and (**B)** the effect of exercise on bone strength in young and adult rats of both genders.

Regarding the respective effects of mechanical loading and estrogen on bone geometry and mechanical competence (IV), our data shows that mechanical loading, according to its locomotive role, is the principal determinant of bone geometry and strength. In addition, the loading effect was shown to be direction-specific as loading was found to have a significant stimulatory effect on the periosteal surface in the mediolateral plane while simultaneously removing bone from the endocortical surface, and consequently through this redistribution of bone mass, significantly increasing section modulus (bending strength) of the midshaft in this apparent primary loading direction. In the orthogonal

anteroposterior direction, the loading-effects were not significant. Estrogen, in turn, as a primarily reproductive hormone was shown not to have an effect in the structural particulars of bone (i.e. bone cross-sectional geometry or strength), but rather, in the accrual of bone mass to possibly provide readily accessible calcium reservoir for reproductive purposes. Furthermore, the most striking evidence for the actions of the two factors to be completely independent and also very distinct within the bone structure was provided by our micro-CT analysis of the trabecular bone texture of the distal femoral metaphysis; mechanical loading alone resulted in thickening of individual trabeculae whereas the estrogen-effect was discernible as a denser trabecular network. Finally, when the two factors were combined, the effects were perfectly additive. The apparent functional dimorphism is in perfect agreement with a recent cell culture study showing additive effects of estrogen and mechanical stress on the promotion of paracrine factors (NO, PGE_2) by bone cells (Bakker et al. 2005). This is, to the very best of our knowledge, the first time that it has been shown that the endocrine and locomotive control of bone tissue concern distinct structural particulars.

As an attempt to extrapolate our findings regarding the effect of estrogen on mechanosensitivity of bone to clinical practise, our results implicate that the postmenopausal skeleton preserves its ability to adapt to increased loading. However, exercise has been shown to induce only marginal (~1-2%) bone mineral gains in the clinical trials evaluating the effectiveness of exercise for the prevention of bone loss in postmenopausal women (Kohrt et al. 1995 and 1997, Bérard et al. 1997). Thus, the clinical data suggest that exercise intervention in the postmenopausal women can, at best, maintain bone mineral, but rarely serves to add substantial amounts of bone de novo. In contrast, our results suggest that the postmenopausal skeleton is actually more responsive to loading than premenopausal, and women without ERT would show greater exercise-induced bone benefits compared to women using ERT. Unfortunately, current literature provides only few clinical studies (Kohrt et al. 1995 and 1998, Heikkinen et al. 1997) exploring the effect of ERT and exercise on postmenopausal skeleton. In each of these studies, the subjects with both ERT and exercise had the highest BMD (lumbar spine and proximal femur) compared with the other study groups after the completion of the intervention. Moreover, the anabolic effects of estrogen and exercise were shown to be additive in nature suggesting that these two influences on the skeleton act via different mechanisms, a finding in full concordance with the results of study IV of this thesis. However, the actual within-group *responses* to exercise (change in BMD/BMC, controls versus exercise) was not done in any of these original studies. Therefore, in our recent perspective article (Järvinen et al. 2003) we reanalyzed the data of these papers, and in concordance with the results of study I, a statistically significant BMD response was seen in the hip region of the estrogen-deplete women in all three studies, but no response in the estrogen-replete (ERT) women was found.

Our findings regarding the lack of *direct* estrogen-effect on bone geometry or periosteal apposition (IV) are of utmost importance as they challenge the prevailing view which attributes the changes occurring in the morphology of female bones at both ends of reproductive life - the smaller periosteal apposition in fertile-aged women than men after puberty (Bass et al. 1999) and the

accelerated periosteal apposition in women after menopause (Smith and Walker 1964, Ruff and Hayes 1982, Beck et al. 2000, Ahlborg et al. 2003) - to effects of the hormone *per se* (Seeman 2003). However, in the presence of mechanical loading (E^+L^+ vs. E^-L^+) the estrogen effect on bone cross-sectional geometry became visible, i.e. the estrogen-induced inhibition of periosteal apposition (IV). Our data thus provides support to the original view put forward as early as over 40 years ago by Smith and Walker (1964) for the mechanical stress being responsible for the activation of periosteal accretion of bone as an attempt to maintain its mechanical competence at menopause and during aging, even with less bone, through displacement of bone further from the neutral axis of bone, i.e. increased cross-sectional moment of inertia, section modulus and consequently, bone strength. Further evidence suggesting age-related periosteal expansion being mechanical loading-driven rather than direct estrogen deficiency-related was provided in a study by Ruff and Hayes (1982) in which regions within the same bone (femur and tibia) experiencing relatively high mechanical stress during locomotion showed the greatest increases with age in total subperiosteal area and cross-sectional moment of inertia and the smallest decreases in cortical bone area. In addition, in the study by Ahlborg et al. (2003) women experiencing greater endocortical bone loss had also greater periosteal bone formation suggesting that as bone is lost on the endocortical surface, mechanical stresses in the bone tissue are increased, thus stimulating periosteal bone formation. Likewise, in view of our data, the smaller periosteal apposition occurring in females compared to males during reproductive phase of life can be attributed to the estrogen-driven condensation of the female skeleton, as the more rigid female bones experience lower strains on the periosteal surface exerted by the equivalent level of mechanical loads and thus, not necessitating the need for the periosteum to enlarge.

In addition, the skeletal effects of estrogen appeared to be loading-dependent and direction-specific, as the estrogen-induced anabolic effect on the endocortical surface of the femoral midshaft was evident only in the loaded bones and occurring rather exclusively on surfaces experiencing principal loading, i.e. mediolateral surfaces (IV). The only plausible explanation for the observed loading-dependency and direction-specificity of the anabolic skeletal effect of estrogen we could think of is that loading somehow triggers estrogen's signal transduction pathway, at least, in some bone regions. In perfect agreement with our results, similar loading-dependency was recently shown to exist for the bone actions of parathyroid hormone (PTH) (Burr et al. 2001, Lotinun et al. 2004, Turner et al. 2006). In these studies, elevated levels of the PTH stimulated intracortical remodelling, and consequently, increased the porosity of the tibial diaphysis mostly on the endocortical surfaces. More interestingly, the PTH-effect was direction-specific, as the increase in the porosity occurred virtually exclusively in the mediolateral regions of the tibial diaphysis (Lotinun et al. 2004). Furthermore, the PTH-induced detrimental effects on the mechanical strength of the bone were later followed by an addition of new bone onto the endocortical and periosteal surfaces, an obvious loading-driven compensatory response to maintain the mechanical competence of bones. Finally, the permissive role of loading on the anabolic actions of PTH was persuasively

suggested by a very recent study (Turner et al. 2006) in which the PTH-induced anabolic effects on cortical bone structure were readily apparent in the weight-bearing bones, but absent in bones subjected to hindlimb suspension. It is also recalled here that such relationship (loading-dependent hormone-tissue effect) has already been proven between the anabolic effects androgens and muscle hypertrophy (Zachwieja 1999, Harjola et al. 2000, Joumaa et al. 2002).

In addition to estrogen, age is considered an important factor in modulating the response of bone to mechanical loading (Forwood and Burr 1993). The previous human studies have suggested that main function of mechanical loading in the adult skeleton is to conserve or maintain existing bone, as exercise interventions have been shown to result only in small bone gains of a few percent (Dalsky et al. 1988, Kannus et al. 1995, Heinonen et al. 1996, Bérard et al. 1997, Ernst 1998, Haapasalo et al. 1998, Seeman 2002). The reduced capacity of the aged skeleton to respond to changes in the loading environment has been attributed to a reduction in osteogenic potential on a cellular level with aging (Pearson and Lieberman 2004). In contrast to this view, we showed the lack of age-specificity in the responsiveness of bone to exercise since no quantitative exercise-induced differences was observed between young and adult rats (III). However, our data supports the previous notion for the aged skeleton to be less capable of responding through geometrical changes (Forwood and Burr 1993) as significant exercise-induced geometrical changes (increase in bone size) were observed in young rats whereas the adult skeleton showed no exercise-induced effect on bone size. In turn, adult skeleton displayed marked increase in bone density providing further evidence for the assumption that additional bone acquired after skeletal maturity is deposited along the existing bone structures (Kannus et al. 1995, Kontulainen et al. 2002). Furthermore, age did not seem to modulate the ability of the skeleton to maintain the exercise-induced bone benefits, since no difference was observed in the loss of bone in the growing and adult rats after cessation of the exercise (deconditioning).

Prompted by the suggestions that the exercise-induced structural changes obtained during the skeletal growth period could be, at least, partially maintained even if the exercise is decreased or completely ceased (Kontulainen et al. 1999 and 2001), we extended the deconditioning period of the previously exercised young rats to rather old age (61 weeks) (II). Although exercise through the period of the fastest skeletal growth resulted in significant improvements in size, mineral mass, and strength of the femoral neck, a deconditioning-induced gradual disappearance of the exercise-induced positive effects on the femoral neck characteristics was observed. Thus, our results indicate that continued training is probably needed to maintain the positive effects of youth exercise into adulthood and further studies should focus on assessing the minimal level of activity needed to maintain the exercise-induced bone gains.

There are several factors that may contribute to the apparent controversy between the results of this thesis and previous studies. Regarding the human studies, prospective human studies possess many confounding factors such as differences in study designs (exercise protocols, etc.), poor compliance, small sample sizes, failure to control other bone-affecting factors (e.g. nutrition) and failure to document the possible intervention-induced geometrical bone changes

(e.g. bone mass distribution) with current non-invasive measuring techniques (DXA-derived BMC/aBMD). Furthermore, in evaluating the age-spesificity of bone sensitivity to mechanical loading, it should be acknowledged that skeletal response to increased loading is probably slower in adult skeleton compared to growing skeleton. Thus, long-term longitudinal studies may be necessary to document the possible exercise-induced effects in adults.

The greatest attributable factor to the controversy between the results of this thesis and previous studies is probably the fact that previous studies have virtually exclusively focused on surrogates or determinants of whole-bone strength (e.g. cellular activities, histomorphometric parameters, bone mineral status alone), neglecting to actually test the mechanical competence of the bones. However, it should be recalled here that although these more detailed parameters can provide an important insight into the underlying adaptation mechanisms of bone to mechanical loading and may ultimately (not necessarily) lead to altered whole bone strength, conclusions regarding bone's mechanical function based solely on these surrogates or determinants of whole bone strength are inappropriate and likely misleading (van der Meulen 2001). For example, if we had chosen BMC as our main outcome parameter in the study IV, the conclusion of the study would have been totally opposite i.e. estrogen increases the sensitivity of bones to mechanical loading. Furthermore, recent studies have strongly suggested that bones prefer geometric adaptation over changes in BMC and/or BMD (the two most commonly used outcome parameters in skeletal research) as a means to cope with changes occurring in their functional environment (Järvinen et al. 1998b, Järvinen et al. 1999, Robling et al. 2002). In addition, extrapolation of *in vitro* data to tissue-organ level phenomenon has to be made with caution, as the *in vivo* reality tends to be far more complex when the responses are also modified by local and/or systemic factors not present *in vitro*. For example, despite the rather persuasive previous evidence suggesting an inverse relationship between donor age and proliferative potential of fibroblasts, a relatively recent study clearly shows that if health status and biopsy conditions are controlled, the replicative lifespan of fibroblasts in culture does not correlate with donor age (Cristofalo et al. 1998). Regarding bones, if we do not know whether the bone as an organ has truly strengthened, we have no certainty of knowing whether a change in any of the intermediate or surrogate measures of bone strength denote only a transient phenomenon – like a "snap-shot" of a dynamic movement eventually fading away - or actually a strengthened bone structure as a response to the stimulus of interest.

In this context, it is also noteworthy that the adaptive response of whole bone architecture to mechanical loading is very complex (Ruff et al. 2006). The correspondence between bone strain patterns and bone structure is variable, depending on the skeletal location and the general mechanical environment (e.g. distal vs. proximal limb elements, mediolateral vs. anteroposterior bone surfaces within the bone region) (Hsieh et al. 2001, Robling et al. 2002). This creates an issue that requires consideration as we chose not to characterize the definitive strain environment and its relation to structural adaptation of bone to loading in our studies employing increased loading model (treadmill training) (I-III). The only possible method for measuring strains and applying loading-induced strains

of certain level in certain direction on bone is the use of strain gauging and controlled (isolated) external loading models (Goodship et al 1979, Lanyon et al. 1982, O'Connor and Lanyon 1982, Rubin and Lanyon 1984, Mosley and Lanyon 1998, Hsieh et al. 2001, Robling et al. 2002). However, in view of our results in study IV and the very recent study (Leppänen et al. 2006), in which the physiological loading-induced new bone formation was found almost exclusively in the apparent direction of principal loading in the femoral midshaft (the mediolateral axis), one could challenge the observed relationship between artificially applied external loading and structural adaptation of bone in strain gauging studies. Although definitive measure of strain in certain direction of bone cross-section can be applied by using isolated external loading, the bone's response to loading is totally dependent on the direction of applied loads. For example, the loading-induced structural changes are observed primarily in the plane of least bending rigidity (I_{min}) in bone cross-section in the studies using isolated loading model of rat ulna (Hsieh et al. 2001, Robling et al. 2002). Accordingly it can be argued that the isolated external loading model of a rat ulna induces greatest strains in a direction which the bones are not accustomed to during the normal physiological loading (I_{max} plane) and thus, these controlled external loading models do not represent an appropriate model to simulate the loading environment bones experience in nature. This pinpoints the methodological strength of using unloading (study IV) as a model determining the independent and possible interactive influence of loading and other factors (e.g. hormones) on structural adaptation of bone, as no strains are induced on unloaded bones while the contralateral bones are subjected to normal physiological loading. Thus, we have replaced the increased loading model with unloading (neurectomy) in future studies in an attempt to assess the loading-hormone interactions.

The greatest limitation of our study can be attributed to the use of a rat as the experimental animal. In the 1970s and 1980s, rats were considered unsuitable for skeletal research because of the continuous growth of the skeleton throughout the life and because of not having secondary Haversian remodelling (intracortical remodelling) in the cortical bone. Actually some of these arguments may be flawed as it has been shown that the linear growth of the female Sprague-Dawley rat becomes trivial after 12 months of age and they have been shown to even cease bone elongation at 18 months of age (Kimmel 1991, Li et al. 1991). However, in male rats, anatomically identical growth cartilages close later than in females and many growth cartilages do not close before the age of 30 months (Dawson 1925, Spark and Dawson 1928, Joss et al. 1963). Furthermore, regarding the previous findings that rats do not exhibit much intracortical remodeling in normal conditions, the recent observations actually suggest that if rats of sufficient age are used, they actually display intracortical porosity (WSS Jee, personal communication). Despite these suggested disadvantages, the similarities in rats and humans in the principal biological mechanisms controlling bone mass gains (longitudinal bone growth and modeling drifts) and losses (BMU-based remodeling), as well as in responses to mechanical loading, hormones, drugs and other agents, have made rat the most widely used experimental animal in skeletal research (Frost and Jee 1992).

Nevertheless, despite the above noted similarities in the control of bone homeostasis in rats and humans, the problem attributable to the continued (although slowing) growth of (male) rat skeleton and body weight through virtually the entire lifespan of the animal is present in all study designs using young growing rats, especially when employing long-term prospective studies (study II in the present series). The situation is basically further complicated in exercise studies of male rats, as the exercise-induced weight-loss (as opposed to weight-gain in females) is a well-documented phenomenon in males (Pitts 1984, Cortright et al. 1997). This exercise-related retardation of body weight gain in male rats (~10%) was also apparent in the present study series (I-III) as the control rats were significantly heavier compared to the corresponding exercised rats after the exercise periods. To eliminate this imminent bias introduced by variation in body weights of the animals between the study groups, we adjusted all the data pertaining to mechanical competence of the rat femora by including the muscle weight as a covariate, i.e. the bones were made comparable with each other in terms of their natural loading, the primary regulator of size, shape and geometry of bone (Burr 1997, Frost 1997). Analogically, it would be unreasonable to directly compare the bones of a 40-kg female gymnast to those of a 75-kg woman living contemporary life without taking into account the different functional environments the bones act upon (i.e. predominant skeletal loads produced by the involved skeletal muscles). Naturally, the gymnast's bones would have lower absolute mass (BMC), smaller size, and probably but not necessarily, lower mechanical strength than those of the much heavier woman. However, considering these bones within their appropriate locomotive scope, the superiority of the gymnast's bones becomes readily apparent.

The finding of direction-specific effect of loading on the femoral midshaft (mediolateral axis) (IV) reveals apparent limitations of our current methods for mechanically testing the bone samples and may, at least partly, explain the inability to show exercise-induced effect on the mechanical strength of femoral midshaft in studies I-III since the mechanical testing was executed, according to current concept, in the anteroposterior direction. This observation similarly challenges the appropriateness of using vertical loading (directed parallel to the axis of femoral shaft) in measuring the femoral neck strength as in a rat (as a quadrupedal animal) the support of weight is on a flexed hip joint so that the load applied is probably at some 45° to the femoral shaft. In order to mechanically test the femoral neck in a more appropriate direction, the natural loading direction of the femoral neck should be assessed. Unfortunately, we were not able to perform this as the resolution of the pQCT machine used in our studies sets a limitation to the precise structural characterization of small skeletal regions as the rat femoral neck. However, it should be noted that significant exercise/loading-induced benefits were observed in the mechanical strength of femoral neck in each study and more importantly, the strength results were consistent with other measured femoral neck parameters. Thus, it appears that the current method for mechanically testing the femoral neck of a rat is a useful test in assessing the potential exercise/loading-induced effects. However, there seems to be an urgent need to re-evaluate the appropriateness of the current methodology in structural testing of bone samples and the importance of

considering the natural loading direction of bone is emphasized hereby when structural testing is performed in future studies. Indeed, prompted by the results of study IV, we have very recently introduced a new protocol for testing the rat femoral midshaft in the primary loading direction, i.e. mediolateral direction (Leppänen et al. 2006).

SUMMARY AND CONCLUSIONS

The primary findings and conclusions of the present series of studies are summarised as follows:

I. The results of study I showed that there was a sex-specific and age-independent difference in the mechanosensitivity of bone, the female rats exhibiting a clearly lower responsiveness to exercise than male rats. Removal of normal secretion of estrogen resulted in enhanced mechanosensitivity of bone to exercise compared to estrogen-replete female rats, suggesting that estrogen decreases the mechanosensitivity of bone. However, relative to the mechanical demands placed on the skeleton, the bones of the estrogen-replete female rats were considerably denser than those of the estrogen-deplete counterparts and males, thus indicating that rather than directly taking part in the mechanical loading-dependent control of bone integrity, estrogen has a distinct role of depositing mechanically excess mineral to the growing female bones in puberty, most likely to act as a calcium storage for the development of the fetal skeleton and milk production for breast-feeding. This estrogen-driven extra condensation of female skeleton consequently increases the rigidity of bone structure and indirectly damps the responsiveness of the female skeleton to exercise.

II In study II, it was shown that exercise through the entire period of rapid skeletal growth resulted in significant improvements in size, mineral mass, and strength of the femoral neck of growing male rats. However, these exercise-induced bone benefits were eventually lost when exercise was completely ceased, and thus continued training is probably needed to maintain the positive effects of youth exercise into adulthood.

III. The comprehensive analysis of femoral midshaft and neck showed that there was no quantitative differences in the responsiveness of bone to exercise in young and adult rats indicating that aging is not related to reduction in the mechanosensitivity of bone (study III). However, an apparent trend for different mechanisms of adaptation to exercise was observed so that the bones of the young rats mainly adapted through geometrical changes (increase in bone size) whereas adult rats seemed to adapt mainly through increase in apparent bone density. Likewise, the ability of the bone to preserve

the exercise-induced bone benefits did not seem to be related to age, since the loss of bone in the young and adult rats was identical after cessation of exercise.

IV. The results of study IV indicate that mechanical loading, according to its locomotive role, is the principal determinant of bone geometry and strength. The loading effect was shown to be direction-specific as loading was found to have a significant stimulatory effect on the bone surfaces in the primary loading direction. Estrogen, in turn, as a primarily reproductive hormone was shown not to have its primary target in the structural particulars of bone (i.e., bone cross-sectional geometry or strength), but rather, in accrual of bone mass to possibly provide readily accessible calcium reservoir for reproductive purposes. Furthermore, the skeletal actions of mechanical loading and estrogen were shown to be completely independent and also very distinct within the bone structure.

ACKNOWLEDGMENTS

This study was carried out at the Department of Surgery, Medical School, University of Tampere, and at the UKK Institute, Tampere, Finland, from 1999 to 2006.

First of all, I wish to thank Professor Markku Järvinen, M. D., Professor and Head of the Department of Surgery, University of Tampere, for providing me with the best facilities to work in the field of bone research.

My sincere gratitude is extended to my two supervisors, Docent Teppo Järvinen, M. D., and Docent Harri Sievänen, D. Sc, for their excellent supervision, guidance and support during these years. I am especially indebted to Teppo for recruiting me to your research group, and for your everlasting enthusiasm towards the research, innovative way of thinking, and your unbelievable persistency and patience during the several years it took me to finish this thesis. I am also very grateful for your friendship and all the unforgettable moments I had the priviledge in sharing with you during these years. Most of all, it's been fun to work with you! I will also like to express my warmest gratitude to docent Sievänen especially for your astonishing capability to interpret the results and overall understanding in the field of bone research.

I sincerely appreciate the contribution of all the co-authors of the original studies. My special thanks goes to Professor Pekka Kannus for your excellent comments, guidance and contribution regarding the original studies. I also wish to express my warmest thanks to Tuomo Vuohelainen, M. D., for your assistance in conducting the experiments and especially for your friendship and all the fun we've had. The assistance in pQCT measurements of Docent Juha Tuukkanen, D. D. S., Ph. D., Department of Anatomy, University of Oulu, is also acknowledged. The excellent technical assistance of Mrs. Elina Selkälä and Mrs Minna Vanhala is also ackowledged.

The rest of the "crüe", Jarkko Jokihaara, M. D., and Olli Leppänen, B. M., are sincerely thanked for your help, support, friendship and fun we've had during these years (and undoubtedly will have).

My sincere thanks goes to the official reviewers of this thesis, Professor Tim Skerry, Ph. D., University of Sheffield, UK, and Professor Petri Lehenkari, M. D., Ph. D., University of Oulu, for their valuable comments and suggestions for the manuscript of the thesis. I am especially grateful for their speed to complete the review process.

I also wish to express my warmful thanks to all my friends, especially to Jani, Jukka and Timo for your everlasting friendship, support and for all the unforgettable moments we've shared in the past, and will share!

I devote my cordial thanks to my dear parents Jorma and Pirjo for your unconditional support in my life. Your encouragement made it possible for me to

complete this project. My dear sisters, Nelli and Tytti, are sincerely thanked for your very important background support.

Finally, I owe my deepest gratitude to my wife Paula and my lovely children, Elias and Siiri. Paula has unselfishly sacrificed her own interest over those of mine and provided me invaluable support during the course of this study, and in my life in general. Elias and Siiri, you are the joy of my life and have an amazing ability to brighten up my days despite occasional tiredness or sorrow I may have. Love you all!

This study was financially supported by the Medical Research Fund of Tampere University Hospital, National Graduate School for Musculoskeletal Diseases, The Pirkanmaa Regional Fund of the Finnish Cultural Foundation, Ministry of Education, and the AO Research Fund, Switzerland.

REFERENCES

Ahlborg HG, Johnell O, Turner CH, Rannevik G and Karlsson MK (2003): Bone loss and bone size after menopause. N Engl J Med 349:327-334.

Albright F, Bloomberg E and Smith PH (1940): Postmenopausal osteoporosis. Trans Assoc Am Phys 55:298-305.

Albright F, Smith PH and Richardson AM (1941): Postmenopausal osteoporosis: its clinical features. JAMA 116:2465-2474.

Bakker AD, Klein-Nulend J, Tanck E, Albers GH, Lips P and Burger EH (2005): Additive effects of estrogen and mechanical stress on nitric oxide and prostaglandin E2 production by bone cells from osteoporotic donors. Osteoporos Int 16:983-989.

Baron R, Neff L, Tran Van P, Nefussi JR and Vignery A (1986): Kinetic and cytochemical identification of osteoclast precursors and their differentiation into multinucleated osteoclasts. Am J Pathol 122:363-378.

Bass S, Pearce G, Bradney M, Hendrich E, Delmas PD, Harding A and Seeman E (1998): Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. J Bone Miner Res 13:500-507.

Bass S, Delmas PD, Pearce G, Hendrich E, Tabensky A and Seeman E (1999): The differing tempo of growth in bone size, mass and density in girls is region specific. J Clin Invest 104:795-804.

Beck TJ, Looker AC, Ruff CB, Sievanen H and Wahner HW (2000): Structural trends in the aging femoral neck and proximal shaft: analysis of NHANES III DXA data. J Bone Miner Res 15:2297–2304.

Beck TJ, Stone KL, Oreskovic TL, Hochberg MC, Nevitt MC, Genant HK and Cummings SR (2001): Effects of current and discontinued estrogen replacement therapy on hip structural geometry: the study of osteoporotic fractures. J Bone Miner Res 16:2103-2110.

Bell KL, Loveridge N, Jordan GR, Power J, Constant CR and Reeve J (2000): A novel mechanism for induction of increased cortical porosity in cases of intracapsular hip fracture. Bone 27:297-304.

Bérard A, Bravo G and Gauthier P (1997): Meta-analysis of the effectiveness of physical activity for the prevention of bone loss in postmenopausal women. Osteoporos Int 7: 331-337.

Bikle DD, Harris J, Halloran BP and Morey-Holton ER (1994): Skeletal unloading induces resistance to insulin-like growth factor I. J Bone Miner Res 9:1789-1796.

Bilezikian JP, Morishima A, Bell J and Grumbach MM (1998): Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 339:599–603.

Boppart MD, Kimmel DB, Yee JA and Cullen DM (1998): Time course of osteoblast appearance after in vivo mechanical loading. Bone 23:409-415.

Boskey AL, van der Meulen MC and Wright TM (2003): Guidelines for describing mouse skeletal phenotype. J Orthop Res 21:1-5.

Bouvier M (1985): Application of in vivo bone strain measurement techniques to problems of skeletal adaptations. Yrbk Phys Anthropol 28:237-247.

Bouxsein ML, Myburgh KH, van der Meulen MC, Lindenberger E and Marcus R (1994): Age-related differences in cross-sectional geometry of the forearm bones in healthy women. Calcif Tissue Int 54:113-118.

Bowman BM and Miller SC (1999): Skeletal mass, chemistry, and growth during and after multiple reproductive cycles in the rat. Bone 25:553–559.

Braidman I, Baris C, Wood L, Selby P, Adams J, Freemont A and Hoyland J (2000): Preliminary evidence for impaired estrogen receptor-α protein expression in osteoblasts and osteocytes from men with idiopathic osteoporosis. Bone 26:423-427.

Brockstedt H, Kassem M, Eriksen EF, Mosekilde L and Melson F (1993): Age- and sex-related changes in iliac cortical bone mass and remodeling. Bone 14:681-691.

Buckwalter JA, Glimcher MJ, Cooper RR and Recker R (1995): Bone biology. Part I. Structure, blood supply, cells, matrix, and mineralization. J Bone Joint Surg 77-A:1256-1275.

Buhl KM, Jacobs CR, Turner RT, Evans GL, Farrell PA and Donahue HJ (2001): Aged bone displays an increased responsiveness to low-intensity resistance exercise. J Appl Physiol 90:1359-1364.

Burr DB (1997): Muscle strength, bone mass, and age-related bone loss. Commentary. J Bone Miner Res 12:1547-1551.

Burr DB, Hirano T, Turner CH, Hotchkiss C, Brommage R and Hock JM (2001): Intermittently administered human parathyroid hormone (1-34) treatment increases intracortical bone turnover and porosity without reducing bone strength in the humerus of ovariectomized cynomolgus monkeys. J Bone Miner Res 16:157-165.

Cameron N, Tanner JM and Whitehouse RH (1982): A longitudinal analysis of the growth of limb segments in adolescence. Ann Human Biol 9:211-220.

Canalis E (1983): The hormonal and local regulation of bone formation. Endocr Rev 4:62-77.

Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS and Simpson ER (1997): Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 337:91–95.

Chakkalakal DA (1989): Mechanoelectric transduction in bone. J Mater Res 4:1034-1036.

Cheng MZ, Zaman G, Rawlinson SCF, Suswillo RFL and Lanyon LE (1996): Mechanical loading and sex hormone interactions in organ cultures of rat ulna. J Bone Miner Res 11:502-511.

Cheng MZ, Zaman G, Rawlinson SCF, Pitsillides AA, Suswillo RFL and Lanyon LE (1997): Enhancement by sex hormones of the osteoregulatory effects of mechanical loading and prostaglandins in explants of rat ulnae. J Bone Miner Res 12:1424-1430.

Compston JE (2001): Sex steroids and bone. Physiol Rev 81:419-447.

Cortright RN, Chandler MP, Lemon PW and DiCarlo SE (1997): Daily exercise reduces fat, protein and body mass in male but not female rats. Physiol Behav 62:105-111.

Cowin SC, Moss-Salentijn L and Moss ML (1991): Candidates for the mechanosensory system in bone. J Biomech Eng 113:191-197.

Cristofalo VC, Allen RG, Pignolo RJ, Martin BG and Beck JC (1998): Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proc Natl Acad Sci 95:10614-10619.

Currey JD (1984): The mechanical adaptations of bones. Princeton University Press, Princeton.

Currey JD (2001): Bone strength: what are we trying to measure? Calcif Tissue Int 68:205-210.

Dalsky GP, Stocke KS, Ehsani AA, Slatopolsky E, Lee WC and Birge SJ Jr (1988): Weight-bearing exercise training and lumbar bone mineral content in postmenopausal women. Ann Intern Med 108:824-828.

Damien E, Price JS and Lanyon LE (1998): The estrogen receptor's involvement in osteoblasts' adaptive response to mechanical strain. J Bone Miner Res 13:1275-1282.

Damien E, Price JS and Lanyon LE (2000): Mechanical strain stimulates osteoblast proliferation through the estrogen receptor in males as well as females. J Bone Miner Res 15:2169-2177.

Dawson AB (1925): The age order of epiphyseal union in the long bones of the albino rat. Anat Rec 31:1-17.

DeCherney A (1993): Physiologic and pharmacologic effects of estrogen and progestins on bone. J Reprod Med 38 (suppl.):S1007-S1014.

Dehority W, Halloran BP, Bikle DD, Curren T, Kostenuik PJ, Wronski TJ, Shen Y, Rabkin B, Abderrahman B and Morey-Holton E (1999): Bone and hormonal changes induced by skeletal unloading in the mature male rat. Am J Physiol 276:E62-E69.

DeMoss DL and Wright GL (1998): Sex and strain differences in whole skeletal development in the rat. Calcif Tissue Int 62:153-157.

Einhorn TA (1992): Bone strength: the bottom line. Calcif Tissue Int 51:333-339.

Einhorn TA (1996): The bone organ system: Form and function. In: Osteoporosis, pp 3-22. Eds. R Marcus, D Feldman and J Kelsey, Academic Press, New York.

Emans SJ, Grace E, Hoffer FA, Gundberg C, Ravnikar V and Woods ER (1990): Estrogen deficiency in adolescents and young adults: impact on bone mineral content and effects of estrogen replacement therapy. Obstet Gynecol 76:585-592.

Eriksen EF, Colvard DS, Berg NJ, Graham ML, Mann KG, Spelsberg TC and Riggs BL (1988): Evidence of estrogen receptors in normal human osteoblast-like cells. Science 241:84-86.

Ernst E (1998): Exercise for female osteoporosis: a systematic review of randomised clinical trials. Sports Med 25: 359-368.

Faulkner KG, Glüer C-C, Majumdar S, Lang P, Engelke K and Genant HK (1991): Noninvasive measurement of bone mass, structure, and strength: current methods and experimental techniques. Am J Roentgenol 157:1229-1237.

Feik SA, Thomas CD, Bruns R and Clement JG (2000): Regional variations in cortical modeling in the femoral mid-shaft: sex and age differences. Am J Phys Anthropol 112:191–205.

Forwood MR and Burr DB (1993): Physical activity and bone mass: exercises in futility? Bone Miner 21:89-112.

Frost HM (1961): Postmenopausal osteoporosis: a disturbance in osteoclasia. J Am Geriatr Soc 9:1078-1085.

Frost HM (1987a): Bone "mass" and the "mechanostat": a proposal. Anat Rec 219:1-9.

Frost HM (1987b): The mechanostat: a proposed pathogenetic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone Miner 2:73-85.

Frost HM (1990a): Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the modeling problem. Anat Rec 226:403-413.

Frost HM (1990b): Skeletal structural adaptations to mechanical usage (SATMU): 2. Redefining Wolff's law: the remodeling problem. Anat Rec 226:414-422.

Frost HM (1992): The role of changes in mechanical usage set points in the pathogenesis of osteoporosis. J Bone Miner Res 7:253-261.

Frost HM (1997): On our age-related bone loss: Insights from a new paradigm. Perspective. J Bone Miner Res 12:1539-1546.

Frost HM (2003): Bone's mechanostat: a 2003 update. Anat Rec 275A:1081-1101.

Frost HM and Jee WSS (1992): On the rat model of human osteopenias and osteoporoses. Bone Miner 18:227-236.

Garn S (1970): The earlier gain and later loss of cortical bone. In: Nutritional perspectives, pp. 3-120. Ed. CC Thomas, Springfield.

Garn SM, Rohmann CG and Bloomenthal P (1966): Ossification sequence polymorphism and sexual dimorphism in skeletal development. Am J Phys Anthropol 24:101-115.

Goodship AE, Lanyon LE ans McFie H (1979): Functional adaptation of bone to increased stress. J Bone Joint Surg 61-A:263-276.

Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P and Chambon P (1986): Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature 320:134-139.

Gustavsson A, Olsson T and Nordström P (2003): Rapid loss of bone mineral density of the femoral neck after cessation of ice hockey training: a 6-year longitudinal study in males. J Bone Miner Res 18:1964-1969.

Haapasalo H, Kannus P, Sievänen H, Pasanen M, Uusi-Rasi K, Heinonen A, Oja P and Vuori I (1998): Effetc of long-term unilateral activity on bone mineral density of female junior tennis players. J Bone Mner Res 13:310-319.

Haapasalo H, Kontulainen S, Sievänen H, Kannus P, Järvinen M and Vuori I (2000): Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. Bone 27:351-357.

Halloran BP, Bikle DD, Harris J, Autry CP, Currier PA, Tanner S, Patterson-Buckendahl P and Morey-Holton E (1995): Skeletal unloading induces selective resistance to the anabolic actions of growth hormone on bone. J Bone Miner Res 10:1168-1176.

Han Y, Cowin SC, Schaffler MB and Weinbaum S (2004): Mechanotransduction and strain amplification in osteocyte cell processes. Proc Natl Acad Sci USA 101: 16689-16694.

Hansson LI, Menander-Sellman K, Stenström A and Thongren KG (1972): Rate of normal longitudinal bone growth in the rat. Calcif Tissue Res 10:238-251.

Harjola VP, Jankala H and Harkonen M (2000): The effect of androgen status on skeletal muscle myosin heavy chain mRNA and protein levels in rats recovering from immobilization. Eur J Appl Physiol 83:427-433.

Heaney RP (1965): A unified concept of osteoporosis. Am J Med 39:377-380.

Heaney RP (1969): A unified concept of osteoporosis: A second look. In: Osteoporosis, pp. 257-265. Ed. US Barzel, Grune and Stratton, New York.

Heaney RP (1974): Pathophysiology of osteoporosis: implications for treatment. Tex Med 70:37-45.

Heaney RP and Whedon GD (1958): Radiocalcium studies of bone formation rate in human metabolic bone disease. J Clin Endocrinol Metab 18:1246-1267.

Heaney RP, Barger-Lux MJ, Davies KM, Ryan RA, Johnson ML and Gong G (1997): Bone dimensional change with age: Interactions of genetic, hormonal, and body size variables. Osteoporosis Int 7:426-431.

Heikkinen J, Kyllönen E, Kurttila-Matero E, Wilen-Rosenqvist G, Lankinen KS, Rita H and Väänänen HK (1997): HRT and exercise: effects on bone density, muscle strength and lipid metabolism. A placebo controlled 2-year prospective trial on two estrogen-progestin regimens in healthy postmenopausal women. Maturitas 26:139–149.

Heinonen A, Sievänen H, Kannus P, Oja P and Vuori I (1996): Effects of unilateral strength training and detraining on bone mineral mass and estimated mechanical characteristics of the upper limb bones in young women. J Bone Miner Res 11:490-501.

Honda A, Umemura Y and Nagasawa S (2001): Effect of high-impact and low-repetition training on bones in ovariectomized rats. J Bone Miner Res 16:1688-1693.

Honda A, Sogo N, Nagasawa S, Shimizu T and Umemura Y (2003): High-impact exercise strengthens bone in osteopenic ovariectomized rats with the same outcome as sham rats. J Appl Physiol 95:1032-1037.

Hoyland JA, Baris C, Wood L, Baird P, Selby PL, Freemont AJ and Braidman IP (1999): Effect of ovarian steroid deficiency on oestrogen receptor alpha expression in bone. J Pathol 188:294-303.

Hsieh YF, Robling AG, Ambrosius WT, Burr DB and Turner CH (2001): Mechanical loading of diaphyseal bone in vivo: the strain threshold for an osteogenic response varies with location. J Bone Miner Res 16:2291-2297.

Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR and Boyce BF (1996): Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-ß. Nat Med 2:1132-1136.

Hustmyer FG, Walker E, Yu XP, Girasole G, Sakagami Y, Peacock M and Manolagas SC (1993): Cytokine production and surface antigen expression by peripheral blood mononuclear cells in postmenopausal osteoporosis. J Bone Miner Res 8:51-59.

Högler W, Blimkie CJ, Cowell CT, Kemp AF, Briody J, Wiebe P, Farpour-Lambert N, Duncan CS and Woodhead HJ (2003): A comparison of bone geometry and cortical density at the midfemur between prepuberty and young adulthood using magnetic resonance imaging. Bone 33:771–778.

Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, Langdahl B, van Meurs JB, Mosekilde L, Scollen S, Albagha OM, Bustamante M, Carey AH, Dunning AM, Enjuanes A, van Leeuwen JP, Mavilia C, Masi L, McGuigan FE, Nogues X, Pols HA, Reid DM, Schuit SC, Sherlock RE and Uitterlinden AG (2004): Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. JAMA. 292:2105-2114.

Iwamoto J, Yeh JK and Aloia JF (2000) Effect of deconditioning on cortical and cancellous bone growth in the exercise trained young rats. J Bone Miner Res 15:1842-1849.

Jagger CJ, Chow JW and Chambers TJ (1996): Estrogen suppresses activation but enhances formation phase of osteogenic response to mechanical stimulation in rat bone. J Clin Invest 98:2351-2357.

Jasani C, Nordin BEC, Smith DA and Swanson I (1965): Spinal osteoporosis and the menopause. Proc R Soc Med 58:441–444.

Jessop HL, Sjoberg M, Cheng MZ, Zaman G, Wheeler Jones CP and Lanyon LE (2001): Mechanical strain and estrogen activate estrogen receptor alpha in bone cells. J Bone Miner Res 16:1045-1055.

Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H and Manolagas SC (1992): Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257:88-91.

Jilka RL, Takahashi K, Munshi M, Williams DC, Roberson PK and Manolagas SC (1998): Loss of estrogen upregulates osteoblastogenesis in the murine marrow. J Clin Invest 101:1942-1950.

Johnson DL, McAllister TN and Frangos JA (1996): Fluid flow stimulates rapid and continuous release of nitric oxide in osteoblasts. Am J Physiol 271:E205-208.

Johnston CC, Slemenda CW and Melton LJ (1991): Clinical use of bone densitometry. N Engl J Med 324:1105-1109.

Joldersma M, Klein-Nulend J, Oleksik AM, Heyligers IC and Burger EH (2001): Estrogen enhances mechanical stress-induced prostaglandin production by bone cells from elderly women. Am J Endocrinol Metab 280:E436-E442.

Joss EE, Sobel EH and Zuppinger KA (1963): Skeletal maturation in rats with special reference to order and time of epiphyseal closure. Endocrinology 72:117-122.

Joumaa WH, Bouhlel A, Bigard X and Leoty C (2002): Nandrolone decanoate pre-treatment attenuates unweighting-induced functional changes in rat soleus muscle. Acta Physiol Scand 176:301-309.

Järvinen TLN, Sievänen H, Kannus P and Järvinen M (1998a): Dual-energy X-ray absorptiometry in predicting mechanical characteristics of rat femur. Bone 22:551-558.

Järvinen TLN, Kannus P, Sievänen H, Jolma P, Heinonen A and Järvinen M (1998b): Randomized controlled study of effects of sudden impact loading on rat femur. J Bone Miner Res 13:1475-1482.

Järvinen TLN, Kannus P and Sievänen H (1999): Have the DXA-based exercise studies seriously underestimated the effects of mechanical loading on bone? J Bone Miner Res 14:1634-1635.

Järvinen TLN, Kannus P and Sievänen H (2003): Estrogen and bone- a reproductive and locomotive perspective. J Bone Miner Res 18:1921-1931.

Järvinen TL, Sievänen H, Jokihaara J and Einhorn TA (2005): Revival of bone strength: the bottom line. J Bone Miner Res 20:717-720.

Kalu DN (1984): Evaluation of the pathogenesis of skeletal changes in ovariectomized rats. Endocrinology 115:507-512.

Kalu DN (1990): Proliferation of tartrate-resistant acid phosphatase positive multinucleate cells in ovariectomized animals. Proc Soc Exp Biol Med 195:70-74.

Kalu DN (1991): The ovariectomized rat model of postmenopausal bone loss. Bone Miner 15:175-192.

Kanis JA and Gluer CC (2000): An update on the diagnosis and assessment of osteoporosis with densitometry. Committee of Scientific Advisors, International Osteoporosis Foundation. Osteoporos Int 11:192–202.

Kannus P, Haapasalo H, Sankelo M, Sievänen H, Pasanen M, Heinonen A, Oja P and Vuori I (1995): Effect of starting-age of physical activity on bone mass in the dominant arm of tennis and squash players. Ann Intern Med 123:27-31.

Karlsson MK, Johnell O and Obrant KJ (1995): Is bone mineral density advantage maintained long-term in previous weight lifters? Calcif Tissue Int 57:325-328.

Karlsson MK, Hasserius R and Obrant KJ (1996): Bone mineral density in athletes during and after career: a comparison between loaded and unloaded skeletal regions. Calcif Tissue Int 59:245-248.

Karlsson MK, Linden C, Karlsson C, Johnell O, Obrant K and Seeman E (2000): Exercise during growth and bone mineral density and fractures in old age. Lancet 355:469-470.

Khan KM, Green RM, Saul L, Bennell KL, Crichton KJ, Hopper JL and Wark JD (1996): Retired elite female ballet dancers and nonathletic controls have similar bone mineral density at weightbearing sites. J Bone Miner Res. 11:1566-1574.

Khan KM, McKay HA, Haapasalo H, Bennell KL, Forwood MR, Kannus P and Wark JD (2000): Does childhood and adolescence provide a unique opportunity for exercise to strengthen the skeleton? J Sci Med Sport 3:150-164.

Khosla S, Melton LJ 3rd and Riggs BL (1998): Estrogen and the male skeleton. J Clin Endocrinol Metab 87:1443-1450.

Kimmel DB (1991): Quantitative histologic changes in the proximal tibial growth cartilage of aged female rats. Cells Mater 1(suppl.):S11-S18.

Kimmel DB (1993): A paradigm for skeletal strength homeostasis. J Bone Miner Res 8(suppl.):S515-S522.

Kiuchi A, Arai Y and Katsuta S (1998): Detraining effects on bone mass in young male rats. Int J Sports Med 19:245-249.

Klein-Nulend J, Van der Plas A, Semeins CM, Ajubi NE, Frangos JA, Nijweide PJ and Burger EH (1995): Sensitivity of osteocytes to biomechanical stress in vitro. FASEB J 9:441-445.

Klein-Nulend J, Sterck JG, Semeins CM, Lips P, Joldersma M, Baart JA and Burger E (2002): Donor age and mechanosensitivity of human bone cells. Osteoporos Int 13:137-146.

Kohrt WM, Snead DB, Slatopolsky E and Birge SJJ (1995): Additive effects of weight-bearing exercise and estrogen on bone mineral density in older women. J Bone Miner Res 10:1303–1311.

Kohrt WM, Ehsani AA and Birge SJ (1997): Effects of exercise involving predominantly either joint-reaction or ground-reaction forces on bone mineral density in older women. J Bone Miner Res 12:1253-1261.

Kohrt WM, Ehsani AA and Birge SJJ (1998): HRT preserves increases in bone mineral density and reductions in body fat after a supervised exercise program. J Appl Physiol 84:1506–1512.

Komm BS, Terpening CM, Benz NJ, Graeme KA, Gallegos A, Korc M, Greene GL, O'Malley BW and Haussler MR (1988): Estrogen binding, receptor mRNA, and biologic response in osteoblast-like osteosarcoma cells. Science 241:81-84.

Kontulainen S, Kannus P, Haapasalo H, Heinonen A, Sievänen H, Oja P and Vuori I (1999): Changes in bone mineral content with decreased training in competitive young adult tennis players and controls: a prospective 4-yr follow-up. Med Sci Sports Exer 31:646-652.

Kontulainen S, Kannus P, Haapasalo H, Sievänen H, Pasanen M, Heinonen A, Oja P and Vuori I (2001): Good maintenance of exercise-induced bone gain with decreased training of female tennis and squash players: a prospective 5-year follow-up study of young and old starters and controls. J Bone Miner Res 16:195-201.

Kontulainen S, Sievänen H, Kannus P, Pasanen M and Vuori I (2002): Effect of long-term impact-loading on mass, size, and estimated strength of humerus and radius of female raquet-sports players: a peripheral quantitative computed tomography study between young and old starters and controls. J Bone Miner Res 17:2281-2289.

Kontulainen S, Macdonald HM, Khan KM and McKay HA (2005): Examining bone surfaces across puberty: a 20-month pQCT trial. J Bone Miner Res 20:1202-1207.

Korenstein R, Somjen D, Fischler H and Binderman I (1984): Capacitative pulsed electric stimulation of bone cells. Induction of cyclic-AMP changes and DNA synthesis. Biochim Biophys Acta 803:302-307.

Kostenuik PJ, Harris J, Halloran BP, Turner RT, Morey-Holton ER and Bikle DD (1999): Skeletal unloading causes resistance of osteoprogenitor cells to parathyroid hormone and to insulin-like growth factor-I. J Bone Miner Res 14:21-31.

Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S and Gustafsson JA (1996): Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925-5930.

Lanyon LE (1973): Analysis of surface bone strain in the sheep during normal locomotion. J Biomech 6:41-49.

Lanyon LE (1984): Functional strain as a determinant for bone remodeling. Calcif Tissue Int 36(suppl):S56-S61.

Lanyon LE (1993): Osteocytes, strain detection, bone modeling and remodeling. Calcif Tissue Int 53(suppl 1):S102-S106.

Lanyon LE and Smith RN (1970): Bone strain in the tibia during normal quadrupedal locomotion. Acta Orthopaedica Scand 42:238-248.

Lanyon L and Skerry T (2001): Postmenopausal osteoporosis as a failure of bone's adaptation to functional loading: a hypothesis. J Bone Miner Res 16:1937-1947.

Lanyon LE, Hampson WGJ, Goodship AE and Shah JS (1975): Bone deformation recorded in vivo from strain gauges atteched to the human tibial shaft. Acta Orthopaedica Scand 46:256-268.

Lanyon LE, Goodship AE, Pye CJ and MacFie JH (1982): Mechanically adaptive bone remodeling. J Biomech 15:141-154.

Lee K, Jessop H, Suswillo R, Zaman G and Lanyon L (2003): Bone adaptation requires oestrogen receptor-alpha. Nature 424:389.

Lee KC, Jessop H, Suswillo R, Zaman G and Lanyon LE (2004): The adaptive response of bone to mechanical loading in female transgenic mice is deficient in the absence of oestrogen receptor-alpha and –beta. J Endocrinol 182:193-201.

Leppänen O, Sievänen H, Jokihaara J, Pajamäki I and Järvinen TLN (2006): Three-point bending of rat femur in mediolateral direction: introduction and validation of a novel biomechanical testing protocol. J Bone Miner Res 21:1231-1237.

Li XJ, Jee WSS, Ke HZ, Mori S and Akamine T (1991): Age-related changes of cancellous and cortical bone histomorphometry in female Sprague-Dawley rats. Cells Mater 1(suppl.): S25-S35.

Li Z, Kong K and Qi W (2006): Osteoclast and its roles in calcium metabolism and bone development and remodeling. Biochem Biophys Res Commun. 343:345-350.

Lindsay R (1987): The menopause: sex steroids and osteoporosis. Clin Obstet Gynecol 30:847-859.

Liu YZ, Liu YJ, Recker RR and Deng HW (2003): Molecular studies of identification of genes for osteoporosis: the 2002 update. J Endocrinol 177:147-196.

Lotinun S, Evans GL, Bronk JT, Bolander ME, Wronski TJ, Ritman EL and Turner RT (2004): Continuous parathyroid hormone induces cortical porosity in the rat: effects on bone turnover and mechanical properties. J Bone Miner Res 19:1165-1171.

Lu PW, Briody JN, Ogle GD, Morley K, Humphries IR, Allen J, Howman-Giles R, Sillence D and Cowell CT (1994): Bone mineral density of total body, spine, and femoral neck in children and young adults: a cross-sectional and longitudinal study. J Bone Miner Res 9:1451-1458.

Lu PW, Cowell CT, Lloyd-Jones SA, Briody JN and Howman-Giles R (1996): Volumetric bone mineral density in normal subjects, aged 5-27 years. J Clin Endocrinol Metab 81:1586-1590.

MacKelvie KJ, Khan KM and McKay HA (2002): Is there a critical period for bone response to weight-bearing exercise in children and adolescents? A systematic review. Br J Sports Med 36:250-257.

Manolagas SC (1994): Estrogens, cytokines, and the pathophysiology of postmenopausal osteoporosis. Ther Res 15:82-99.

Manolagas SC (2000): Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21:115-137.

Manolagas SC and Jilka RL (1995): Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 332:305-311.

Manolagas SC, Kousteni S and Jilka RL (2002): Sex steroids and bone. Recent Prog Horm Res 57:385-409.

McLean FC and Urist MR (1961): Bone, 2nd Ed. University of Chicago Press, Chicago.

Morishima A, Grumbach MM, Simpson ER, Fisher C and Qin K (1995): Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689–3698.

Mosekilde L and Mosekilde L (1990): Sex differences in age-related changes in vertebral body size, density and biomechanical competence in normal individuals. Bone 11:67-73.

Mosley JR and Lanyon LE (1998): Strain rate as a controlling influence on adaptive modeling in response to dynamic loading of the ulna in growing male rats. Bone 23:313-318.

Mullender MG and Huiskes R (1997): Osteocytes and bone lining cells: which are the best candidates for mechano-sensors in cancellous bone? Bone 20:527-532.

Mundy GR (1993): Role of cytokines in bone resorption. J Cell Biochem 53:296-300.

Mundy GR and Roodman GD (1987): Osteoclast ontogeny and function. In: Bone and Mineral Research, pp. 209-279. Ed. A Peck, Elsevier, Amsterdam.

Neu CM, Manz F, Rauch F, Merkel A and Schoenau E (2001a): Bone densities and bone size at the distal radius in healthy children and adolescents: a study using peripheral quantitative computed tomography. Bone 28:227-232.

Neu CM, Rauch F, Manz F and Schoenau E (2001b): Modeling of cross-sectional bone size, mass and geometry at the proximal radius: a study of normal bone development using peripheral quantitative computed tomography. Osteoporos Int 12:538-547.

NIH consensus development Panel (2001): Osteoporosis prevention, diagnosis, and therapy. JAMA 285:785-795.

Nordin BEC (1960): Osteomalacia, osteoporosis and calcium deficiency. Clin Orthop 17:235-258.

Nordin BEC (1961): The pathogenesis of osteoporosis. Lancet 1:1011-1014.

Nordin BEC (1964): Osteoporosis. Adv Metab Dis 1:125-151.

Nordström A, Karlsson C, Nyquist F, Olsson T, Nordström P and Karsson M (2005): Bone loss and fracture risk after reduced physical activity. J Bone Miner Res 20:202-207.

O'Connor JA and Lanyon LE (1982): The influence of strain rate on adaptive bone remodeling. J Biomech 15:767-781.

Oursler MJ, Osdoby P, Pyfferoen J, Riggs BL and Spelsberg TC (1991): Avian osteoclasts as estrogen target cells. Proc Natl Acad Sci USA 88:6613-6617.

Oursler MJ, Pederson L, Fitzpatrick L and Riggs BL (1994): Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. Proc Natl Acad Sci USA 91:5227-5231.

Pacifici R (1993): Cytokine production and surface antigen expression by peripheral blood mononuclear cells in postmenopausal osteoporosis. J Bone Miner Res 8:775-778.

Pacifici R (1998): Estrogen and bone loss. Adv Organ Biol 5C:641-659.

Pacifici R, Rifas L, Teitelbaum S, Slatopolsky E, McCracken R, Bergfeld M, Lee W, Avioli LV and Peck WA (1987): Spontaneous release of interleukin 1 from human blood monocytes reflects bone formation in idiopathic osteoporosis. Proc Natl Acad Sci USA 84:4616-4620.

Pacifici R, Brown C, Puscheck E, Friedrich E, Slatopolsky E, Maggio D, McCracken R and Avioli LV (1991): Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proc Natl Acad Sci USA 88:5134-5138.

Parfitt AM (1984): The cellular basis of bone remodelling: the quantum concept reexamined in light of recent advances in the cell biology of bone. Calcif Tissue Int 36(suppl):S37-S45.

Parfitt AM (1998): A structural approach to renal bone disease. J Bone Miner Res 13:1213-1220.

Pearson OM and Lieberman DE (2004): The aging of Wolff's "law:" ontogeny and responses to mechanical loading in cortical bone. Yrbk Phys Anthropol 47:63-99.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S and Marshak DR (1999): Multilineage potential of adult human mesenchymal stem cells. Science 284:143-147.

Pitts GC (1984): Body composition in the rat: interactions of exercise, age, sex, and diet. Am J Physiol 246:R495-R501.

Raab DM, Smith EL, Crenshaw TD and Thomas DP (1990): Bone mechanical properties after exercise training in young and old rats. J Appl Physiol 68:130-134.

Rapuri PB, Gallagher JC, Knezetic JA and Haynatzka V (2006): Estrogen receptor alpha gene polymorphisms are associated with changes in bone remodeling markers and treatment response to estrogen. Maturitas 53:371-379.

Rawlinson SCF, El-Haj AJ, Minter SL, Tavares IA, Bennett A and Lanyon LE (1991): Loading related increases in prostaglandin production in cores of adult canine cancellous bone in vitro: a role for prostacyclin in adaptive bone remodelling. J Bone Miner Res 6:1345-1351.

Reich KM, Gay CV and Frangos JA (1990): Fluid shear stress as a mediator of osteoblast cyclic adenosine monophosphate production. J Cell Physiol 143:100-104.

Rico H, Revilla M, Villa LF, Ruiz-Contreras D, Hernandez ER and Alvarez de Buergo M (1994): The four-compartment models in body composition: data from a study

with dual-energy X-ray absorptiometry and near-infrared interactance on 815 normal subjects. Metabolism 43:417-422.

Riggs BL and Melton LJ 3rd (1983): Evidence for two distinct syndromes of involutional osteoporosis. Am J Med 75:899-901.

Riggs BL and Melton LJ 3rd (1986): Involutional osteoporosis. N Engl J Med 314:1676-1686.

Riggs BL, Khosla S and Melton LJ 3rd (1998): A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 13:763-773.

Riggs BL, Khosla S and Melton LJ 3rd (2002): Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279-302.

Riggs BL, Melton LJ 3rd, Robb RA, Camp JJ, Atkinson EJ, Peterson JM, Rouleau PA, McCollough CH, Bouxsein ML and Khosla S (2004): Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. J Bone Miner Res 19:1945-1954.

Rizzoli R, Bonjour JP and Ferrari SL (2001): Osteoporosis, genetics and hormones. J Mol Endocrinol 26:79-94.

Robling AG and Turner CH (2002): Mechanotransduction in bone: genetic effects on mechanosensitivity in mice. Bone 31:562-569.

Robling AG, Hinant FM, Burr DB and Turner CH (2002): Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into shourt bouts. J Bone Miner Res 17:1545-1554.

Ross MH, Romrell LJ and Kaye GI (1995): Bone. In: Histology: a text and atlas, pp. 150-187. Ed. PA Coryell, Williams and Wilkins, Baltimore.

Rubin CT and Lanyon LE (1984): Regulation of bone formation by applied dynamic loads. J Bone Joint Surg 66-A:397-402.

Rubin CT and McLeod KJ (1994): Promotion of bony ingrowth by frequency-specific, low-amplitude mechanical strain. Clin Orthop Relat Res 298:165-174.

Rubin CT, Bain SD and McLeod KJ (1992): Suppression of the osteogenic response in the aging skeleton. Calcif Tissue Int 50:306-313.

Ruff CB and Hayes WC (1982): Subperiosteal expansion and cortical remodeling of the human femur and tibia with aging. Science 217:945-948.

Ruff CB and Hayes WC (1988): Sex differences in age related remodeling of the femur and tibia. J Orthop Res 6:886–896.

Ruff CB, Holt B and Trinkaus E (2006): Who's afraid the big bad Wolff?: "Wolff's law" and bone functional adaptation. Am J Phys Anthropol 129:484-498.

Schiessl H, Frost HM and Jee WSS (1998): Estrogen and bone-muscle strength and mass relationships. Bone 22:1-6.

Schot LPC and Schuurs AHWM (1990): Sex steroids and osteoporosis: effects of deficiencies and susbstitutive treatments. J Steroid Biochem Mol Biol 37:167-182.

Schöenau E, Neu CM, Mokov E, Wassmer G and Manz F (2000): Influence of puberty on muscle area and cortical bone area of the forearm in boys and girls. J Clin Endocrinol Metab 85:1095-1098.

Schöenau E, Neu CM, Rauch F and Manz F (2001): The development of bone strength at the proximal radius during childhood and adolescence. J Clin Endocrinol Metab 86:613-618.

Schöenau E, Neu CM, Rauch F and Manz F (2002): Gender-specific pubertal changes in volymetric cortical bone density at the proximal radius. Bone 31:110-113.

Seeman E (1997): From density to structure: growing up and growing old on the surfaces of bone. J Bone Miner Res 12:509-21.

Seeman E (2001): Sexual dimorphism in skeletal size, density, and strength. J Clin Endocrinol Metab 86:4576-4584.

Seeman E (2002): An exercise in Geometry. J Bone Miner Res 17:373-380.

Seeman E (2003): Periosteal bone formation- a neglected determinant of bone strength. N Engl J Med 349:320-323.

Sherman HC and MacLeod FL (1925): The calcium content of the body in relation to age, growth, and food. J Biol Chem 64**:**429–459.

Sietsma WK (1995): Animal models of cortical porosity. Bone 17(suppl.):S297-S305.

Sievänen H (2000): A physical model for dual-energy X-ray absorptiometry-derived bone mineral density. Invest Radiol 35:325-330.

Sievänen H, Koskue V, Rauhio A, Kannus P, Heinonen A and Vuori I (1998): Peripheral quantitative computed tomography in human long bones: evaluation in vitro and in vivo precision. J Bone Miner Res 13:871-882.

Silbermann M, Schapira D, Leichter I and Steinberg R (1991): Moderate physical exercise throughout adulthood increases peak bone mass at middle age and maintains higher trabecular bone density in vertebrae of senescent female rats. Cells Mater 1(suppl):S151-S158.

Sims NA, Dupont S, Krust A, Clement-Lacroix P, Minet D, Resche-Rigon M, Gaillard-Kelly M and Baron R (2002): Deletion of estrogen receptors reveals a regulatory role for estrogen receptors-ß in bone remodeling in females but not in males. Bone 30:18-25.

Sims NA, Clement-Lacroix P, Minet D, Fraslon-Vanhulle C, Gaillard-Kelly M, Resche-Rigon M and Baron R (2003): A functional androgen receptor is not sufficient to allow estradiol to protect bone after gonadectomy in estradiol receptor-deficient mice. J Clin Invest 111:1319-1327.

Singh R, Umemura Y, Honda A and Nagasawa S (2002): Maintenance of bone mass and mechanical properties after short-term cessation of high impact exercise in rats. Int J Sports Med 23:77-81.

Skerry TM (2006): One mechanostat or many? Modifications of the site-specific response of bone to mechanical loading by nature and nurture. J Musculoskelet Neuronal Interact 6:122-127.

Smith EP*,* Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB and Korach KS (1994): Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331:1056-1061.

Smith RW Jr and Walker RR (1964): Femoral expansion in aging women: implications for osteoporosis and fractures. Science 145:156-157.

Snapper I (1957): Bone diseases in medical practise. Grune and Stratton, New York.

Somjen D, Binderman I, Berger E and Harell A (1980): Bone remodeling induced by physical stress is prostaglandin E_2 mediated. Biochim Biophys Acta 627:91-100.

Spark C and Dawson AB (1928): The order and time appearance of centers of ossification in the fore and hind limbs of the albino rat, with special reference to the possible influence of the sex factor. Amer J Anat 41:411-445.

Stein M, Thomas C, Feik S, Wark J and Clement J (1998): Bone size and mechanics at the femoral diaphysis across age and sex. J Biomech 31:1101–1110.

Stevenson JC, Abeyasekera G, Hillyard CJ, Phang KG, MacIntyre I, Campbell S, Townsend PT, Young O and Whitehead MI (1981): Calcitonin and the calcium-regulating hormones in postmenopausal women: effect of oestrogens. Lancet 1:693-695.

Suuriniemi M, Mahonen A, Kovanen V, Alén M, Lyytikäinen A, Wang Q, Kröger H and Cheng S (2004): Association between exercise and puvertal BMD is modulated by estrogen receptor-α genotype. J Bone Miner Res 19:1758-1765.

Taichman RS (2005): Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood 150:2631-2639.

Tiegs RD, Body JJ, Wahner HW, Barta J, Riggs BL and Heath HR (1985): Calcitonin secretion in postmenopausal osteoporosis. N Engl J Med 312:1097-1100.

Tomkinson A, Gevers EF, Wit JM, Reeve J and Noble BS (1998): The role of estrogen in the control of rat osteocyte apoptosis. J Bone Miner Res 13:1243-1250.

Turner CH and Burr DB (1993): Basic biomechanical measurements of bone: a tutorial. Bone 14:595-608.

Turner CH and Pavalko FM (1998): Mechanotransduction and functional response of the skeleton to physical stress: the mechanisms and mechanics of bone adaptation. J Orthop Sci 3:346-355.

Turner CH, Forwood MR and Otter MW (1994): Mechanotransduction in bone: do bone cells act as sensors of fluid flow. FASEB J 8:875-878.

Turner CH, Takano Y and Owan I (1995): Aging changes mechanical loading thresholds for bone formation in rats. J Bone Miner Res 10:1544-1549.

Turner RT (1999): Mechanical signaling in the development of postmenopausal osteoporosis. Lupus 8:388-392.

Turner RT, Vandersteenhoven JJ and Bell NH (1987a): The effects of ovariectomy and 17b-estradiol on cortical bone histomorphometry in growing rats. J Bone Miner Res 2:115-122.

Turner RT, Wakley GK, Hannon KS and Bell NH (1987b): Tamoxifen prevents the skeletal effects of ovarian hormone deficiency in rats. J Bone Miner Res 2:449-456.

Turner RT, Hannon KS, Demers LM, Buchanan J and Bell NH (1989): Differential effects of gonadal function on bone histomorphometry in male and female rats. J Bone Miner Res 4:557-563.

Turner RT, Colvard DS and Spelsberg TC (1990): Estrogen inhibition of periosteal bone formation in rat long bones: down-regulation of gene expression for bone matrix proteins. Endocrinology 127:1346-1351.

Turner RT, Backup P, Sherman PJ, Hill E, Evans GL and Spelsberg TC (1992): Mechanism of action of estrogen on intramembranous bone formation: regulation of osteoblast differentiation and activity. Endocrinology 131:883-889.

Turner RT, Lotinun S, Hefferan TE and Morey-Holton E (2006): Disuse in adult male rats attenuates the bone anabolic response to a therapeutic dose of parathyroid hormone. J Appl Physiol 101:881-886.

Turner AS (2001): Animal models of osteoporosis- necessity and limitations. European Cells and Materials 1:66-81.

Ulrich D, van Rietbergen B, Laib A and Ruegsegger P (1999): The ability of three-dimensional structural indices to reflect mechanical aspects of trabecular bone. Bone 25:55-60.

Umemura Y, Ishiko T, Tsujimoto H, Miura H, Mokushi N and Suzuki H (1995): Effects of jump training on bone hypertrophy in young and old rats. Int J Sports Med 16:364-367.

Walker DG (1975): Control of bone resorption by hematopoietic tissue. The induction and reversal of congenital osteopetrosis in mice through use of bone marrow and splenic transplants. J Exp Med 142:651-663.

van der Meulen MCH, Jepsen KJ and Mikic B (2001): Understanding bone strength: size isn't everything. Perspective. Bone 29:101-104.

Wang L, McMahan CA, Banu J, Okafor MC and Kalu DN (2003): Rodent model for investigating the effects of estrogen on bone and muscle relationship during growth. Calcif Tissue Int 72:151-155.

Wang Q, Alén M, Nicholson PHF, Lyytikäinen A, Suuriniemi M, Helkala E, Suominen H and Cheng S (2005): Growth patterns at distal radius and tibial shaft in pubertal girls: a 2-year longitudinal study. J Bone Miner Res 20:954-961.

Waynforth HB (1988): Experimental and surgical techniques in the rat. Academic Press, San Diego.

Weinbaum S, Cowin SC and Zeng Y (1993): A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stress. J Biomech 27:339-360.

Weinmann JP and Sicher H (1955): Bone and bones, CV Mosby Co., St Louis.

Westerlind KC, Wronski TJ, Ritman EL, Luo ZP, An KN, Bell NH and Turner RT (1997): Estrogen regulates the rate of bone turnover but bone balance in

ovariectomized rats is modulated by prevailing mechanical strain. Proc Natl Acad Sci U.S.A. 15:4199-4204.

Whitson SW, Dawson LR and Jee WSS (1978): A tetracycline study of cyclic longitudinal bone growth in the female rat. Endocrinology 103:2006-2010.

Wolff J (1892): Das Gesetz der Transformation der Knochen (Hirchwild, Berlin), translated as The Law of Bone Remodeling (trans. P. Maquet, R. Furlong), Springer, Berlin

World Health Organization (1994): Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group. World Health Organization Technical Report Series, Report No. 843. World Health Organization, Geneva.

Wronski TJ and Yen CF (1991): The ovariectomized rat as an animal model for postmenopausal bone loss. Cells and Materials (suppl. 1):S69-S74.

Wronski TJ, Lowry PL, Walsh CC and Ignaszewski LA (1985): Skeletal alterations in ovariectomized rats. Calcif Tissue Int 37:324-328.

Wronski TJ, Walsh CC and Ignaszewski LA (1986): Histologic evidence for osteopenia and increased bone turnover in ovariectomized rats. Bone 7:119-123.

Wronski TJ, Schenck PA, Cintron M and Walsh CC (1987): Effect of body weight on osteopenia in ovariectomized rats. Calcif Tissue Int 40:155-159.

Wronski TJ, Cintron M and Dann LM (1988): Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. Calcif Tissue Int 43:179-183.

Wronski TJ, Dann LM, Scott KS and Cintron M (1989): Long-term effects of ovariectomy and aging on the rat skeleton. Calcif Tissue Int 45:360-366.

Wu K, Jett S and Frost HM (1967): Bone resorption rates in rib in physiological, senile, and postmenopausal osteoporoses. J Lab Clin Med 5:810-818.

Vuori I, Heinonen A, Sievänen H, Kannus P, Pasanen M and Oja P (1994): Effects of unilateral strength training and detraining on bone mineral density and content in young women: a study of mechanical loading and deloading on human bones. Calcif Tissue Int 55:59-67.

Yeh JK and Aloia JF (1990): Deconditioning increases bone resorption and decreases bone formation in the rat. Metabolism 39: 659-663.

Zachwieja JJ, Smith SR, Lovejoy JC, Rood JC, Windhauser MM and Bray GA (1999): Testosterone administration preserves protein balance but not muscle strength during 28 days of bed rest. J Clin Endocrinol Metab 84:207-212.

Zaman G, Suswillo RFL, Cheng MZ, Tavares IA and Lanyon LE (1997): Early responses to dynamic strain change and prostaglandins in bone-derived cells in culture. J Bone Miner Res 12:769-777.

Zanchetta JR, Plotkin H and Alvarez Filgueira ML (1995): Bone mass in children: normative values for the 2–20-year old population. Bone 16(suppl):S393–S399.

Zhang XZ, Kalu DN, Erbas B, Hopper JL and Seeman E (1999): The effect of gonadectomy on bone size, mass and volumetric density in growing rats may be gender-site-, and growth hormone-dependent. J Bone Miner Res 14:802-809.

ORIGINAL COMMUNICATIONS

The following copyright owners are acknowledged for permission to reprint the original communications:

I Järvinen TLN, Kannus P, Pajamäki I, Vuohelainen T, Tuukkanen J, Järvinen M, Sievänen H (2003): Estrogen deposits extra mineral into bones of female rats in puberty, but simultaneously seems to suppress the responsiveness of female skeleton to mechanical loading. Bone 32:642-651. Reprinted with permission from Elsevier.

Seeman E and Zebaze RMD (2004): On Jarvinen et al. Letter to the editor. Bone 34:231-232. Reprinted with permission from Elsevier.

Pajamäki I, Sievänen H, Kannus P and Järvinen TLN (2004): Response to Seeman and Zebaze. Reply to letter to the editor. Bone 34:233-235. Reprinted with permission from Elsevier.

II Pajamäki I, Kannus P, Vuohelainen T, Sievänen H, Tuukkanen J, Järvinen M, Järvinen TLN (2003): The bone gain induced by exercise is not preserved through a virtually life-long deconditioning: A randomized controlled experimental study in male rats. J Bone Miner Res 18:544-552. Reprinted with permission of the American Society for Bone and Mineral Research.

III Järvinen TLN, Pajamäki I, Sievänen H, Vuohelainen T, Tuukkanen J, Järvinen M, Kannus P (2003): Femoral neck response to exercise and subsequent deconditioning in young and adult rats. J Bone Miner Res 18:1292-1299. Reprinted with permission of the American Society for Bone and Mineral Research.

IV Pajamäki I, Sievänen H, Kannus P, Jokihaara J, Vuohelainen T, Järvinen TLN: Anabolic skeletal effects of estrogen and loading are structurally distinct. Submitted for publication.