STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
155

Tuija Mikkola

Genetic and Environmental Contributions to Bone Structural Strength in Postmenopausal Women

JYVÄSKYLÄN YLIOPISTO

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 155

Tuija Mikkola

Genetic and Environmental Contributions to Bone Structural Strength in Postmenopausal Women

Esitetään Jyväskylän yliopiston liikunta- ja terveystieteiden tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212
marraskuun 5. päivänä 2010 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
in Auditorium S212, on November 5, 2010 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

Genetic and Environmental Contributions to Bone Structural Strength in Postmenopausal Women

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 155

Tuija Mikkola

Genetic and Environmental Contributions to Bone Structural Strength in Postmenopausal Women

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

Editor
Harri Suominen
Department of Health Sciences, University of Jyväskylä
Pekka Olsbo, Sini Rainivaara
Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-4069-0
ISBN 978-951-39-4069-0 (PDF)

ISBN 978-951-39-4058-4 (nid.)
ISSN 0356-1070

Copyright © 2010, by University of Jyväskylä

Jyväskylä University Printing House, Jyväskylä 2010

ABSTRACT

Mikkola, Tuija
Genetic and environmental contributions to bone structural strength in postmenopausal women
Jyväskylä: University of Jyväskylä, 2010, 77 p.
(Studies in Sport, Physical Education, and Health
ISSN 0356-1070; 155)
ISBN 978-951-39-4069-0 (PDF), 978-951-39-4058-4 (nid.)
Finnish summary
Diss.

The purpose of this study was to investigate the genetic and environmental contributions to estimated bone structural strength in postmenopausal women. Specifically, the association of hip fracture history and postmenopausal hormone therapy (HT) to bone strength and its determinants were studied. In addition, the association of loading environment with bone properties was assessed.

This study utilized three different datasets comprising bone measurements obtained using peripheral quantitative computed tomography. The participants in the first dataset were 103 monozygotic and 114 dizygotic 63- to 76-year-old female twin pairs. The participants in the second dataset were 60- to 85-year-old women with a hip fracture history (n=38) and control women without hip fracture history (n=22). The participants in the third dataset were 24 monozygotic female twin pairs aged 54 to 72 years and discordant for HT.

Genetic effects were found to account for 40 to 60% of the inter-individual differences in the estimated bone strength of the body weight-loaded lower limb and 80% of that of the non weight-loaded upper limb. The association between muscle cross-sectional area and bone strength was explained by both common genetic and common environmental factors. In women with hip fracture history, bone strength was significantly impaired in the tibia of the fractured leg, which impairment was largely explained by reduced muscle cross-sectional area and muscle strength. The HT users had significantly higher bone strength in the upper and lower limb than their non-HT-using co-twins.

This study shows that while genetic factors are important for bone strength in postmenopausal women environmental factors have considerable influence especially in the lower limbs. Bone strength is negatively influenced by hip fracture and positively by HT. The results also suggest that loading environment modifies the heritability of bone strength but not the influence of HT on bone strength. Muscle-induced loading may preserve bone strength after hip fracture.

Keywords: Bone strength, postmenopausal women, genetic effect, twin, hip fracture, postmenopausal hormone therapy, mechanical loading

Author's address

Tuija Mikkola
Department of Health Sciences
Gerontology Research Centre
University of Jyväskylä
P.O. Box 35 (Viv)
FI-40014 University of Jyväskylä, Finland

Supervisors

Professor Ari Heinonen, PhD
Department of Health Sciences
University of Jyväskylä
Jyväskylä, Finland

Docent Sarianna Sipilä, PhD
Gerontology Research Centre
University of Jyväskylä
Jyväskylä, Finland

Docent Harri Sievänen, ScD
UKK-institute for Health Promotion Research
Tampere, Finland

Reviewers

Professor Raija Korpelainen, PhD
Department of Sports and Exercise Medicine
Deaconess Institute of Oulu,
Oulu, Finland

Professor Tuan Nguyen, PhD
Garvan Institute of Medical Research
The University of New South Wales
Sydney, Australia

Opponent

Associate Professor Mattias Lorentzon, MD, PhD
Institute of Medicine at the Sahlgrenska Academy
University of Gothenburg
Gothenburg, Sweden

ACKNOWLEDGEMENTS

This study was carried out at the Gerontology Research Centre, Department of Health Sciences, University of Jyväskylä. I feel privileged to have had the opportunity to work there surrounded by skillful researchers and to have been able to use excellent datasets in this thesis.

I wish to express my sincere gratitude to my supervisors, Professor Ari Heinonen, PhD, Research Director Sarianna Sipilä, PhD and Research Director Harri Sievänen, ScD. I am grateful to Ari for introducing the world of research to me and guiding my work with expertise. I am grateful to Sarianna that she has always been there to guide and encourage me whenever I have needed. Her quick and explicit answers have helped me tackle numerous problems. I am also grateful to Harri for sharing his vast understanding on bone research. I am thankful that you all have trusted in me during this project and I sincerely appreciate that you have also understood the challenges in my personal life during the doctoral studies.

I am grateful to the official reviewers of this thesis, Professor Raija Korpelainen, PhD and Professor Tuan V. Nguyen, PhD for their valuable comments and thorough revision of this thesis. I am also grateful to Associate Professor Mattias Lorentzon, MD, PhD for agreeing to be the opponent in the public defense of this dissertation.

I want to thank all the coauthors of the original papers for their collaboration and valuable comments during the writing processes of the papers: Professor Taina Rantanen, PhD, Professor Harri Suominen, PhD, Professor Markku Alén, MD, PhD, Professor Jaakko Kaprio, MD, PhD, Professor Markku Koskenvuo, MD, PhD, Professor Sulin Cheng, PhD, Specialist in Physical Medicine and Rehabilitation Mauri Kallinen, MD, PhD, Professor Ilkka Kiviranta, MD, PhD, Statistician Markku Kauppinen, MSc, Laboratory Chief Vuokko Kovanen, PhD, Professor Urho Kujala, MD, PhD, Professor Jukka Puolakka, MD, PhD, Director of Medical Services Mika Pekkonen, MD, PhD and Researchers Kristina Tiainen, PhD, Erja Portegijs, PhD, Carina Ankarberg-Lindgren, PhD and Paula Ronkainen, MSc. Michael Freeman is acknowledged for revising the language of this thesis and the original papers.

I express my warmest thanks to my colleagues and the staff at the Gerontology Research Centre and the Department of Health Sciences. I thank them for the help, support and friendship during these years. Especially, I want to thank Minna Mänty, PhD and Inka Pakkala, MSc for their friendship. The support I have received from you is invaluable.

I am grateful for the financial support I have received for carrying out the doctoral studies. This work was supported by the Peurunka Rehabilitation and Exercise Foundation, University of Jyväskylä, Ministry of Education, Finnish Cultural Foundation, Juho Vainio Foundation, Yrjö Jahnsson Foundation, Aging, Well-Being and Technology Graduate School and National Graduate School of Musculoskeletal Disorders and Biomaterials. The Asymmetria research project was supported by the Ministry of Education, and the FITSA and

the SAWEs were supported by the Ministry of Education and the Academy of Finland.

I want to express my most loving thanks to my family. Pekka, you have stood by my side and made compromises because of my work. I am indebted for that. Leevi, I thank you for bringing joy into my daily life. You two have the ability to put everything into perspective. I am also grateful to my parents, Sirkka and Seppo. Thank you for believing in me and supporting me whatever choices I have made.

Jyväskylä, September 2010

Tuija Mikkola

ORIGINAL ARTICLES

This thesis is based on the following original papers, which are referred to in the text by their roman numerals:

I. Mikkola, T. M., Sipilä, S., Rantanen, T., Sievänen, H., Suominen, H., Kaprio, J., Koskenvuo, M., Kauppinen, M., Heinonen, A. 2008. Genetic and environmental influence on structural strength of weight-bearing and non-weight-bearing bone: a twin study. Journal of Bone and Mineral Research 23, 492-498.

II. Mikkola, T. M., Sipilä, S., Rantanen, T., Sievänen, H., Suominen, H., Tiainen, K., Kaprio, J., Koskenvuo, M., Kauppinen, M., Heinonen, A. 2009. Muscle cross-sectional area and structural bone strength share genetic and environmental effects in older women. Journal of Bone and Mineral Research 24, 338-345.

III. Mikkola, T., Sipilä, S., Portegijs, E., Kallinen, M., Alén, M., Kiviranta, I., Pekkonen, M., Heinonen, A. 2007. Impaired geometric properties of tibia in older women with hip fracture history. Osteoporosis International 18, 1083-1090.

IV. Mikkola, T.M., Heinonen, A., Kovanen, V., Cheng, S., Kujala, U. M., Suominen, H., Alén, M., Puolakka, J., Ankarberg-Lindgren, C., Ronkainen, P. H. A., Koskenvuo, M., Kaprio, J., Rantanen, T., Sipilä, S. Influence of long-term postmenopausal hormone replacement therapy on bone structural strength: a study in discordant monozygotic twins. Journal of Bone and Mineral Research. Published online September 27, 2010. DOI: 10.1002/jbmr.255

In addition some previously unpublished results are presented.

ABBREVIATIONS

-2LL	- 2 times log-likelihood
A	additive genetic effect
AIC	Akaike's information criterion
aBMD	areal bone mineral density
ANCOVA	analysis of covariance
Ant	anterior
BMC	bone mineral content
BMU	basic multicellular unit
BSIbend	bone bending strength index
BSIcomp	bone compressive strength index
C	shared environmental effect
CI	confidence interval
CoA	cortical bone area
CoD	cortical bone mineral density
D	dominant genetic effect
df	degrees of freedom
DNA	deoxyribonucleic acid
DZ	dizygotic
Dom	dominant
E	individual environmental effect
FITSA	Finnish Twin Study on Ageing
Fx	fractured
HT	hormone therapy
Lat	lateral
mCSA	muscle cross-sectional area
Med	medial
MRI	magnetic resonance imaging
MZ	monozygotic
NA	not applicable
Non-dom	non-dominant
Non-fx	non-fractured
pQCT	peripheral quantitative computed tomography
Post	posterior
RCT	randomized controlled trial
SAWEs	Sarcopenia—Skeletal Muscle Adaptation to Postmenopausal Hypogonadism and Effects of Hormone Replacement Therapy and Physical Activity in Older Women: a Genetic and Molecular Biological Study on Estrogen-related Pathways -study
ToA	total cross-sectional area of bone
ToD	total volumetric bone mineral density
TrD	trabecular volumetric bone mineral density
vBMD	volumetric bone mineral density

CONTENTS

1 INTRODUCTION

With aging, the skeleton undergoes inevitable changes in bone mineral mass and structure which weaken the bones (Seeman 2002, Russo et al. 2003, Lauretani et al. 2008). As the increase in fragility proceeds, the bones become more susceptible to fractures (Kanis 2002, Lacroix et al. 2009). Therefore, osteoporotic fractures are very common among older people (Cummings & Melton 2002). In Finland, for instance, the annual number of hip fractures in people over 50 years is 7000 and is predicted to rise due to the aging of the population (Kannus et al. 2006). Fractures, especially hip fractures, constitute a threat to the health and functioning of older population. Hip fractures lead to increased mortality, morbidity and disability (Johnell & Kanis 2004) and the treatment of hip fractures places a great financial burden on the community (Borgström et al. 2006). Therefore, prevention of these fractures is essential.

Postmenopausal women are a group at a particularly high risk for fractures (Cummings et al. 2002, Banks et al. 2009). Generally, the fracture risk is higher in women than in men, at least partly due to lower bone strength caused by smaller bone size (Seeman 2002). In addition, menopause accelerates the age-related deterioration of bone in women (Seeman 2002, Riggs et al. 2002). It has been estimated that almost half of the women 60 years or older sustain a bone fracture during their residual life-time (Nguyen et al. 2007).

At the population level, poor bone properties together with falls increase the risk of bone fracture (Kanis 2002, Lacroix et al. 2009, Sievänen et al. 2007, Järvinen et al. 2008). Areal bone mineral density is a widely used predictor of bone fractures and is used in osteoporosis diagnostics (Kanis 2002). Until recently, the research on osteoporosis has been focused on areal bone mineral density while other aspects of bone strength have received less attention. However, bone strength is a combination of several factors, such as material properties and micro- and macrostructure (van der Meulen et al. 2001, Currey 2002, Järvinen et al. 2005). Research on these aspects is important for a comprehensive understanding of bone as an organ whose main function is load-bearing.

In order to develop effective strategies for the prevention of bone fragility, detailed knowledge on the factors influencing bone properties is crucial. Genet-

ic research reveals the importance of genetic factors in determining differences between individuals and further increases knowledge on the regulation mechanisms at the cell level while the investigation of environmental factors indicates the importance of potentially modifiable factors and reveals targets for preventive actions.

This study focuses on the genetic and environmental influences on bone structural strength in postmenopausal women. The purpose was to estimate the relative proportions of genetic and environmental effects underlying bone structural strength. In addition, the association of two specific factors, hip fracture and postmenopausal hormone therapy, to bone strength and its determinants were investigated.

2 REVIEW OF THE LITERATURE

2.1 Bone biology and structure

The primary function of the skeleton is load-bearing. In addition, bones participate in mineral homeostasis and hematopoiesis (Parfitt et al. 2008). The human skeleton can be divided in two parts: the axial skeleton comprising the skull and spinal column, and the appendicular skeleton, which includes the limb bones and pelvic girdle. Bone tissue is dynamic tissue which undergoes constantly breakdown of old tissue and formation of new (Morgan et al. 2008).

2.1.1 Bone tissue

Bone tissue is composed of an organic and an inorganic phase. The organic phase consists of collagen, some other proteins, polysaccharides, living bone cells and blood vessels, whereas calcium phosphate makes up the inorganic phase. In addition, by weight, 10% of bone is water (Currey 2002, Morgan et al. 2008). Type I collagen is the most common form of collagen in bone and it makes up 80% of the protein in bone (Viguet-Carrin et al. 2006). Tropocollagen molecules consist of three polypeptides which are held together by hydrogen bonds and form a triple-helix structure. These molecules aggregate to form microfibrils which further bond to form larger entities, fibrils (Currey 2002). The collagen is filled and surrounded by plates of bone mineral, impure hydroxyapatite, which is one type calcium phosphate (Currey 2002, Morgan et al. 2008). The mineral plates tend to be oriented along the collagen fibrils (Currey 2002, Viguet-Carrin et al. 2006) and contribute substantially to the strength of bone (Bono & Einhorn 2003).

During the first years of life, woven bone, which is considered immature bone, is replaced by mature, lamellar bone. (Morgan et al. 2008) In lamellar bone, the collagen is arranged as sheets (lamellae) (Currey 2002). Lamellae can be found wrapped as large concentric rings in the outer part of a long bone shaft. Lamellar bone can also be arranged as Haversian systems, i.e. secondary

osteons, which are the results of replacing old bone with new. Haversian systems have a canal, the Haversian canal, that is surrounded by concentric lamellae and occupied by blood vessels and nerves. A Haversian system also has transverse canals, known as Volkmann's canals, for blood vessels (Morgan et al. 2008).

Bone cells make up 2% of the organic phase of bone (Morgan et al. 2008). There are four different types of mature bone cells: osteoblasts, osteoclasts, osteocytes and lining cells. *Osteoblasts* are cells that form new bone. They are able to produce and secrete proteins which form bone matrix. They also control the mineralization of the matrix. Osteoblasts originate from precursor cells in the bone marrow which may also differentiate to, e.g., muscle or fat cells (Manolagas 2000). Those osteoblasts, which are buried in the osteoid that they have formed, become osteocytes and other may become flattened lining cells (Martin & Seeman 2008). *Osteocytes* are the most abundant of the bone cells. They are former osteoblasts and are embedded in the bone tissue (Currey 2002). They send out tens of processes via canaliculi connecting with other osteocytes and lining cells. Osteocytes are currently thought to be responsible for mechanosensory function and perceiving damage in bone (Manolagas 2000, Currey 2002, Martin & Seeman 2008). *Osteoclasts* are multinucleated bone-resorbing cells. They derive from hematopoietic cells of the monocyte/macrophage lineage and are able to form an acidic environment, which dissolves bone mineral. Osteoclasts also secrete matrix metalloproteinases and cathepsins to break down the proteins of the matrix (Manolagas 2000, Morgan et al. 2008). *Bone lining cells* are former osteoblasts and they cover the surface of bone. The purpose of lining cells is believed to be the guidance of osteoclasts to the desired location and participation in making the bone surface suitable for osteoclast to attach to (Manolagas 2000).

Deposition of bone without previous bone resorption is called bone *modeling* whereas in *remodeling*, bone resorption precedes bone formation. Bone modeling and remodeling sculpt the contours and internal structure of bone to optimize bone properties to achieve light yet sufficiently strong structure (Seeman & Delmas 2006, Martin & Seeman 2008). Modeling and remodeling take place in endocortical, intracortical and trabecular components and to lesser extent in the periosteum (Seeman & Delmas 2006). Modeling occurs mainly during growth and it changes bone shape and increases bone size according to genetic regulation and also to obtain the optimal shape of bone for load-bearing (Martin & Seeman 2008). The main purpose of bone remodeling is to prevent impairment of the main function of bone: load-bearing. Bone that is damaged by micro cracks has to be removed and replaced by new undamaged bone so that the load-bearing function will not be threatened (Parfitt 2002, Martin & Seeman 2008). Bone remodeling also participates in the regulation of plasma calcium homeostasis (Parfitt 2002).

Mostly, bone is in a quiescent state. However, part of the bone surface is constantly under remodeling (Morgan et al. 2008). Bone remodeling is performed by basic multicellular units (BMUs) which are composed of a group of

osteoclasts, a group of osteoblasts, blood supply and associated connective tissue (Parfitt 2002). In remodeling, bone resorption and formation are tightly coupled: bone resorption by osteoclasts is followed by the formation of new bone of a comparable amount by osteoblasts (Pogoda et al. 2005, Martin & Seeman 2008). This coupling is caused by osteoblast and osteoclasts influencing the differentiation and activity of each other. An important link between osteoblasts and osteoclasts is the receptor activator of NF-κB (RANK) in osteoclasts and its ligand (RANKL), which is secreted by osteoblast lineage cells. Binding of RANKL to RANK is required for osteoclast differentiation. Osteoprotegerin, which is also secreted by osteoblasts, inhibits the action of RANKL. Osteoblast lineage cells can thus promote or inhibit the formation of mature osteoclasts (Robling et al. 2006, Morgan et al. 2008).

Remodeling takes place at several points simultaneously, and at any moment, about one million BMUs are working. In a remodeling cycle, osteocyte death is thought to be a signal to bone-lining cells of bone damage. Bone-lining cells can transmit this information for the purpose of recruiting precursor cells from the bone marrow, blood or bone remodeling compartment needed for remodeling (Martin & Seeman 2008). Mature osteoclasts resorb damaged bone by excavating tunnels in cortical bone and trenches on the surface of trabecular bone (Manolagas 2000, Martin & Seeman 2008). Within one to two weeks after the completion of resorption, osteoblasts start filling the resorbed volume with osteoid (Morgan et al. 2008, Martin & Seeman 2008). The resorption phase takes only two to three weeks whereas the formation phase lasts two to three months. At the time of deposition, the new bone material, osteoid, undergoes a rapid primary mineralization, with secondary mineralization occuring during the succeeding months or even years (Martin & Seeman 2008). Since the secondary mineralization time is long, the adaptation of bone to different stimuli is slow.

2.1.2 Structure of long bones

Most of the limb bones are long bones also known as tubular bones. Since the function of bones determines their structure, long bones as load-bearing bones are thick-walled but hollow. This is the optimal shape because long bones need to be stiff and strong enough to withstand large bending moments yet light enough for efficient locomotion (Currey 2002).

Longitudinally, long bones can be divided into three parts. The diaphysis is the central part of the bone. The expanded ends of the bones are called epiphyses and they are separated from the rest of the bone by growth plates, or physes, which are responsible for the longitudinal growth of bone (Schoenau et al. 2004, Morgan et al. 2008). The area between the physis and diaphysis is the metaphysis (Morgan et al. 2008). Bones consist of an outer or periosteal and inner or endosteal envelope (Figure 1). The endosteal envelope can be divided into intracortical, endocortical and trabecular components. On these surfaces, bone resorption and formation takes place (Seeman 2007). The endosteum lines the inner surface of bone and consists of osteoblasts and lining cells. The periosteum consists of two layers: the outer layer is fibrous connective tissue and the

inner layer harbours osteoblast and chondrocyte progenitors (Morgan et al. 2008). The periosteum enables the increase in diameter during growth and aging (Seeman 2007, Morgan et al. 2008).

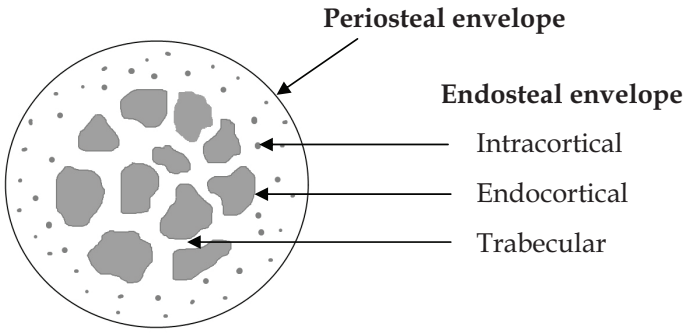

FIGURE 1 Schematic picture of the cross-section of the lower leg showing the envelopes of bone. The periosteal envelope is the outer surface whereas the endosteal envelope, including intracortical, endocortical and trabecular compartments, comprises the rest of the bone. Adapted from Seeman 2002.

The diaphysis of long bone is composed almost entirely of cortical bone in order to resist high bending and torsional loads (Bono & Einhorn 2003). Cortical bone is dense. Its porosity ranges between 5 to 20 % and is mainly due to Haversian and Volkmann's canals. The epi- and metaphyses also have a thin cortical shell, but these regions are mainly composed of trabecular bone (Morgan et al. 2008). Trabecular bone consists of plate- and rod-shaped trabeculae that are from 50 to 300 µm thick. The spaces between the trabeculae are from 100 to 500 µm and filled with bone marrow. The porosity of trabecular bone can be from 40 to 95% (Morgan et al. 2008). The structure of trabecular bone is good in resisting compressive loads (Bono & Einhorn 2003).

2.1.3 Mechanical properties of bone

The mechanical properties of whole bones are determined both by material properties and bone structure. The material properties of bone can be described by a stress-strain curve, and the behavior of bone structure by a load-deformation curve (Figure 2). These curves describe how a material or structure undergoes deformation under a given load. Strain is defined as the proportional change in length and stress as the force per area unit (Currey 2002). The modulus of elasticity is a measure of the material's resistance to deformation under the applied stress. It can be calculated using the slope of the stress-strain curve. In structural properties, the corresponding parameter is stiffness. The linear part of the curves represents the elastic area, in which the deformation caused is

reversible when the stress or load is restored to 0. Beyond the linear area, more specifically after the yield point, deformations are irreversible, i.e. plastic (van der Meulen et al. 2001). Tough material has a reasonable amount of post yield deformation, which absorbs a lot of energy before breaking, whereas brittle material breaks without post yield deformation (Currey 2002). In the case of structural properties, strength is the ultimate load needed to break the whole structure (van der Meulen et al. 2001).

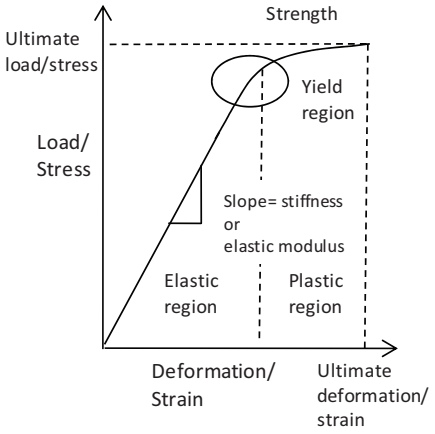

FIGURE 2 Stress-strain and load-deformation curve. Adapted from Borer 2005.

The material properties of bone are influenced by material composition, mainly bone mineralization and collagen content, and other factors such as the microstructural organization of material, collagen fiber orientation and collagen cross-linking (van der Meulen et al. 2001). Basically, collagen determines the ability of bone to absorb energy, i.e. toughness, whereas the mineral provides the stiffness (Viguet-Carrin et al. 2006).

The macrostructure of bone also influences greatly whole bone strength. In compression, cross-sectional area is an important parameter since it is directly proportional to strength (Martin 1991, Currey 2001). Areal and polar moment of inertia reflect the distribution of material on a cross-section. They increase exponentially as the material is distributed farther away from the neutral axis or the center of mass, respectively (Morgan et al. 2008). The section modulus, which describes bending strength, is directly proportional to the moment of inertia and inversely proportional to the maximum distance to the outer border (Beck 2007). Although a larger diameter increases the section modulus, increasing the diameter with the same amount of material results eventually in thinner walls, which increases the risk of buckling (Currey 2002). In addition, it should be noticed that bone is anisotropic. This means that the mechanical properties of bone depend on the loading direction and, owing to its main function i.e. load-bearing, bone tends to be strongest in the direction of primary loading (Bouxsein 2008).

2.1.4 Non-invasive methods of measuring bone mineral density, structure and strength

*Dual-energy x-ray absorptiometry (*DXA) measures the relative absorption of the dual-energy x-ray spectrum in tissues. (Griffith & Genant 2008) For measuring aBMD, two different x-ray energies and information on the fat-to-lean ratio adjacent to bone are used to differentiate bone-related absorption from soft tissue-related absorption (Ellis 2000, Beck 2007). DXA is a planar imaging technique and its density parameter aBMD gives the mass of bone mineral on a projectional area unit (g/cm^2) (Griffith & Genant 2008). In adults, DXA-derived aBMD relative to the mean aBMD of young adults is used for the diagnosis of osteoporosis (Anonymous 2003, Binkley et al. 2008). Several axial and appendicular bone sites can be scanned, although the lumbar spine and hip are the most commonly measured sites (Schoenau et al. 2004, Griffith & Genant 2008). Mathematical models have been developed to extract bone structural parameters, such as bone cross-sectional area, cross-sectional moment of inertia and section modulus, from two-dimensional DXA data (Beck 2007). Also, determination of lean and fat mass is possible using DXA (Griffith & Genant 2008). The advantages of DXA are low radiation dose (a few μSv), good accessibility (Griffith & Genant 2008, Binkley et al. 2008) and good precision (Sievänen et al. 1992, Sievänen et al. 1996a). On the other hand, the limitations of DXA are its inability to separate cortical and trabecular bone and a strong effect of bone size on aBMD results (Sievänen 2000, Griffith & Genant 2008, Binkley et al. 2008), let alone the compromised accuracy due to soft tissue disparities within the measured bone sites (Bolotin et al. 2003). Futher, due to the planar nature of DXA, sructural parameters are very sensitive to changes in positioning and they can be estimated only on the image plane (Sievänen 2000, Sievänen et al. 2007, Beck 2007).

Quantitative computed tomography (QCT), the attenuation of x-rays passing through the body at various angles in the horizontal plane is measured and algorithms are then used to construct a cross-sectional image from this attenuation information, with each pixel containing information on the density of the tissue of interest (Ellis 2000). Peripheral quantitative computed tomography (pQCT) is a device for measuring bone properties in peripheral bone sites, usually the forearm and lower leg (Binkley et al. 2008), whereas QCT is mainly used in measuring volumetric bone mineral density (vBMD) in the vertebrae (Adams 2009). The radiation dose in pQCT is considerably lower than in QCT and is usually less than 3 μSv compared to tens or hundreds of μSv in QCT (Griffith & Genant 2008, Binkley et al. 2008, Adams 2009). pQCT enables the analysis of trabecular and cortical bone separately except in sites with very thin cortices relative to resolution (Schoenau et al. 2004, Binkley et al. 2008). pQCT can be used in determining vBMD and structural parameters such as cross-sectional area and cortical thickness. Also, various strength indices, such as the stress-strain index, moments of inertia and section modulus have been calculated based on pQCT data (Sievänen et al. 1998, Schoenau et al. 2004, Binkley et al. 2008). pQCT also enables determination of muscle and fat cross-sectional

areas (Schoenau et al. 2004, Adams 2009). pQCT is a precise method for measuring bone properties: coefficient of variation ranges from less than 1% to 8% (Sievänen et al. 1998). Further advantages of pQCT devices, compared to standard CT, are the small size of the device and low costs (Griffith & Genant 2008). High-resolution QCT or pQCT can be used to determine trabecular structures (Genant et al. 2008).

Magnetic resonance imaging (MRI) can be used for measuring bone macrostructure. Using a high-resolution MRI, trabecular parameters, such as trabecular spacing and thickness, can be assessed. Micro-MRI may yield trabecular parameters similar to histological parameters. The advantage of MRI is the absence of ionizing radiation (Griffith & Genant 2008).

Quantitative ultrasound (QUS) techniques measure ultrasonic wave propagation in bone. Speed of sound is a measure of ultrasonic velocity (m/s) in bone. It is affected by the density, micro- and macrostructure and elastic modulus of bone. Ultrasound attenuation in bone reflects frequency dependence of signal attenuation (dB/MHz). QUS devices are usually site-specific but multi-site devices also exist (Binkley et al. 2008, Guglielmi et al. 2009). The advantages of QUS are the absence of ionizing radiation, and portable scanners. However, the use of QUS techniques is limited because QUS variables reflect the sum of various properties of bone and are not thus unequivocal (Binkley et al. 2008).

2.2 Development and aging of the skeleton

During growth, long bones increase their length through a process that takes place in the growth plates at the bone ends. First, cartilage tissue is formed which is then transformed into bone tissue in the adjacent metaphyseal bone region (Schoenau et al. 2004, Riggs et al. 2008). Simultaneously, cross-sectional shape changes when periosteal apposition deposits bone on the outer surface while endocortical resorption simultaneously enlarges the marrow cavity. The cortex thickens, however, since periosteal apposition is greater than endocortical resorption (Wang et al. 2005, Seeman 2008). Longitudinal growth is terminated by the closure of the epiphyseal plates at the end of puberty. In women, earlier termination of longitudinal growth and lower rate of periosteal apposition results in smaller bones than in men (Riggs et al. 2002, Seeman 2008). After the cessation of longitudinal growth, bones continue to gain mass and size by periosteal apposition and possibly by trabecular thickening. Women reach their peak bone mass around the end of the second or third decade of life (Riggs et al. 2002).

After completion of growth, the rate of remodeling slows down (Martin & Seeman 2008). In young adulthood the amount of bone resorbed begins to exceed the amount of bone formed in each BMU due to decline in bone formation. This creates a negative BMU balance resulting in continuous net bone loss (Seeman 2008). In young adulthood, this is not very deleterious since the remodeling rate is low and the loss affects trabecular thickness rather than con-

nectivity. Also, bone loss is compensated by an increase in bone diameter by periosteal apposition, which improves bone strength (Martin & Seeman 2008). Before age 50, 30 to 40% of the total lifetime trabecular bone loss has taken place, whereas cortical bone loss is very small before that age (Riggs et al. 2008, Seeman 2008).

At menopause, bone loss accelerates temporarily for 4 to 8 years. This is due to increase in the rate of remodeling together with the negative BMU balance (Martin & Seeman 2008, Riggs et al. 2008). It has been suggested that the temporary rapid decline in bone mass and its reversal is caused by a temporary, large imbalance in the number of acting bone formation and resorbing units. The high remodeling rate increases first the amount of bone resorption, which is not balanced until bone formation by the same BMUs begins during the next years. The lag in the mineralization of new bone also contributes to the rapid net loss (Martin & Seeman 2008). According to cross-sectional studies using bone biopsies (Han et al. 1996) and high-resolution pQCT (Khosla et al. 2006), older women lose their trabecular bone by a complete removal of trabecular elements rather than by trabecular thinning. Also, the porosity of cortical bone increases with aging (Han et al. 1996, Cooper et al. 2007).

Longitudinal studies have suggested that in premenopausal women, endocortical resorption is accompanied with periosteal apposition. This results in the cortex being placed farther from the bone center and becoming thinner. Due to this displacement, no loss in whole bone bending strength indices before menopause has been found in previous studies (Szulc et al. 2006, Lauretani et al. 2008). However, it seems that although there is some amount of periosteal apposition after menopause, it is unable to offset the effect of increased bone resorption and consequently, bone strength declines (Ahlborg et al. 2003, Szulc et al. 2006).

2.3 Genetic influences on bone properties

The basic unit for inheritance is a gene. A gene is a section of deoxyribonucleic acid (DNA) and it encodes a protein. DNA is packed into chromosomes which are located in the cell nucleus. A gene may have different forms, known as alleles, which may have different expression. A trait may be determined by a single gene or several genes (Lewin 1997). Quantitative genetic methods can be used to investigate the relative proportions of genetic and environmental effects on the differences between individuals in different traits. Such methods can be applied to family, twin or adoption data (Rijsdijk & Sham 2002).

2.3.1 Quantitative genetic method in twins

Quantitative genetic method can be applied on twin data consisting of monozygotic (MZ) and dizygotic (DZ) twin pairs. In this method, individual differences, i.e. variation in a trait, is considered to arise from four sources: additive

genetic effects (A), non-additive genetic effects i.e. dominance (D), shared environmental effects (C) and individual environmental effects (E). A refers to the sum of the effects of the individual alleles, whereas D refers to interactions between alleles of the same or different genes. C includes factors that are shared by both twins, such as those related to their childhood environment, and E consists of exposures that are not shared by the co-twins, such as diseases and accidents that have affected only one sibling (Rijsdijk & Sham 2002, Posthuma et al. 2003).

MZ co-twins have identical genes, which means that the correlations for both A and D is 1 (Figure 3). In DZ co-twins these correlations are 0,5 and 0,25, respectively, since DZ co-twins share on average half of their genes as do ordinary siblings. The correlations of C and E are similar in both MZ and DZ co-twins: 1 for C and 0 for E. E also contains measurement error (Rijsdijk & Sham 2002). Using these correlations and the observed variance and co-variance between co-twins in a trait, structural equation modeling can be used to estimate the effects of latent factors A, D, C and E on a trait as regression coefficients (Rijsdijk & Sham 2002, Boomsma et al. 2002).

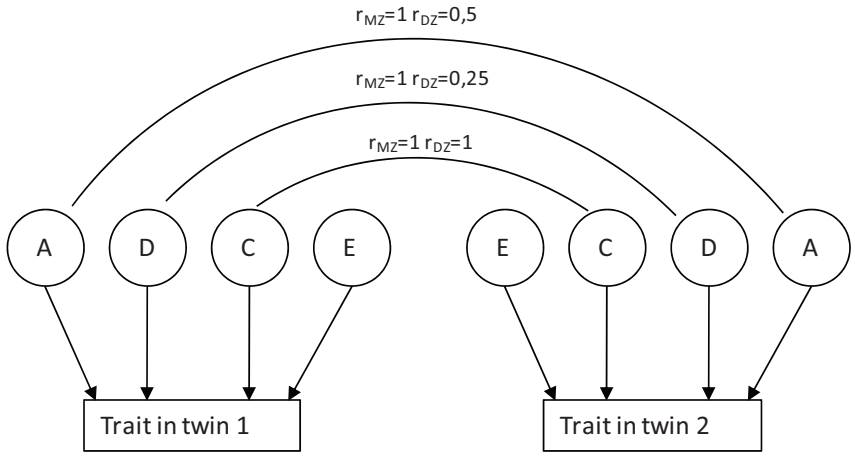

FIGURE 3 Correlations between monozygotic (r_{MZ}) and dizygotic (r_{DZ}) co-twins in quantitative genetic models. A, additive genetic effects; D, dominant genetic effects; C, shared environmental effects; E, individual environmental effects. Adapted from Rijsdijk & Sham (2002).

Multivariate quantitative genetic models reveal to what extent the traits of interest are influenced by the same and to what extent by different genetic and environmental factors (Posthuma et al. 2003). In multivariate models, the observed covariance between two or more traits is decomposed into genetic and environmental components using information on the cross-twin cross-trait correlations in MZ and DZ twins (Boomsma et al. 2002).

An advantage of twin data over family data is that the effects of genes and shared environment can be discriminated. However, the classical twin method includes several assumptions that should be met. First, MZ co-twins are as-

sumed to share their environment to same extent as DZ co-twins. Second, it assumes that genetic factors do not influence environmental factors and vice versa. This leads to the assumption that there is no assortative mating with respect to the studied trait meaning that people would choose a partner who is phenotypically similar to themselves. Further, the method assumes that genetic factors do not influence which environments people select themselves into. Finally, the proportion of gene-environment interactions is assumed to be minimal. Violation of these assumptions leads to over- or underestimation of the effects of the different latent factors A, D, C or E. Also, with respect to the generalizability of the results, it is important that twins do not differ from the general population in the trait that is being studied (Rijsdijk & Sham 2002).

2.3.2 Heritability of bone properties

The heritability of aBMD has been studied rather widely in nuclear families, pedigrees and also in twins. In general, heritability estimates of DXA-derived aBMD in different bone sites vary between 60 and 83% (Howard et al. 1998, Harris et al. 1998, Hunter et al. 2001, Yang et al. 2005, Deng et al. 2006, Yang et al. 2006, Ng et al. 2006, Videman et al. 2007, Zhang et al. 2008a, Sigurdsson et al. 2008). The heritability of vBMD has been little studied. Based on a study by Lenchik et al. (2004), its heritability may be similar to that of aBMD, approximately 70% (Lenchik et al. 2004). Heritability estimates of QUS parameters vary between 19 and 74%(Howard et al. 1998, Knapp et al. 2003, Lee et al. 2004). The heritability of bone properties may not be similar between the sexes. However, the results concerning heritability in men and women are contradictory: similar, higher and lower heritability estimates have been obtained for women than for men in different pedigree and family studies (Yang et al. 2006, Ng et al. 2006, Zhang et al. 2008a). Further, the heritability of bone loss seem to be lower (from 42 to 57%) than that of aBMD (Zhai et al. 2009). In line with this observation it has been reported that the heritability of aBMD is lower in postmenopausal women than premenopausal women (Hunter et al. 2001).

Bone structure may be less heritable than aBMD. Heritability estimates of DXA-derived bone size have varied between 46 and 83%, and they have been rather consistently lower than heritability estimates of aBMD in the same studies (Yang et al. 2005, Ng et al. 2006). Only a few studies have investigated the proportion of genetic effects on other structural bone parameters or estimated bone strength. In a pedigree study with 4489 individuals, heritability of DXA-derived femoral neck width, cross-sectional area, cortical thickness, section modulus and buckling ratio ranged from 50 to 60% (Sun et al. 2006). In a nuclear family study, the same estimates ranged from 41 to 67% (Xu et al. 2006).

Research on heritability in different bone sites has also focused on aBMD. However, there seems to be no consistent pattern in the studies reporting upper and lower limb bone heritability estimates. Some studies have obtained lower heritability estimates for upper limb aBMD (53 to 63%) than lower limb aBMD (over 70%) (Knapp et al. 2003) whereas other studies have reported rather similar heritabilites for upper and lower limb aBMD (Hunter et al. 2001, Zhang et al.

2008a). Also, the heritability of lumbar spine aBMD seems to vary from lower to higher than the heritability of hip aBMD in different studies (Seeman et al. 1996, Howard et al. 1998, Harris et al. 1998, Hunter et al. 2001, Yang et al. 2005, Deng et al. 2006, Yang et al. 2006, Videman et al. 2007, Sigurdsson et al. 2008).

Only a few studies have investigated the common background of bone properties in different bone sites as well as between bone and other tissues. These studies have reported that the association between different bone sites derives from both genetic and environmental factors (Howard et al. 1998, Nguyen et al. 1998, Yang et al. 2006). It has also been suggested that bone properties share genetic effects with fat mass (Nguyen et al. 1998) and lean mass (Seeman et al. 1996, Sun et al. 2006).

According to the widely accepted view, bone properties are polygenic traits, i.e. they are influenced by multiple genes with the majority making a modest contribution to the trait (Peacock et al. 2002, Shen et al. 2005). Accordingly, a large number of genes have been suggested to be associated with aBMD (Shen et al. 2005, Huang & Kung 2006). However, the majority of gene identification studies have been underpowered and therefore definitive conclusions on the importance of candidate genes have been lacking (Shen et al. 2005).

2.4 Environmental influences on bone properties

Although the contribution of genetic effects to bone properties appears to dominate, bone properties are also influenced by multiple environmental factors. Mechanical loading is considered as the most important environmental factor modifying postnatal bone but also factors such as previous fractures, hormone therapy (HT) and nutrition influence bone mineral mass.

2.4.1 Mechanical loading

Harold Frost's mechanostat theory describes the adaptation of bone to *strains* caused by mechanical loading. According to the theory, bone adapts its mechanical properties to keep the highest habitual loads to which it is subjected to within a safe range. If bone is subjected to higher than normal strain, it improves its properties until the same load induces strain that is within the safe range. Similarly, if the customary peak strains decrease, as happens in disuse, bone adapts to the prevailing environment by decreasing its excessive structural rigidity through the process of remodeling. The strain magnitudes at which increases and decreases in rigidity are initiated are thought to be set by genes (Frost 2003). Strain thresholds at which osteogenesis is initiated have been observed to vary between bone sites according to their typical strain level (Hsieh et al. 2001).

However, the osteogenic effect of loading is not dependent only on strain magnitude, but on other aspects of strain also. According to the first of the three rules for bone adaptation by Turner (1998), bone adapts to dynamic rather than

static loads. Secondly, a stimulus of short duration is adequate for bone response and longer duration gives only little benefit. Thirdly, osteogenic effects can also be obtained with unusual loading pattern. This means that the stresses should be oriented in different directions than in the customary loading. Further, strain frequency influences bone response: bone properties can be enhanced also by low-magnitude but high frequency strains (Turner 1998). Higher rates of change in strain increase bone formation more than lower rates (Mosley & Lanyon 1998).

The largest customary loads on bone derive from *muscle* contractions, and thus muscle-induced loads are considered as a major regulator of bone properties (Currey 2002). This is supported by studies indicating that bone and muscle properties are strongly associated (Sievänen et al. 1996b, Ferretti et al. 1998, Rittweger et al. 2000, Schoenau et al. 2002, Lauretani et al. 2006, Rantalainen et al. 2008). More specifically, muscle cross-sectional area is a determinant of muscle force (Jones et al. 2008), and it seems that muscle cross-sectional area is associated with bone cross-sectional area and strength but not with volumetric BMD (Rittweger et al. 2000, Heinonen et al. 2001, Lauretani et al. 2006, Daly et al. 2004). However, it may be that these associations cannot be interpreted merely as the influence of muscle force on bone traits, since a few earlier studies suggest that lean mass and bone properties also are related through common genes (Seeman et al. 1996, Nordström & Lorentzon 1999, Sun et al. 2006).

According to meta-analyses, both pre- and post menopausal women benefit from exercise programs that contain resistance, weight-bearing or impact exercises, although the effect on aBMD remains small, less than 2% (Wolff et al. 1999, Kelley et al. 2001). Some studies have shown that impact exercise improves cortical structure, leading to higher bone strength in both pre- and postmenopausal women (Cheng et al. 2002, Uusi-Rasi et al. 2003b, Vainionpää et al. 2005, Vainionpää et al. 2007). The response of bone macrostructure to exercise seem to be associated with the number of high impacts at least in premenopausal women (Vainionpää et al. 2007). In postmenopausal women, even low-intensity exercise, such as walking, may have a small positive effect on lower limb aBMD (Martyn-St James & Carroll 2008). However, bone in postmenopausal women may undergo adaptations to exercise in structure rather than bone mass (Kaptoge et al. 2003, Adami et al. 1999), and therefore exercise may induce larger increases in bone strength than could be concluded from aBMD. However, a meta-analysis based on four randomized controlled trials showed that resistance or impact exercise lasting 12 months or less has no effect on bone structural strength in postmenopausal women (Nikander et al. 2010). This may be due to the duration of the interventions since it has been suggested that longer duration of exercise is required to show positive changes in the skeletons of postmenopausal women (Zehnacker & Bemis-Dougherty 2007). Also in individuals with low bone mineral density, strength and aerobic training, which include weight-bearing exercises, have been found to be beneficial for bone mineral mass (de Kam et al. 2009). In this group, impact exercise may also reduce fall-related fractures (Korpelainen et al. 2006).

According to the mechanostat theory, decrease in strain, as happens with *disuse*, leads to decline in bone strength (Frost 2003). In bed rest experiments, a rapid decline in bone mass has been observed in young, healthy subjects (Shackelford et al. 2004, Rittweger et al. 2005, Berg et al. 2007, Rittweger et al. 2009). In only 35 days, bed rest results in a 0.5 to 3% decline in tibial bone mineral content (BMC). This loss appears to be mainly of cortical origin: the relative bone mineral loss in the cortex may be as high as 17% (Rittweger et al. 2009). Resistance or flywheel exercise may attenuate bone loss but only partly (Shackelford et al. 2004, Rittweger et al. 2005). Recovery after bed rest takes many times longer than the bone loss period although the initial rate of bone mineral accrual during recovery is rapid (Rittweger & Felsenberg 2009). It seems that besides immobilization, reduction in limb weight-bearing also leads to significant bone loss in less than a month (Rittweger et al. 2006).

2.4.2 Fractures

It has been shown that a previous fracture increases the risk of a new fracture (van Staa et al. 2002, Chapurlat et al. 2003, Colon-Emeric et al. 2003). This is likely to be related to impaired bone properties after an injury, since bone fracture and other major injuries lead to decline in aBMD in the injured extremity. After a tibial fracture, significant bone loss in the ipsilateral trochanter is observed already 2 months after the fracture and the decline seems to be steep during the first three to six months post fracture (Magnusson et al. 2001, Veitch et al. 2006). During the first six months after the fracture, the concentrations of biochemical bone markers, which reflect the bone formation and resorption of the entire skeleton, are clearly elevated (Åkesson et al. 2005, Veitch et al. 2006). A hip fracture appears to induce a similar amount of bone loss, 10 to 20%, as a tibial fracture in the fractured limb during the first year post-fracture (Zerahn et al. 1998, Magnusson et al. 2001). Some recovery of bone mass may take place after the first year following fracture (van der Poest Clement et al. 1999), but according to cross-sectional studies comparing aBMD between the fractured and non-fractured leg a decade after the trauma, some proportion of bone loss is usually permanent (Kannus et al. 1994a, Kannus et al. 1994b, Leppälä et al. 1999b).

The effect of fracture on cortical and trabecular bone properties has been little studied. A cross-sectional study in 10 subjects 2 years after femoral shaft fracture found no significant side-to-side differences in tibial cortical thickness or density (Bråten et al. 1992). However, another study in 12 subjects with a previous femoral shaft fracture found the cortex in the tibia of the fractured leg to be 2 % thinner (Terjesen et al. 1985). Trabecular bone mineral density may respond more to fracture than cortical density. A larger deficit in trabecular density than in cortical density in the fractured leg has been found 24 weeks (Veitch et al. 2006) and 65 weeks (Findlay et al. 2002) after a tibial fracture.

Bone loss occurs not only on the ipsilateral side, but also in the contralateral leg. However, this loss is considerably smaller than that on the ipsilateral side (Petersen et al. 1997, van der Poest Clement et al. 1999), but larger than the normal age-related loss (Magaziner et al. 2006). During the first year after a tibi-

al fracture, bone loss on the contralateral femoral neck was found to be 2,5 % while on the ipsilateral side the loss was 5% (van der Poest Clement et al. 1999).

It has been suggested that there are three causes of the post-traumatic bone loss. First, the injury itself and second, injury-related surgical treatment induces catabolic reactions. Third, disuse of the injured limb contributes to bone loss (Järvinen & Kannus 1997). The role of reduced use is supported by the observation that lean mass declines after a fracture (Karlsson et al. 1996, Fox et al. 2000, Magnusson et al. 2001). Bone loss after a fracture has also been shown to be associated with the duration of unloading (Van der Wiel et al. 1994) and immobilization time (Kannus et al. 1994b). However, a more important factor than the period of total immobilization may be disuse in the long-term after fracture since fracture-induced bone loss has found to be larger in individuals with a lower level of function or muscle strength (Kannus et al. 1994b, Leppälä et al. 1999c).

In addition to the use of the injured limb, several other factors may modify bone loss or recovery after an injury. A more severe injury may lead to greater bone loss while the treatment used may also contribute to the degree of bone loss (Leppälä et al. 1999a, Veitch et al. 2006). Also, increasing age and smoking have found to have a negative association with the recovery of aBMD (Leppälä et al. 1999c).

2.4.3 Postmenopausal hormone therapy

Among other hormones, estrogen plays an important role in regulating bone properties in both sexes. Generally, estrogen is considered to preserve bone mineral mass. It suppresses bone remodeling by influencing both osteoblasts and osteoclasts (Riggs et al. 2002). At puberty, estrogen contributes to the termination of longitudinal growth of appendicular bones and is thought to restrict periosteal apposition (Seeman 2004). Moreover, in pubertal girls estrogen is related to the accrual of bone mineral endocortically (Seeman 2004, Wang et al. 2006). The withdrawal of estrogen at menopause induces rise in the remodeling rate and consequently a phase of rapid bone mineral loss (Riggs et al. 2002, Martin & Seeman 2008).

Exogenous estrogen can be used as postmenopausal hormone therapy (HT) to alleviate the symptoms, most frequently vasomotor symptoms, related to menopause-induced estrogen deficiency. In HT, estrogen can be accompanied by progestin in non-hysterectomized women. Also tibolone, which has weak estrogenic, progestinic and androgenic actions, can be used as HT (Garefalakis & Hickey 2008).

HT has previously been used in the treatment of osteoporosis but due to increased risk of cardiovascular problems and breast cancer observed in randomized controlled trials (RCTs), its use for osteoporosis has decreased (McGowan & Stefanik 2008). However, HT has beneficial effects on bone fractures. During a few years of use, HT decreases fracture risk by 25 to 45% (Cauley et al. 2003, Cummings et al. 2008). HT is able to increase aBMD and the beneficial effect seems to increase with the duration of use (Cauley et al. 2003,

Cummings et al. 2008, McGowan & Stefanik 2008). A meta-analysis of RCTs has shown that the two-year effect of HT on aBMD is 4 to 7 %, varying between different bone sites (Wells et al. 2002). RCTs have also shown that HT improves bone macrostructure and strength (Cheng et al. 2002, Greenspan et al. 2005).

The results of observational studies using DXA technology have suggested that hormone therapy prevents endocortical resorption but may simultaneously restrict periosteal apposition (Beck et al. 2001, Szulc et al. 2006). Restriction of periosteal apposition would be disadvantageous for bone bending strength. More complex relationships between estrogen and periosteal apposition have also been proposed, including modificatory influences of age or hormone concentration on the relationship (Vanderschueren et al. 2006). Furthermore, estrogen and its receptors have been proposed to occupy a central role in the mechanosensory system, suggesting that estrogen enables exercise-induced changes in the skeleton (Lanyon & Skerry 2001). However, an opposing view considers estrogen to serve purely reproductive purposes in the skeleton. This means that estrogen induces accrual of bone mineral to the skeleton at puberty, which is unpacked at menopause as the reproductive period ends (Järvinen et al. 2003).

2.4.4 Other environmental factors

Calcium is a building material of the skeleton. It has been shown that calcium alone and in combination with vitamin D prevents bone loss in persons over 50 years of age. It also prevents fractures and the effect is most prominent in those with low baseline calcium intake (Tang et al. 2007). The effect of calcium supplementation in healthy children has been questioned (Winzenberg et al. 2006) but calcium supplementation may increase bone mass in children who have low baseline calcium intake (Huncharek et al. 2008).

Vitamin D has several roles in calcium homeostasis. For example, it promotes the intestinal absorption of calcium (Rizzoli 2008). In elderly persons, vitamin D deficiency may lead to increase in bone loss via hyperparathyroidism (Bouillon & Reid 2008). In this age group, vitamin D seems to be effective in fracture prevention (Bischoff-Ferrari et al. 2005). Besides calcium and vitamin D, other nutritional factors, such as protein intake, various vitamins and minerals, also affect bone properties (Rizzoli 2008, Tucker 2009).

Bone properties seem to be negatively influenced by smoking and heavy alcohol use (Law & Hackshaw 1997). Also a large number of diseases, e.g. rheumatoid arthritis, and drugs, e.g. glucocorticoids, have adverse influences on bone (Lowe & Shane 2008).

2.5 Summary of the literature

The skeleton is an organ whose primary function is load-bearing (Currey 2002, Bouxsein 2008). Although bone turnover is slow, groups of bone cells are constantly replacing old bone with new in concerted fashion in order to improve

the quality of the bone material and to reshape the bone structure. Aging has an adverse influence on the balance between the removal of old bone and deposition of new bone, leading to constant net bone loss (Martin & Seeman 2008). This can be seen as declining bone mineral density and deteriorating bone structure, leading to decline in bone strength with aging (Russo et al. 2003, Lauretani et al. 2008, Martin & Seeman 2008). In women, menopause further accelerates the increasing fragility of bone, rendering postmenopausal women especially susceptible to bone fractures (Cummings & Melton 2002, Riggs et al. 2002, Banks et al. 2009,).

A large body of knowledge exists on the influence of various factors on aBMD. Although genes appear to determine the majority of the individual differences in aBMD (Hunter et al. 2001, Yang et al. 2006, Ng et al. 2006) multiple environmental factors also have an effect on aBMD. Of the environmental factors, mechanical loading is considered to be one of the most important ones (Currey 2002, Frost 2003). It has been shown that physical activity is beneficial for aBMD (Wolff et al. 1999, Borer 2005, Uusi-Rasi et al. 2008) whereas disuse leads to a decline in aBMD (Rittweger et al. 2005, Berg et al. 2007). Also injury, such as a fracture, results in bone mineral loss in the affected limb (Kannus et al. 1994a, Zerahn et al. 1998). In postmenopausal women, HT is an agent which improves bone properties (Cauley et al. 2003, Greenspan et al. 2005). Further, aBMD is also influenced by various nutritional agents (Tucker 2009). However, aBMD alone cannot reflect the multifaceted nature of bone strength and therefore it is important to search for information also on bone structure and bone strength. Compared to aBMD, to date rather few studies have been conducted to investigate the influence of genetic and environmental factors on bone structure or bone strength.

3 PURPOSE OF THE STUDY

The objective of this thesis was to investigate the contributions of genetic and environmental factors to estimated bone structural strength in postmenopausal women. Specifically, the associations of hip fracture history and postmenopausal hormone therapy to bone structural strength were studied. In detail, the aims were:

1. To determine the relative contributions of genetic and environmental influences on bone structural strength (studies I and II)
2. To examine to what extent bone structural strength in epiphyseal bone sites share genetic and environmental influences (study I)
3. To examine whether the association between muscle cross-sectional area and bone structural strength has its origin in common genetic or common environmental effects (study II)
4. To evaluate the association of hip fracture history with tibial bone structural strength and its determinants (study III)
5. To estimate the association of long-term postmenopausal hormone therapy with bone structural strength and its determinants (study IV)
6. To evaluate whether loading environment modifies
    a. the heritability of bone structural strength (study I)
    b. bone structural strength after hip fracture (study III)
    c. the association of HT with bone structural strength (study IV)

4 MATERIAL AND METHODS

This thesis consists of data from three different datasets gathered in the Department of Health Sciences, University of Jyväskylä. All the analyses use cross-sectional data.

Using a classical twin design and the data from the Finnish Twin Study on Aging (FITSA), we aimed at estimating the relative proportions of genetic and environmental influences on bone strength (Study I & II). A case-control design was applied to the Asymmetry dataset to investigate the association between hip fracture history and bone strength (Study III). Further, using a co-twin control design and the FITSA and SAWEs datasets, the association of HT to bone strength was studied (Study IV). Co-twin control design is a very powerful design since the genetic effects on a trait can be controlled for (Duffy 2000).

TABLE 1 Datasets, designs and numbers of participants in the different studies

Study	Dataset	Design	N
I	FITSA	Classical twin design	217 twin pairs 103 MZ 114DZ
II	FITSA	Classical twin design	215 twin pairs 102 MZ 113 DZ
III	Asymmetry	Case-control design	38 with hip fracture history 22 controls
IV	FITSA and SAWEs	Co-twin control design	24 MZ twin pairs

MZ, monozygotic; DZ, dizygotic; HT, hormone therapy

4.1 Study subjects

The first dataset, FITSA, was originally collected to investigate the genetic and environmental influences on the disablement process in older women. The participants were recruited from the nationwide Finnish Twin Cohort which comprises all same-sex twin pairs born before 1958 and with both co-twins alive in 1975 (N=13 888 pairs) (Kaprio et al. 1978, Kaprio & Koskenvuo 2002). An invitation to participate in the study was sent, on the basis of age and zygosity, to 414 twin pairs aged 63-76 years. The baseline cohort consisted of 1260 respondent female pairs in this age group. To be included in the study, both co-twins had to agree to participate. Finally 103 monozygotic (MZ) and 114 dizygotic (DZ) twin pairs arrived at the laboratory for assessments. Zygosity was confirmed using a battery of 10 highly polymorphic gene markers in DNA extracted from a venous blood sample.

The second dataset, Asymmetry, was collected to study the health, functional capacity and rehabilitation of older persons with hip fracture history. The patient records of Jyväskylä Central Hospital were searched during 2004–2005 to recruit all community-living 60 to 85-year-old men and women who had sustained a femoral neck or trochanteric fracture within the period from 6 months to 7 years earlier and were living independently in the catchment area of the Central Finland Health Care District. Persons who were willing to participate were interviewed in telephone by one trained person using a structured questionnaire (N=132). Exclusion criteria were: inability to move outside without assistance from another person, neurological or severe progressive illnesses, and lower limb amputations. Altogether, 79 persons participated in the laboratory assessments. In addition, a control group of 31 persons without previous hip fractures was recruited through advertisements in the same health care district as the hip fracture patients. For the present analysis, we wished to minimize the effect of other previous injuries. Therefore, we excluded hip fracture patients who had a history of bilateral hip fracture, and also those patients and controls who had undergone hip or knee replacement surgery (other than due to hip fracture) or sustained other major lower limb traumas within 15 years prior to the examinations. Men were also excluded from the analysis, resulting in a sample of 38 women with hip fracture history and 22 control women. The women with hip fracture history had sustained their fracture on average 3.5 (standard deviation 2.0) years earlier.

The third dataset used in the study, "Sarcopenia—Skeletal Muscle Adaptation to Postmenopausal Hypogonadism and Effects of Hormone Replacement Therapy and Physical Activity in Older Women: a Genetic and Molecular Biological Study on Estrogen-related Pathways" (SAWEs), comprised 15 monozygotic female twin pairs aged 54 to 62 years and discordant for postmenopausal hormone therapy (HT). The participants were recruited from the same twin cohort as FITSA by asking MZ female twin pairs with suitable birth years to self-identify themselves as discordant for HT use. Only pairs in which one sister

had been a current HT user for at least the previous 12 months and the other sister had never used HT were included in the study. In addition, using the same criterion, monozygotic pairs discordant for HT were identified from the FITSA participants (n=9). Subjects with bone diseases or medication affecting bone metabolism were excluded. Thus, the total sample of monozygotic HT discordant pairs was 24. Mean age at initiation of HT use was 53 (SD 6.5) and the mean duration of HT use was 8.3 years (SD 5.7). Twelve women used estradiol only, eight estradiol and progesterone, and four products containing tibolone. Mean age at last menstruation was 50 (SD 3.6) for the HT users and 50 (3.5) for the non-users.

The characteristics of the study subjects are presented in Table 2. All the subjects provided a written informed consent. The original research projects were approved by the ethics committee of the Central Finland Health Care District.

4.2 Measurements

4.2.1 Bone measurements

Bone properties were assessed using peripheral quantitative computed tomography, i.e. pQCT (XCT 2000, Stratec Medizintechnik GmbH, Pforzheim, Germany). pQCT scans were obtained from the lower leg and forearm on the side of the dominant hand. In the Asymmetry material, both legs and dominant forearm were scanned. In the lower leg, the scanning sites were 55% (tibial shaft) and 5% (distal tibia) of the length of the tibia proximal to the distal end of the tibia. In the forearm, the scanning site was 4% (distal radius) of the length of the forearm proximal to the distal end of the radius. The distal bone sites were epiphyseal sites consisting of both trabecular and cortical bone, and the tibial shaft is a diaphyseal bone site that is almost entirely composed of cortical bone. Pixel size was 0.8×0.8 mm in the measurements of the tibia and 0.59×0.59 mm in the measurement of the radius.

The analysis of the pQCT images was performed with software designed for analyzing cross-sectional CT images (Geanie 2.1, Commit; Ltd, Espoo, Finland). To separate the bone from the surrounding soft tissues, a density threshold of 130 mg/cm^3 was used in the distal sites. In the Asymmetry dataset, a threshold of 169 mg/cm^3 was used for the distal sites, except for cases in which the threshold was too high for the whole bone area to be included in the analysis. In these cases the threshold was set at 120 mg/cm^3 (in the distal tibia this was done for 14 subjects with hip fracture and two control subjects, and in the distal radius for one subject with hip fracture and one control subject). However, in the distal tibia the same threshold was used for both legs. Bone marrow was included in the analyses. Separation of trabecular and cortical bone was performed in the distal tibia by peeling 30% and in the distal radius by peeling 50% from the outer edge of the bone's cross-sectional area and considering the re-

maining area as trabecular bone. In the tibial shaft, a density threshold of 280 mg/cm^3 was used to separate the bone from the surrounding soft tissues. Cortical bone was separated from trabecular bone by using an automatic contour detection algorithm. Bone marrow was excluded from the analysis of the tibial shaft by applying a density threshold of 100 mg/cm^3, except in the analysis of total cross-sectional area (ToA). Inclusion of bone marrow signifies that ToA comprises the area that is inside the contours of the bone.

The bone variables analyzed are presented in Table 2. A compressive bone strength index (BSIcomp) was calculated as the product of volumetric bone mineral density squared and total cross-sectional area, where the first term denotes the apparent compressive strength of the bone tissue (~ a material property) and the latter the load-bearing cross-sectional area (Carter & Hayes 1976, Martin 1991). Bone mineral content (BMC) was calculated for a 1-mm-thick slice by multiplying the total volumetric bone mineral density by the total cross-sectional area and 1 mm. A bending strength index (BSIbend), i.e. the polar section modulus, was determined as the density-weighted polar moment of inertia divided by the square-root of the total cross-sectional area of the bone. The radial distribution of volumetric BMD and the polar distribution of bone mineral mass were analyzed in Study IV. The former expresses volumetric BMD as a function of the distance from the center of bone mass and the latter gives bone mineral mass as an angular distribution for 72 sectors around the center of bone mass. In the radial distribution, 9 out of 10 regions are presented due to the partial volume effect on the outermost region, and in the polar distribution, the sectors were grouped into 8 sectors.

The precision of bone mineral density, structure and strength index measurements in our laboratory ranges from 0.4 to 1.6% root mean square coefficient of variation (Rantalainen et al. 2008). The main reasons for missing bone and muscle data were: substantial movement artifacts during scanning, previous fracture in the scanned region, inability to fit the leg into the gantry of the pQCT device, and inaccurate positioning of the leg.

4.2.2 Health status, medication and physical activity

Self-reports of acute and chronic diseases, medication, smoking and physical activity were obtained by a questionnaire which was sent home before the laboratory visit. The questions on diseases were formulated: “Do you have X disease diagnosed by a physician?”. These self-reports of diseases and medication were confirmed by a physician during the clinical examination.

Those reporting no other physical activity but light walking no more than twice a week at the most were rated as sedentary in the classification of current physical activity. Those reporting walking or other light exercise at least three times a week, or exercise of moderate intensity up to two times a week, were rated as moderately active. If a participant reported moderate or vigorous exercise at least three times per week, she was rated as physically active (Grimby 1986).

TABLE 2 Bone variables reported in the different studies.

Bone site	Variable	Unit	Study
Distal radius	BMC	mg	III, IV
	ToD	mg/cm^3	I,IV
	TrD	mg/cm^3	IV
	ToA	mm^2	I, IV
	BSIcomp	g^2/cm^4	I,II,IV
	radial and polar distribution	NA	IV
Distal tibia	BMC	mg	III, IV
	ToD	mg/cm^3	I,IV
	TrD	mg/cm^3	III, IV
	ToA	mm^2	I, III, IV
	BSIcomp	g^2/cm^4	I,II,IV
	radial and polar distribution	NA	IV
Tibial shaft	BMC	mg	III,IV
	CoD	mg/cm^3	III, IV
	ToA	mm^2	III, IV
	CoA	mm^2	III,IV
	CoA/ToA	NA	IV
	BSIbend	NA	II,IV
	radial and polar distribution	NA	IV

BMC, bone mineral content; ToD, total volumetric bone mineral density; TrD, trabecular volumetric bone mineral density, ToA, total bone cross-sectional area; BSIcomp, bone compressive strength index; CoD, cortical volumetric bone mineral density, CoA, cortical bone cross-sectional area; BSIbend, bone bending strength index; NA, not applicable

4.2.3 Muscle strength, muscle cross-sectional area and walking speed

Lower leg muscular properties and maximal walking speed were measured since they plausibly reflect the mechanical loading on the tibia.

The cross-sectional area of the lower leg muscles (mCSA) was measured from the pQCT images (55% site) by manually defining the boundaries between muscle and bone and between muscle and subcutaneous fat (Studies II, III). Maximal isometric knee extension strength (Study III) was measured from both sides in a sitting position using an adjustable dynamometer chair (Good Strength, Metitur, Palokka, Finland). The measurements were performed at a knee angle of 60° from full extension with the ankle fastened by a belt to a strain-gauge system. The subjects were allowed to familiarize themselves with the method by doing two to three submaximal efforts. Three to five maximal trials of 2-3 seconds, separated by rest intervals of 30 seconds, were conducted. During the measurements, the subjects were verbally encouraged to produce their maximal effort. For each subject, the best performance with the highest value was accepted as the result. In our laboratory, the coefficient of variation

(CV) for the measurement of isometric knee extension strength is 6% (Sipilä et al. 1996).

In the maximal walking speed test the subjects were asked to walk 10 meters as fast as possible without compromising their safety (Study III). The subjects were allowed 3 meters for acceleration. Time was measured with photocells. The subjects were allowed to use their habitual walking aids. In our laboratory, CV for the measurement of walking speed is less than 5% (Sipilä et al. 1996).

4.3 Statistical methods

Quantitative genetic analyses were used in Studies I and II whereas Studies III and IV utilized other statistical methods.

4.3.1 Quantitative genetic analyses

The equality of the means of the continuous variables and the equality of the distributions of the categorical variables between the MZ and DZ individuals were analyzed with the Wald test, and the equality of variances was tested with the variance ratio test, taking into account the dependence of observations between co-twins (Stata 8.0). The cross-twin cross-trait correlations were calculated separately for the MZ and DZ groups using Pearson's correlation coefficient (SPSS 14.0).

In structural equation models, the effects are latent variables which represent the summed effect of distinct genetic effects (A, D) or distinct unmeasured environmental factors (C, E) (Boomsma et al. 2002). The possible combinations of the different effects that can be tested in the genetic models are the full models (ACE and ADE) and their submodels (AE, CE and E). The model with D but not A (DE) is biologically implausible and hence was not tested, while D and C cannot be estimated in the same model (ADCE) using data that consists of twin pairs reared together (Posthuma et al. 2003). Structural equation modeling programs aim at finding estimates for the components which minimize the discrepancy between the observed and expected covariance matrices (Rijsdijk & Sham 2002).

Univariate genetic analyses were carried out to evaluate the genetic and environmental contributions to each phenotype separately (mCSA, tibial shaft BSIbend, distal tibia BSIcomp and distal radius BSIcomp). A bivariate Cholesky model (Neale & Cardon 1992) was used to evaluate to what extent common and site-specific genetic and environmental factors influence radial and tibial BSIcomp (Study I). This structural equation model consists of genetic and environmental effects (A_1, C_1, E_1) that are common to both variables (radial and tibial BSIcomp) and of genetic and environmental effects (A_2, C_2, E_2) that are specific to the second variable (tibial BSIcomp). In addition, genetic and environmental correlations between mCSA, tibial shaft BSIbend, distal tibia BSIcomp and

tween each pair of variables (Study II). A trivariate independent pathway model (Neale & Cardon 1992, Rijsdijk & Sham 2002) was used to investigate whether the three traits - mCSA, tibial shaft BSIbend and distal tibia BSIcomp - share genetic and/or environmental effects (Study II). The full trivariate independent pathway model consists of genetic and environmental effects that are common to all three traits (A_c, C_c, E_c) and of genetic (A_1, A_2, A_3), shared environmental (C_1, C_2, C_3), and individual environmental (E_1, E_2, E_3) effects that are specific to each trait.

The univariate and multivariate genetic analyses were performed with Mx software (Neale et al. 2003) using the full information maximum likelihood method with raw data input. In all the genetic analyses, age was used as a covariate. The analyses were started with the hypothetical full ACE model. To obtain a more parsimonious model, the full model was modified by dropping the nonsignificant or very small parameters one by one. The aim was a model which fits the data well but has the least possible number of explanatory components. The alternative univariate and multivariate models obtained were compared against the full model by Akaike's information criterion (AIC = -2 x log-likelihood - 2 x degrees of freedom), which is smaller for better fitting models, and by the p-value of the χ^2 difference between the models. Significant χ^2 test values indicate poorer fit of the data to the tested model than to the comparison model.

4.3.2 Other statistical analyses

In Study III, the differences between the hip fracture and control groups in age, weight and height were analyzed using t-test for independent samples. The side-to-side differences in bone variables, knee extension strength and mCSA between the legs were defined in the hip fracture patients as (fractured side - non-fractured side) / non-fractured side * 100%, and in the controls as (dominant side - non-dominant side) / non-dominant side * 100%. The dominant leg of the control subjects was determined as the leg preferred for kicking a football. The side-to-side differences between the legs were analyzed using paired samples t-tests, separately for hip fracture subjects and controls. Comparison of the side-to-side differences in the bone variables between the groups was made using the analysis of covariance (ANCOVA) with age and weight as covariates. To evaluate the determinants of side-to-side differences in tibial shaft BSIbend and distal tibia BSIcomp among the subjects with hip fracture history, Pearson's correlation coefficient was used. The correlations of age, time since fracture, side-to-side difference in isometric knee extension strength, side-to-side difference in mCSA, and maximal walking speed with side-to-side difference in bone strength were analyzed. Further, all the variables which correlated significantly with the bone variables were entered as independent variables in linear regression models in which the side-to-side differences in the bone variables were

dependent variables. The regression models were adjusted for time since fracture.

The influence of exposure to HT was evaluated by comparing differences in bone properties within the twin pairs using paired-samples t-test (Study IV). In cases of non-normal distribution in the intra-pair differences, the Wilcoxon signed ranks test was used. Mean intra-pair differences [=(HT user - non-user)/non-user*100] with 95% confidence intervals (CI) were calculated.

In studies III and IV, the statistical analyses were done using SPSS software versions 12.0.1 to 15.0.

5 RESULTS

5.1 Characteristics of the participants

Table 3 shows the background characteristics and Table 4 the bone characteristics of the study participants. The MZ and DZ groups did not differ in the means or variances of age, height, weight, distal radius BSIcomp, distal tibia BSIcomp, tibial shaft BSIbend or mCSA, except for the variances of age (p=0.004) and distal tibia BSIcomp (p=0.028) (Study I and II). No significant differences were observed between women with hip fracture history and controls in mean age, height or weight (Study III). Also, the HT users did not differ from their non-using co-twins in height or weight (Study IV).

TABLE 3 Background characteristics of the study subjects.

	Study I & II				Study III				Study IV			
	MZ twins (N=206)		DZ twins (N=228)		Subjects with hip fracture (N=37)		Control subjects (N=22)		HT users (N=24)		HT non-users (N=24)	
Variable	Mean(SD)		Mean(SD)		Mean(SD)		Mean(SD)		Mean(SD)		Mean(SD)	
Age (years)	68(3.7)		69(3.1)		76(6.1)		74(6.4)		61(6.0)		61(6.0)	
Height (cm)	158(6.4)		160(5.8)		160(6.2)		158(6.0)		162(5.5)		161(5.1)	
Body weight (kg)	69.6(11.8)		70.6(12.2)		66.6(11.6)		68.7(9.2)		71.2(9.6)		72.6(12.5)	
	N	%	N	%	N	%	N	%	N	%	N	%
Physical activity												
High	64	31	84	37	3	8	13	59	13	54	12	50
Moderate	123	60	122	54	14	38	6	27	9	38	10	42
Low	18	9	20	9	20	54	3	14	2	8	2	8
HT use	41	20	40	18	1	3	1	5	24	100	0	0
Corticosteroid use	4	2	10	4	1	3	0	0	0	0	0	0
Smoking	7	3	11	5	1	3	0	0	5	21	3	13

MZ, monozygotic; DZ, dizygotic; HT, hormone therapy

TABLE 4 Bone characteristics of the participants. For subjects with previous hip fracture, lower limb bone characteristics are presented for fractured (Fx side) and non-fractured side (Non-fx side), and for non-fracture control subjects for dominant (Dom side) and nondominant side (Non-dom side). *previously unpublished data

	Study I & II				Study III						Study IV			
	MZ twins		DZ twins		Subjects with hip fracture			Control subjects			HT users		HT non-users	
Variable	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	Mean (SD)	N	Mean (SD)	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Distal radius	191		210		33			18			22		22	
BMC (mg)		96 (17)*		95 (20)*		91 (15)			94 (14)			106 (14)		97 (19)
ToD (mg/cm^3)		281 (56)		276 (60)		253 (52)*			292 (66)*			329 (59)		303 (75)
TrD (mg/cm^3)		168 (50)*		162 (53)*		127 (42)*			149 (43)*			178 (31)		157 (50)
ToA (mm^2)		344 (45)		346 (45)		365 (50)*			328 (43)*			329 (51)		328 (54)
BSIcomp (g^2/cm^4)		0.28 (0.10)		0.27 (0.11)		0.24 (0.08)*			0.28 (0.10)*			0.35 (0.09)		0.30 (0.12)
						Fx side	Non-fx side		Dom side	Non-dom side				
Tibial shaft	197		220		37			21			21		21	
BMC (mg)		327 (43)*		328 (50)*		297 (55)	311 (49)		337 (41)	339 (45)		365 (28)		333 (44)
CoD (mg/cm^3)		999 (55)*		1003 (60)*		985 (82)	996 (69)		1018 (38)	1018 (43)		1049 (46)		1002 (72)
ToA (mm^2)		479 (52)*		474 (49)*		473 (48)	478 (54)		485 (43)	481 (39)		490 (49)		482 (39)
CoA (mm^2)		275 (37)*		275 (40)*		240 (41)	253 (37)		278 (38)	278 (41)		299 (22)		280 (32)
CoA/ToA		0.58 (0.07)*		0.58 (0.08)*		0.51 (0.09)*	0.53 (0.08)*		0.58 (0.07)*	0.58 (0.08)*		0.61 (0.05)		0.58 (0.08)
BSIbend		1.59 (0.25)		1.59 (0.26)		1.47 (0.27)*	1.56 (0.25)*		1.64 (0.22)*	1.66 (0.25)*		1.76 (0.20)		1.63 (0.21)
Distal tibia	196		216		37			21			23		23	
BMC (mg)		251 (39)*		251 (48)*		228 (44)	240 (44)		264 (40)	268 (40)		276 (34)		255 (46)
ToD (mg/cm^3)		259 (42)		251 (47)		210 (44)*	216 (40)*		255 (40)*	252 (41)*		276 (30)		255 (51)
TrD (mg/cm^3)		216 (42)*		208 (46)*		178 (50)	181 (49)		209 (44)	208 (42)		218 (27)		202 (45)
ToA (mm^2)		981 (119)		1009 (125)		1094 (121)	1116 (115)		1055 (107)	1060 (98)		1004 (102)		1012 (122)
BSIcomp (g^2/cm^4)		0.66 (0.19)		0.65 (0.23)		0.49 (0.19)*	0.53 (0.19)*		0.70 (0.20)*	0.68 (0.20)*		0.77 (0.16)		0.67 (0.23)

BMC, bone mineral content; ToD, total volumetric bone mineral density; TrD, trabecular volumetric bone mineral density, ToA, total bone cross-sectional area; BSIcomp, bone compressive strength index; CoD, cortical volumetric bone mineral density, CoA, cortical bone cross-sectional area; BSIbend, bone bending strength index

5.2 Genetic and environmental effects on bone structural strength (Studies I and II)

The univariate genetic ACE models and their submodels adjusted for age for BSIcomp of the distal radius, BSIcomp of the distal tibia, BSIbend of the tibial shaft and mCSA are presented in Table 5. The effect of age explained 10% of the variance in BSIcomp of the distal radius, 3% of that in BSIcomp of the distal tibia and less than 1% of the variance in BSIbend of the tibial shaft and in mCSA. According to AIC, the best fitting models were the AE models for BSIcomp of the distal radius and mCSA. For BSIcomp of the distal tibia the ACE model gave the best fit. The AE model showed slightly better fit for BSIbend of the tibial shaft than the ACE model. For the BSIcomp of the distal tibia, A explained 49%, C explained 26% and E explained 24% of the variance. For the other variables, A explained 75 to 80% of the variance, the rest of the variance being explained by E.

TABLE 5 Univariate genetic models (adjusted for age) for bone strength indices at three different bone sites using data from 100-102 monozygotic and 112-113 dizygotic twin pairs.

		Model fit		Standardized estimates (95% CI)					
Variable	Model	AIC	p[a]	A		C		E	
Distal radius	ACE	259.8		0.79	(0.50-0.85)	0.01	(0.00-0.28)	0.20	(0.15-0.28)
BSIcomp	AE	257.8	0.94	0.80	(0.72-0.85)			0.20	(0.15-0.28)
	CE	285.1	p<0.001			0.57	(0.46-0.65)	0.43	(0.35-0.54)
	E	358.2	p<0.001					1.00	(1.00-1.00)
Distal tibia	ACE	852.9		0.49	(0.22-0.79)	0.26	(0.00-0.49)	0.24	(0.18-0.34)
BSIcomp	AE	854.3	0.063	0.77	(0.68-0.83)			0.23	(0.17-0.32)
	CE	863.1	p<0.001			0.61	(0.52-0.69)	0.39	(0.31-0.48)
	E	956.4	p<0.001					1.00	(1.00-1.00)
Tibial shaft	ACE	1030.5		0.53	(0.25-0.80)	0.21	(0.00-0.45)	0.26	(0.19-0.36)
BSIbend	AE	1030.4	0.16	0.75	(0.66-0.81)			0.25	(0.19-0.34)
	CE	1041.3	<0.001			0.59	(0.50-0.67)	0.41	(0.33-0.50)
	E	1127.9	<0.001					1.00	(1.00-1.00)
mCSA	ACE	254.7		0.71	(0.40-0.81)	0.03	(0.00-0.31)	0.26	(0.19-0.35)
	AE	252.7	0.83	0.75	(0.65-0.81)			0.25	(0.19-0.35)
	CE	272.1	<0.001			0.54	(0.44-0.63)	0.46	(0.37-0.56)
	E	341.6	<0.001					1.00	(1.00-1.00)

AIC, Akaike's information criterion. The lower value reflects better fit.
[a]p-value of the χ^2 test between the model and ACE model. p<0.05 indicates poorer fit of the model than the fit of the ACE model.
BSIcomp, bone compressive strength; BSIbend, bone bending strength; mCSA cross-sectional area of the lower leg muscles

5.3 Shared genetic and environmental effects on bone structural strength (Studies I and II)

A bivariate Cholesky model was constructed to evaluate to what extent estimated bone strength in non-weight-loaded radius and body weight-loaded tibia share common genetic and environmental effects (Study I). This analysis began from the full ACE model (AIC=744.5). The final model is presented in Figure 4 with proportions of variance explained by each factor and their confidence intervals. The final model (AIC=744.2, p-value of the χ^2 difference compared to the full model=0.188) included additive genetic effects common to both traits. These effects explained 83% of the variance in BSIcomp of the distal radius and 61% of that in BSIcomp of the distal tibia. Shared environmental effects accounted for 15% of the variance in BSIcomp of the distal tibia. The traits had common individual environmental effects which accounted for 17% of the variance in BSIcomp of the distal radius and 10% of that in BSIcomp of the distal tibia. In addition, trait-specific individual environmental effects explained 14% of the variance in BSIcomp of the distal tibia. From the original full model, the following paths (estimate, CI) were dropped: common C to BSIcomp of the distal radius (0%, 0-1%), tibia-specific A (0%, 0-0%), and tibia-specific C (0%, 0-19%). Dropping C completely from the model worsened the fit significantly (AIC=791.8, p<0.001).

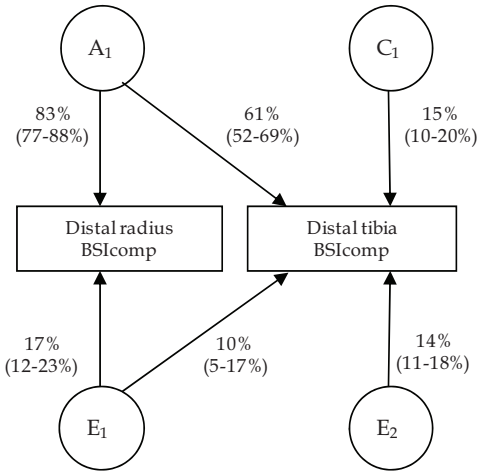

FIGURE 4 The final, reduced Cholesky model for compressive strength (BSIcomp) of the distal radius and distal tibia. The percentages (95% CI) are the proportions of the total variance of each variable explained by each genetic (A), shared environmental (C) or individual environmental (E) factor.

In order to investigate whether muscle cross-sectional area and lower limb bone strength share common genetic and environmental effects, a trivariate independent pathway model was constructed (Study II). AIC of the full ACE model was

-1.2. Figure 5 shows the final trivariate independent pathway model for mCSA, BSIbend of the tibial shaft and BSIcomp of the distal tibia with the proportions accounted for by each factor and their confidence intervals. The final model (AIC=-11.2, p=1.00) included genetic effects influencing all three variables. These common genetic effects explained 75% of the variance in mCSA, 17% of that in BSIbend and 8% of that in BSIcomp. Trait-specific genetic factors accounted for 38% of the variance in BSIbend and 32% of that in BSIcomp. All three variables were also influenced by same individual environmental factors. These factors explained 5% of the variance in mCSA, 22% of that in BSIbend of the tibial shaft and 13% of that in BSIcomp of the distal tibia. In addition, the bone variables were influenced by same shared environmental effects which accounted for 20% of the variance in BSIbend and 37% of that in BSIcomp. The rest of the variances were explained by trait-specific individual environmental effects. The paths that were dropped were the following in the original full model: the path of common C to mCSA (estimate 0%, 95% CI: 0-7%), specific A to mCSA (0%, 0-51%), specific C to mCSA (1%, 0-24%), specific C to BSIbend (0%, 0-18%) and specific C to BSIcomp (0%, 0-26%). Dropping C completely from this model worsened the fit significantly (AIC=28.3, p<0.001).

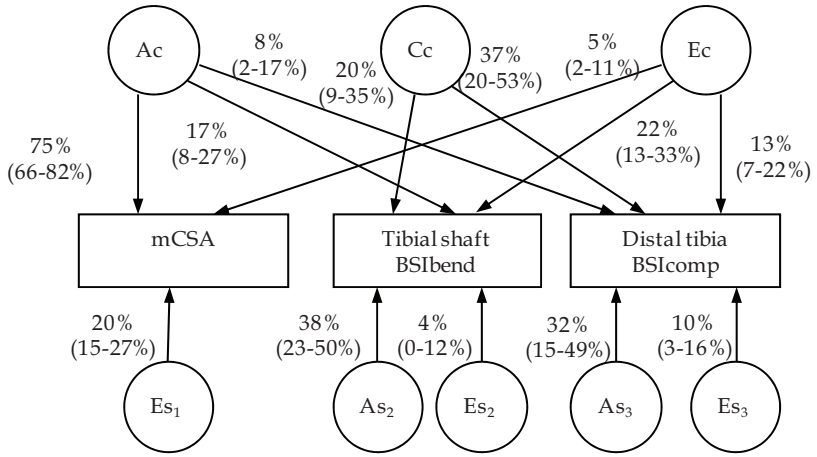

FIGURE 5 The final, reduced independent pathway model for muscle cross-sectional area (mCSA), bending strength (BSIbend) of the tibial shaft and compressive strength (BSIcomp) of the distal tibia. The percentages (95% CI) are the proportions of the total variance of each variable explained by each genetic (A), shared environmental (C) or individual environmental (E) factor. In the factors, c refers to a factor common to multiple variables and s to a trait-specific factor.

The genetic and environmental correlations between the pairs of variables are shown in Table 6 (Study I and II). The highest genetic correlation was observed between BSIcomp of the distal radius and BSIcomp of the distal tibia (1.00) and the highest environmental correlations between BSIcomp of the distal tibia and BSIbend of the tibial shaft (0.68) and between BSIcomp of the distal radius and distal tibia (0.64).

TABLE 6 Genetic (above the diagonal) and environmental correlations (below the diagonal) with 95% confidence intervals derived from the bivariate AE models.

	Distal radius BSIcomp	Distal tibia BSIcomp	Tibial shaft BSIbend	mCSA
Distal radius BSIcomp	NA	1.00 (1.00-1.00)	0.45 (0.32-0.56)*	NA
Distal tibia BSIcomp	0.64 (0.51-0.74)	NA	0.51 (0.20-0.72)	0.43 (0.23-0.66)
Tibial shaft BSIbend	0.53 (0.36-0.66)*	0.68 (0.55-0.78)	NA	0.46 (0.33-0.57)
mCSA	NA	0.34 (0.15-0.50)	0.42 (0.25-0.57)	NA

BSIcomp, compressive bone strength index, BSIbend bone bending strength index, mCSA, muscle cross-sectional area, NA, not applicable.
*previously unpublished

5.4 Association of hip fracture history with bone structural strength and its determinants (Study III)

The associations of history of hip fracture with estimated bone strength and its determinants were analyzed by comparing bone properties in the tibia of the fractured leg to those in the tibia of the non-fractured leg in women with hip fracture history. In the tibial shaft of the fractured leg, BMC and BSIbend were significantly lower, and CoA and CoA/ToA were significantly smaller than in the non-fractured leg (Figure 6). In the distal tibia of the fractured leg, BMC, ToD and BSIcomp were lower and ToA smaller. The smaller CoA/ToA in the tibial shaft of the fractured than that of the non-fractured leg indicate thinner cortices in the fractured leg. Further, the smaller CoA in the fractured leg but similar ToA in both legs suggest that the cortex has lost mineral on the endocortical surface. In the controls without hip fracture, the side-to-side differences were not statistically significant. Side-to-side differences in the distal tibia BMC (p-value of ANCOVA=0.041), ToD (p=0.006) and BSIcomp (p<0.001) and in the tibial shaft BMC (p=0.018) and CoA (p=0.005) were significantly different between the women with hip fracture history and the controls.

Side-to-side difference in mCSA and isometric knee extension strength correlated positively with side-to-side difference in BSIbend of the tibial shaft and BSIcomp of the distal tibia. The side-to-side differences in muscle accounted for 38% and 41% of the variance in side-to-side difference of BSIbend of the tibial shaft and BSIcomp of the distal tibia, respectively (Table 7).

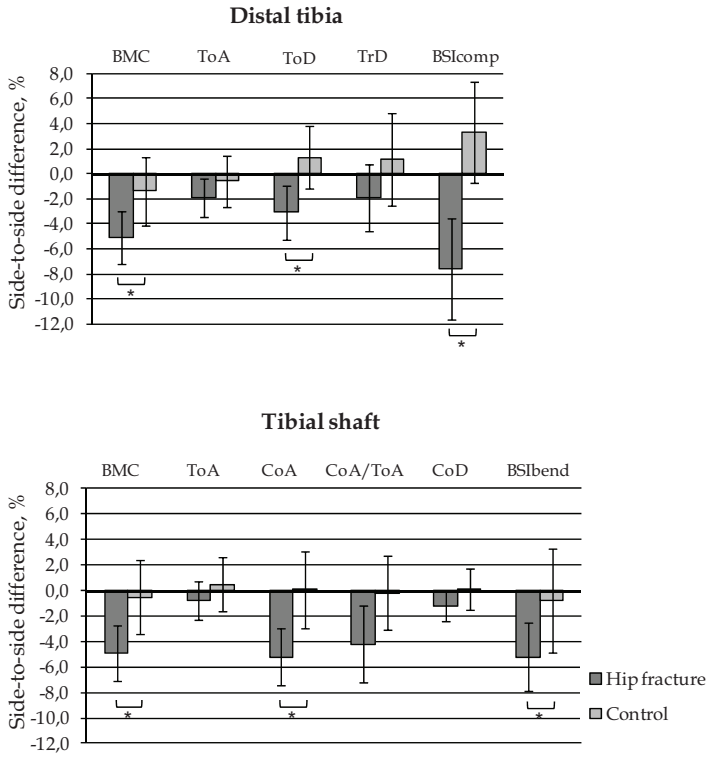

FIGURE 6 Relative mean side-to-side differences (with error bars indicating 95% CI) in bone structural strength and its determinants in women with hip fracture history (fractured side compared to non-fractured) (N=37) and control subjects (dominant side compared to non-dominant) (N=22). *statistically significant difference between the groups.

TABLE 7 Regression models, adjusted for time since fracture, for side-to-side differences in bone properties in subjects with hip fracture history. Results previously unpublished.

Dependent variables[a]	Predictors[a]	β	p	R^2
Tibial shaft BSIbend	Knee extension strength	0.39	0.019	
	mCSA	0.51	0.005	0.41
Distal tibia BSIcomp	Knee extension strength	0.44	0.011	
	mCSA	0.36	0.046	0.38

[a] Side-to-side difference variables

5.5 Association of hormone therapy with bone structural strength and its determinants (Study IV)

When analyzing the association between HT use and estimated bone strength, it was found that the HT users had significantly higher bone compressive strength of the distal tibia and distal radius and bone bending strength of the tibial shaft than their non-using co-twins (Figure 7). Also, BMC, ToD, and TrD were higher in the HT users in the epiphyseal bone sites, distal radius and distal tibia. In the diaphyseal bone site, the tibial shaft, the HT users had significantly higher BMC, CoA/ToA, and CoD and larger CoA than their co-twins. The HT users and non-users did not differ significantly in ToA in any bone site. The larger cortical to total area ratio in the HT users together with similar total cross-sectional areas in the users and non-users indicates thicker cortices in the HT users due to additional bone mineral on the endocortical surface.

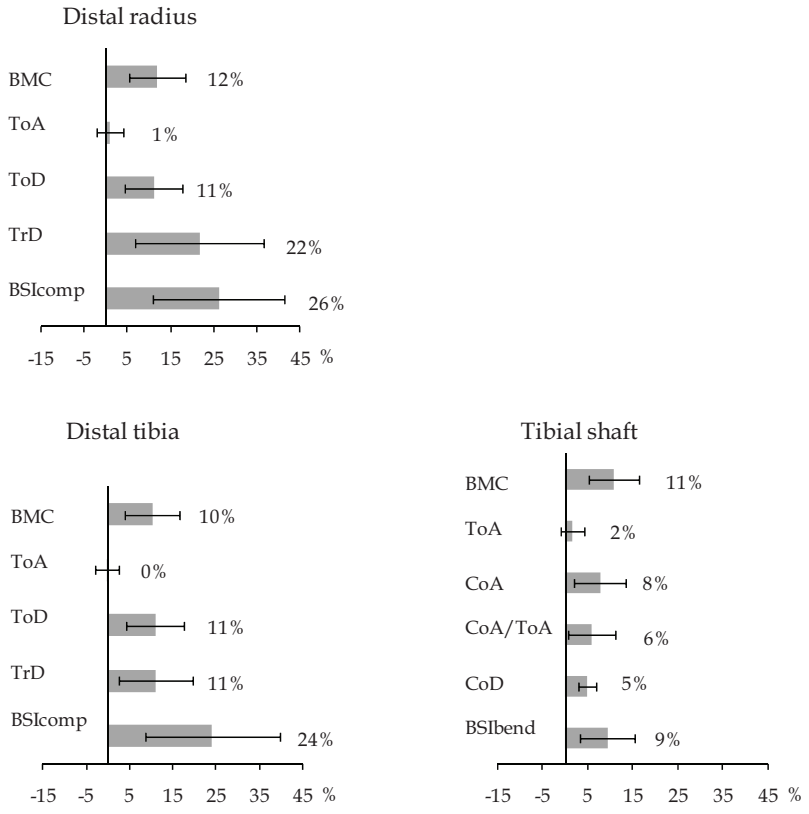

FIGURE 7 Properties of bone in women using HT compared to their monozygotic non-using co-twins. The bars represent mean intra-pair differences [(HT user - non-user)/non-user*100] and the error bars represent 95% CI.

In the epiphyseal bone sites, volumetric BMD was significantly higher throughout the bone's cross-section in the HT users compared to non-users except in the innermost area and in the trabecular area next to the cortical wall (Figure 8). Bone mineral mass was significantly higher in all eight sectors around the center of bone mass in both bone sites except for one anterior sector in the distal tibia and two anterior sectors in the distal radius (Figure 9).

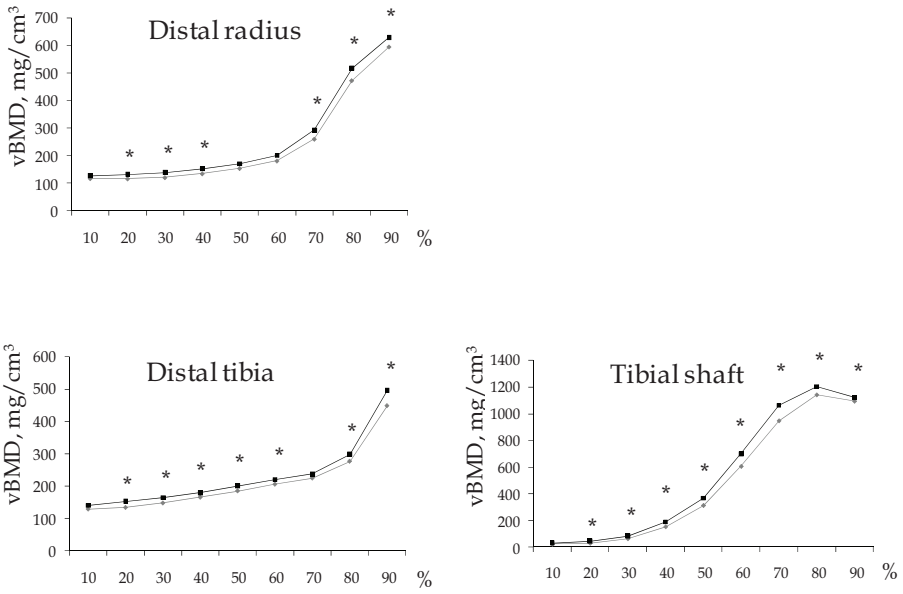

FIGURE 8 Bone mineral density as a function of relative distance from the center of bone mass to the periosteum in the HT users (black line with squares) and their non-using monozygotic co-twins (grey line with diamonds). *statistically significant difference between HT users and non-users ($p<0.05$)

In the diaphyseal bone site, volumetric BMD was significantly higher throughout the bone's cross-section, from the centre to the outer edge, in the HT users compared to non-users, except in the innermost area (Figure 8). The HT users had significantly higher bone mineral mass than the non-users in all directions (Figure 9).

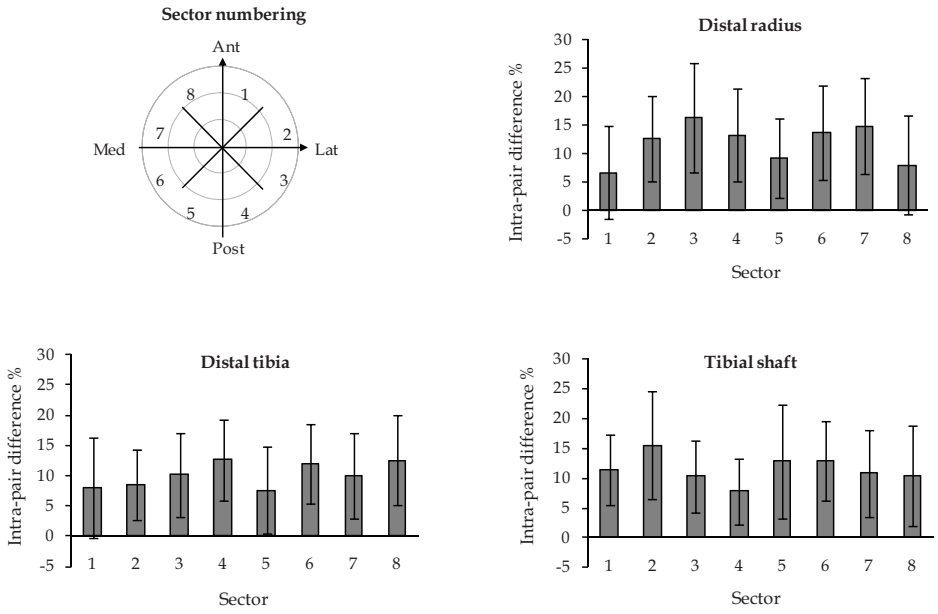

FIGURE 9 Mean intra-pair differences [=(HT user - non-user)/non-user*100] with 95% CI in bone mineral mass in 8 sectors around the center of bone mass. Sector numbering of the bar charts is shown relative to the anterior (Ant), lateral (Lat), posterior (Post) and medial (Med) directions.

6 DISCUSSION

This study investigated the genetic and environmental influences on estimated bone structural strength in postmenopausal women. In these women, genetic factors seem to explain the majority of the inter-individual differences. However, the influence of environmental factors is larger in the body weight-loaded lower limb than non-weight-loaded upper limb. Bone strength in structurally similar bone sites in the upper and lower limb were found to share all their genetic effects. In structurally different types of bones, genetic effects were only partly shared. In addition, bone strength shared genetic and environmental factors with muscle cross-sectional area. Of the distinct factors, hip fracture history was negatively and HT was positively associated with estimated bone strength. Women with a hip fracture on average 3.5 years earlier had significantly reduced bone strength in the lower leg of the fractured side compared to the non-fractured side. This was mainly due to reduced cortical cross-sectional area in diaphyseal bone and reduced bone mineral density in epiphyseal bone. The impairments in bone strength were largely explained by reductions in muscle cross-sectional area and muscle strength in the fractured leg. Long-term HT use, on average 8 years, was associated with higher bone strength both in the upper and lower limb. Higher bone strength in the HT users was due to higher bone mineral density in all bone sites and larger cortical cross-sectional area in diaphyseal bone.

6.1 Genetic influences on bone structural strength and muscle cross-sectional area

Genetic factors are considered as determining the majority of the individual differences in bone properties (Peacock et al. 2002, Ralston & de Crombrugghe 2006). This conclusion is mainly based on the findings on the heritability of aBMD, since other bone properties, e.g. bone strength, have been studied far less. In our study, the heritability of bone strength varied from 40 to 80%, being

40 to 60 % in the lower limb and 80% in the upper limb. Previous studies, among Chinese families (Xu et al. 2006) and Caucasian pedigrees (Sun et al. 2006), have obtained rather similar heritabilities, from 40 to 55%, in DXA-derived section modulus (i.e. estimated bending strength) of the femoral neck compared to that observed in tibial bending strength in our study. However, to our knowledge no previous studies have investigated the heritability of bone strength in different bone sites. The heritability estimates of aBMD vary from one bone site to another (Seeman et al. 1996, Howard et al. 1998, Harris et al. 1998, Hunter et al. 2001, Yang et al. 2005, Deng et al. 2006, Yang et al. 2006, Videman et al. 2007, Sigurdsson et al. 2008), but there does not seem to be any consistent pattern as to which bone site has the highest heritability. For example, in a large twin study in over 1000 female pairs, lumbar spine aBMD had the highest heritability (76%) and femoral neck had the lowest heritability (60%) (Hunter et al. 2001) whereas in females in a pedigree study, spine aBMD had the lowest heritability (63%) and hip had the highest heritability (71%) (Yang et al. 2006).

The results of our study further showed a total genetic overlap between radial and tibial compressive strength, i.e., strength in these structurally similar bone sites are regulated by the same genes or genes that are in close linkage to each other. There appear to be no other studies that have evaluated the heritability of bone properties in structurally similar but differently loaded bones. In a previous study in Caucasian pedigrees, the genetic correlation between proximal femur and distal radius aBMD was clearly lower (0.64) (Yang et al. 2006), than between the upper and lower limb bone sites in our study. Between the most widely studied bone sites, lumbar spine and proximal femur aBMD, which both contain cortical and trabecular bone but are structurally dissimilar, genetic correlations have ranged from 0.64 to 0.74 (Howard et al. 1998, Nguyen et al. 1998, Yang et al. 2005, Yang et al. 2006). In the present study, the genetic correlation between epiphyseal and diaphyseal bone strength was considerably lower than between the epiphyseal bone sites. It is plausible that the distal tibia and radius, which have a similar structure and have had a similar function in the early phase of human evolution, share their genes totally whereas structurally dissimilar sites share only part of their genes.

A large variety of genes have been proposed to influence bone properties. However, until recently most studies have been underpowered and some previously found candidate genes are likely to be false positive (Shen et al. 2005). A recent large meta-analysis of genome-wide association studies in over 19 000 individuals (Rivadeneira et al. 2009) confirmed previously found associations of aBMD to e.g. estrogen receptor 1, Lrp5, RANK and RANKL genes. Also the type 1 collagen (COLIA1) gene polymorphism has been studied in a sample of 20 0000 individuals and it was found to associate with aBMD and fractures (Ralston et al. 2006). There are likely to be a large number of other genes that influence bone properties, since the proportion of the variance in aBMD explained by the genes found in the association studies is small. For example, in

the previously mentioned meta-analysis, 20 genes explained less than 3% of the genetic variance in aBMD (Rivadeneira et al. 2009).

Previous studies have found genetic effects to explain from 56% to 84% of the variance in lean mass, which is in agreement with the heritability estimate of muscle cross-sectional area in the present study (Nguyen et al. 1998, Sun et al. 2006). Knowledge on the heritability of muscle cross-sectional area, however, is sparse. Although investigating a different muscle group, paraspinal muscles, a study in MZ male twins found a comparable proportion of genetic and shared environmental effects, 66% to 73%, underlying muscle cross-sectional area (Gibbons et al. 1998). Also lower leg muscles have been studied. Prior et al. (Prior et al. 2007) reported that the heritability of the cross-sectional area of calf muscles is 23% but this estimate is not comparable with ours because the authors included several covariates in the analysis.

The finding that muscle cross-sectional area and bone strength were influenced by common genetic factors support previous pedigree, family and twin studies which have found lean mass and aBMD to share genetic effects (Seeman et al. 1996, Nordström & Lorentzon 1999, Sun et al. 2006,). However, a previous study in 112 female twin pairs aged 20 to 83 years yielded different result. The study suggested that the association between lean mass and aBMD is wholly explained by common environmental effects (Nguyen et al. 1998). The genetic association between muscle and bone may be difficult to find, because according to our study, the absolute size of the genetic effects shared by muscle and bone is modest. Despite this, based on the results of genome-wide linkage analyses, it is likely that the genetic association between muscle and bone is caused by the influence of several different genes. These linkage analyses have found lean mass and bone strength to share several chromosomal areas (Deng et al. 2007, Karasik et al. 2009). The genes influencing both bone and muscle phenotypes may include the vitamin D receptor (VDR) gene, since the same polymorphism (Fok I) has been found to be associated with bone mineral density (Falchetti et al. 2007) and lean mass (Roth et al. 2004). In addition, polymorphisms of myostatin which is a known negative regulator of muscle mass, have also been found to be associated with aBMD (Zhang et al. 2008b). Other possible candidate mechanisms, among others, underlying the genetic association are IGF-I (Rivadeneira et al. 2004, Niu & Rosen 2005, Kostek et al. 2005, Adamo & Farrar 2006) and androgen receptors (Walsh et al. 2005, Stiger et al. 2008, Karasik & Kiel 2010).

6.2 Environmental factors underlying bone structural strength

In the present study, environmental factors accounted for 40 to 60 % of the variance in lower limb bone strength and 20% of that in upper limb bone strength. As the size of the genetic effects influencing bone strength in the lower limbs was similar to that found in previous studies, the total sum of environmental influences, i.e. shared and individual environmental effects combined, agrees

with previous findings. However, the results disagree in the proportion of shared environmental effects. The previous family studies on bone strength indices have found no (Xu et al. 2006) or a smaller than 5% (Sun et al. 2006) shared environmental effect, whereas in the present analyses the proportion of shared environmental factors in lower leg bone strength was considerable, 15-37%. This difference between the previous and our present results may be due to the data: twin data is considered to be more powerful in discriminating shared environmental effects from additive genetic effects than any other practical family designs (MacGregor et al. 2000). However, many previous twin studies using aBMD as an outcome have found no indication of shared environmental influences (Nguyen et al. 1998, Hunter et al. 2001, Videman et al. 2007). A twin study in young, 10-to 26-year-old, female twin pairs found a significant shared environmental effect in girls who were still living together, but as they moved apart from one another, the effect diminished and, with advancing age, finally disappeared (Hopper et al. 1998). Also, one twin study in women on average 50 years old, found suggestive but not statistically significant evidence of shared environmental effects on radius aBMD (Knapp et al. 2003). Therefore, the discrepancy between previous and our study may lie also in part in the variables used. Our results support this, since the genetic analyses showed no evidence of shared environmental effects on vBMD in our study (Study I).

The results of the present study further showed that tibial strength is partly influenced by the same individual environmental factors as radial strength. This accords with a previous pedigree study that has shown that common and site-specific environmental factors to underlie aBMD in the upper and lower limbs (Yang et al. 2006). In that study, the environmental correlation between hip and radius aBMD in women was 0.43 which is slightly lower than that found in bone strength in the present study. Earlier studies investigating environmental influences common to multiple bone sites in women have mainly focused on spinal and proximal femur aBMD, which have been found to share a part of their environmental effects, the environmental correlations ranging from 0.50 to 0.57 (Howard et al. 1998, Nguyen et al. 1998, Yang et al. 2006). These estimates are similar to the environmental correlation found in our study between bone sites with a different structure, i.e. the tibial shaft and distal radius.

Quantitative genetic analyses conducted without information on specific environmental factors cannot, of course, specify the environmental factors included in the latent factors of the genetic models. The shared environmental factors in a twin analysis may consist of effects of childhood environment along with environmental factors that are similar in the co-twins in adulthood. The shared environmental effects in the present study may well include factors from childhood since it has been shown that the influences at least of exercise and nutrition on bone in childhood may partly be maintained into adulthood and, in women, even into the postmenopausal years (Khan et al. 1998, Kalkwarf et al. 2003, Opotowsky & Bilezikian 2003). The environmental factors common to all bone sites are likely to include factors that act systemically, thus influencing the

whole skeleton, such as nutrition (Matkovic et al. 2004), medication (Martin et al. 1997, Cranney et al. 2002), smoking (Ward & Klesges 2001) and some diseases (Christiansen et al. 1997, Martin et al. 1997, Ward & Klesges 2001, Cranney et al. 2002). In the tibia, physical activity-related mechanical loading is another plausible factor contributing to the observed environmental association between distal tibia and tibial shaft strength. Physical activity is also likely to play role in the environmental association between muscle cross-sectional area and bone. In a few exercise studies, where both bone and muscle properties have been measured, and muscle mass and bone traits have been found to improve in response to the same exercise program (Daly et al. 2004, Borer et al. 2007). In addition, periods of disuse lead to impairment in both muscle mass and bone structure (Natri et al. 1999, Rittweger et al. 2005). Further, nutrition (Uusi-Rasi et al. 2002) and some medicines, such as glucocorticoid treatment (Natsui et al. 2006) and hormone therapy (Wells et al. 2002, Sipilä 2003), may add to common environmental effects between muscle and bone.

6.2.1 Hip fracture history

The present study showed that hip fracture is followed by significantly impaired bone strength. The impairment of bone properties was also seen in bone mineral mass, which was 5% lower in the tibia of the affected leg than in the healthy leg. This finding is in line with previous studies, which have reported significant differences between legs in DXA-derived aBMD between the legs several years post fracture. In older persons who had sustained a lower leg fracture on average nine years earlier, the mean difference between the hips was found to be 3 to 5 % (van der Poest Clement et al. 1999). Both tibial fractures and femoral shaft fractures seem to result in side-to-side differences ranging from 2 to 11% a decade after the fracture in 30-to 40-year-old men (van der Poest Clement et al. 1999, Kannus et al. 1994a, Kannus et al. 1994b). Since significant side-to-side differences have been observed such a long time after the injury, at least part of the injury-induced impairment of bone is likely to be permanent.

According to the present study, the cortex appears to be the main site of mineral loss after a fracture. The results suggested that in the diaphysis (tibial shaft), bone loss is mainly due to endocortical resorption, since in the fractured leg, the cortical area was clearly reduced while the total area did not differ between the legs. Also in the epiphysis (distal tibia) of the fractured leg, the cortex is a plausible site of bone loss. Since trabecular density between the legs was equal, most of the bone mineral loss must have taken place outside this trabecular area. Due to the method of analysis, this area consisted of cortical bone together with a varied amount of trabecular bone next to the cortical wall. Deficit in cortical bone may be caused by disuse, as disuse has been suggested to result in greater bone loss from the cortical than trabecular compartment (Allen & Bloomfield 2003, Rittweger et al. 2009). Our results on trabecular bone deficit contradict previous findings in longitudinal (Veitch et al. 2006) and cross-sectional (Findlay et al. 2002) studies, which have found significant losses in

trabecular density within a year and a half after tibial shaft fracture. The discrepancy between the previous studies and our study may be partly due to the post fracture time point studied: trabecular bone loss may have recovered in our subjects. However, time point is not likely to explain the discrepancy totally. The subjects in our study were older than those in the previous studies and the loss of cortical bone may be typical in the age-group studied. With advancing age, as trabeculae deteriorate, the surface for resorption increases in cortical bone while it decreases in trabecular bone (Martin & Seeman 2008). Therefore, increased resorption after a fracture may focus on cortical rather than trabecular bone in older persons.

The marked reductions in bone strength which were observed in the present study, have to be considered clinically important. According to Russo et al. (Russo et al. 2003), the annual decline in the tibial shaft strength index (minimum moment of inertia) is 0.5% in 60-year-old and older women. Consequently, the bone fragility in the fractured leg of hip fracture subjects is several years ahead of the reported age-related decline. This higher fragility is likely to contribute to the increased risk of a new fracture.

6.2.2 Hormone therapy

This study showed that hormone therapy is one of the environmental factors that has a considerable contribution to bone structural strength in postmenopausal women. The observed differences in bone strength between HT users and non-users were substantial: 9% in the diaphyseal bone site and over 20% in the epiphyseal bone sites. The observed influences of HT on bone strength in the present study are larger than those reported in previous RCT studies using DXA (Greenspan et al. 2005, Chen et al. 2008) and CT techniques (Cheng et al. 2002) in which one to six years of use of HT resulted in an increase of 2.5% to 4% in bending strength.

In the present study, higher compressive strength of the epiphyseal bone sites in the HT users than non-users was due to higher volumetric BMD, since there was no difference between the co-twins in cross-sectional area, which together with total density determines compressive strength. According to the radial distribution analysis, HT increased volumetric BMD rather uniformly in the trabecular and cortical compartments. The higher trabecular density in the HT users may be due to the proposed ability of estrogen to prevent trabecular thinning (Cano et al. 2008) and to preserve the trabecular structures (Pajamäki et al. 2008).

Also in the diaphyseal bone, higher bone strength in the HT users resulted from additional bone mineral mass. Mineral mass was distributed uniformly throughout the cortex. The higher cortical volumetric BMD in HT users compared to non-users has also been observed in unrelated women (Uusi-Rasi et al. 1999, Uusi-Rasi et al. 2003a) and is likely to reflect a lower level of intracortical porosity, which normally increases with aging (Cooper et al. 2007). The present results further suggested that the HT users had more bone mineral in the endocortical region than the non-users. It is likely, that endocortical resorption

occurred in the non-users, as happens normally after menopause (Russo et al. 2003, Seeman 2003, Szulc et al. 2006), and that HT has attenuated this resorption in the HT-users, as suggested by observational DXA studies (Beck et al. 2001, Szulc et al. 2006). Also in an RCT by Cheng et al. (2002), HT had positive effect on bone mass at the endocortical region.

It has been proposed that periosteal apposition takes place in women also after menopause and partly compensates for the loss of strength following age-related mineral loss (Szulc et al. 2006). Previous findings in DXA studies have suggested that this periosteal apposition is smaller in HT users than non-users (Beck et al. 2001, Szulc et al. 2006, Chen et al. 2008). If HT restricted periosteal apposition it could offset the positive effects of increased mineral density and mass on bone bending strength. However, in our genetically controlled study, cross-sectional areas in the HT users and non-users were similar, which suggests, in contrast to previous findings, that HT does not restrict periosteal apposition.

6.2.3 Loading environment

Examination of bone structural strength in the upper and lower limb in the quantitative genetic analyses aimed at evaluating whether the loading environment modifies heritability. It was assumed that if such a modifying influence exists, the proportions of genetic and environmental effects would be different in the body weight-loaded tibia than in the non-weight-loaded radius. We found that loading increases the proportion of environmental effects on estimated bone strength. In the distal tibia, which is mainly subjected to compressive loads generated by body weight-bearing locomotion, the proportion of environmental influences was substantially larger than in the non-weight-loaded radius. A previous study in over 200 female twin pairs, obtained a lower heritability estimate for upper limb aBMD than lower limb aBMD (Knapp et al. 2003) while a larger twin study and a pedigree study have reported rather similar heritability estimates for upper and lower limb aBMD (Hunter et al. 2001, Zhang et al. 2008a). The previous and the present results do not necessarily conflict as it is not likely to be bone mass per se but the strength of the whole bone that adapts to the demands of the loading environment. This is supported by studies which have found larger differences in bone strength indices than in bone mineral density between athletes and non-athletes (Adami et al. 1999, Heinonen et al. 2001, Nikander et al. 2006). In addition, according to our results the heritability of vBMD is similar in the upper and lower limbs (Study I).

We also aimed at investigating whether loading environment and the influence of HT interact. It has been proposed that estrogen modulates the effect of mechanical loading on bone (Lanyon & Skerry 2001). The results of the present study do not support this view since HT had a similar influence on bone strength and BMC in both weight-loaded and non-weight-loaded bone. Further, HT users had higher mineral mass than non-users in all directions in the polar distribution analysis. If HT modulated the effect of loading, the differences between HT users and non-users would be expected to vary in different directions

since exercise seems to increase bone mineral mass direction specifically (Ma et al. 2009). Although the present results reflect the influence of the customary daily loading on to the lower limbs, the results seem to agree with previous exercise intervention studies. In these previous studies, exercise regimens have contained exercises that are considered osteogenic, such as jumping, weight-bearing and weight-lifting activities. However, no interaction have been found between HT and exercise in these studies (Cheng et al. 2002, Going et al. 2003).

The main source of loading, which bone is subjected to, is considered to originate from muscle contractions (Frost 2003). Therefore the finding that side-to-side differences in muscle strength and muscle cross-sectional area explained the side-to-side differences in bone strength in the persons with previous hip fracture is expected. In line with this, previous studies have shown bone loss after a fracture to be associated with the level of limb function (Kannus et al. 1994a, Kannus et al. 1994b) and muscle strength (Kannus et al. 1994b, Leppälä et al. 1999c). Therefore, disuse of the injured limb seems to be an important determinant of posttraumatic bone loss (Järvinen & Kannus 1997). With disuse, muscle tissue degrades rapidly (Rittweger et al. 2005) and the reduced muscle strength is unable to maintain the strain level in bone, which, according to the mechanostat theory, leads to reduction in bone rigidity (Frost 2003). Although to our knowledge no intervention studies have been carried out, the present findings suggest that part of the bone loss after a hip fracture could be prevented by rehabilitative strategies aiming at muscle preservation. However, it is likely that these strategies are not able to prevent bone impairment totally due to the strong effect of posttraumatic catabolic reactions on bone (Sievänen et al. 1994, Järvinen & Kannus 1997).

Although muscle-induced loading is an important determinant of bone properties, the results of the genetic analyses showed that the association observed between muscle and bone derives partly from shared genes. This means that generally the association between muscle and bone properties cannot be straightforwardly interpreted as the influence of muscle loading on bone.

6.3 Methodological considerations

An important strength of this study was that the samples, excluding the control group for the hip fracture subjects, were population-based. However, the inclusion criteria may have led to the exclusion of persons with poor health which could plausibly have reduced the overall variance. In the genetic studies, twin pairs with one sister unable to participate due to poor health were excluded and thus the participating co-twins may have been more alike than co-twins in general. The higher similarity between co-twins in both zygocities may overestimate the proportion of shared environmental effects in the quantitative genetic analyses.

The classical twin method used in the present genetic studies is considered as a valid way to estimate the total effect of genetic and environmental factors

on a trait. The advantage of twin data over family data in quantitative genetic analyses is that it enables discrimination of genetic effects from shared environmental effects (MacGregor et al. 2000). Still, it has been discussed whether the results of twin studies are generalizable. The representativeness of twins of the general population has been questioned mainly due to the special biology that characterizes twin pregnancies. However, evidence that would compromise the interpretation of twin studies has not been presented (Kyvik 2000). In any case, caution must be exercised when applying the results to other populations than Caucasian older women since heritability estimates are always population-specific.

The rather strict assumptions of the classical twin method may not be met in the true world and this may influence the results obtained from the genetic analyses. Contrary to the assumptions, MZ co-twins may share their environment to larger extent than DZ co-twins due to the fact that the majority of MZ co-twins share their placenta and therefore have more similar intrauterine environment during pregnancy than do DZ co-twins who all have separate placentas (Phillips 2000). It is, however, unclear to what extent this phenomenon might influence bone properties. Violation of the equal environments assumption would lead to overestimation of genetic effects (Rijsdijk & Sham 2002). The assumption that genes and environment should not interact is likely to be unrealistic in terms of bone since it has been found that at least in some populations certain genotypes are more responsive to the effects of physical activity (Lorentzon et al. 2007, Suuriniemi et al. 2007) and calcium (Stathopoulou et al. 2010). Depending on the nature of the environmental factor, shared or individual, the proportion of genetic or individual environmental effects, respectively, is overestimated in the genetic models (Rijsdijk & Sham 2002).

A cross-sectional design is not an ideal way to estimate the influence of a factor. Nevertheless, the designs used, the side-to-side comparison and co-twin control design, have the advantage of controlling for the effect of many confounding factors. In the analysis of the influence of previous hip fracture, by comparing limbs in the same subject, the influence of genes, age and all systemic factors can be controlled for. However, a longitudinal design would have allowed the true changes taking place after a hip fracture to be measured. For evaluation of the effect of a drug, such as HT, a randomized controlled trial is generally considered the most appropriate design. However, long-term interventions are difficult to conduct and approval for an HT intervention study might be hard to obtain due to the reported side-effects of HT. In a co-twin control design, comparison of MZ co-twins controls for the influence of age, genes and multiple environmental factors that are shared by the co-twins. As shown in studies I and II, such shared environmental influences account for more than 70% of the individual differences in bone strength. Thus, the number of participants needed is low and consequently, the design is very efficient. The design also enabled the investigation of long-term influences, which is rarely possible in RCTs. In addition, an observational design is more likely to reflect real-life circumstances than highly-controlled RCTs.

Among the advantages of this study, was the method of bone measurement used. pQCT is able to give precise information on the volumetric mineral density and structural properties of limb bones (Sievänen et al. 1998) and also enables the assessment of muscle cross-sectional area. We were able to measure different types of bone sites, epiphyseal and diaphyseal bone sites, and upper and lower limb bone sites, although the hip, which is considered a clinically very important bone site, could not be measured. A smaller pixel size in the pQCT scans could have enabled more precise investigation of cortical bone in the epiphyseal bone sites. It is also noted that the bone strength indices used in this study were not directly measured but calculated from different pQCT-measured traits. Being an aggregate measure of several traits, the uncertainty in bone strength indices may be increased compared to directly measured traits. However, all data were analyzed in a similar way and it is thus unlikely that the results of genetic or other analyses would have been biased because of using these traits. Furthermore, the strength indices, compressive and bending strength index, were considered reasonable phenotypes as they reflect the structural strength of whole bones (Kontulainen et al. 2008), the other major determinant of fracture risk besides the loads imposed on bones.

This study aimed at evaluating whether loading environment modulates selected phenomena. However, loading environment could only be evaluated indirectly, using comparison between bone sites that customarily are loaded differently and using muscle properties as a surrogate of mechanical loading. Such approaches are, however, simplifications of the true loading environment and do not take into account for example different levels and types of loading.

6.4 Implications and future studies

This thesis provides information on the overall importance of genetic and environmental factors on bone strength in postmenopausal women. The moderate to high heritability of bone strength observed in this study suggests that it is worthwhile to search for genes influencing bone strength. Detection of specific genes predisposing to low bone strength may, in the future, help to identify persons at risk for osteoporotic fractures and to target preventive interventions more accurately. Also, gene identification leads to further understanding of the cellular regulation of bone tissue and advances the development of therapeutic agents. On the other hand, the considerable size of the environmental effects on bone strength emphasizes the role of factors that are potentially modifiable. While genetic analyses produce population-level information on the relative contribution of genetic and environmental effects, this study also suggests that specific environmental factors, such as previous hip fracture and HT, may have important influences on the skeleton of individual women. In addition, local factors, such as injuries, may result in large differences even within the skeleton. Consequently, this thesis highlights the importance of initiating appropriate

therapeutic actions after a hip fracture to prevent further increase in bone fragility.

Further studies on different factors influencing bone structural strength and its components are needed in order to gain more precise information on the mechanisms underlying bone fragility. The effect of mechanical loading on bone strength in postmenopausal women should be further studied using randomized trials to determine the characteristics of optimal osteogenic exercises for this specific group. Since our results found an association between impaired muscle properties and reduced bone strength after hip fracture, it should be evaluated, using an experimental design, whether intensive rehabilitation aiming at improving muscle strength prevents decline in bone strength after hip fracture. Also, due to the considerable influence of long-term HT observed in the present study, it should be investigated whether the fracture preventive effects of HT outweigh the side-effects in certain groups of postmenopausal women.

7 MAIN FINDINGS AND CONCLUSIONS

The main findings of the present study can be summarized as follows:

1. Genetic effects accounted for 40 to 60% of the variance in lower limb bone strength and 80% of the variance in upper limb bone strength, while the remaining variances were explained by environmental effects.
2. Bone strength in the epiphyseal bone sites shared their genetic effects fully and environmental effects partly.
3. The association between muscle cross-sectional area and bone strength has its origin in both common genetic and common environmental effects.
4. Hip fracture history is associated with reduced bone strength in the tibia of the affected leg through reduced bone mineral density and impaired bone structure.
5. Long-term postmenopausal hormone therapy is positively associated with bone structural strength. Hormone therapy may prevent the decline in bone mineral density in epiphyseal and diaphyseal bone and may also prevent the impairment of bone structure in diaphyseal bone.
6. Loading environment modifies the heritability of bone strength: in a body-weight-loaded bone, the proportion of environmental effects is larger than in a non-weight-loaded bone. However, loading environment does not modify the influence of HT on bone strength. Muscle-induced loading may preserve bone structural strength after hip fracture.

YHTEENVETO

Perimän ja ympäristötekijöiden vaikutus luun lujuuteen vaihdevuosi-iän ohittaneilla naisilla

Ikääntyessä luuston mineraalitiheyden lasku ja rakenteen muutokset johtavat luuston heikkenemiseen. Tämä heikkeneminen lisää murtumariskiä ja voi lopulta johtaa luunmurtumiin. Luunmurtumat, etenkin lonkkamurtumat, saattavat olla kohtalokkaita iäkkäille, koska ne voivat huonontaa tuntuvasti toimintakykyä ja terveyttä. Vaihdevuosi-iän ohittaneet naiset ovat luustonsa rakenteen vuoksi erityisen alttiita murtumille. Luututkimus on aiemmin keskittynyt luun mineraalimassaan vaikuttavien tekijöiden selvittämiseen, vaikka luun lujuuteen vaikuttavat useat muutkin tekijät, kuten luun mikro- ja makrorakenne. Luun lujuutta ja siihen vaikuttavia tekijöitä on kuitenkin tutkittu niukasti.

Tämän väitöskirjatutkimuksen tarkoituksena oli selvittää vaihdevuosi-iän ohittaneiden naisten laskennallisen luun lujuuden taustalla olevien perintö- ja ympäristötekijöiden vaikutuksia. Tutkimuksessa määritettiin perimän ja ympäristötekijöiden suhteelliset osuudet yksilöiden välisistä eroista sekä tarkasteltiin kahden ympäristötekijän, aiemman lonkkamurtuman ja hormonikorvaushoidon käytön, yhteyttä luun lujuuteen ja lujuuden osatekijöihin. Lisäksi tarkoituksena oli tarkastella kuormitusympäristön yhteyttä luun lujuuteen vertaamalla kehon painoa kantavaa luuta (alaraajan luu) luuhun, johon ei kohdistu kehon painoa (yläraajan luu) sekä arvioimalla kuormitusta lihaksen ominaisuuksien avulla.

Tutkimuksessa käytettiin kolmea eri aineistoa. Aineistoa, joka koostui 103 identtisestä ja 114 epäidenttisestä 63–76-vuotiaasta naiskaksosparista, käytettiin määritettäessä perimän ja ympäristön vaikutusten suhteellisia osuuksia. Toinen aineisto koostui 38:sta iältään 60—85-vuotiaasta naisesta, joilla oli ollut lonkkamurtuma keskimäärin 3,5 vuotta aiemmin ja 22 vastaavanikäisestä naisesta, joilla ei ollut aiempia alaraajamurtumia. Kolmas aineisto sisälsi 24 identtistä naiskaksosparia, joissa toinen kaksonen oli käyttänyt hormonikorvaushoitoa pitkäaikaisesti (keskimäärin 8 vuotta), ja toinen ei ollut käyttänyt sitä koskaan. Tutkimuksen luustomittaukset tehtiin perifeerisellä tietokonetomografialaitteella ylä- ja alaraajaan.

Tämän tutkimuksen geneettisen mallinnuksen tulokset osoittivat, että perimä selittää 40—60 % yksilöiden välisistä alaraajan luun lujuuden eroista. Yläraajan luun lujuuden eroista 80 % selittyi perimällä. Tarkasteltaessa eri luukohtia havaittiin, että luun lujuutta rakenteeltaan samankaltaisissa ylä- ja alaraajan luukohdissa säätelevät täysin samat geenit ja osittain samat ympäristötekijät. Alaraajan luun lujuutta säätelevät myös osin samat geenit ja ympäristötekijät kuin alaraajan lihaksen poikkipinta-alaa. Kun luun ominaisuuksia tutkittiin naisilla, joilla oli aiemmin ollut lonkkamurtuma, havaittiin, että luun lujuus on alentunut murtuneen puolen sääriluussa. Alentunut luun lujuus johtui sekä alentuneesta mineraalitiheydestä että heikentyneestä luun rakenteesta, kuten kuoriluun seinämän ohenemisesta. Lisäksi havaittiin, että murtuneen alaraajan

lihasvoima ja lihaksen poikkipinta-ala selittivät noin 40 % luun lujuuden alenemasta. Hormonikorvaushoidon käyttäjien luun lujuus oli puolestaan merkittävästi, 9—26 %, suurempi sekä ylä- että alaraajassa kuin niiden, jotka eivät käyttäneet hormonikorvaushoitoa. Suurempi luun lujuus johtui luun ääreisosissa suuremmasta luun mineraalitiheydestä. Luun varsiosissa suurempi lujuus johtui puolestaan sekä suuremmasta mineraalitiheydestä että tukevammasta kuoriluun seinämästä. Luustoerot hormonikorvaushoidon käyttäjien ja eikäyttäjien välillä olivat samanlaiset ylä- ja alaraajassa.

Tutkimuksen tulokset viittaavat siihen, että perintötekijät määräävät suurelta osin vaihdevuosi-iän ohittaneiden naisten luun lujuuden. Ympäristötekijöillä on kuitenkin suuri merkitys etenkin alaraajojen luun lujuudelle, mikä viittaa siihen, että elämäntapatekijöillä voidaan vaikuttaa merkittävästi luuhun. Yksittäisillä ympäristötekijöillä, kuten lonkkamurtumalla ja hormonikorvaushoidolla, voi olla merkittävä vaikutus yksittäisen naisen luustoon. Lonkkamurtuma heikentää luuston rakennetta vuosiksi, mikä saattaa lisätä uuden murtuman riskiä. Hormonikorvaushoidolla on puolestaan myönteinen vaikutus vaihdevuosi-iän ohittaneiden naisten luun lujuuteen. Liikkumisen aiheuttaman kuormituksen merkitys luustolle on suuri alaraajan luussa. Kuormitus ei näytä säätelevän hormonikorvaushoidon luustovaikutuksia, mutta lihasten aiheuttama kuormitus näyttäisi olevan tärkeä tekijä luun heikkenemisen ehkäisyssä lonkkamurtuman jälkeen.

REFERENCES

Prevention and management of osteoporosis. Report of a WHO scientific group. 2003. WHO Tech Rep Ser 921. Geneva.

Adami, S., Gatti, D., Braga, V., Bianchini, D. & Rossini, M. 1999. Site-specific effects of strength training on bone structure and geometry of ultradistal radius in postmenopausal women. J.Bone Miner.Res. 14 (1), 120-124.

Adamo, M. L. & Farrar, R. P. 2006. Resistance training, and IGF involvement in the maintenance of muscle mass during the aging process. Ageing Res.Rev. 5 (3), 310-331.

Adams, J. E. 2009. Quantitative computed tomography. Eur.J.Radiol. 71 (3), 415-424.

Ahlborg, H. G., Johnell, O., Turner, C. H., Rannevik, G. & Karlsson, M. K. 2003. Bone loss and bone size after menopause. N.Engl.J.Med. 349 (4), 327-334.

Allen, M. R. & Bloomfield, S. A. 2003. Hindlimb unloading has a greater effect on cortical compared with cancellous bone in mature female rats. J.Appl.Physiol. 94 (2), 642-650.

Banks, E., Reeves, G. K., Beral, V., Balkwill, A., Liu, B., Roddam, A. & Million Women Study Collaborators 2009. Hip fracture incidence in relation to age, menopausal status, and age at menopause: prospective analysis. PLoS Med. 6 (11), e1000181.

Beck, T. J. 2007. Extending DXA beyond bone mineral density: understanding hip structure analysis. Curr.Osteoporos Rep. 5 (2), 49-55.

Beck, T. J., Stone, K. L., Oreskovic, T. L., Hochberg, M. C., Nevitt, M. C., Genant, H. K. & Cummings, S. R. 2001. Effects of current and discontinued estrogen replacement therapy on hip structural geometry: the study of osteoporotic fractures. J.Bone Miner.Res. 16 (11), 2103-2110.

Berg, H. E., Eiken, O., Miklavcic, L. & Mekjavic, I. B. 2007. Hip, thigh and calf muscle atrophy and bone loss after 5-week bedrest inactivity. Eur.J.Appl.Physiol. 99 (3), 283-289.

Binkley, T. L., Berry, R. & Specker, B. L. 2008. Methods for measurement of pediatric bone. Rev.Endocr Metab.Disord. 9 (2), 95-106.

Bischoff-Ferrari, H. A., Willett, W. C., Wong, J. B., Giovannucci, E., Dietrich, T. & Dawson-Hughes, B. 2005. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA 293 (18), 2257-2264.

Bolotin, H. H., Sievänen, H. & Grashuis, J. L. 2003. Patient-specific DXA bone mineral density inaccuracies: quantitative effects of nonuniform extraosseous fat distributions. J.Bone Miner.Res. 18 (6), 1020-1027.

Bono, C. M. & Einhorn, T. A. 2003. Overview of osteoporosis: pathophysiology and determinants of bone strength. Eur.Spine J. 12 Suppl 2, S90-6.

Boomsma, D., Busjahn, A. & Peltonen, L. 2002. Classical twin studies and beyond. Nat.Rev.Genet. 3 (11), 872-882.

Borer, K. T. 2005. Physical activity in the prevention and amelioration of osteoporosis in women: interaction of mechanical, hormonal and dietary factors. Sports Med. 35 (9), 779-830.

Borer, K. T., Fogleman, K., Gross, M., La New, J. M. & Dengel, D. 2007. Walking intensity for postmenopausal bone mineral preservation and accrual. Bone 41 (4), 713-721.

Borgström, F., Zethraeus, N., Johnell, O., Lidgren, L., Ponzer, S., Svensson, O., Abdon, P., Ornstein, E., Lunsjo, K., Thorngren, K. G., Sernbo, I., Rehnberg, C. & Jonsson, B. 2006. Costs and quality of life associated with osteoporosis-related fractures in Sweden. Osteoporos.Int. 17 (5), 637-650.

Bouillon, R. & Reid, I. R. 2008. Vitamin D and its metabolites and analogs in the management of osteoporosis. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (3.th edition) Burlington MA USA: Elsevier, 1659-1686.

Bouxsein, M. L. 2008. Biomechanics of age-related fractures. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (3.th edition) Burlington MA USA: Elsevier, 601.

Bråten, M., Nordby, A., Terjesen, T. & Rossvoll, I. 1992. Bone loss after locked intramedullary nailing. Computed tomography of the femur and tibia in 10 cases. Acta Orthop.Scand. 63 (3), 310-314.

Cano, A., Dapia, S., Noguera, I., Pineda, B., Hermenegildo, C., del Val, R., Caeiro, J. R. & Garcia-Perez, M. A. 2008. Comparative effects of 17beta-estradiol, raloxifene and genistein on bone 3D microarchitecture and volumetric bone mineral density in the ovariectomized mice. Osteoporos.Int. 19 (6), 793-800.

Carter, D. R. & Hayes, W. C. 1976. Bone compressive strength: the influence of density and strain rate. Science 194 (4270), 1174-1176.

Cauley, J. A., Robbins, J., Chen, Z., Cummings, S. R., Jackson, R. D., LaCroix, A. Z., LeBoff, M., Lewis, C. E., McGowan, J., Neuner, J., Pettinger, M., Stefanick, M. L., Wactawski-Wende, J., Watts, N. B. & Women's Health Initiative Investigators 2003. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA 290 (13), 1729-1738.

Chapurlat, R. D., Bauer, D. C., Nevitt, M., Stone, K. & Cummings, S. R. 2003. Incidence and risk factors for a second hip fracture in elderly women. The Study of Osteoporotic Fractures. Osteoporos.Int. 14 (2), 130-136.

Chen, Z., Beck, T. J., Cauley, J. A., Lewis, C. E., LaCroix, A., Bassford, T., Wu, G., Sherrill, D. & Going, S. 2008. Hormone therapy improves femur geometry among ethnically diverse postmenopausal participants in the Women's Health Initiative hormone intervention trials. J.Bone Miner.Res. 23 (12), 1935-1945.

Cheng, S., Sipilä, S., Taaffe, D. R., Puolakka, J. & Suominen, H. 2002. Change in bone mass distribution induced by hormone replacement therapy and high-impact physical exercise in post-menopausal women. Bone 31 (1), 126-135.

Christiansen, P., Steiniche, T., Brixen, K., Hessov, I., Melsen, F., Charles, P. & Mosekilde, L. 1997. Primary hyperparathyroidism: biochemical markers and

bone mineral density at multiple skeletal sites in Danish patients. Bone 21 (1), 93-99.

Colon-Emeric, C., Kuchibhatla, M., Pieper, C., Hawkes, W., Fredman, L., Magaziner, J., Zimmerman, S. & Lyles, K. W. 2003. The contribution of hip fracture to risk of subsequent fractures: data from two longitudinal studies. Osteoporos.Int. 14 (11), 879-883.

Cooper, D. M., Thomas, C. D., Clement, J. G., Turinsky, A. L., Sensen, C. W. & Hallgrimsson, B. 2007. Age-dependent change in the 3D structure of cortical porosity at the human femoral midshaft. Bone 40 (4), 957-965.

Cranney, A., Guyatt, G., Griffith, L., Wells, G., Tugwell, P., Rosen, C. & Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group 2002. Meta-analyses of therapies for postmenopausal osteoporosis. IX: Summary of meta-analyses of therapies for postmenopausal osteoporosis. Endocr.Rev. 23 (4), 570-578.

Cummings, S. R., Ettinger, B., Delmas, P. D., Kenemans, P., Stathopoulos, V., Verweij, P., Mol-Arts, M., Kloosterboer, L., Mosca, L., Christiansen, C., Bilezikian, J., Kerzberg, E. M., Johnson, S., Zanchetta, J., Grobbee, D. E., Seifert, W., Eastell, R. & LIFT Trial Investigators 2008. The effects of tibolone in older postmenopausal women. N.Engl.J.Med. 359 (7), 697-708.

Cummings, S. R. & Melton, L. J. 2002. Epidemiology and outcomes of osteoporotic fractures. Lancet 359 (9319), 1761-1767.

Currey, J. D. 2002. Bones: Structure and Mechanics. Princeton, NY, USA: Princeton University Press.

Currey, J. D. 2001. Bone strength: what are we trying to measure? Calcif.Tissue Int. 68 (4), 205-210.

Daly, R. M., Saxon, L., Turner, C. H., Robling, A. G. & Bass, S. L. 2004. The relationship between muscle size and bone geometry during growth and in response to exercise. Bone 34 (2), 281-287.

de Kam, D., Smulders, E., Weedersteyn, V. & Smits-Engelsman, B.M.C. 2009 Exercise interventions to reduce fall-related fractures and their risk factors in individuals with low bone density: a systematic review of randomized controlled trials. Osteoporos. Int. 20 (12), 2111-2125.

Deng, F. Y., Lei, S. F., Li, M. X., Jiang, C., Dvornyk, V. & Deng, H. W. 2006. Genetic determination and correlation of body mass index and bone mineral density at the spine and hip in Chinese Han ethnicity. Osteoporos.Int. 17 (1), 119-124.

Deng, F. Y., Xiao, P., Lei, S. F., Zhang, L., Yang, F., Tang, Z. H., Liu, P. Y., Liu, Y. J., Recker, R. R. & Deng, H. W. 2007. Bivariate whole genome linkage analysis for femoral neck geometric parameters and total body lean mass. J.Bone Miner.Res. 22 (6), 808-816.

Duffy, D. L. 2000. The co-twin control study. In T. D. Spector, H. Snieder & A. J. MacGregor (Eds.) Advances in twin and sib-pair analysis.London: Greenwich Medical Media, 53--66.

Ellis, K. J. 2000. Human body composition: in vivo methods. Physiol.Rev. 80 (2), 649-680.

Falchetti, A., Sferrazza, C., Cepollaro, C., Gozzini, A., Del Monte, F., Masi, L., Napoli, N., Di Fede, G., Cannone, V., Cusumano, G., Pandolfo, M. C., Rini, G. B., Tanini, A. & Brandi, M. L. 2007. FokI polymorphism of the vitamin D receptor gene correlates with parameters of bone mass and turnover in a female population of the Italian island of Lampedusa. Calcif.Tissue Int. 80 (1), 15-20.

Ferretti, J. L., Capozza, R. F., Cointry, G. R., Garcia, S. L., Plotkin, H., Alvarez Filgueira, M. L. & Zanchetta, J. R. 1998. Gender-related differences in the relationship between densitometric values of whole-body bone mineral content and lean body mass in humans between 2 and 87 years of age. Bone 22 (6), 683-690.

Findlay, S. C., Eastell, R. & Ingle, B. M. 2002. Measurement of bone adjacent to tibial shaft fracture. Osteoporos.Int. 13 (12), 980-989.

Fox, K. M., Magaziner, J., Hawkes, W. G., Yu-Yahiro, J., Hebel, J. R., Zimmerman, S. I., Holder, L. & Michael, R. 2000. Loss of bone density and lean body mass after hip fracture. Osteoporos.Int. 11 (1), 31-35.

Frost, H. M. 2003. Bone's mechanostat: a 2003 update. Anat.Rec.A.Discov.Mol.Cell.Evol.Biol. 275 (2), 1081-1101.

Garefalakis, M. & Hickey, M. 2008. Role of androgens, progestins and tibolone in the treatment of menopausal symptoms: a review of the clinical evidence. Clin.Interv.Aging 3 (1), 1-8.

Genant, H. K., Engelke, K. & Prevrhal, S. 2008. Advanced CT bone imaging in osteoporosis. Rheumatology (Oxford) 47 Suppl 4, iv9-16.

Gibbons, L. E., Videman, T., Battie, M. C. & Kaprio, J. 1998. Determinants of paraspinal muscle cross-sectional area in male monozygotic twins. Phys.Ther. 78 (6), 602-10; discussion 611-2.

Going, S., Lohman, T., Houtkooper, L., Metcalfe, L., Flint-Wagner, H., Blew, R., Stanford, V., Cussler, E., Martin, J., Teixeira, P., Harris, M., Milliken, L., Figueroa-Galvez, A. & Weber, J. 2003. Effects of exercise on bone mineral density in calcium-replete postmenopausal women with and without hormone replacement therapy. Osteoporos.Int. 14 (8), 637-643.

Greenspan, S. L., Beck, T. J., Resnick, N. M., Bhattacharya, R. & Parker, R. A. 2005. Effect of hormone replacement, alendronate, or combination therapy on hip structural geometry: a 3-year, double-blind, placebo-controlled clinical trial. J.Bone Miner.Res. 20 (9), 1525-1532.

Griffith, J. F. & Genant, H. K. 2008. Bone mass and architecture determination: state of the art. Best Pract.Res.Clin.Endocrinol.Metab. 22 (5), 737-764.

Grimby, G. 1986. Physical activity and muscle training in the elderly. Acta Med.Scand.Suppl. 711, 233-237.

Guglielmi, G., Adams, J. & Link, T. M. 2009. Quantitative ultrasound in the assessment of skeletal status. Eur.Radiol. 19 (8), 1837-1848.

Han, Z. H., Palnitkar, S., Rao, D. S., Nelson, D. & Parfitt, A. M. 1996. Effect of ethnicity and age or menopause on the structure and geometry of iliac bone. J.Bone Miner.Res. 11 (12), 1967-1975.

Harris, M., Nguyen, T. V., Howard, G. M., Kelly, P. J. & Eisman, J. A. 1998. Genetic and environmental correlations between bone formation and bone mineral density: a twin study. Bone 22 (2), 141-145.

Heinonen, A., McKay, H. A., Whittall, K. P., Forster, B. B. & Khan, K. M. 2001. Muscle cross-sectional area is associated with specific site of bone in prepubertal girls: a quantitative magnetic resonance imaging study. Bone 29 (4), 388-392.

Heinonen, A., Sievänen, H., Kyröläinen, H., Perttunen, J. & Kannus, P. 2001. Mineral mass, size, and estimated mechanical strength of triple jumpers' lower limb. Bone 29 (3), 279-285.

Hopper, J. L., Green, R. M., Nowson, C. A., Young, D., Sherwin, A. J., Kaymakci, B., Larkins, R. G. & Wark, J. D. 1998. Genetic, common environment, and individual specific components of variance for bone mineral density in 10- to 26-year-old females: a twin study. Am.J.Epidemiol. 147 (1), 17-29.

Howard, G. M., Nguyen, T. V., Harris, M., Kelly, P. J. & Eisman, J. A. 1998. Genetic and environmental contributions to the association between quantitative ultrasound and bone mineral density measurements: a twin study. J.Bone Miner.Res. 13 (8), 1318-1327.

Hsieh, Y. F., Robling, A. G., Ambrosius, W. T., Burr, D. B. & Turner, C. H. 2001. Mechanical loading of diaphyseal bone in vivo: the strain threshold for an osteogenic response varies with location. J.Bone Miner.Res. 16 (12), 2291-2297.

Huang, Q. Y. & Kung, A. W. 2006. Genetics of osteoporosis. Mol.Genet.Metab. 88 (4), 295-306.

Huncharek, M., Muscat, J. & Kupelnick, B. 2008. Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis. Bone 43 (2), 312-321.

Hunter, D. J., de Lange, M., Andrew, T., Snieder, H., MacGregor, A. J. & Spector, T. D. 2001. Genetic variation in bone mineral density and calcaneal ultrasound: a study of the influence of menopause using female twins. Osteoporos.Int. 12 (5), 406-411.

Järvinen, M. & Kannus, P. 1997. Injury of an extremity as a risk factor for the development of osteoporosis. J.Bone Joint Surg.Am. 79 (2), 263-276.

Järvinen, T. L., Kannus, P. & Sievänen, H. 2003. Estrogen and bone--a reproductive and locomotive perspective. J.Bone Miner.Res. 18 (11), 1921-1931.

Järvinen, T. L., Sievänen, H., Jokihaara, J. & Einhorn, T. A. 2005. Revival of bone strength: the bottom line. J.Bone Miner.Res. 20 (5), 717-720.

Järvinen, T. L., Sievänen, H., Khan, K. M., Heinonen, A. & Kannus, P. 2008. Shifting the focus in fracture prevention from osteoporosis to falls. BMJ 336 (7636), 124-126.

Johnell, O. & Kanis, J. A. 2004. An estimate of the worldwide prevalence, mortality and disability associated with hip fracture. Osteoporos.Int. 15 (11), 897-902.

Jones, E. J., Bishop, P. A., Woods, A. K. & Green, J. M. 2008. Cross-sectional area and muscular strength: a brief review. Sports Med. 38 (12), 987-994.

Kalkwarf, H. J., Khoury, J. C. & Lanphear, B. P. 2003. Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. Am.J.Clin.Nutr. 77 (1), 257-265.

Kanis, J. A. 2002. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359 (9321), 1929-1936.

Kannus, P., Niemi, S., Parkkari, J., Palvanen, M., Vuori, I. & Järvinen, M. 2006. Nationwide decline in incidence of hip fracture. J.Bone Miner.Res. 21 (12), 1836-1838.

Kannus, P., Järvinen, M., Sievänen, H., Järvinen, T. A., Oja, P. & Vuori, I. 1994a. Reduced bone mineral density in men with a previous femur fracture. J.Bone Miner.Res. 9 (11), 1729-1736.

Kannus, P., Järvinen, M., Sievänen, H., Oja, P. & Vuori, I. 1994b. Osteoporosis in men with a history of tibial fracture. J.Bone Miner.Res. 9 (3), 423-429.

Kaprio, J. & Koskenvuo, M. 2002. Genetic and environmental factors in complex diseases: the older Finnish Twin Cohort. Twin Res. 5 (5), 358-365.

Kaprio, J., Sarna, S., Koskenvuo, M. & Rantasalo, I. 1978. The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program. Prog.Clin.Biol.Res. 24 Pt B, 179-184.

Kaptoge, S., Dalzell, N., Jakes, R. W., Wareham, N., Day, N. E., Khaw, K. T., Beck, T. J., Loveridge, N. & Reeve, J. 2003. Hip section modulus, a measure of bending resistance, is more strongly related to reported physical activity than BMD. Osteoporos.Int. 14 (11), 941-949.

Karasik, D. & Kiel, D. P. 2010. Evidence for pleiotropic factors in genetics of the musculoskeletal system. Bone 46 (5), 1226-1237.

Karasik, D., Zhou, Y., Cupples, L. A., Hannan, M. T., Kiel, D. P. & Demissie, S. 2009. Bivariate genome-wide linkage analysis of femoral bone traits and leg lean mass: Framingham study. J.Bone Miner.Res. 24 (4), 710-718.

Karlsson, M., Nilsson, J. A., Sernbo, I., Redlund-Johnell, I., Johnell, O. & Obrant, K. J. 1996. Changes of bone mineral mass and soft tissue composition after hip fracture. Bone 18 (1), 19-22.

Kelley, G. A., Kelley, K. S. & Tran, Z. V. 2001. Resistance training and bone mineral density in women: a meta-analysis of controlled trials. Am.J.Phys.Med.Rehabil. 80 (1), 65-77.

Khan, K. M., Bennell, K. L., Hopper, J. L., Flicker, L., Nowson, C. A., Sherwin, A. J., Crichton, K. J., Harcourt, P. R. & Wark, J. D. 1998. Self-reported ballet classes undertaken at age 10-12 years and hip bone mineral density in later life. Osteoporos.Int. 8 (2), 165-173.

Khosla, S., Riggs, B. L., Atkinson, E. J., Oberg, A. L., McDaniel, L. J., Holets, M., Peterson, J. M. & Melton, L. J.,3rd 2006. Effects of sex and age on bone microstructure at the ultradistal radius: a population-based noninvasive in vivo assessment. J.Bone Miner.Res. 21 (1), 124-131.

Knapp, K. M., Andrew, T., MacGregor, A. J., Blake, G. M., Fogelman, I. & Spector, T. D. 2003. An investigation of unique and shared gene effects on speed of sound and bone density using axial transmission quantitative ultrasound and DXA in twins. J.Bone Miner.Res. 18 (8), 1525-1530.

Kontulainen S. A., Johnston, J. D., Liu, D., Leung, C., Oxland, T. R. & McKay, H. A. 2008. Strength indices from pQCT imaging predict up to 85% of variance in bone failure properties at tibial epiphysis and diaphysis. J.Musculoskelet.Neuronal Interact. 8 (4), 401-409.

Korpelainen, R., Keinänen-Kiukaanniemi, S., Heikkinen, J., Väänänen, K. & Korpelainen, J. 2006. Effect of impact exercise on bone mineral density in elderly women with low BMD: a population-based randomized controlled 30-month intervention. Osteoporos.Int. 17 (1), 109-118.

Kostek, M. C., Delmonico, M. J., Reichel, J. B., Roth, S. M., Douglass, L., Ferrell, R. E. & Hurley, B. F. 2005. Muscle strength response to strength training is influenced by insulin-like growth factor 1 genotype in older adults. J.Appl.Physiol. 98 (6), 2147-2154.

Kyvik, K. O. 2000. Generalizability and assumptions of twin studies. In T. D. Spector, H. Snieder & A. J. MacGregor (Eds.) Advances in twin and sib-pair analysis.London: Greenwich Medical Media, 67--78.

Lacroix, A. Z., Beck, T. J., Cauley, J. A., Lewis, C. E., Bassford, T., Jackson, R., Wu, G. & Chen, Z. 2009. Hip structural geometry and incidence of hip fracture in postmenopausal women: what does it add to conventional bone mineral density? Osteoporos.Int. 21 (6), 919-929.

Lanyon, L. & Skerry, T. 2001. Postmenopausal osteoporosis as a failure of bone's adaptation to functional loading: a hypothesis. J.Bone Miner.Res. 16 (11), 1937-1947.

Lauretani, F., Bandinelli, S., Griswold, M. E., Maggio, M., Semba, R., Guralnik, J. M. & Ferrucci, L. 2008. Longitudinal changes in BMD and bone geometry in a population-based study. J.Bone Miner.Res. 23 (3), 400-408.

Lauretani, F., Bandinelli, S., Russo, C. R., Maggio, M., Di Iorio, A., Cherubini, A., Maggio, D., Ceda, G. P., Valenti, G., Guralnik, J. M. & Ferrucci, L. 2006. Correlates of bone quality in older persons. Bone 39 (4), 915-921.

Law, M. R. & Hackshaw, A. K. 1997. A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. BMJ 315 (7112), 841-846.

Lee, M., Czerwinski, S. A., Choh, A. C., Towne, B., Demerath, E. W., Chumlea, W. C., Sun, S. S. & Siervogel, R. M. 2004. Heritability of calcaneal quantitative ultrasound measures in healthy adults from the Fels Longitudinal Study. Bone 35 (5), 1157-1163.

Lenchik, L., Hsu, F. C., Register, T. C., Lohman, K. K., Freedman, B. I., Langefeld, C. D., Bowden, D. W. & Carr, J. J. 2004. Heritability of spinal trabecular volumetric bone mineral density measured by QCT in the Diabetes Heart Study. Calcif.Tissue Int. 75 (4), 305-312.

Leppälä, J., Kannus, P., Natri, A., Pasanen, M., Sievänen, H., Vuori, I. & Järvinen, M. 1999a. Effect of anterior cruciate ligament injury of the knee on bone mineral density of the spine and affected lower extremity: a prospective one-year follow-Up study. Calcif.Tissue Int. 64 (4), 357-363.

Leppälä, J., Kannus, P., Niemi, S., Sievänen, H., Vuori, I. & Järvinen, M. 1999b. An early-life femoral shaft fracture and bone mineral density at adulthood. Osteoporos.Int. 10 (4), 337-342.

Leppälä, J., Kannus, P., Sievänen, H., Vuori, I. & Järvinen, M. 1999c. A tibial shaft fracture sustained in childhood or adolescence does not seem to interfere with attainment of peak bone density. J.Bone Miner.Res. 14 (6), 988-993.

Lewin, B. 1997. Genes VI. New York, USA: Oxford University Press.

Lorentzon, M., Eriksson, A. L., Nilsson, S., Mellström, D. & Ohlsson, C. 2007. Association between physical activity and BMD in young men is modulated by cathecol-o-methyltransferase (COMT) genotype: The GOOD study. J.Bone Miner.Res. 22 (8), 1165-1172.

Lowe, H. & Shane, E. 2008. Osteoporosis associated with illnesses and medications. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (3.th edition) Burlington MA USA: Elsevier, 1283-1314.

Ma, H., Leskinen, T., Alen, M., Cheng, S., Sipilä, S., Heinonen, A., Kaprio, J., Suominen, H. & Kujala, U. M. 2009. Long-term leisure time physical activity and properties of bone: a twin study. J.Bone Miner.Res. 24 (8), 1427-1433.

MacGregor, A. J., Snieder, H., Schork, N. J. & Spector, T. D. 2000. Twins. Novel uses to study complex traits and genetic diseases. Trends Genet. 16 (3), 131-134.

Magaziner, J., Wehren, L., Hawkes, W. G., Orwig, D., Hebel, J. R., Fredman, L., Stone, K., Zimmerman, S. & Hochberg, M. C. 2006. Women with hip fracture have a greater rate of decline in bone mineral density than expected: another significant consequence of a common geriatric problem. Osteoporos.Int. 17 (7), 971-977.

Magnusson, H. I., Linden, C., Obrant, K. J., Johnell, O. & Karlsson, M. K. 2001. Bone mass changes in weight-loaded and unloaded skeletal regions following a fracture of the hip. Calcif.Tissue Int. 69 (2), 78-83.

Manolagas, S. C. 2000. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr.Rev. 21 (2), 115-137.

Martin, J. C., Munro, R., Campbell, M. K. & Reid, D. M. 1997. Effects of disease and corticosteroids on appendicular bone mass in postmenopausal women with rheumatoid arthritis: comparison with axial measurements. Br.J.Rheumatol. 36 (1), 43-49.

Martin, R. B. 1991. Determinants of the mechanical properties of bones. J.Biomech. 24 Suppl 1, 79-88.

Martin, T. J. & Seeman, E. 2008. Bone remodelling: its local regulation and the emergence of bone fragility. Best Pract.Res.Clin.Endocrinol.Metab. 22 (5), 701-722.

Martyn-St James, M. & Carroll, S. 2008. Meta-analysis of walking for preservation of bone mineral density in postmenopausal women. Bone 43 (3), 521-531.

Matkovic, V., Landoll, J. D., Badenhop-Stevens, N. E., Ha, E. Y., Crncevic-Orlic, Z., Li, B. & Goel, P. 2004a. Nutrition influences skeletal development from

childhood to adulthood: a study of hip, spine, and forearm in adolescent females. J.Nutr. 134 (3), 701S-705S.

McGowan, J. A. & Stefanik, M. L. 2008. Estrogen therapy: prevention and treatment of osteoporosis. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (3.th edition) Burlington MA USA: Elsevier, 1687-1704.

Morgan, W. R., Barnes, G. L. & Einhorn, T. A. 2008. The bone organ system: form and function. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (3.th edition) Burlington MA USA: Elsevier, 3-25.

Mosley, J. R. & Lanyon, L. E. 1998. Strain rate as a controlling influence on adaptive modeling in response to dynamic loading of the ulna in growing male rats. Bone 23 (4), 313-318.

Natri, A., Sievänen, H., Parkkari, J., Kannus, P., Vihtonen, K., Koskela, J. & Järvinen, M. 1999. Spontaneous shaft fracture of the tibia in weightlifting. A case report with dual-energy X-ray absorptiometry and peripheral quantitative computed tomography measurements. Am.J.Sports Med. 27 (2), 238-240.

Natsui, K., Tanaka, K., Suda, M., Yasoda, A., Sakuma, Y., Ozasa, A., Ozaki, S. & Nakao, K. 2006. High-dose glucocorticoid treatment induces rapid loss of trabecular bone mineral density and lean body mass. Osteoporos.Int. 17 (1), 105-108.

Neale, M. C., Boker, S. M., Xie, G. & Maes, H. H. 2003. Mx: Statistical Modeling. (6th edition) Richmond VA, USA: Department of Psychiatry, Virginia Commonwealth University.

Neale, M. C. & Cardon, L. R. 1992. Methodology for Genetic Studies of Twins and Families. Dordrecht, Netherlands: Kluwer.

Ng, M. Y., Sham, P. C., Paterson, A. D., Chan, V. & Kung, A. W. 2006. Effect of environmental factors and gender on the heritability of bone mineral density and bone size. Ann.Hum.Genet. 70 (Pt 4), 428-438.

Nguyen, N. D., Ahlborg, H. G., Center, J. R., Eisman, J. A. & Nguyen, T. V. 2007. Residual lifetime risk of fractures in women and men. J.Bone Miner.Res. 22 (6), 781-788.

Nguyen, T. V., Howard, G. M., Kelly, P. J. & Eisman, J. A. 1998. Bone mass, lean mass, and fat mass: same genes or same environments? Am.J.Epidemiol. 147 (1), 3-16.

Nikander, R., Sievänen, H., Heinonen, A., Daly, R.M., Uusi-Rasi, K. & Kannus, P. 2010. Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life. BMC Med. 8, 47.

Nikander, R., Sievänen, H., Uusi-Rasi, K., Heinonen, A. & Kannus, P. 2006. Loading modalities and bone structures at nonweight-bearing upper extremity and weight-bearing lower extremity: a pQCT study of adult female athletes. Bone 39 (4), 886-894.

Niu, T. & Rosen, C. J. 2005. The insulin-like growth factor-I gene and osteoporosis: a critical appraisal. Gene 361, 38-56.

Nordström, P. & Lorentzon, R. 1999. Influence of heredity and environment on bone density in adolescent boys: a parent-offspring study. Osteoporos.Int. 10 (4), 271-277.

Opotowsky, A. R. & Bilezikian, J. P. 2003. Racial differences in the effect of early milk consumption on peak and postmenopausal bone mineral density. J.Bone Miner.Res. 18 (11), 1978-1988.

Pajamäki, I., Sievänen, H., Kannus, P., Jokihaara, J., Vuohelainen, T. & Järvinen, T. L. 2008. Skeletal effects of estrogen and mechanical loading are structurally distinct. Bone 43 (4), 748-757.

Parfitt, A. M. 2008. Skeletal heterogeneity and the purposes of bone remodeling; implications for the understanding of osteoporosis. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (3.th edition) Burlington MA USA: Elsevier, 837-860.

Parfitt, A. M. 2002. Targeted and nontargeted bone remodeling: relationship to basic multicellular unit origination and progression. Bone 30 (1), 5-7.

Peacock, M., Turner, C. H., Econs, M. J. & Foroud, T. 2002. Genetics of osteoporosis. Endocr.Rev. 23 (3), 303-326.

Petersen, M. M., Gehrchen, P. M., Nielsen, P. K. & Lund, B. 1997. Loss of bone mineral of the hip assessed by DEXA following tibial shaft fractures. Bone 20 (5), 491-495.

Phillips, D. I. 2000. Fetal programming or genes? In T. D. Spector, H. Snieder & A. J. MacGregor (Eds.) Advances in twin and sib-pair analysis.London: Greenwich Medical Media, 67--78.

Pocock, N. A., Eisman, J. A., Hopper, J. L., Yeates, M. G., Sambrook, P. N. & Eberl, S. 1987. Genetic determinants of bone mass in adults. A twin study. J.Clin.Invest. 80 (3), 706-710.

Pogoda, P., Priemel, M., Rueger, J. M. & Amling, M. 2005. Bone remodeling: new aspects of a key process that controls skeletal maintenance and repair. Osteoporos.Int. 16 Suppl 2, S18-24.

Posthuma, D., Beem, A. L., de Geus, E. J., van Baal, G. C., von Hjelmborg, J. B., Iachine, I. & Boomsma, D. I. 2003. Theory and practice in quantitative genetics. Twin Res. 6 (5), 361-376.

Prior, S. J., Roth, S. M., Wang, X., Kammerer, C., Miljkovic-Gacic, I., Bunker, C. H., Wheeler, V. W., Patrick, A. L. & Zmuda, J. M. 2007. Genetic and environmental influences on skeletal muscle phenotypes as a function of age and sex in large, multigenerational families of African heritage. J.Appl.Physiol. 103 (4), 1121-1127.

Ralston, S. H. & de Crombrugghe, B. 2006. Genetic regulation of bone mass and susceptibility to osteoporosis. Genes Dev. 20 (18), 2492-2506.

Ralston, S. H., Uitterlinden, A. G., Brandi, M. L., Balcells, S., Langdahl, B. L., Lips, P., Lorenc, R., Obermayer-Pietsch, B., Scollen, S., Bustamante, M., Husted, L. B., Carey, A. H., Diez-Perez, A., Dunning, A. M., Falchetti, A., Karczmarewicz, E., Kruk, M., van Leeuwen, J. P., van Meurs, J. B., Mangion, J., McGuigan, F. E., Mellibovsky, L., del Monte, F., Pols, H. A., Reeve, J., Reid, D. M., Renner, W., Rivadeneira, F., van Schoor, N. M., Sherlock, R. E.,

Ioannidis, J. P. & GENOMOS Investigators 2006. Large-scale evidence for the effect of the COLIA1 Sp1 polymorphism on osteoporosis outcomes: the GENOMOS study. PLoS Med. 3 (4), e90.

Rantalainen, T., Heinonen, A., Komi, P. V. & Linnamo, V. 2008. Neuromuscular performance and bone structural characteristics in young healthy men and women. Eur.J.Appl.Physiol. 102 (2), 215-222.

Riggs, B. L., Khosla, S. & Melton, L. J.,3rd 2002. Sex steroids and the construction and conservation of the adult skeleton. Endocr.Rev. 23 (3), 279-302.

Riggs, L. B., Khosla, S. & Melton, L. J. 2008. Estrogen, bone homeostasis, and osteoporosis. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (Third edition edition) Elsevier Academic Press, 1011-1040.

Rijsdijk, F. V. & Sham, P. C. 2002. Analytic approaches to twin data using structural equation models. Brief Bioinform 3 (2), 119-133.

Rittweger, J. & Felsenberg, D. 2009. Recovery of muscle atrophy and bone loss from 90 days bed rest: results from a one-year follow-up. Bone 44 (2), 214-224.

Rittweger, J., Simunic, B., Bilancio, G., De Santo, N. G., Cirillo, M., Biolo, G., Pisot, R., Eiken, O., Mekjavic, I. B. & Narici, M. 2009. Bone loss in the lower leg during 35 days of bed rest is predominantly from the cortical compartment. Bone 44 (4), 612-618.

Rittweger, J., Winwood, K., Seynnes, O., de Boer, M., Wilks, D., Lea, R., Rennie, M. & Narici, M. 2006. Bone loss from the human distal tibia epiphysis during 24 days of unilateral lower limb suspension. J.Physiol. 577 (Pt 1), 331-337.

Rittweger, J., Frost, H. M., Schiessl, H., Ohshima, H., Alkner, B., Tesch, P. & Felsenberg, D. 2005. Muscle atrophy and bone loss after 90 days' bed rest and the effects of flywheel resistive exercise and pamidronate: results from the LTBR study. Bone 36 (6), 1019-1029.

Rittweger, J., Beller, G., Ehrig, J., Jung, C., Koch, U., Ramolla, J., Schmidt, F., Newitt, D., Majumdar, S., Schiessl, H. & Felsenberg, D. 2000. Bone-muscle strength indices for the human lower leg. Bone 27 (2), 319-326.

Rivadeneira, F., Houwing-Duistermaat, J. J., Beck, T. J., Janssen, J. A., Hofman, A., Pols, H. A., Van Duijn, C. M. & Uitterlinden, A. G. 2004. The influence of an insulin-like growth factor I gene promoter polymorphism on hip bone geometry and the risk of nonvertebral fracture in the elderly: the Rotterdam Study. J.Bone Miner.Res. 19 (8), 1280-1290.

Rivadeneira, F., Styrkarsdottir, U., Estrada, K., Halldorsson, B. V., Hsu, Y. H., Richards, J. B., Zillikens, M. C., Kavvoura, F. K., Amin, N., Aulchenko, Y. S., Cupples, L. A., Deloukas, P., Demissie, S., Grundberg, E., Hofman, A., Kong, A., Karasik, D., van Meurs, J. B., Oostra, B., Pastinen, T., Pols, H. A., Sigurdsson, G., Soranzo, N., Thorleifsson, G., Thorsteinsdottir, U., Williams, F. M., Wilson, S. G., Zhou, Y., Ralston, S. H., van Duijn, C. M., Spector, T., Kiel, D. P., Stefansson, K., Ioannidis, J. P., Uitterlinden, A. G. & Genetic Factors for Osteoporosis (GEFOS) Consortium 2009. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat.Genet. 41 (11), 1199-1206.

Rizzoli, R. 2008. Nutrition: its role in bone health. Best Pract.Res.Clin.Endocrinol.Metab. 22 (5), 813-829.

Robling, A. G., Castillo, A. B. & Turner, C. H. 2006. Biomechanical and molecular regulation of bone remodeling. Annu.Rev.Biomed.Eng. 8, 455-498.

Roth, S. M., Zmuda, J. M., Cauley, J. A., Shea, P. R. & Ferrell, R. E. 2004. Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. J.Gerontol.A Biol.Sci.Med.Sci. 59 (1), 10-15.

Russo, C. R., Lauretani, F., Bandinelli, S., Bartali, B., Di Iorio, A., Volpato, S., Guralnik, J. M., Harris, T. & Ferrucci, L. 2003. Aging bone in men and women: beyond changes in bone mineral density. Osteoporos.Int. 14 (7), 531-538.

Schoenau, E., Neu, C. M., Beck, B., Manz, F. & Rauch, F. 2002. Bone mineral content per muscle cross-sectional area as an index of the functional muscle-bone unit. J.Bone Miner.Res. 17 (6), 1095-1101.

Schoenau, E., Saggese, G., Peter, F., Baroncelli, G. I., Shaw, N. J., Crabtree, N. J., Zadik, Z., Neu, C. M., Noordam, C., Radetti, G. & Hochberg, Z. 2004. From bone biology to bone analysis. Horm.Res. 61 (6), 257-269.

Seeman, E. 2008. Structural basis of growth-related gain and age-related loss of bone strength. Rheumatology (Oxford) 47 Suppl 4, iv2-8.

Seeman, E. 2007. The periosteum--a surface for all seasons. Osteoporos.Int. 18 (2), 123-128.

Seeman, E. 2004. Estrogen, androgen, and the pathogenesis of bone fragility in women and men. Curr.Osteoporos Rep. 2 (3), 90-96.

Seeman, E. 2003. Periosteal bone formation--a neglected determinant of bone strength. N.Engl.J.Med. 349 (4), 320-323.

Seeman, E. 2002. Pathogenesis of bone fragility in women and men. Lancet 359 (9320), 1841-1850.

Seeman, E. & Delmas, P. D. 2006. Bone quality--the material and structural basis of bone strength and fragility. N.Engl.J.Med. 354 (21), 2250-2261.

Seeman, E., Hopper, J. L., Young, N. R., Formica, C., Goss, P. & Tsalamandris, C. 1996. Do genetic factors explain associations between muscle strength, lean mass, and bone density? A twin study. Am.J.Physiol. 270 (2 Pt 1), E320-7.

Shackelford, L. C., LeBlanc, A. D., Driscoll, T. B., Evans, H. J., Rianon, N. J., Smith, S. M., Spector, E., Feeback, D. L. & Lai, D. 2004. Resistance exercise as a countermeasure to disuse-induced bone loss. J.Appl.Physiol. 97 (1), 119-129.

Shen, H., Liu, Y., Liu, P., Recker, R. R. & Deng, H. W. 2005. Nonreplication in genetic studies of complex diseases--lessons learned from studies of osteoporosis and tentative remedies. J.Bone Miner.Res. 20 (3), 365-376.

Sievänen, H., Kannus, P. & Järvinen, T. L. 2007. Bone quality: an empty term. PLoS Med. 4 (3), e27.

Sievänen, H. 2000. A physical model for dual-energy X-ray absorptiometry--derived bone mineral density. Invest.Radiol. 35 (5), 325-330.

Sievänen, H., Koskue, V., Rauhio, A., Kannus, P., Heinonen, A. & Vuori, I. 1998. Peripheral quantitative computed tomography in human long bones: evaluation of in vitro and in vivo precision. J.Bone Miner.Res. 13 (5), 871-882.

Sievänen, H., Kannus, P., Nieminen, V., Heinonen, A., Oja, P. & Vuori, I. 1996a. Estimation of various mechanical characteristics of human bones using dual energy X-ray absorptiometry: methodology and precision. Bone 18 (1 Suppl), 17S-27S.

Sievänen, H., Heinonen, A. & Kannus, P. 1996b. Adaptation of bone to altered loading environment: a biomechanical approach using X-ray absorptiometric data from the patella of a young woman. Bone 19 (1), 55-59.

Sievänen, H., Kannus, P., Heinonen, A., Oja, P. & Vuori, I. 1994. Bone mineral density and muscle strength of lower extremities after long-term strength training, subsequent knee ligament injury and rehabilitation: a unique 2-year follow-up of a 26-year-old female student. Bone 15 (1), 85-90.

Sievänen, H., Oja, P. & Vuori, I. 1992. Precision of dual-energy x-ray absorptiometry in determining bone mineral density and content of various skeletal sites. J.Nucl.Med. 33 (6), 1137-1142.

Sigurdsson, G., Halldorsson, B. V., Styrkarsdottir, U., Kristjansson, K. & Stefansson, K. 2008. Impact of genetics on low bone mass in adults. J.Bone Miner.Res. 23 (10), 1584-1590.

Sipilä, S. 2003. Body composition and muscle performance during menopause and hormone replacement therapy. J.Endocrinol.Invest. 26 (9), 893-901.

Sipilä, S., Multanen, J., Kallinen, M., Era, P. & Suominen, H. 1996. Effects of strength and endurance training on isometric muscle strength and walking speed in elderly women. Acta Physiol.Scand. 156 (4), 457-464.

Stathopoulou, M. G., Dedoussis, G. V., Trovas, G., Katsalira, A., Hammond, N., Deloukas, P. & Lyritis, G. P. 2010 Low-density lipoprotein receptor-related protein 5 polymorphisms are associated with bone mineral density in Greek postmenopausal women: An interaction with calcium intake. J.Am.Diet.Assoc. 110 (7), 1078-1083.

Stiger, F., Brandstrom, H., Gillberg, P., Melhus, H., Wolk, A., Michaelsson, K. & Kindmark, A. 2008. Association between repeat length of exon 1 CAG microsatellite in the androgen receptor and bone density in men is modulated by sex hormone levels. Calcif.Tissue Int. 82 (6), 427-435.

Sun, X., Lei, S. F., Deng, F. Y., Wu, S., Papacian, C., Hamilton, J., Recker, R. R. & Deng, H. W. 2006. Genetic and environmental correlations between bone geometric parameters and body compositions. Calcif.Tissue Int. 79 (1), 43-49.

Suuriniemi M., Suominen, H., Mahonen, A., Alén, M. & Cheng, S. 2007. Estrogen receptor α polymorphism modifies the association between childhood exercise and bone mass: follow-up study. Pediatr.Exerc.Sci. 19 (4), 444-458.

Szulc, P., Seeman, E., Duboeuf, F., Sornay-Rendu, E. & Delmas, P. D. 2006. Bone fragility: failure of periosteal apposition to compensate for increased endocortical resorption in postmenopausal women. J.Bone Miner.Res. 21 (12), 1856-1863.

Tang, B. M., Eslick, G. D., Nowson, C., Smith, C. & Bensoussan, A. 2007. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet 370 (9588), 657-666.

Terjesen, T., Nordby, A. & Arnulf, V. 1985. Bone atrophy after plate fixation. Computed tomography of femoral shaft fractures. Acta Orthop.Scand. 56 (5), 416-418.

Tucker, K. L. 2009. Osteoporosis prevention and nutrition. Curr.Osteoporos Rep. 7 (4), 111-117.

Turner, C. H. 1998. Three rules for bone adaptation to mechanical stimuli. Bone 23 (5), 399-407.

Uusi-Rasi, K., Kannus, P. & Sievänen, H. 2008. Physical activity in prevention of osteoporosis and associated fractures. In R. Marcus, D. Feldman, D. A. Nelson & C. J. Rosen (Eds.) Osteoporosis. (3.th edition) Burlington MA USA: Elsevier, 837-860.

Uusi-Rasi, K., Beck, T. J., Sievänen, H., Heinonen, A. & Vuori, I. 2003a. Associations of hormone replacement therapy with bone structure and physical performance among postmenopausal women. Bone 32 (6), 704-710.

Uusi-Rasi, K., Kannus, P., Cheng, S., Sievänen, H., Pasanen, M., Heinonen, A., Nenonen, A., Halleen, J., Fuerst, T., Genant, H. & Vuori, I. 2003b. Effect of alendronate and exercise on bone and physical performance of postmenopausal women: a randomized controlled trial. Bone 33 (1), 132-143.

Uusi-Rasi, K., Sievänen, H., Pasanen, M., Oja, P. & Vuori, I. 2002. Associations of calcium intake and physical activity with bone density and size in premenopausal and postmenopausal women: a peripheral quantitative computed tomography study. J.Bone Miner.Res. 17 (3), 544-552.

Uusi-Rasi, K., Sievänen, H., Vuori, I., Heinonen, A., Kannus, P., Pasanen, M., Rinne, M. & Oja, P. 1999. Long-term recreational gymnastics, estrogen use, and selected risk factors for osteoporotic fractures. J.Bone Miner.Res. 14 (7), 1231-1238.

Vainionpää, A., Korpelainen, R., Leppaluoto, J. & Jämsä, T. 2005. Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. Osteoporos.Int. 16 (2), 191-197.

Vainionpää, A., Korpelainen, R., Sievänen, H., Vihriala, E., Leppaluoto, J. & Jämsä, T. 2007. Effect of impact exercise and its intensity on bone geometry at weight-bearing tibia and femur. Bone 40 (3), 604-611.

van der Meulen, M. C., Jepsen, K. J. & Mikic, B. 2001. Understanding bone strength: size isn't everything. Bone 29 (2), 101-104.

van der Poest Clement, E., van der Wiel, H., Patka, P., Roos, J. C. & Lips, P. 1999. Long-term consequences of fracture of the lower leg: cross-sectional study and long-term longitudinal follow-up of bone mineral density in the hip after fracture of lower leg. Bone 24 (2), 131-134.

Van der Wiel, H. E., Lips, P., Nauta, J., Patka, P., Haarman, H. J. & Teule, G. J. 1994. Loss of bone in the proximal part of the femur following unstable fractures of the leg. J.Bone Joint Surg.Am. 76 (2), 230-236.

van Staa, T. P., Leufkens, H. G. & Cooper, C. 2002. Does a fracture at one site predict later fractures at other sites? A British cohort study. Osteoporos.Int. 13 (8), 624-629.

Vanderschueren, D., Venken, K., Ophoff, J., Bouillon, R. & Boonen, S. 2006. Clinical Review: Sex steroids and the periosteum--reconsidering the roles of androgens and estrogens in periosteal expansion. J.Clin.Endocrinol.Metab. 91 (2), 378-382.

Veitch, S. W., Findlay, S. C., Hamer, A. J., Blumsohn, A., Eastell, R. & Ingle, B. M. 2006. Changes in bone mass and bone turnover following tibial shaft fracture. Osteoporos.Int. 17 (3), 364-372.

Videman, T., Levalahti, E., Battie, M. C., Simonen, R., Vanninen, E. & Kaprio, J. 2007. Heritability of BMD of femoral neck and lumbar spine: a multivariate twin study of Finnish men. J.Bone Miner.Res. 22 (9), 1455-1462.

Viguet-Carrin, S., Garnero, P. & Delmas, P. D. 2006. The role of collagen in bone strength. Osteoporos.Int. 17 (3), 319-336.

Walsh, S., Zmuda, J. M., Cauley, J. A., Shea, P. R., Metter, E. J., Hurley, B. F., Ferrell, R. E. & Roth, S. M. 2005. Androgen receptor CAG repeat polymorphism is associated with fat-free mass in men. J.Appl.Physiol. 98 (1), 132-137.

Wang, Q., Alen, M., Nicholson, P., Lyytikainen, A., Suuriniemi, M., Helkala, E., Suominen, H. & Cheng, S. 2005. Growth patterns at distal radius and tibial shaft in pubertal girls: a 2-year longitudinal study. J.Bone Miner.Res. 20 (6), 954-961.

Wang, Q., Alen, M., Nicholson, P. H., Halleen, J. M., Alatalo, S. L., Ohlsson, C., Suominen, H. & Cheng, S. 2006. Differential effects of sex hormones on peri- and endocortical bone surfaces in pubertal girls. J.Clin.Endocrinol.Metab. 91 (1), 277-282.

Ward, K. D. & Klesges, R. C. 2001. A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcif.Tissue Int. 68 (5), 259-270.

Wells, G., Tugwell, P., Shea, B., Guyatt, G., Peterson, J., Zytaruk, N., Robinson, V., Henry, D., O'Connell, D., Cranney, A. & Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group 2002. Meta-analyses of therapies for postmenopausal osteoporosis. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. Endocr.Rev. 23 (4), 529-539.

Winzenberg, T., Shaw, K., Fryer, J. & Jones, G. 2006. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomised controlled trials. BMJ 333 (7572), 775.

Wolff, I., van Croonenborg, J. J., Kemper, H. C., Kostense, P. J. & Twisk, J. W. 1999. The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre- and postmenopausal women. Osteoporos.Int. 9 (1), 1-12.

Xu, H., Long, J. R., Yang, Y. J., Deng, F. Y. & Deng, H. W. 2006. Genetic determination and correlation of body weight and body mass index (BMI) and cross-sectional geometric parameters of the femoral neck. Osteoporos.Int. 17 (11), 1602-1607.

Yang, T. L., Zhao, L. J., Liu, Y. J., Liu, J. F., Recker, R. R. & Deng, H. W. 2006. Genetic and environmental correlations of bone mineral density at different skeletal sites in females and males. Calcif.Tissue Int. 78 (4), 212-217.

Yang, Y. J., Dvornyk, V., Jian, W. X., Xiao, S. M. & Deng, H. W. 2005. Genetic and environmental correlations between bone phenotypes and anthropometric indices in Chinese. Osteoporos.Int. 16 (9), 1134-1140.

Zehnacker, C.H. & Bemis-Dougherty, A. 2007. Effect of weighted exercises on bone mineral density in post menopausal women. A systematic review. J.Geriatr.Phys.Ther. 30 (2), 79-88.

Zerahn, B., Olsen, C., Stephensen, S., Kanstrup, I. L. & Schwarz Lausten, G. 1998. Bone loss after hip fracture is correlated to the postoperative degree of mobilisation. Arch.Orthop.Trauma.Surg. 117 (8), 453-456.

Zhai, G., Andrew, T., Kato, B. S., Blake, G. M. & Spector, T. D. 2009. Genetic and environmental determinants on bone loss in postmenopausal Caucasian women: a 14-year longitudinal twin study. Osteoporos.Int. 20 (6), 949-953.

Zhang, F., Xiao, P., Yang, F., Shen, H., Xiong, D. H., Deng, H. Y., Papasian, C. J., Drees, B. M., Hamilton, J. J., Recker, R. R. & Deng, H. W. 2008a. A whole genome linkage scan for QTLs underlying peak bone mineral density. Osteoporos.Int. 19 (3), 303-310.

Zhang, Z. L., He, J. W., Qin, Y. J., Hu, Y. Q., Li, M., Zhang, H., Hu, W. W., Liu, Y. J. & Gu, J. M. 2008b. Association between myostatin gene polymorphisms and peak BMD variation in Chinese nuclear families. Osteoporos.Int. 19 (1), 39-47.

Åkesson, K., Käkönen, S. M., Josefsson, P. O., Karlsson, M. K., Obrant, K. J. & Pettersson, K. 2005. Fracture-induced changes in bone turnover: a potential confounder in the use of biochemical markers in osteoporosis. J.Bone Miner.Metab. 23 (1), 30-35.

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH

1 KIRJONEN, JUHANI, On the description of a human movement and its psychophysical correlates under psychomotor loads. 48 p. 1971.

2 KIRJONEN, JUHANI JA RUSKO, HEIKKI, Liikkeen kinemaattisista ominaispiirteistä, niiden psykofyysisistä selitysyhteyksistä ja näiden muutoksista psykomotorisen kuormituksen ja kestävyysharjoittelun vaikutuksesta. - On the kinematic charac-teristics and psychophysical correlates of a human movement and their changes during psychomotor loading and endurace conditioning. 156 p. 1971.

3 SARVIHARJU, PEKKA J., Effects of psycho-physical loading and progressive endurance conditioning on selected biochemical correlates of adaptive responses in man. 95 p. 1973.

4 KIVIAHO, PEKKA, Sport organizations and the structure of society. 54 p. 1973.

5 KOMI, PAAVO V., NELSON, RICHARD C. AND PULLI, MATTI, Biomechanics of skijumping. 53 p. 1974.

6 METELI, Työolot, terveys ja liikuntakäyttäytyminen metallitehtaissa. Kartoittavan kyselyn aineistot ja toteuttaminen. 178 p. 1974.

7 TIAINEN, JORMA M., Increasing physical education students' creative thinking. 53 p. 1976.

8 RUSKO, HEIKKI, Physical performance characteristics in Finnish athletes. 40 p. 1976.

9 KIISKINEN, ANJA, Adaptation of connective tissues to physical training in young mice. 43 p. 1976.

10 VUOLLE, PAULI, Urheilu elämänsisältönä. Menestyneiden urheilijoiden elämänura kilpailuvuosina - Top sport as content of life. 227 p. 1977.

11 SUOMINEN, HARRI, Effects of physical training in middle-aged and elderly people with special regard to skeletal muscle, connective tissue, and functional aging. 40 p. 1978.

12 VIITASALO, JUKKA, Neuromuscular performance in voluntary and reflex contraction with special reference to muscle structure and fatigue. 59 p. 1980.

13 LUHTANEN, PEKKA, On the mechanics of human movement with special reference to walking, running and jumping. 58 p. 1980.

14 LAAKSO, LAURI, Lapsuuden ja nuoruuden kasvuympäristö aikuisiän liikuntaharrastusten selittäjänä: retrospektiivinen tutkimus. - Socialization environment in childhood and youth as determinant of adult-age sport involvement: a retrospective study. 295 p. 1981.

15 BOSCO, CARMELO, Stretch-schortening cycle inskeletal muscle function with special reference to elastic energy and potentiation of myoelectrical activity. 64 p. 1982.

16 OLIN, KALEVI, Päätöksentekijöiden viiteryhmät kaupunkien liikuntapolitiikassa. - Reference groups of decision-makers in the sport politics of cities. 155 p. 1982.

17 KANNAS, LASSE, Tupakointia koskeva terveyskasvatus peruskoulussa. - Health education on smoking in the Finnish comprehensive school. 251 p. 1983.

18 Contribution of sociology to the study of sport. Festschrift Book in Honour of Professor Kalevi Heinilä. Ed. by OLIN, K. 243 p. 1984.

19 ALÉN, MARKKU, Effects of self-administered, high-dose testosterone and anabolic steroids on serum hormones, lipids, enzymes and on spermatogenesis in power athletes. 75 p. 1985.

20 HÄKKINEN, KEIJO, Training and detraining adaptations in electromyographic, muscle fibre and force production characteristics of human leg extensor muscles with special reference to prolonged heavy resistance and explosive type strength training. 106 p. 1986.

21 LAHTINEN, ULLA, Begåvningshandikappad ungdom i utveckling. En uppföljningstudie av funktionsförmåga och fysisk aktivitet hos begåvningshandikappade ungdomar i olika livsmiljöer. 300 p. 1986.

22 SILVENNOINEN, MARTTI, Koululainen liikunnanharrastajana: liikuntaharrastusten ja liikuntamotiivien sekä näiden yhteyksien muuttuminen iän mukana peruskoululaisilla ja lukiolaisilla. - Schoolchildren and physically active interests: The changes in interests in and motives for physical exercise related to age in Finnish comprehensive and upper secondary schools. 226 p. 1987.

23 POHJOLAINEN, PERTTI, Toimintakykyisyys, terveydentila ja elämäntyyli 71-75-vuotiailla miehillä. - Functional capacity, health status and life-style among 71-75 year-old men. 249 p. Summary 13 p. 1987.

24 MERO, ANTTI, Electromyographic acticity, force and anaerobic energy production in sprint running; with special reference to different constant speeds ranging from submaximal to supramaximal. 112 p. Tiivistelmä 5 p. 1987.

25 PARKATTI, TERTTU, Self-rated and clinically measured functional capacity among women and men in two age groups in metal industry. 131 p. Tiivistelmä 2 p. 1990.

26 HOLOPAINEN, SINIKKA, Koululaisten liikuntataidot. - The motor skills of schoolboys and girls. 217 p. Summary 6 p. 1990.

27 NUMMINEN, PIRKKO, The role of imagery in physical education. 131 p. Tiivistelmä 10 p. 1991.

28 TALVITIE, ULLA, Aktiivisuuden ja omatoimivuuden kehittäminen fysioterapian tavoitteena. Kehittävän työntutkimuksen sovellus lääkintävoimistelijan työhön. - The development of activity and self-motivation as the aim of physiotherapy. The application of developmental work research in physiotherapy. 212 p. Summary 8 p. 1991.

29 KAHILA, SINIKKA, Opetusmenetelmän merkitys prososiaalisessa oppimisessa - auttamis-

käyttäytymisen edistäminen yhteistyöskentelyn avulla koululiikunnassa. - The role of teaching method in prosocial learning - developing helping behavior by means of the cooperative teaching method in physical education. 132 p. Summary 2 p. 1993.

30 LIIMATAINEN-LAMBERG, ANNA-ESTER, Changes in student smoking habits at the vocational institutions and senior secondary schools and health education. 195 p. Yhteenveto 5 p. 1993.

31 KESKINEN, KARI LASSE, Stroking characteristics of front crawl swimming. 77 p. Yhteenveto 2 p. 1993.

32 RANTANEN, TAINA, Maximal isometric strength in older adults. Cross-national comparisons, background factors and association with Mobility. 87 p. Yhteenveto 4 p. 1994.

33 LUSA, SIRPA, Job demands and assessment of the physical work capacity of fire fighters. 91 p. Yhteenveto 4 p. 1994.

34 CHENG, SULIN, Bone mineral density and quality in older people. A study in relation to exercise and fracture occurrence, and the assessment of mechanical properties. 81 p. Tiivistelmä 1 p. 1994.

35 KOSKI, PASI, Liikuntaseura toimintaympäristössään. - Sports club in its organizational environment. 220 p. Summary 6 p. 1994.

36 JUPPI, JOEL, Suomen julkinen liikuntapolitiikka valtionhallinnon näkökulmasta vuosina 1917-1994. - Public sport policy in Finland from the viewpoint of state administration in 1917-1994. 358 p. Summary 7 p. 1995.

37 KYRÖLÄINEN, HEIKKI, Neuromuscular performance among power- and endurance-trained athletes. 82 p. Tiivistelmä 3 p. 1995.

38 NYANDINDI, URSULINE S., Evaluation of a school oral health education programme in Tanzania: An ecological perspective. 88 p. Tiivistelmä 2 p. 1995.

39 HEIKINARO-JOHANSSON, PILVIKKI, Including students with special needs in physical education. 81 p. Yhteenveto 4 p. 1995.

40 SARLIN, EEVA-LIISA, Minäkokemuksen merkitys liikuntamotivaatiotekijänä. - The significance of self perception in the motivational orientation of physical education. 157 p. Summary 4 p. 1995.

41 LINTUNEN, TARU, Self-perceptions, fitness, and exercise in early adolescence: a four-year follow-up study. 87 p. Yhteenveto 5 p.1995.

42 SIPILÄ, SARIANNA, Physical training and skeletal muscle in elderly women. A study of muscle mass, composition, fiber characteristics and isometric strength. 62 p. Tiivistelmä 3 p. 1996.

43 ILMANEN, KALERVO, Kunnat liikkeellä. Kunnallinen liikuntahallinto suomalaisen yhteiskunnan muutoksessa 1919-1994. - Municipalities in motion. Municipal sport administration in the changing Finnish society 1919-1994. 285 p. Summary 3 p. 1996.

44 NUMMELA, ARI, A new laboratory test method for estimating anaerobic performance characteristics with special reference to sprint running. 80 p. Yhteenveto 4 p. 1996.

45 VARSTALA, VÄINÖ, Opettajan toiminta ja oppilaiden liikunta-aktiivisuus koulun liikuntatunnilla. - Teacher behaviour and students' motor engagement time in school physical education classes. 138 p. Summary 4 p. 1996.

46 POSKIPARTA, MARITA, Terveysneuvonta, oppimaan oppimista. Videotallenteet hoitajien terveysneuvonnan ilmentäjinä ja vuorovaikutustaitojen kehittämismenetelmänä. - Health counselling, learning to learn. Videotapes expressing and developing nurses´ communication skills. 159 p. Summary 6 p. 1997.

47 SIMONEN, RIITTA, Determinants of adult psychomotor speed. A study of monozygotic twins. - Psykomotorisen nopeuden determinantit identtisillä kaksosilla. 49 p. Yhteenveto 2 p. 1997.

48 NEVALA-PURANEN, NINA, Physical work and ergonomics in dairy farming. Effects of occupationally oriented medical rehabilitaton and environmental measures. 80 p. (132 p.) 1997.

49 HEINONEN, ARI, Exercise as an Osteogenic Stimulus. 69 p. (160 p.) Tiivistelmä 1 p. 1997.

50 VUOLLE, PAULI (Ed.) Sport in social context by Kalevi Heinilä. Commemorative book in Honour of Professor Kalevi Heinilä. 200 p. 1997.

51 TUOMI, JOUNI, Suomalainen hoitotiede-keskustelu. - The genesis of nursing and caring science in Finland. 218 p. Summary 7 p. 1997.

52 TOLVANEN, KAIJA, Terveyttä edistävän organisaation kehittäminen oppivaksi organisaatioksi. Kehitysnäytökset ja kehittämistehtävät terveyskeskuksen muutoksen virittäjänä. - Application of a learning organisation model to improve services in a community health centre. Development examples and development tasks are the key to converting a health care. 197 p. Summary 3 p. 1998.

53 OKSA, JUHA, Cooling and neuromuscular performance in man. 61 p. (121 p.) Yhteenveto 2 p. 1998.

54 GIBBONS, LAURA, Back function testing and paraspinal muscle magnetic resonance image parameters: their associations and determinants. A study on male, monozygotic twins. 67 p (128 p.) Yhteenveto 1p. 1998.

55 NIEMINEN, PIPSA, Four dances subcultures. A study of non-professional dancers´ socialization, participation motives, attitudes and stereotypes. - Neljä tanssin alakulttuuria. Tutkimus tanssinharrastajien tanssiin sosiaalistumisesta, osallistumismotiiveista, asenteista ja stereotypioista. 165 p. Yhteenveto 4 p. 1998.

56 LAUKKANEN, PIA, Iäkkäiden henkilöiden selviytyminen päivittäisistä toiminnoista. - Carrying

out the activities of daily living among elderly people. 130 p. (189 p.). Summary 3 p. 1998.

57 AVELA, JANNE, Stretch-reflex adaptation in man. Interaction between load, fatigue and muscle stiffness. 87 p. Yhteenveto 3 p. 1998.

58 SUOMI, KIMMO, Liikunnan yhteissuunnittelumetodi. Metodin toimivuuden arviointi Jyväskylän Huhtasuon lähiössä. - Collaborative planning method of sports culture. Evaluation of the method in the Huhtasuo suburb of the city of Jyväskylä. 190 p. Summary 8 p. 1998.

59 PÖTSÖNEN, RIIKKA, Naiseksi, mieheksi, tietoiseksi. Koululaisten seksuaalinen kokeneisuus, HIV/AIDS-tiedot, -asenteet ja tiedonlähteet. - Growing as a woman, growing as a man, growing as a conscious citizen. 93 p. (171 p.). Summary 3 p. 1998.

60 HÄKKINEN, ARJA, Resistance training in patients with early inflammatory rheumatic diseases. Special reference to neuromuscular function, bone mineral density and disease activity. - Dynaamisen voimaharjoittelun vaikutukset nivelreumaa sairastavien potilaiden lihasvoimaan, luutiheyteen ja taudin aktiivisuuteen. 62 p. (119 p.) Yhteenveto 1 p. 1999.

61 TYNJÄLÄ, JORMA, Sleep habits, perceived sleep quality and tiredness among adolescents. A health behavioural approach. - Nuorten nukkumistottumukset, koettu unen laatu ja väsyneisyys. 104 p. (167 p.) Yhteenveto 3 p. 1999.

62 PÖNKKÖ, ANNELI, Vanhemmat ja lastentarhanopettajat päiväkotilasten minäkäsityksen tukena. - Parents´ and teachers´ role in self-perception of children in kindergartens. 138 p. Summary 4 p. 1999.

63 PAAVOLAINEN, LEENA, Neuromuscular characteristics and muscle power as determinants of running performance in endurance athletes with special reference to explosive-strength training. - Hermolihasjärjestelmän toimintakapasiteetti kestävyyssuorituskykyä rajoittavana tekijänä. 88 p. (138 p.) Yhteenveto 4 p. 1999.

64 VIRTANEN, PAULA, Effects of physical activity and experimental diabetes on carbonic anhydrace III and markers of collagen synthesis in skeletal muscle and serum. 77 p. (123 p.) Yhteenveto 2 p. 1999.

65 KEPLER, KAILI, Nuorten koettu terveys, terveyskäyttäytyminen ja sosiaalistumisympäristö Virossa. - Adolescents' perceived health, health behaviour and socialisation enviroment in Estonia. - Eesti noorte tervis, tervisekäitumine ja sotsiaalne keskkond. 203 p. Summary 4p. Kokkuvõte 4 p. 1999.

66 SUNI, JAANA, Health-related fitness test battery for middle-aged adults with emphasis on musculoskeletal and motor tests. 96 p. (165 p.) Yhteenveto 2 p. 2000.

67 SYRJÄ, PASI, Performance-related emotions in highly skilled soccer players. A longitudinal study based on the IZOF model. 158 p. Summary 3 p. 2000.

68 VÄLIMAA, RAILI, Nuorten koettu terveys kyselyaineistojen ja ryhmähaastattelujen valossa. - Adolescents' perceived health based on surveys and focus group discussions. 208 p. Summary 4 p. 2000.

69 KETTUNEN, JYRKI, Physical loading and later lower-limb function and findings . A study among male former elite athletes. - Fyysisen kuormituksen yhteydet alaraajojen toimintaan ja löydöksiin entisillä huippu-urhelijamiehillä. 68 p. (108 p.) Yhteenveto 2 p. 2000.

70 HORITA, TOMOKI, Stiffness regulation during stretch-shortening cycle exercise. 82 p. (170 p.) 2000.

71 HELIN, SATU, Iäkkäiden henkilöiden toimintakyvyn heikkeneminen ja sen kompensaatioprosessi. - Functional decline and the process of compensation in elderly people. 226 p. Summary 10 p. 2000.

72 KUUKKANEN, TIINA, Therapeutic exercise programs and subjects with low back pain. A controlled study of changes in function, activity and participation. 92 p. (154 p.) Tiivistelmä 2 p. 2000.

73 VIRMAVIRTA, MIKKO, Limiting factors in ski jumping take-off. 64 p. (124 p.) Yhteenveto 2 p. 2000.

74 PELTOKALLIO, LIISA, Nyt olisi pysähtymisen paikka. Fysioterapian opettajien työhön liittyviä kokemuksia terveysalan ammatillisessa koulutuksessa. - Now it's time to stop. Physiotherapy teachers' work experiences in vocational health care education. 162 p. Summary 5 p. 2001.

75 KETTUNEN, TARJA, Neuvontakeskustelu. Tutkimus potilaan osallistumisesta ja sen tukemisesta sairaalan terveysneuvonnassa. - Health counseling conversation. A study of patient participation and its support by nurses during hospital counseling. 123 p. (222 p.) Summary 6 p. 2001.

76 PULLINEN, TEEMU, Sympathoadrenal response to resistance exercise in men, women and pubescent boys. With special reference to interaction with other hormones and neuromuscular performance. 76 p. (141 p.) Yhteenveto 2 p. 2001.

77 BLOMQVIST, MINNA, Game understanding and game performance in badminton. Development and validation of assessment instruments and their application to games teaching and coaching. 83 p. Yhteenveto 5 p. 2001.

78 FINNI, TAIJA, Muscle mechanics during human movement revealed by *in vivo* measurements of tendon force and muscle length. 83 p. (161 p.) Yhteenveto 3 p. 2001.

79 KARIMÄKI, ARI, Sosiaalisten vaikutusten arviointi liikuntarakentamisessa. Esimerkkinä Äänekosken uimahalli. - Social impact

assessment method in sports planning. - The case of Äänekoski leisure pool. 194 p. Summary 3 p. 2001.

80 PELTONEN, JUHA, Effects of oxygen fraction in inspired air on cardiorespiratory responses and exercise performance. 86 p. (126 p.) Yhteenveto 2 p. 2002.

81 HEINILÄ, LIISA, Analysis of interaction processes in physical education. Development of an observation instrument, its application to teacher training and program evaluation. 406 p. Yhteenveto 11 p. 2002.

82 LINNAMO, VESA, Motor unit activation and force production during eccentric, concentric and isometric actions. - Motoristen yksiköiden aktivointi ja lihasten voimantuotto eksentrisessä, konsentrisessa ja isometrisessä lihastyössä. 77 p. (150 p.) Yhteenveto 2 p. 2002.

83 PERTTUNEN, JARMO, Foot loading in normal and pathological walking. 86 p. (213 p.) Yhteenveto 2 p. 2002.

84 LEINONEN, RAIJA, Self-rated health in old age. A follow-up study of changes and determinants. 65 p. (122 p.) Yhteenveto 2 p. 2002.

85 GRETSCHEL, ANU, Kunta nuorten osallisuusympäristönä. Nuorten ryhmän ja kunnan vuorovaikutussuhteen tarkastelu kolmen liikuntarakentamisprojektin laadunarvioinnin keinoin. - The municipality as an involvement environment - an examination of the interactive relationship between youth groups and municipalities through the quality assessment of three sports facilities construction projects. 236 p. Summary 11 p. 2002.

86 PÖYHÖNEN, TAPANI, Neuromuscular function during knee exercises in water. With special reference to hydrodynamics and therapy. 77 p. (124 p.) Yhteenveto 2 p. 2002.

87 HIRVENSALO, MIRJA, Liikuntaharrastus iäkkäänä. Yhteys kuolleisuuteen ja avuntarpeeseen sekä terveydenhuolto liikunnan edistäjänä. - Physical activity in old age - significance for public health and promotion strategies. 106 p. (196 p.) Summary 4 p. 2002.

88 KONTULAINEN, SAIJA, Training, detraining and bone - Effect of exercise on bone mass and structure with special reference to maintenance of exercise induced bone gain. 70 p. (117 p.) Yhteenveto 2 p. 2002.

89 PITKÄNEN, HANNU, Amino acid metabolism in athletes and non-athletes. - With Special reference to amino acid concentrations and protein balance in exercise, training and aging. 78 p. (167 p.) Yhteenveto 3 p. 2002.

90 LIIMATAINEN, LEENA, Kokemuksellisen oppimisen kautta kohti terveyden edistämisen asiantuntijuutta. Hoitotyön ammattikorkeakouluopiskelijoiden terveyden edistämisen oppiminen hoitotyön harjoittelussa. - Towards health promotion expertise through experiential learning. Student nurses' health promotion learning during clinical practice. 93 p. (164 p.) Summary 4 p. 2002.

91 STÅHL, TIMO, Liikunnan toimintapolitiikan arviointia terveyden edistämisen kontekstissa. Sosiaalisen tuen, fyysisen ympäristön ja poliittisen ympäristön yhteys liikunta-aktiivisuuteen. - Evaluation of the Finnish sport policy in the context of health promotion. Relationships between social support, physical environment, policy environment and physical activity 102 p. (152 p.) Summary 3 p. 2003.

92 OGISO, KAZUYUKI, Stretch Reflex Modulation during Exercise and Fatigue. 88 p. (170 p.) Yhteenveto 1 p. 2003.

93 RAUHASALO, ANNELI, Hoitoaika lyhenee – koti kutsuu. Lyhythoitoinen kirurginen toiminta vanhusten itsensä kokemana. - Care-time shortens – home beckons. Short term surgical procedures as experienced by elderly patients. 194 p. Summary 12 p. 2003.

94 PALOMÄKI, SIRKKA-LIISA, Suhde vanhenemiseen. Iäkkäät naiset elämänsä kertojina ja rakentajina. - Relation to aging. Elderly women as narrators and constructors of their lives. 143 p. Summary 6 p. 2004.

95 SALMIKANGAS, ANNA-KATRIINA, Nakertamisesta hanketoimintaan. Tapaustutkimus Nakertaja-Hetteenmäen asuinalueen kehittämistoiminnasta ja liikunnan osuudesta yhteissuunnittelussa. - From togetherness to project activity. A case study on the development of a neighbourhood in Kainuu and the role of physical activity in joint planning. 269 p. Summary 8 p. 2004.

96 YLÖNEN, MAARIT E., Sanaton dialogi. Tanssi ruumiillisena tietona. - Dialogue without words. Dance as bodily knowledge. 45 p. (135 p.) Summary 5 p. 2004.

97 TUMMAVUORI, MARGAREETTA, Long-term effects of physical training on cardiac function and structure in adolescent cross-country skiers. A 6.5-year longitudinal echocardiographic study. 151 p. Summary 1 p. 2004.

98 SIROLA, KIRSI, Porilaisten yhdeksäsluokkalaisten ja kasvattajien käsityksiä nuorten alkoholinkäytöstä ja alkoholinkäytön ehkäisystä. - Views of ninth graders, educators and parents in Pori, Finland on adolescent alcohol use and on preventing alcohol use. 189 p. Summary 3 p. 2004.

99 LAMPINEN, PÄIVI, Fyysinen aktiivisuus, harrastustoiminta ja liikkumiskyky iäkkäiden ihmisten psyykkisen hyvinvoinnin ennustajina. 65–84-vuotiaiden jyväskyläläisten 8-vuotisseuruututkimus. - Activity and mobility as associates and predictors of mental well-being among older adults. 94 p. (165 p.) Summary 2 p. 2004.

100 RANTA, SARI, Vanhenemismuutosten eteneminen. 75-vuotiaiden henkilöiden antropometristen ominaisuuksien, fyysisen toimintakyvyn ja kognitiivisen kyvykkyyden muutokset viiden ja kymmenen vuoden seuranta-aikana. - The progress of aging processes. A 5- and 10-year follow-up study of the changes in anthropometrical characteristics and physical and cognitive capacities among 75-year-old persons. 186 p. Summary 2 p. 2004.

101 SIHVONEN, SANNA, Postural balance and aging. Cross-sectional comparative studies and a balance training intervention. - Ikääntyminen ja tasapaino. Eri ikäisten tasapaino ja tasapainoharjoittelun vaikuttavuus ikääntyneillä palvelukodissa asuvilla naisilla. 65 p. (106 p.) Yhteenveto 2 p. 2004.

102 RISSANEN, AARO, Back muscles and intensive rehabilitation of patients with chronic low back pain. Effects on back muscle structure and function and patient disability. - Selkälihakset ja pitkäaikaista selkäkipua sairastavien potilaiden intensiivinen kuntoutus. Vaikutukset selkälihasten rakenteeseen ja toimintaan sekä potilaiden vajaakuntoisuuteen. 90 p. (124 p.) Yhteenveto 2 p. 2004.

103 KALLINEN, MAURI, Cardiovascular benefits and potential hazards of physical exercise in elderly people. - Liikunnan hyödylliset ja mahdolliset haitalliset vaikutukset ikääntyneiden verenkiertoelimistöön. 97 p. (135 p). Yhteenveto 2 p. 2004.

104 SÄÄKSLAHTI, ARJA, Liikuntaintervention vaikutus 3–7-vuotiaiden lasten fyysiseen aktiivisuuteen ja motorisiin taitoihin sekä fyysisen aktiivisuuden yhteys sydän- ja verisuonitautien riskitekijöihin. - Effects of physical activity Intervention on physical activity and motor skills and relationships between physical activity and coronary heart disease risk factors in 3–7-year-old children. 153 p. Summary 3 p. 2005.

105 HÄMÄLÄINEN, PIIA, Oral health status as a predictor of changes in general health among elderly people. 76 p. (120 p.) Summary 2 p. 2005.

106 LIINAMO, ARJA, Suomalaisnuorten seksuaalikasvatus ja seksuaaliterveystiedot oppilaan ja koulun näkökulmasta. Arviointia terveyden edistämisen viitekehyksessä. - Sexual education and sexual health knowledge among Finnish adolescents at pupil and school level. Evaluation from the point of view of health promotion. 111 p. (176 p.) Summary 5 p. 2005.

107 ISHIKAWA, MASAKI, *In vivo* muscle mechanics during human locomotion. Fascicle-tendinous tissue interaction during stretch-shortening cycle exercises. - Venytysrefleksin muutokset liikkeessä ja väsymyksessä. 89 p. (228 p.) Yhteenveto 1 p. 2005.

108 KÄRKI, ANNE, Physiotherapy for the functioning of breast cancer patients. Studies of the effectiveness of physiotherapy methods and exercise, of the content and timing of post-operative education and of the experienced functioning and disability . - Rintasyöpäleikattujen toimintakyky ja siihen vaikuttaminen fysioterapiassa ja harjoittelussa. 70 p. (138 p.) Yhteenveto 3 p. 2005.

109 RAJANIEMI, VESA, Liikuntapaikkarakentaminen ja maankäytön suunnittelu. Tutkimus eri väestöryhmät tasapuolisesti huomioon ottavasta liikuntapaikkasuunnittelusta ja sen kytkemisestä maankäyttö- ja rakennuslain mukaiseen kaavoitukseen. - Sports area construction and land use planning – Study of sports area planning that considers all the population groups even-handedly and integrates sports area planning with land use planning under the land use and building act. 171 p. Summary 6 p. 2005.

110 WANG, QINGJU, Bone growth in pubertal girls. Cross-sectional and lingitudinal investigation of the association of sex hormones, physical activity, body composition and muscle strength with bone mass and geometry. 75 p. (117 p.) Tiivistelmä 1 p. 2005.

111 ROPPONEN, ANNINA, The role of heredity, other constitutional structural and behavioral factors in back function tests.- Perimä, muut synnynnäiset rakenteelliset tekijät ja käyttäytymistekijät selän toimintakyky-testeissä. 78 P. (125 p.) Tiivistelmä 1 p. 2006.

112 ARKELA-KAUTIAINEN, MARJA, Functioning and quality of life as perspectives of health in patients with juvenile idiopathic arthritis in early adulthood. Measurement and long-term outcome. - Toimintakyky ja elämänlaatu terveyden näkökulmina lastenreumaa sairastaneilla nuorilla aikuisilla. Mittaaminen ja pitkäaikaistulokset. 95 p. (134 p.) Tiivistelmä 2 p. 2006.

113 RAUTIO, NINA, Seuruu- ja vertailututkimus sosioekonomisen aseman yhteydestä toimintakykyyn iäkkäillä henkilöillä. - A follow-up and cross-country comparison study on socio-economic position and its relationship to functional capacity in elderly people. 114 p. (187 p.) Summary 3 p. 2006.

114 TIIKKAINEN, PIRJO, Vanhuusiän yksinäisyys. Seuruutukimus emotionaalista ja sosiaalista yksinäisyyttä määrittävistä tekijöistä. - Loneliness in old age – a follow-up study of determinants of emotional and social loneliness. 76 p. (128 p.) Summary 2 p. 2006.

115 AHTIAINEN, JUHA, Neuromuscular, hormonal and molecular responses to heavy resistance training in strength trained men; with special reference to various resistance exercise protocols, serum hormones and gene expression of androgen receptor and insulin-like growth factor-I. - Neuromuskulaariset,

hormonaaliset ja molekulaariset vasteet voimaharjoittelussa voimaurheilijoilla. 119 p. (204 p.) Yhteenveto 2 p. 2006.

116 Pajala, Satu, Postural balance and susceptibility to falls in older women. Genetic and environmental influences in single and dual task situations. - Iäkkäiden naisten tasapainokyky yksinkertaisissa sekä huomion jakamista vaativissa tilanteissa ja kaatumisriskiperimän merkitys yksilöiden välisten erojen selittäjinä. 78 p. (120 p.) Yhteenveto 3 p. 2006.

117 Tiainen, Kristina, Genetics of skeletal muscle characteristics and maximal walking speed among older female twins. - Lihasvoiman ja kävelynopeuden periytyvyys iäkkäillä naiskaksosilla. 77 p. (123 p.) Yhteenveto 2 p. 2006.

118 Sjögren, Tuulikki, Effectiveness of a workplace physical exercise intervention on the functioning, work ability, and subjective well-being of office workers – a cluster randomised controlled cross-over trial with one-year follow-up. - Työpaikalla tapahtuvan fyysisen harjoitteluintervention vaikuttavuus toimistotyöntekijöiden toimintakykyyn, työkykyyn ja yleiseen subjektiiviseen elämänlaatuun – ryhmätasolla satunnaistettu vaihtovuorokoe ja vuoden seuranta. 100 p. (139 p.) Tiivistelmä 3 p. 2006.

119 Lyyra, Tiina-Mari, Predictors of mortality in old age. Contribution of self-rated health, physical functions, life satisfaction and social support on survival among older people. - Kuolleisuuden ennustetekijät iäkkäässä väestössä. Itsearvioidun terveyden, fyysisten toimintojen, elämään tyytyväisyyden ja sosiaalisen tuen yhteys iäkkäiden ihmisten eloonjäämiseen. 72 p. (106 p.) Tiivistelmä 2 p. 2006.

120 Soini, Markus, Motivaatioilmaston yhteys yhdeksäsluokkalaisten fyysiseen aktiivisuuteen ja viihtymiseen koulun liikuntatunneilla. - The relationship of motivational climate to physical activity intensity and enjoyment within ninth grade pupils in school physical education lessons. 91 p. 2006.

121 Vuorimaa, Timo, Neuromuscular, hormonal and oxidative stress responses to endurance running exercises in well trained runners. - Neuromuskulaariset, hormonaaliset ja hapettumisstressiin liittyvät vasteet kestävyysjuoksuharjoituksiin hyvin harjoitelleilla juoksijoilla. 93 p. (152 p.) Yhteenveto 3 p. 2007.

122 Mononen, Kaisu, The effects of augmented feedback on motor skill learning in shooting. A feedback training intervention among inexperienced rifle shooters. - Ulkoisen palautteen vaikutus motoriseen oppimiseen ammunnassa: Harjoittelututkimus kokemattomilla kivääriampujilla. 63 p. Yhteenveto 4 p. 2007.

123 Sallinen, Janne, Dietary Intake and Strength Training Adaptation in 50–70 -year old Men and Women. With special reference to muscle mass, strength, serum anabolic hormone concentrations, blood pressure, blood lipids and lipoproteins and glycemic control. - Ravinnon merkitys voimaharjoittelussa 50–70 -vuotiailla miehillä ja naisilla 103 p. (204 p.) Yhteenveto 3 p. 2007.

124 Kasila Kirsti, Schoolchildren's oral health counselling within the organisational context of public oral health care. Applying and developing theoretical and empirical perspectives. 96 p. (139 p.) Tiivistelmä 3 p. 2007.

125 Pyöriä, Outi, Reliable clinical assessment of stroke patients' postural control and development of physiotherapy in stroke rehabilitation. - Aivoverenkiertohäiriöpotilaiden toimintakyvyn luotettava kliininen mittaaminen ja fysioterapian kehittäminen Itä-Savon sairaanhoitopiirin alueella. 94 p. (143 p.) Yhteenveto 6 p. 2007.

126 Valkeinen, Heli, Physical fitness, pain and fatigue in postmenopausal women with fibromyalgia. Effects of strength training. - Fyysinen kunto, kipu- ja väsymysoireet ja säännöllisen voimaharjoittelun vaikutukset menopaussi-iän ohittaneilla fibromyalgiaa sairastavilla naisilla. 101 p. (132 p.) Yhteenveto 2 p. 2007.

127 Hämäläinen, Kirsi, Urheilija ja valmentaja urheilun maailmassa. Eetokset, ihanteet ja kasvatus urheilijoiden tarinoissa. - An athlete and a coach in the world of sports. Ethos, ideals and education in athletes' narratives. 176 p. Tiivistelmä 2 p. 2008.

128 Aittasalo, Minna, Promoting physical activity of working aged adults with selected personal approaches in primary health care. Feasibility, effectiveness and an example of nationwide dissemination. - Työikäisten liikunnan edistäminen avoterveydenhuollossa – työtapojen toteuttamiskelpoisuus ja vaikuttavuus sekä esimerkki yhden työtavan levittämisestä käytäntöön. 105 p. (161 p.) Yhteenveto 3 p. 2008.

129 Portegijs, Erja, Asymmetrical lower-limb muscle strength deficit in older people. - Alaraajojen lihasvoiman puoliero iäkkäillä ihmisillä. 105 p. (155 p.) Yhteenveto 3 p. 2008.

130 Laitinen-Väänänen, Sirpa, The construction of supervision and physiotherapy expertise: A qualitative study of physiotherapy students' learning sessions in clinical education. - Opiskelijan ohjauksen ja fysioterapian asiantuntijuuden rakentuminen: Laadullinen tutkimus fysioterapiaopiskelijan oppimistilanteista työharjoittelussa. 69 p. (118 p.) Yhteenveto 3 p. 2008.

131 Iivonen, Susanna, Early Steps -liikunta-ohjelman yhteydet 4–5-vuotiaiden päiväkotilasten motoristen perustaitojen kehitykseen. - The associations between an Early Steps physical education curriculum and the fundamental motor skills development of 4–5-year-old preschool children. 157 p. Summary 4 p. 2008.

132 Ortega-Alonso, Alfredo, Genetic effects on mobility, obesity and their association in older female twins. 87 p. 2009.

133 Hulmi, Juha, Molecular and hormonal responses and adaptation to resistance exercise and protein nutrition in young and older men. - Voimaharjoittelun fysiologiset ja molekyylibiologiset vaikutukset lihaskasvunsäätelyssä lisäproteiinia nautittaessa tai ilman. 109 p. (214 p.) Yhteenveto 2 p. 2009.

134 Martinmäki, Kaisu, Transient changes in heart rate variability in response to orthostatic task, endurance exercise and training. With special reference to autonomic blockades and time-frequency analysis. - Sykevaihtelun muutokset ortostaattisessa testissä, kestävyysliikunnassa ja kestävyysharjoittelussa käyttäen hyväksi autonomisen säätelyn salpauskokeita ja aika-taajuusanalyysiä . 99 p. (151 p.) Yhteenveto 2 p. 2009.

135 Sedliak, Milan, Neuromuscular and hormonal adaptations to resistance training. Special effects of time of day of training. 84 p. (175 p.) 2009.

136 Nikander, Riku, Exercise loading and bone structure. 97 p. (141 p.) Yhteenveto 1 p. 2009.

137 Korhonen, Marko T., Effects of aging and training on sprint performance, muscle structure and contractile function in athletes. - Ikääntymisen ja harjoittelun vaikutukset nopeussuorituskykyyn, lihasten rakenteeseen ja voimantuotto-ominaisuuksiin urheilijoilla. 123 p. (211 p.) Tiivistelmä 5 p. 2009.

138 Javanainen-Levonen, Tarja, Terveydenhoitajat liikunnanedistäjinä lastenneuvolatyössä. - Public Health Nurses as Physical Activity Promoters in Finnish Child Health Clinics. 104 p. (148 p.) Summary 6 p. 2009.

139 Klemola, Ulla, Opettajaksi opiskelevien vuorovaikutustaitojen kehittäminen liikunnan aineenopettajakoulutuksessa.- Developing student teachers´ social interaction skills in physical education teacher education. 92 p. (138 p.) Summary 4 p. 2009.

140 Niemi, Reetta, Onks tavallinen koe vai sellanen, missä pitää miettii? Ympäristölähtöisen terveyskasvatuspedagogiikan kehittäminen narratiivisena toimintatutkimuksena. - Is this a normal test or do we have to think? Developing environmentally oriented health education pedagogy through narrative action research . 215 p. 2009.

141 von Bonsdorff, Mikaela, Physical activity as a predictor of disability and social and health service use in older people. - Fyysinen aktiivisuus toiminnanvajauden ja sosiaali- ja terveyspalvelujen käytön ennustajana iäkkäillä henkilöillä 101 p. (134 p.) Yhteenveto 2 p. 2009.

142 Palomäki, Sanna, Opettajaksi opiskelevien pedagoginen ajattelu ja ammatillinen kehittyminen liikunnanopettajakoulutuksessa. - Pre-service teachers' pedagogical thinking and professional development in physical education teacher education. 118 p. (163 p.) Summary 3 p. 2009.

143 Vehmas, Hanna, Liikuntamatkalla Suomessa. Vapaa-ajan valintoja jälkimodernissa yhteiskunnassa. - Sport tourism in Finland – leisure choices in the post-modern society. 205 p. Summary 10 p. 2010.

144 Kokko, Sami, Health promoting sports club. Youth sports clubs' health promotion profiles, guidance, and associated coaching practice, in Finland. 147 p. (230 p.) Yhteenveto 5 p. 2010.

145 Kääriä, Sanna, Low back disorders in the long term among employees in the engineering industry. A study with 5-, 10- and 28-year follow-ups. - Metalliteollisuuden työntekijöiden alaselän sairaudet ikääntyessä: METELI–tutkimuksen 5–, 10– ja 28–vuotisseurantatutkimus. 76 p. (102 p.) Yhteenveto 2 p. 2010.

146 Santtila, Matti, Effects of added endurance or strength training on cardiovascular and neuromuscular performance of conscripts during the 8-week basic training period. - Lisätyn voima- ja kestävyysharjoittelun vaikutukset varusmiesten hengitys- ja verenkiertoelimistön sekä hermo-lihas järjestelmän suorituskykyyn kahdeksan viikon peruskoulutuskauden aikana. 85 p. (129 p.) Yhteenveto 2 p. 2010.

147 Mänty, Minna, Early signs of mobility decline and physical activity counseling as a preventive intervention in older people. - Liikkumiskyvyn heikkenemistä ennakoivat merkit ja liikuntaneuvonta liikkumisvaikeuksien ehkäisyssä iäkkäillä henkilöillä. 103 p. (149 p.) Yhteenveto 2 p. 2010.

148 Rantalainen, Timo, Neuromuscular function and bone geometry and strength in aging. - Neuromuskulaarinen suorituskyky luun geometrian ja voiman selittäjänä ikääntymisen yhteydessä. 87 p. (120 p.) Yhteenveto 1 p. 2010.

149 Kuitunen, Sami, Muscle and joint stiffness regulation during normal and fatiguing stretch-shortening cycle exercise . - Lihas- ja niveljäykkyyden säätely normaalin sekä väsyttävän venymis-lyhenemissykli – tyyppisen harjoituksen aikana . 76 p. (142 p.) Yhteenveto 1 p. 2010.