Aki Vainionpää

BONE ADAPTATION TO IMPACT LOADING—SIGNIFICANCE OF LOADING INTENSITY

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Bone Adaptation to Impact Loading—Significance of Loading Intensity

Academic dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, for public defence in the Auditorium of the Department of Physiology, on June 15th, 2007, at 12 noon.
Vainionpää, Aki, Bone adaptation to impact loading—Significance of loading intensity
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Abstract
Ageing populations have made osteoporosis and fragility fractures a major public health concern worldwide. Half of all women and 30% of all men will suffer a fracture related to osteoporosis during their lifetime. While medical prevention of this immense problem is impossible at population level, it is necessary to find efficient preventive strategies. Exercise is one of the major prevention approaches because one reason behind the increasing burden of osteoporosis is the modern sedentary lifestyle. However, the optimal type, intensity, frequency, and duration of exercise that best enhances skeletal integrity are still largely unknown.

We conducted a 12-month population-based randomized controlled exercise intervention in 120 premenopausal women. The aim was to investigate the effect of impact exercise on bone mineral density, geometry and metabolism in healthy women with the intention of assessing the intensity and amount of impact loading with a novel accelerometer-based measurement device. Training effects on risk factors of osteoporotic fractures, physical performance and risk factors of cardiovascular diseases were also evaluated.

This study demonstrated that 12 months of regular impact exercise favoured bone formation, increased bone mineral density in weight-bearing bones, especially at the hip, and led to geometric adaptations by increasing periosteal circumference. Bone adaptations had a dose- and intensity-dependent relationship with measured impact loading. Changes in proximal femur were threshold-dependent, indicating the importance of high impacts exceeding acceleration of 4 g as an osteogenic stimulus. The number of impacts needed to achieve this stimulation was 60 per day. Impact exercise also had a favourable effect on physical performance and cardiorespiratory risk factors by increasing maximal oxygen uptake, dynamic leg strength and decreasing low-density lipoproteins and waist circumference. Changes were dose-dependent with impact loading at wide intensity range.

Bone adapts to impact loading through various mechanisms to ensure optimal bone strength. The number of impacts needed to achieve bone stimulation appeared to be 60 per day, comparable to the same number of daily jumps. If done on a regular basis, impact exercise may be an efficient and safe way of preventing osteoporosis.

Keywords: acceleration, bone density, cardiovascular diseases, exercise, female, fragility fractures, impact intensity, intervention studies, osteoporosis, premenopause
Tiivistelmä

Väestön ikääntymisen ja elintapojen muutosten myötä osteoporoosista ja osteoporoottisista murtumista on tullut maailmanlaajuinen terveysongelma. Ongelmasta aiheutuvien murtumien lääketieteellinen ehkäisy ei ole mahdollista kattavasti väestötasolla, joten vaihtoehtoisista ehkäisymenetelmiä käytettävä on välttämätön. Liikunta on yksi potentiaalinen ehkäisykeino, koska yksi tärkeä tekijä ongelman taustalla on arkiliikunnan vähentyminen. Liikunnan tieletään hyödyttävän luustoa, mutta optimaalisen liikunnan tyyppi, intensiteetti, määrä ja kesto ovat kuitenkin selvitettävissä.


Tutkimus osoitti luun mukautumisen ja mukautumisen vaikutuksia kuormitukseen useiden teknisten määrittelyten mukautuneista harjoittelupäivityksistä ja mukautumisen olevan kuormitusten intensiteettistä riippuvasta. Osteoporoosin ehkäisyyn kannalta tehokas ja turvallinen kuormitusmäärä näyttää olevan 60 hyppyä päivässä.

Asiasanat: ehkäisy, kiitettävyys, liikunta, luunmurtumat, luuntiheyys, luusto, nainen, osteoporoosi, sydän- ja verisuonisairaudet
To my family
Acknowledgements

This study was carried out in the years 2001-2006 at the Department of Medical Technology and Department of Physiology, University of Oulu, and the Department of Sports Medicine, Deaconess Institute of Oulu. I wish to thank former and present heads of these departments, professor Timo Jämsä, professor Juhani Leppälä, professor Timo Takala and professor Olli Vuolteenaho, for providing excellent conditions for doing research.

I wish to express my deepest gratitude to my supervisor professor Timo Jämsä for his guidance, enthusiasm and optimism. Despite several other projects and work he has always had the time to help and guide me in the most exemplary and friendly way. I want to express my gratitude to my second supervisor, professor Juhani Leppälä. His incredible scientific experience, expert guidance and never-ending enthusiasm have been extremely valuable during these years. I also wish to thank Raija Korpelainen for her practical supervision and assistance that have many times made my life much easier.

I express my sincere gratitude to my co-authors Erkki Vihriälä, Anneli Rintapäävola, docent Harri Sievänen, professor Kalervo Väänänen, Jouko Haapalahti, Hannu Kaikkonen, and professor Mikael Knip for their contribution and expertise during the study.

My sincere thanks go to Minna Tervo, physiotherapist in our team. Her contribution during the intervention was invaluable. I also wish to thank the entire staff in the Department of Sports Medicine, Deaconess Institute of Oulu, and Department of Medical Technology, University of Oulu for their assistance, encouragement and friendship. Special thanks go to Arja Retsu and Sarianna Vaara for their expertise in performing bone measurements and to docent Pentti Nieminen for his expert statistical assistance.

I am very thankful for professor Petri Lehenkari, the former co-ordinator of the M.D./Ph.D. programme in Faculty of Medicine in University of Oulu, who introduced and encouraged me to the field of research.

I am very grateful to the official referees of the thesis, docent Klaus Engelke and docent Pekka Kannus, for their valuable comments and careful revisions of the manuscript.

I want to thank my friends Janne P., Aleksi, Tuomas, Hermanni, Janne L. and Antti for the joyful moments in the back rows of lectures as well as in leisure time. I hope that our relaxing adventures will continue in the future.
I owe my very deep gratitude to my parents Armi and Asko, for their encouragement and unconditional love and care. I cannot imagine better parents! I want to thank my brother Ari for his encouragement, support and being an exemplary role model. I wish to acknowledge also my in-laws Eeva and Mauri for their unlimited support in daily life. Finally, my most loving thanks go to my wife Jaana for her love and patience during these years. On difficult days, she always gave me faith and was there for me.

This work was supported by the Finnish Funding Agency for Technology and Innovation, the Juho Vainio Foundation, the Instrumentarium Foundation for Science, the Research Foundation of the Institutes of Sports, the Finnish Cultural Foundation, the Finnish Foundation for Sports Research, the Finnish Medical Society Duodecim, Newtest Ltd., CCC Group, and Fastrax Ltd.
## Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>ε</td>
<td>strain</td>
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<tr>
<td>σ</td>
<td>stress</td>
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<tr>
<td>g</td>
<td>acceleration of gravity</td>
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<td>BMC</td>
<td>bone mineral content</td>
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<td>BMD</td>
<td>bone mineral density</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>BMU</td>
<td>basic multicellular unit</td>
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<td>BUA</td>
<td>broadband ultrasound attenuation</td>
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<tr>
<td>BW</td>
<td>body weight</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CSA</td>
<td>cross-sectional area</td>
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<td>CSMI</td>
<td>cross-sectional moment of inertia</td>
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<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DXA</td>
<td>dual-energy x-ray absorptiometry</td>
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<td>GH</td>
<td>growth hormone</td>
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<td>GRF</td>
<td>ground reaction force</td>
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<td>HDL-C</td>
<td>high-density lipoprotein cholesterol</td>
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<td>HSA</td>
<td>hip structural analysis</td>
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<td>IGF</td>
<td>insulin-like growth factor</td>
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<td>LDL-C</td>
<td>low-density lipoprotein cholesterol</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>PINP</td>
<td>procollagen type I aminoterminal propeptide</td>
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<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
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<tr>
<td>QCT</td>
<td>quantitative computed tomography</td>
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<tr>
<td>QUS</td>
<td>quantitative ultrasound measurement</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SOS</td>
<td>speed of sound</td>
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<tr>
<td>TRACP5b</td>
<td>tartrate-resistant acid phosphatase 5b</td>
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<tr>
<td>VO₂max</td>
<td>maximal oxygen uptake</td>
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<tr>
<td>Z</td>
<td>Z-score</td>
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<tr>
<td>25OHD</td>
<td>serum 25-hydroxyvitamin D</td>
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List of original articles

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


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1 Introduction

Bone is a vital connective tissue that makes up the solid foundation for the human body. As the primary function of bone is to make possible efficient locomotion of human beings, it has to resist continuous mechanical load without breaking. To enable this, it optimises its structure with subtle adaptations to maintain rigidity along with lightness (Wolff 1892, Turner 1998, Currey 2003). However, decreases in bone mass are inevitable and physiological with age, leading eventually to skeletal fragility. When the bone mass drops to a critical level below which fracture risk is substantially increased, the state is considered pathological and referred to as osteoporosis (World Health Organisation 1994). Osteoporosis is currently defined as a systemic disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and increased fracture risk (Consensus development conference 1993).

Osteoporosis, especially osteoporotic fractures, have become a serious global public health concern as a result of increased life expectancy leading to a more ageing population (Kanis & Johnell 1999, Reginster & Burlet 2006). It is currently estimated that over 200 million people worldwide suffer from osteoporosis (Cooper 1999) and that about 50% of all women and 30% of all men will suffer a fracture related to osteoporosis during their lifetime (Cooper et al. 1992). As these injuries are associated with increased morbidity, mortality and impose a financial burden on the community, it is crucial to prevent these types of fractures (Johnell & Kanis 2004, Johnell & Kanis 2006). Modern osteoporosis medication with antiresorptive drugs can reduce the fracture risk by about 50% (Seeman 1997b). Nevertheless, medical prevention and treatment of this immense problem is not economically efficient or even possible at population level (Stevenson et al. 2005, Sanders et al. 2006). Thus, alternative strategies are crucial.

One of the most important prevention approaches is exercise, as one reason behind the increasing burden of osteoporosis is the modern sedentary lifestyle (Cummings & Melton 2002). The potential of exercise in prevention is increased by its wide spectrum of targets in promotion of public health, from osteoporosis to cardiovascular disease (Fentem 1994, NIH Consensus Development Panel on Physical Activity and Cardiovascular Health 1996).

Although the importance of mechanical loading-induced bone strains as an adaptive stimulus to promote skeletal integrity has been acknowledged since the late 1800s (Wolff 1892), the type, intensity, frequency, and duration of loading
that best enhances bone strength is still largely unknown. It has been
discovered that exercise-induced dynamic loading, especially impact type that
generates high-intensity loading forces, leads to beneficial bone adaptations
(Turner 1998, Turner et al. 2003). However, the optimum loading patterns of bone
that produce clinically significant benefits have not been clearly defined to date,
since it has not been possible to easily quantify exercise intensity in terms of
bone-loading forces in clinical settings (Kohrt et al. 2004).

Direct measurement of bone strains requires invasive surgical procedures
(Hoshaw et al. 1997) and indirect measurement of ground reaction forces are
limited to a fixed place and time, being thus impracticable in long-term clinical
measurements. However, accelerations are another indirect surrogate of bone
strains that are now readily measurable with a novel accelerometer-based
measurement device. They are mathematically related to bone strains with a few
simplifying assumptions (Heikkinen et al. 2007), and accelerations measured
during exercise have also been shown to be related to impact load forces (Servais

The purpose of this population-based randomised controlled study was to
investigate the effect of exercise-induced impact loading on bone mineral density,
geometry and metabolism in healthy premenopausal women with the intention of
assessing the intensity and amount of impact loading required to gain adaptive
effects. In addition, our aim was to evaluate the effect of a bone-targeted exercise
training programme on extra-skeletal risk factors of osteoporotic fractures,
physical performance and main risk factors of cardiovascular diseases.
Determination of quantified intensity thresholds for efficient exercise would
provide tools to promote osteogenic exercise patterns to be included in everyday
routines and personal activities and thus increase the possibility to impact public
health as well.
2 Review of literature

2.1 Bone biology

Bone is a vital connective tissue that balances between versatile functions. It provides mechanical integrity for the human locomotor system, protects the vital internal organs and serves as a mineral reservoir in mineral homeostasis. Bone is also a primary site of haematopoiesis and acts as part of the immune system. Bone structure is characterised by a multilevel architectural organisation that ensures optimal mass, size and shape for structural strength and function. (Einhorn 1995, Seeman & Delmas 2006)

2.1.1 Bone composition

Bone is composed of cells and extracellular matrix having inorganic and organic components. By weight, bone tissue consists of approximately 70% mineral or inorganic matter, 22 to 25% organic matrix, and 5 to 8% water, but there are major variations in the degree of mineralisation depending on function. The inorganic component is the mixture of calcium and phosphorus in crystalline hydroxyapatite (Ca_{10} (PO_{4})_{6} (OH)_{2}) composing 95% of the mineral phase. Type I collagen constitutes approximately 90% of the organic matrix, and the rest is composed of proteoglycans and non-collagenous proteins. (Bono & Einhorn 2003), (Seeman & Delmas 2006)

Four different cell types can be distinguished in bone tissue. Osteoblasts, osteoclasts, and bone-lining cells are present on bone surfaces, whereas osteocytes permeate the mineralised interior. Osteoblasts are the fully differentiated bone-forming cells derived from stromal cell precursors in the bone marrow. Osteoblasts also have the ability to synthesise and secrete unmineralised extracellular matrix (osteoid) and control the mineralisation with secreting vesicles containing alkaline phosphatase. (Lian 1995, Seeman & Delmas 2006) After the bone is mineralised some of the osteoblasts are embedded in lacunae in the bone matrix and are called osteocytes after undergoing morphologic changes. Osteocytes are connected with each other as well as with osteoblasts and bone-lining cells through the cytoplasmic network projecting through small canals between lacunae called canaliculi in the mineralised bone matrix (Burger & Klein-Nulend 1999). Bone-lining cells are inactive cells remaining on the bone
surface that are undergoing neither bone formation nor resorption but are involved in regulation remodelling. Osteoclasts are multinuclear cells derived from haematopoietic stem cells via monocytes that are able to erode and resorb previously formed bone. (Sambrook 2001, Seeman & Delmas 2006).

2.1.2 Bone turnover

The cellular mechanisms responsible for the adaptation of bone tissue are modelling (construction) and remodelling (reconstruction) (Figure 1). Modelling initially forms mineralised bone at developmentally determined sites during growth or as an adaptive response to needs of external mechanical loading by simultaneous resorption and formation at different sites (Frost 1987, Seeman 2003). According to Frost (1990), there are two different modelling types. Micromodelling organises collagen and cells during their formation determining the formed tissue, while macromodelling controls the shape, size, strength and anatomy of an organ (Frost 1990). Bone remodelling is defined as resorption of bone followed by bone formation. It is a bone structure maintaining function and provides access to the skeletal store of minerals, thus contributing to mineral homeostasis. In physiological conditions formation is coupled together with resorption to restore bone loss. (Buckwalter & Cooper 1995b, Sambrook 2001)

In the human skeleton, there are about 2 million basic multicellular units (BMUs) responsible for remodelling at any one time (Raisz 1999, Seeman & Delmas 2006). It is estimated that 20-25% of cancellous bone and 3-4% of cortical bone is renewed annually in mature people (Ganong 2003). The remodelling cycle takes about 200 days in cortical bone and 100 days in cancellous bone (Buckwalter & Cooper 1995b, Väänänen 1996). Disturbances in the system can lead to bone loss. The net amount of bone lost during ageing is determined by the amount of bone that is removed from endocortical, trabecular and intracortical components of its endosteal envelope and formed beneath its periosteal envelope. Endosteal bone loss is determined by the remodelling rate and the negative balance. The negative balance results in trabecular thinning, disappearance and loss of connectivity, cortical thinning and increased intracortical porosity. (Seeman 2003, Ahlborg et al. 2003)
Fig. 1. The remodelling cycle. Signals from osteocytes lead to recruitment and activation of osteoclasts. Activated, multinucleated osteoclasts initiate resorption by the secretion of the hydrogen ions and proteolytic enzymes leading to degradation of collagen, deposition of proteoglycans and release of the growth factors that initiate reversal and formation phases. Activated osteoblasts synthesise osteoids in resorption cavities that are mineralised after a lag time (Einhorn 1995, Raisz 1999). Depending on the osteogenic stimulus, resorption cavities may be overfilled, underfilled or filled equally leading to increased, decreased or maintained bone mass (Turner 1991).

2.1.3 Bone development and structure

In the human skeleton, flat bones (skull bones, scapula, mandibula and ileum) are formed by the ossification of membranes (intramembranous bone formation) independently of cartilage. Long bones are formed by the deposition of mineralised tissue preceded by cartilage analogue (endochondral bone formation or ossification) during the modelling process (Raisz 1999, Sambrook 2001). The external shape of long bones consists of an expansion at both ends (epiphysis), a hollow cylindrical tube in the middle (diaphysis) and the area between these two parts (metaphysis) (Buckwalter & Cooper 1995a). In growing bone, the epiphysis
and metaphysis are separated by cartilage (growth plate), which is responsible for the longitudinal growth of the bone and becomes calcified at the end of longitudinal growth after puberty (Ganong 2003).

Morphologically, there are two contrasting types of bone in the adult human skeleton. Cortical bone is compact and dense, forming approximately 80 percent of the skeleton. It makes up the outer layer of bones and is covered by periosteum, a thin layer of osteogenic and fibroblastic cells in a well-developed nerve and microvascular network (Orwoll 2003, Allen et al. 2004). Cortical bone has a mainly structural, load-bearing function and it prevails in the long bones of the skeleton (Einhorn 1995). It constitutes cylindrical units, each having the Haversian canal in the centre that contains blood and lymph vessels and nerves providing nutrients in cortical bone. Around each Haversian canal, collagen is arranged in concentric layers, forming Haversian systems. Between the layers, osteocytes are located in lacunae that are interconnected through small canals between the lacunae called canaliculi in the mineralised bone matrix so that they come into contact with each other (Einhorn 1995, Sambrook 2001). Trabecular bone is the spongy, porous type of bone located in the interior of cuboid and flat bones and at epiphyseal and metaphyseal ends of long bone. It is made up of spicules or plates that are interconnected to form a network resulting in a cellular solid type of material (Keaveny et al. 2001). Bone marrow is situated within this network. Trabecular bone is mainly responsible for the metabolic function of bone due to its high surface-to-volume ratio. It has a role in increasing bone strength as well. Due to the high surface-to-volume ratio, trabecular bone is more prone than cortical bone to diseases resulting from increased bone remodelling, such as osteoporosis (Einhorn 1995). The proportions of cortical and trabecular bone may vary significantly in different locations of the same bone depending on bone location and function (Einhorn 1992, Borer 2005).

2.2 Bone biomechanics

In normal daily life, bone is subjected to external loading that leads to changes in bone internal resistance. This internal resistance is called stress and it equals the magnitude of external force but is in the opposite direction. External forces lead to deformation in shape and size of the bone, which is called strain in biomechanics. Stress, defined as force per unit area, can be compressive, shear, tension or a combination of these, as is usually the case in vivo. Strain on the other hand is dimensionless proportional change in the length of the bone and is
expressed as a fraction, percentage or microstrain (µε). One percentage change in length is equivalent to 10,000 microstrain. (Currey 2001) Bone mechanical properties can be determined at structural level (extrinsic) when load and deformation are represented in a curve, and at material level (intrinsic) with stress and strain (Figure 2). The curve is similar for compressive, tensile, bending or torsional loading, with only the parameters differing (Turner & Burr 1993, Einhorn 1995). Bone mechanical properties are also strain-rate dependent since bone is viscoelastic material. Bones can thus resist higher stress levels with similar strains when the strain rate increases. However, at very high strain rates bone becomes more brittle. (Frankel & Nordin 2001)

Bone design has been optimised to serve its major functions. When subjected to loading, the ability of bones to carry loads depends on their mechanical properties. Bone mechanical properties are an optimal combination of strength, i.e. the ultimate stress that bone can resist without fracturing, toughness, i.e. amount of energy that bone can absorb before fracturing, stiffness, i.e. the resistance of bone to deformation by an applied force, and fatigue resistance i.e. bone capacity to resist repetitive loading (Currey 2001). Bone should be able to withstand high loads without breaking, resist excessive deformation under submaximal loads (rigidity) and be light in order to facilitate movement with the least possible amount of material. Bone material properties at the tissue level and structural properties at the organ level are linked together by bone geometric properties (Ferretti et al. 2001). Material properties of the bone are mainly determined by the calcification of the bone matrix and microstructural factors such as composition and spatial arrangement of collagen fibres and crystals (Ferretti et al. 2001, Burr 2002). The inorganic matrix determines material stiffness while the organic component is responsible for elasticity. The geometric properties concern mass and spatial distribution as well as the size and architectural design of calcified materials (shape). Structural properties, such as rigidity and structural strength, are a combination of material and geometrical properties and are evident when bone is considered as a whole functional unit (Currey 2001, Currey 2003). Compared with bone mass and architecture, material properties change little with age, gender or species, being thus less important determinants of mechanical integrity (Frost 1997, Currey 2001).
Fig. 2. A typical load/deformation (stress/strain) curve in testing biomechanical structural (material) strength of bone. The height of the curve represents the strength, i.e. the ultimate force (ultimate stress) in the failure point when fracture occurs. The maximum slope of the curve is rigidity (elastic modulus i.e. stiffness), and the width of the curve is ultimate displacement (ultimate strain that are reciprocal of brittleness). The area under curve represent work-to-failure, i.e. absorbed energy (toughness). Prior to the yield point the deformation is elastic and the structure will return to its original shape when the load is removed. After the yield point the deformation is plastic, meaning that the deformation is permanent despite removal of the load.

Bone behaves anisotropically; consequently, its mechanical properties vary depending on loading directions. In a normal situation, bone mechanical properties are optimal in the direction of customary loading (Currey 2001). The major loading types a bone has to resist in normal circumstances are compression, tension, bending and torsion. In vivo, loadings are most often a combination of these, and loading rates may also vary significantly, as in the case of impact loading. Bone cannot always be considered to be in equilibrium, as it can be in quasi-static loading produced by an ordinary testing machine. The ability to absorb kinetic energy is thus one of the major determinants of bone failure resistance. (Currey 2003) A bone with a large mass, density and cross-sectional area is resistant to compressional force simply because the load is distributed over a larger surface area (Einhorn 1996). The dimensions of the bone are more important than its mass or density when it comes into resisting bending or
torsional forces. Ideally, in bending or torsion the bone mass should be distributed as far away from the neutral axis of the load as possible (Keaveny et al. 2001). Hollow and thick-walled diaphysis with longitudinally oriented osteons provides resistance against torsion and bending forces, as the metaphysis is designed to resist compression. It has wide bone ends filled with porous trabecular bone which helps to absorb axial compression and impacts applied across synovial joints (Einhorn 1996). Trabeculae are arranged to provide the greatest strength with the minimum of material in positions of maximum stress (Keaveny et al. 2001, Currey 2003).

However, contrary to material properties, bone structural properties, especially geometry, do not remain unchanged during growth and maturation. Changes in geometry during growth and ageing are crucial for bone strength. During childhood and adolescence, long bones grow in length and diameter, and the bone mass increases in proportion to the enlarging volume of the whole bone. Endosteal resorption and periosteal formation shape the bone cortical geometry and spatial apposition of bone mass during growth. Differences in bone strength between males and females are mainly due to bigger bone size in men, not denser bones. During puberty, males have a high rate of periosteal apposition leading to cortical thickening and wider bones, while periosteal formation is inhibited and endocortical formation stimulated in females leading to cortical thickening and narrowing of the medullary cavity. (Seeman 2001) This difference is related to the onset of oestrogen secretion; in females, oestrogen deposits an extra stock of mineral in the skeleton for the needs of reproduction – pregnancy and lactation. Thus there is no need for periosteal apposition in females to ensure skeletal strength during the premenopausal period. At menopause, withdrawal of oestrogen leads to the unpacking of these reproductive safety deposits of calcium, causing an accelerated phase of bone loss. (Järvinen et al. 2003b)

During ageing, the negative balance of bone remodelling may lead to irreversible bone loss. Bone loss is fastest in trabecular bone due to the high surface-to-volume ratio. In men, this mainly leads to thinning in trabeculae, whereas trabecular connectivity is lost more in women. Trabecular bone loss is also accelerated in women due to menopausal changes in oestrogen levels. (Aaron et al. 1987, Aaron et al. 2000) Continued remodelling may also cause perforation of trabeculae and trabecularisation of cortical bone on the endocortical and intracortical surface (Seeman & Delmas 2006). Periosteal apposition also continues, albeit more slowly than during growth. This may partly offset the bone loss from the endosteal surface at certain bone sites. This redistribution of bone
tissue away from the axis allows the bone to resist bending and torsional loads better. However, the ability to withstand impact loads decreases since the risk of buckling increases with thinning cortices (Currey 2003). Interestingly, this periosteal bone deposition has been found to be greater in men compared to women, leading to better maintained structural strength in men. (Smith & Walker 1964, Ruff & Hayes 1982, Ruff & Hayes 1988, Russo et al. 2003).

2.3 Bone adaptation to mechanical loading

Bones are self-controlled constructions adapting their structure to the prevailing mechanical environment throughout their lifecycle. This ability to adapt to mechanical loading was first recognised over a century ago, and it is referred to as Wolff’s law (Wolff 1892). According to Wolff’s law, changes in the form and function of bones are followed by definite changes in their internal architecture and external conformation in accordance with mathematical laws. In other words, the function of the cells responsible for mechanically adaptive modelling and remodelling is presumably to ensure that the variables which they control, such as mass, architecture, and material properties, are appropriate in relation to the applied load, enabling the primary function of the skeleton – efficient locomotion (Frost 1997, Lanyon & Skerry 2001).

2.3.1 Mechanotransduction

Mechanotransduction is the establishment responsible for ensuring the maintenance of an appropriate match between bone structure and customary loading. The first phase, mechanocoupling, is initiated by mechanical force applied to bone leading to transmission of the local mechanical signal to a sensor cell. Biochemical coupling converts local mechanical signals into a cellular response that alters phenotypic expression and mediates cell signal transduction. These signalling cascades alter the cellular activities of local effector cells (osteoblasts and osteoclasts) leading to appropriate tissue-level response to achieve favourable structural state through maintenance of a target strain environment at each skeletal location (Turner & Pavalko 1998, Zernicke et al. 2006).

The precise nature of the stimuli perceived remains elusive, but proposed mechanisms include cellular deformations as direct stimuli and matrix deformation driven bone fluid as indirect stimuli. Mechanical force induced
deformations in bone matrix generate pressure gradients that cause fluid flow through canalicular spaces (Weinbaum et al. 1994, Turner et al. 1994a, Turner & Pavalko 1998). This fluid flow has been suggested to stimulate bone cells by three different mechanisms that are not mutually exclusive. The flow of an ionic bone fluid over a charged bone matrix surface creates an electrical potential referred to as streaming potential that has been shown to increase with load frequency (Johnson et al. 1982, Turner et al. 1994a). In addition to streaming potentials, fluid flow also increases nutrient flow and waste removal. Bone cells are also extraordinarily sensitive to fluid flow induced shear stresses that have been found to increase the levels of second messengers such as intracellular calcium (Jacobs et al. 1998) prostaglandins (Nauman et al. 2001) and nitric oxide (Johnson et al. 1996). Nevertheless, fluid flow is currently suggested to be the most important mediator of mechanical signal (Turner et al. 1994a, Owan et al. 1997, Han et al. 2004), especially as experimental fluid flow induced shear stress without bone matrix deformation has been found to lead to bone formation (Qin et al. 2003).

An ideal location, interconnection with each other through functional gap junctions, as well as sensitivity to fluid flow makes the osteocytes-bone lining cell complex the best candidate for mechanosensory cells (Lanyon 1993, Turner et al. 1994a, Klein-Nulend et al. 1995, Mullender & Huiskes 1997).

**2.3.2 Mechanostat**

Bone adaptation to prevailing conditions is regulated by mechanical and humoral factors. Bone adaptation to mechanical loading has been suggested to be under regulation of a mechanobiologic feedback system called “Mechanostat”. This theory suggests that a negative feedback mechanism adjusts skeletal structure, corresponding to the present mechanical usage to prevent structural failures of skeletal tissue (Frost 1987, Frost 2003). According to this theory, mechanostat senses and perceives the incident loading-induced strain distribution within the bone and subsequently removes bone tissues from the areas under marginal stresses and increases bone tissue in the areas subjected to the greatest stress. The mechanostat theory has encountered some criticism as it does not conform with some experimental observations, and is probably an oversimplified model that cannot explain all bone adaptation phenomena (Turner 1999, Skerry 2006). However, the concept of certain minimally effective strain threshold levels required for the commencement of bone resorption or bone formation (Figure 3), as suggested in the Mechanostat theory, have a strong pre-clinical background.
In addition to strain magnitude, the nature of the osteogenic stimulus is also affected by strain rate, frequency, rest periods and the number of loading cycles (Turner et al. 2003).

![Mechanostat Theory Diagram](image)

Fig. 3. The mechanostat theory adapted from (Frost 1997). The threshold ranges are believed to be partly genetically determined. However, the current mechanostat theory most likely oversimplifies the threshold levels as they are suggested to vary in different bones and bone regions. The approximated threshold levels presented above may only apply e.g. in the mid-shaft of a long bone (Skerry 2006).

### 2.3.3 Mechanical components of osteogenic loading

In historical perspective, strain magnitude has been acknowledged to be the main bone osteogenic stimulus (Wolff 1892). However, modern understanding has extended the perspective to several tissue-level mechanical parameters including, but not limited to, strain magnitude, strain rate, strain frequency, cycle numbers, bout intervals, strain directions and circumferential strain gradients (Turner 1998, Turner et al. 2003, Kohrt et al. 2004) (Figure 4). However, it has to be pointed out that the vast majority of the previous results are experimental and that separation of these parameters has rarely been done in clinical studies. Furthermore, during normal locomotion, as in the case of clinical studies, all body movements are produced by co-ordinated contractions of skeletal muscles, while the concomitant dynamic muscle work provides the fundamental source of mechanical loading to
the skeleton (Frost 1997, Bassey et al. 1997, Sievanen 2005). Experimental models with external loading sources are thus probably highly simplified.

![Diagram of mechanical parameters affecting osteogenic potential of loading-induced strains in bones.](image)

**Fig. 4.** A schematic diagram of the selected mechanical parameters affecting osteogenic potential of loading-induced strains in bones. Modified from (Kemmler et al. 2004b).

**Strain magnitude**

Strain magnitude has been found to be highly related to new bone formation at the organ level (Rubin & Lanyon 1985, Turner et al. 1994b, Mosley et al. 1997, Cullen et al. 2001). In other words, the larger the generated deformation in the
bone matrix the larger the overall response of the bone. The threshold level for activation of new periosteal and endosteal bone formation was around 1000 µε in turkey ulnae (Rubin & Lanyon 1985). Mosley et al. found geometric adaptation in rat ulnae after a short period of axial loading with 2000 µε and geometric adaptation together with increased bone mass after axial loading with 4000 µε (Mosley et al. 1997). However, more recent data have shown that strain thresholds for osteogenic loading vary significantly even within the same bone (Hsieh et al. 2001). More precisely, these results suggested that osteogenic response depends on the peak strain levels and that the strain threshold is largest distally and smallest proximally where bone strains are lower in normal conditions.

**Strain rate**

If loading is static, even extremely high strains are not always osteogenic. Hert’s pioneering studies conducted over 35 years ago demonstrated that bone tissue in rabbit tibia responds to dynamic rather than static loading (Hert et al. 1969, Hert et al. 1971). This is rational in view of the current understanding in theory of fluid flow behind mechanotransduction. While static loads, even at high strains, do not create any hydrostatic pressure gradients to initiate fluid flow in the bone lacuno-canicular network, they produce a state similar to disuse that has been found to result in bone resorption (Lanyon & Rubin 1984) and suppression of normal appositional growth in bone (Robling & Turner 2002).

It is clear that strain rate-related phenomena are critical for bone adaptive response. Findings by O’Connor et al. (O’Connor et al. 1982) showed the association between strain rate and new bone formation. More recently, it has been shown that strain rate provides a greater osteogenic stimulus for bone formation than strain magnitude (Mosley & Lanyon 1998), and it has been suggested that the bone adaptive response is determined by strain rate alone, i.e. a combination of strain magnitude and frequency (Turner 1998). This deduction was made after finding that increasing the frequency of loading with a constant strain magnitude caused a significant increase in bone formation rate at frequencies of 0.5–2.0 Hz (Turner et al. 1994a, Turner et al. 1995a) However, this is true only for loading frequencies of <2 Hz that nevertheless include most physiologic loading frequencies (Hsieh & Turner 2001).
Number and partition of loading cycles

The number of loading cycles is also essential. Experiments on externally loaded turkey ulnae and tibiae of rats trained to jump onto an elevated platform have shown that few loading cycles are necessary to elicit an osteogenic response (Rubin & Lanyon 1984, Umemura et al. 1997). Moreover, the response to loading diminishes quickly after inception of the loading bout; cycles that occur beyond cycles 50–100 in a single bout are largely ineffective in stimulating any further osteogenic response than that triggered by the first 50–100 cycles (Figure 5A). Even though the bone response is saturated during prolonged loading, mechanical sensitivity has been found to be re-established after a period of unloading. Rest periods of 4 to 8 hours between the loading bouts have restored almost 100% of the bone’s mechanical sensitivity, while a few seconds’ rest between each loading cycle has shown some beneficial effects, and a 14-second rest has almost doubled the osteogenic effect in animal studies (Robling et al. 2001, Umemura et al. 2002, Robling et al. 2002a, Robling et al. 2002b, Srinivasan et al. 2007).

An interesting exception to these consistent findings with moderate loading levels is the results from very high frequency loading. It has been found that even small strains with very high frequency (>30 Hz) with a high number of loading cycles can be osteogenic (Rubin et al. 2001, Rubin et al. 2002). However, some contradictory results also exist (Torvinen et al. 2003). It has also been suggested that there is nonlinear dependence of loading intensity and cycle number associated with the osteogenic stimulus (Figure 5B) (Qin et al. 1998).

Strain distribution and circumferential gradient

In addition to different loading profiles, the osteogenic effect of the loading may also vary due to the direction of loading, as bone is more sensitive to the applied strain distribution (Lanyon 1996). Imposing a strain distribution that produces the same peak strain magnitudes in a bone section but at different locations within the section (i.e. loading from an unusual direction) may initiate new bone formation. Consequently, habitual loading, although creating high strains, may not be osteogenically optimal. The distribution of strain gradients, i.e. changes in deformation across a given volume of tissue also needs to be considered as the sites of new bone formation have been found to vary accordingly (Gross et al. 1997, Judex et al. 1997). This means that strain distribution in a given area is not
uniform and that new bone accretion is linked to specific sites with highest circumferential strain gradients where structural enhancement is most needed.

Fig. 5. A) Only few loading cycles are necessary to elicit an osteogenic response in experimental studies. However, the osteogenic response is quickly saturated if the loading is dosed in one bout. Adapted from (Burr et al. 2002). B) The strain threshold required for osteogenic response in experimental studies is dependent on the magnitude and number of strain cycles. Stimuli above and to the right of the line are osteogenic, indicating that even small strains with very high frequency and a high number of loading cycles can be osteogenic. Adapted from (Qin et al. 1998).

2.4 Non-mechanical regulators of the mechanosensory system

Bone adaptation is also modified by several non-mechanical factors, including parathyroid hormone (PTH), 1,25-dihydroxyvitamin D and calcitonin that are major calcitropic hormones. Also hormones such as oestrogens, androgens, growth hormone (GH), insulin-like-growth factors (IGF), thyroid hormones and glucocorticoids with several paracrine hormones regulate calcium homeostasis and skeletal growth (Raisz 1999, Turner & Robling 2004, Raisz 2005). Two main endocrine hormones, parathyroid hormone (PTH) and oestrogen, are briefly reviewed here as they have been found to alter the osteogenic response to mechanical loading. Paracrine humoral mediators and factors of bone turnover have recently and comprehensively been reviewed by Turner and Robling (Turner & Robling 2004) and Raisz (Raisz 2005). Age has also been found to modulate mechanosensitivity and is thus reviewed along with humoral factors.
2.4.1 Parathyroid hormone

The main action of PTH is to ensure optimal plasma levels of ionised calcium, and it has been found to be under strict feedback regulation (Raisz 2005, Talmage & Talmage 2006). PTH has also been found to amplify the osteogenic effects of loading. It acts synergistically with mechanical loading to enhance periosteal bone formation (Ma et al. 1999a) and has been shown to enhance the anabolic effect of mechanical loading on endocortical and trabecular surfaces in rats (Chow et al. 1998, Li et al. 2003), while the effects on bone resorption are opposite with loading (Bakker et al. 2003). In addition, the anabolic effect of mechanical loading is abolished if the parathyroid glands are removed (Chow et al. 1998). In experimental studies, PTH has been found to sensitize cells to loading, probably by increasing the mobilisation of intracellular calcium (Miyachi et al. 2000, Ryder & Duncan 2001). PTH has been shown to be strongly anabolic if administered intermittently, and while hyperparathyroidism is known to cause secondary osteoporosis (Cranney et al. 2006). The anabolic effects of PTH have been found to be partly site-specific, and the reason for this has been suggested to be the ability of PTH to activate mechanical loading pathways (Bakker et al. 2003). In clinical studies, short-term loading has been found to stimulate transient PTH secretion peaks (Maimoun et al. 2005, Maimoun et al. 2006) while long-term exercise has been associated with low basal levels of serum PTH with high BMD (Brahm et al. 1997), indicating a complex relationship between the loading and PTH.

2.4.2 Oestrogen

The importance of oestrogen in bone biology has been acknowledged since the revolutionary observations of Fuller Albright indicating the vital role of oestrogen in maintaining skeletal integrity after menopause and ovariectomy (Albright et al. 1941). Currently, the principle actions of oestrogen are considered to be suppression of bone turnover, maintaining balanced rates of bone formation and bone resorption (Riggs et al. 2002). Oestrogen may also interact with mechanical loading pathways (Jessop et al. 2001), but the true nature of the interaction remains unclear. In general, oestrogen has been suggested to increase mechanosensitivity of bones by lowering the modelling and remodelling thresholds (Turner 1991, Lanyon & Skerry 2001, Joldersma et al. 2001, Frost 2003). To support this, bone has been found to be more adaptive to mechanical
loading when the number of oestrogen receptors is high, e.g. during adolescence, and less sensitive when the number of oestrogen receptors is low, e.g. postmenopausally (Lee et al. 2003, Lee et al. 2004). Customary loading would thus result in greater bone mass. Conversely, withdrawal of oestrogen reduces the sensitivity to loading and leads to bone loss, as the concurrent loading is not sufficient to counter this change in mechanosensitivity. This theory, however, contradicts the results showing that oestrogen mainly deposits an extra stock of mineral into female bones in puberty and thus, by making female bone mechanically stronger, suppresses the anabolic effect of mechanical loading (Järvinen et al. 2003a). This extra stock would satisfy the anticipated physiological needs of the subsequent reproductive period (Järvinen et al. 2003b, Lee et al. 2004). Furthermore, very recent results by Pajamäki (2007) have indicated that mechanical loading and oestrogen have completely independent skeletal effects, although being additive in nature. Loading was found to affect bone geometry and strength in a direction-specific manner while oestrogen accrued bone mass without specific effects on bone geometry or strength. So far, there are no clinical data to confirm these results in humans as there are only few clinical studies with simultaneous exercise and oestrogen replacement therapies. These studies have shown that combined treatments would lead to the most favourable results (Heikkinen et al. 1991, Kohrt et al. 1995, Heikkinen et al. 1997, Cheng et al. 2002). However, these studies have not provided results of actual group responses showing indisputable benefits of oestrogen-loading interaction. Whether the combined effects of oestrogen and mechanical loading strengthen bones more than mechanical loading alone is thus questionable.

2.4.3 Ageing

Age has been suggested to be one of the factors modulating mechanosensitivity to exercise-induced loading, as the response in exercise has been substantially higher in young and adolescent individuals compared to adults (Kannus et al. 1995, Haapasalo et al. 1998, Wolff et al. 1999, Wallace & Cumming 2000, MacKelvie et al. 2002, Hind & Burrows 2007). This is probably due to activated humoral mechanism during growth, as the peak of growth hormone secretion and peak velocity of bone mass accrual are simultaneous, peaks of the insulin-like growth factor-I and rise of gonadotropins also coinciding probably at the age of 11 to 13 years (MacKelvie et al. 2002). Rapidly induced growth also leads to increases in muscle force and thus elevated strain levels leading to bone
adaptation (Nordstrom et al. 1998). Adolescence thus provides a particularly opportune time to intervene with loading (MacKelvie et al. 2002).

There is also some evidence that postmenopausal women have impaired mechanosensitivity compared to premenopausal women (Bassey et al. 1998). It has even been suggested that osteoporosis is the result of maladaptation to loading caused by oestrogen deficiency or reduction of oestrogen receptors leading to impaired detection of strains (Lanyon & Skerry 2001). However, contradictory results have been presented concerning the role of oestrogen, as was briefly described above. In addition, there are suggestions that the osteogenic responsiveness to loading may differ qualitatively during the lifespan: during the growth period, loading would produce actual structural changes through periosteal expansion, while additional acquired bone after skeletal maturity is deposited along existing structures (Forwood & Burr 1993, Kannus et al. 1995, Haapasalo et al. 2000). Most recent results in animal studies have agreed on the findings suggesting that ageing alters more likely the type of response rather than the responsiveness itself (Järvinen et al. 2003). Similarly, bone’s ability to preserve exercise-induced bone benefits has been found to be independent of age (Järvinen et al. 2003). However, previous experimental studies have provided conflicting results (Raab et al. 1990, Rubin et al. 1992, Forwood & Burr 1993, Umemura et al. 1995, Turner et al. 1995b, Buhl et al. 2001) indicating that mechanisms behind the differences in adaptation during the lifespan are multifactorial, not only due to changes in mechanosensitivity but also to changes in external factors, e.g. muscle forces.

2.5 Measurement of skeletal health

Bone strength is a combination of several mechanical factors including bone size, shape, structure, microarchitecture, material properties and quantity of tissue. These are affected by the rate of bone turnover (Currey 2001, Felsenberg & Boonen 2005). A fracture occurs when the load applied generates an internal stress that exceeds the strength of the bone (Currey 2001). As such, fracture risk is related to bone strength, and in order to understand why bones break, it is necessary to examine the surrogate of bone strength, as we are naturally not able to measure bone strength itself in humans (Currey 2001, Bouxsein 2003). The following is a brief review of the methods that are currently in clinical use or have been suggested to come into clinical use in the near future.
2.5.1 Imaging methods

Dual x-ray absorptiometry

Dual x-ray absorptiometry (DXA) is the most widely used method for the measurement of bone mineral content (BMC) and bone mineral density (BMD). The radiation dose is comparable to daily background radiation exposure (Njeh et al. 1999) and the precision is found to be good (1-2% coefficient of variation, CV%) and stable in clinical use (Fogelman & Blake 2000). Despite the good precision, DXA is prone to several inaccuracies deriving from extra- and intra-osseous soft tissues (Bolotin 1998, Bolotin et al. 2003). Owing to the plane image of DXA, the measurement obtained is the BMC per unit projected area of the bone in the coronal plane, or areal BMD (g/cm²). Thus, DXA only measures areal BMD values, not true volumetric density (g/cm³), and because wider bones are also usually thicker, it overestimates substantially the BMD of larger bones, significantly confounding the interpretation of age- and sex-related changes (Seeman 1997a, Seeman 2001). Furthermore, an increase in the projected area as a result of increased bone size, e.g. from subperiosteal expansion, would lead to a decrease in BMD even if the BMC remains unchanged (Seeman 1997a, Seeman 2001). Thus, it has been suggested that studying geometry along with conventional bone measurements could lead to a better understanding of the processes leading to increased fracture risk.

This need has been responded to by developing a method called hip structural analysis (HSA) that uses areal BMD for measurements made by DXA at the femoral neck to estimate cross-sectional geometry and indices of bone strength. (Beck et al. 1990, Beck et al. 2000) However, due to the planar nature of the basic method this approach provides only a rough approximation of these structural indices (Duan et al. 2003), and it is still under investigation whether HSA provides any significant advantage in predicting fracture risk in clinical practice compared to direct measurement of hip BMD (Melton, III et al. 2005). However, it may provide additional information for research purposes.

Quantitative computed tomography

Quantitative computed tomography (QCT) enables the description of true cross-sections in vivo, and it is able to determine true three-dimensional geometry and
true volumetric bone density of both trabecular and cortical bone separately, providing better prediction of bone failure loads (Genant et al. 1996, Fogelman & Blake 2000, Bousson et al. 2006). In addition, the technique avoids the effect of degenerative disease, a particular drawback of DXA at the spine. The main disadvantages of computed tomography are a relatively high exposure to radiation, availability and higher cost compared to DXA (Kanis 2002). Peripheral QCT has a lower radiation dose and cost, and it has been found to be useful for the measurement of the appendicular skeleton (Genant et al. 1996, Sievänen et al. 1998).

**Magnetic resonance imaging**

Magnetic resonance imaging (MRI) permits visualisation of musculoskeletal tissues without using ionising radiation by imaging the relaxation properties of excited hydrogen nuclei. Clinical high-resolution MRI scanners have recently reached sufficient spatial resolution to quantify microstructural parameters of trabecular bone at peripheral skeletal sites in vivo (Newitt et al. 2002, Wehrli et al. 2006). These parameters typically include apparent bone volume fraction, apparent trabecular thickness, apparent trabecular number, and apparent trabecular separation. Previous studies have reported only moderate correlations between structural indices of trabecular bone and bone density (Newitt et al. 2002, Link et al. 2003, Wehrli et al. 2006), suggesting that microstructural parameters may contribute independent information in predicting bone strength.

**Quantitative ultrasound**

Quantitative ultrasound measurement (QUS) is not a genuine imaging method even though it is a non-invasive method for the assessment of bone properties without radiation exposure. Most QUS devices use the calcaneus as the measurement site, because it encompasses a large volume of trabecular bone and is readily accessible for transmission measurements (Fogelman & Blake 2000), but other bone sites are under intensive research. QUS of the heel has been suggested to yield information about the qualitative mechanical properties of bone and bone strength in addition to the ability to reflect bone density and fracture risk (Njeh et al. 2001). Measurement of bone QUS has been found to be significantly associated with non-spinal fractures in older women (Hans et al. 1996, Marin et al. 2006) even though the cut-off values commonly used in
clinical practice do not exclude or confirm osteoporosis sensitively enough (Nayak et al. 2006). However, the clinical usefulness of the QUS method is still under investigation, as is the method itself (Laugier 2006).

### 2.5.2 Assessment of bone turnover

BMD is a static measure of bone composition, reflecting its "history", and detectable changes take place over an extended period of time (years). Biochemical markers of bone metabolism measured from serum or urine offer inexpensive and generally available tools for detecting short time-frame (months) changes in bone metabolism in the whole skeleton. Biochemical markers reflect enzymatic activity of the bone cells, excess products from the formation or fragments released during the degradation of matrix components (Gerdhem et al. 2004). Rates of bone turnover and changes in bone markers predict improvement in bone density and strength (Nishizawa et al. 2001).

Bone metabolic markers have traditionally been classified into two categories
1) Bone formation markers that are released by osteoblasts and are measured from serum or plasma and 2) Bone resorption markers that are released from bone matrix or osteoclasts during bone resorption and are measured from urine or serum (Delmas et al. 2000, Seibel 2000). There has recently been a suggestion to add a third group – bone turnover, as osteocalcin has been considered to be a marker of bone turnover rather than a marker of formation, as it is also released during bone resorption (Ivaska et al. 2004). Most of the bone metabolic markers are present in tissues other than bone and may therefore be influenced by non-skeletal factors (Delmas et al. 2000). Another limitation is that bone metabolism markers are under the influence of diurnal and seasonal variations that are directly related to variations in the hormonal regulation of skeletal homeostasis (Woitge et al. 1998b, Woitge et al. 2000, Rapuri et al. 2002, Bjarnason et al. 2002, Ivaska et al. 2005). In addition, age, pubertal stage, growth velocity, hormonal regulation, nutritional status, sensitivity and specificity of the marker must be considered when interpreting the results of bone marker measurements as these may have a significantly effect on the results (Delmas et al. 2000, Szulc et al. 2000, Szulc & Delmas 2001). Overall, these factors explain the limited precision of biochemical markers and may limit their clinical use unless least significant changes and monitoring time intervals are carefully considered (Delmas et al. 2000). The list of the main current markers of bone formation, resorption and turnover is given in Table 1.
### Table 1. Currently available bone biochemical marker groups.

<table>
<thead>
<tr>
<th>Formation markers</th>
<th>Turnover markers</th>
<th>Resorption markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatases</td>
<td>Osteocalcin</td>
<td>Hydroxylysines</td>
</tr>
<tr>
<td>Type I collagen propeptides</td>
<td></td>
<td>Hydroxyprolines</td>
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<tr>
<td></td>
<td></td>
<td>Pyridolines</td>
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<td></td>
<td></td>
<td>Deoxypyridolines</td>
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<tr>
<td></td>
<td></td>
<td>Type I collagen telopeptides</td>
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<td></td>
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<td>Bone sialoproteins</td>
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<td></td>
<td></td>
<td>Acid phosphatases</td>
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</tbody>
</table>

### 2.6 Measurement of osteogenic loading

Properties of mechanical loading have been considered a crucial determinant of osteogenic effects. Measurement of these properties would be essential in order to find out the optimal loading patterns in clinical use to enhance osteogenic effects (Snow 1996, Kohrt et al. 2004). External loads create forces focusing on bone that lead to deformation in shape and size of the bone known as strain. This can also be described by a mathematical equation: strain $\varepsilon = \sigma/E$, in which $\sigma$ is the applied stress and $E$ is the elastic modulus. Strain can be directly measured from the bone or estimated with indirect surrogates of loading. Even though there are several methods to measure bone loading, all of them have several limitations.

#### 2.6.1 Bone strain measurements

Direct measurement of bone strains can be performed by bonded or stapled strain gauges implanted into bone. However, these direct measurements are complicated by the relatively invasive surgical technique required for gauge application, not applicable in most clinical settings (Hoshaw et al. 1997). Due to the invasive nature of measurement, most of the measurements have been conducted in laboratory animals, with only few studies in humans with extremely short duration and minimal sample sizes. In human subjects strain has been measured directly from the tibia (Lanyon et al. 1975, Milgrom et al. 1996, Burr et al. 1996, Ekenman et al. 1998, Milgrom et al. 2000a, Milgrom et al. 2000b, Milgrom et al. 2001, Milgrom et al. 2006), upper half of the femur (Bassey et al. 1997), radius (Fodhazy et al. 2005) and metatarsals (Arndt et al. 2002, Milgrom et al. 2002, Arndt et al. 2003).
In these studies, loading properties of single exercise patterns have been tested and compared. High-impact exercises, such as drop jumps and zig-zag jumps, have been found to result in peak compression strains of approximately 1500-2000 microstrain (µε) and shear strains of 4000-5000 µε in female tibia, the corresponding strain rates being 5000-10000 and 15000-30000 µε/s, respectively (Milgrom et al. 2000a, Milgrom et al. 2001). These principal strain magnitudes are about three times higher than strains during normal walking. In distal radius, push-ups have been found to create strains of up to 3000 µε (Mikic & Carter 1995, Fodhazy et al. 2005). However, there are no clinical studies that would have measured osteogenic effects of actual strains, and the results of osteogenic thresholds are thus mostly based on experimental data. The interpretation of strain data is also difficult as the strain magnitude varies greatly between different areas within the same bone (Ekenman et al. 1998). When one side of the bone is under compressive strain, the opposite side is dominated by tension strain (Einhorn 1996). Even though strain measurements produce the most accurate data on loading conditions, the measurement of single exercise patterns is not sufficient to determine optimal osteogenic loading (Mikic & Carter 1995). A more complete record of strain history is required to relate bone biology and morphology to strain. Such records should include the many diverse normal activities of subjects including normal physical activity (Mikic & Carter 1995).

2.6.2 Ground reaction force measurements

The most often used indirect surrogate of loading is ground reaction forces. This is based on Newton's 3rd Law of Motion (Law of Reciprocal Actions): All forces occur in pairs, and these two forces are equal in magnitude and opposite in direction. Due to gravity, we constantly maintain contact with the ground, and in this process, interactions occur between the body and the ground. The reaction force supplied by the ground is called the ground reaction force (GRF). GRF is normally measured by a force plate. During normal locomotion, GRF is generated with each foot strike and during jumping with every take-off and landing. Average ground reaction forces have been 1 to 1.5 times body weight (BW) during walking, 2 to 4 times BW during running and 1.5 to 2.0 times BW during aerobic stepping (Keller et al. 1996, Cole et al. 1996, Bassey et al. 1997, Santos-Rocha et al. 2006). However, significantly higher forces have been observed in athletes during performance. The peak GRF in triple jump has been measured as being up to 22 times BW (Perttunen et al. 2000). Gymnasts have also been found to have
GRF nearly 12 times BW during the landing from horizontal bars (Özguven & Berme 1988, McNitt-Gray 1993). There are also some bone-targeted exercise interventions that have estimated loading with force plate. In prepubescent children, 100 drop landings creating peak vertical GRF 8.5 times BW three times per week for 7 months was found to be osteogenic (Bauer et al. 2001, Fuchs et al. 2001a). In premenopausal women, 10 daily jumps 3 times per week enhanced BMD in weight-bearing bones with GRFs up to 4.8 times BW (Kato et al. 2006). In addition, 18 months of exercise with GRF varying between 2.1 and 5.6 times BW (Heinonen et al. 1996) and a five-month training period with 50 vertical jumps on 6 days per week with GRF 3 times BW (Bassey et al. 1998) enhanced BMD in weight-bearing bone sites. However, a similar training regimen with 50 vertical jumps on 6 days per week with GRF 4 times BW (Bassey et al. 1998) was not efficient in improving BMD in postmenopausal women, while an exercise regimen including GRF between 1.8 and 3.5 times BW maintained, but did not improve lumbar spine and hip BMD in early postmenopausal women with osteopenia (Kemmler et al. 2004a, Kemmler et al. 2004b).

Ground reaction forces measured with force plate are associated with forces measured directly in proximal femur prosthetic implants during jumping patterns, even though the implant forces were about twice the magnitude of GRF (Bassey et al. 1997). In addition, during the jumping forces that create vertical GRF 3.5 times BW have been found to produce strains of approximately 2000 με in tibial bone (Ekenman et al. 1998). The difference between GRF and directly measured bone deformations is partly explained by muscle forces that have also been found to associate with GRF (Bassey et al. 1997). This elucidates the importance of muscle forces as a determinant of osteogenic loading and pinpoints the limitations of indirect surrogates in the measurement of bone loading. Force plate measurements are also limited to a fixed place and time and can be used to measure only single jumps or parts of regimens; they do not provide information on complete loading history during trials.

### 2.6.3 Acceleration measurements

The basic principle of measuring accelerations to determine and categorise movements has been recognised for over a century, as quoted by Lanyon in 1971 (Lanyon 1971). The basic mechanism of measurement of acceleration is similar to a spring mass system where the accelerometer responds to applied accelerations by applying force to a spring leading to stretch or compression. This
displacement of the spring can be measured and used to calculate the applied acceleration (Mathie et al. 2004). In modern applications, this method is most often implemented through piezoelectric or capacitive acceleration sensors.

Currently, accelerometers have mostly been used to assess or classify energy expenditure, physical activity, balance and postural sway, gait, falls, or free-living movements (Mathie et al. 2004). Depending on the purpose of the study, accelerometers have been attached to the part of the body whose movement is under investigation, and some studies have even used several accelerometers to investigate whole body movements. However, the most common place for the accelerometer has been within the pelvis near to the centre of mass (Mathie et al. 2004). The type of the accelerometer has also varied, being either uniaxial recording of single direction forces or triaxial acting along three orthogonal axes providing a three dimensional picture of accelerations (Mathie et al. 2004). Trost et al. (Trost et al. 2005) have recently reviewed commercially available accelerometers. The accelerometer-based measurements have been shown to be reasonably reproducible, as the precision error of the devices typically ranges from 1% to 5% (Trost et al. 2005). There is a wide variety of methods to analyse accelerometer data, including root-mean-square average, power spectrum integral and acceleration count that have been used as measures of activity (Servais et al. 1984, Janz 1994, Eston et al. 1998, Hendelman et al. 2000, Auvinet et al. 2002, Menz et al. 2003, Janz et al. 2004, Kavanagh et al. 2004, Crouter et al. 2006). These physical activity assessments have also been used to evaluate the significance of physical activity levels to enhance bone augmentation (Janz et al. 2001, Janz et al. 2004, Janz et al. 2006). Also very recently, the daily number of steps and the intensity of physical activity measured as metabolic equivalents by accelerometer have been found to be determinants of fracture risk among elderly Japanese (Park et al. 2007). Nevertheless, the major limitation of the analysis methods used in the previous studies is that they are not able to measure precise surrogates of mechanical loads as they use data reduction methods to assess physical activity (Janz et al. 2006).

However, measurement of acceleration provides a surrogate for mechanical loading that can also be mathematically defined with a few simplifying assumptions. For a rigid body undergoing an impact, strain $\varepsilon = \sigma/E$, in which $\sigma$ is the applied stress and $E$ is the elastic modulus. Furthermore, $\sigma = F/A$, i.e. the stress is the force $F$ applied to the cross-sectional area $A$. Finally, the applied force $F$ is the effective mass $m$ multiplied by its acceleration ($F = ma$). Thus,
\[ \varepsilon = \frac{ma}{AE} \]

which supports the association between acceleration and strain at a constant effective mass (Heikkinen et al. 2007). It can therefore be supposed that high peak accelerations are associated with high peak strains. Accelerations during exercise have also been shown to be related to impact load forces (Servais et al. 1984, Janz et al. 2003). However, there have not been any long-term studies measuring accelerations as a surrogate of bone loading.

### 2.7 Effect of impact exercise on bone

In locomotion during activities of daily living, bones are constantly under pressure of a certain amount of load depending on individual lifestyle. These loads are the basal loading level that bones are adapted to resist (Turner 1998, Currey 2003). Exercise has a particular ability to generate loads above basal level in weight-bearing bones. It has thus been suggested to be an important preventive strategy against osteoporosis as it may enhance structural rigidity of bones by providing an osteogenic stimulus. Although all exercise commonly involves some form of loading through joint reaction forces, the frequency, intensity, time and especially type of exercise has been found to have crucial effects on outcome. However, there are numerous studies with different exercise characteristics in a variety of populations, but no optimal exercise regimen has been defined to date.

#### 2.7.1 Bone mineral density

Bone response to exercise has also been shown to be significantly affected by the starting age of exercise (Kannus et al. 1995). It has been suggested that the optimal time to enhance skeletal integrity by maximising the peak bone mass would be during the early pubertal period (MacKelvie et al. 2002). Exercise interventions in children have shown a greater bone mineral change in exercising children over relatively short periods compared with respective controls (Morris et al. 1997, Bradney et al. 1998, McKay et al. 2000, Heinonen et al. 2000, MacKelvie et al. 2001, Fuchs et al. 2001b, McKay et al. 2005). The increases in exercise intervention studies have been found to range from 1.4 to 6.2% in femoral neck BMD and from 0.9 to 5.5% in lumbar spine BMD when adjusted over a 6-month period (Hind & Burrows 2007). Loading quantified as GRFs in
these studies was found to range between 2 to 9 times BW (Hind & Burrows 2007). There were indications that higher GRFs may result in greater osteogenic responses, even though bone mass was significantly enhanced by moderate impact exercise as well (Hind & Burrows 2007).

Bone strength can also be significantly enhanced during adulthood. Women athletes have been found to have approximately 10% higher bone mineral density in weight-bearing bones compared with normal population (Forwood & Burr 1993, Chilibeck et al. 1995, Marcus et al. 1999). In many cases, however, athletes have continued training throughout life, and cross-sectional studies may also include some selection bias. Higher quality information is thus provided by intervention studies, best of all by randomised controlled trials. Randomised controlled intervention studies have shown osteogenic effects in site-specific weight-bearing bones (Table 2). Meta-analyses have indicated that approximately 1-2% of annual bone loss can be prevented with exercise in pre- and postmenopausal women (Chilibeck et al. 1995, Wolff et al. 1999, Marcus et al. 1999, Wallace & Cumming 2000, Petit et al. 2002), even though individual exercise interventions have indicated significantly higher benefits of up to 6% depending on the measured site. BMD responses have been found to be greatest toward the periphery of lower limb. This is consistent with the theory that bone responds most to greatest mechanical loading, as impact loading has been found to attenuate progressively in ankle, knee and hip joints (Bergmann et al. 2001, Bauer et al. 2001). Resistance or weight training interventions have provided inconsistent findings (Gleeson et al. 1990, Rockwell et al. 1990, Lohman et al. 1995, Sinaki et al. 2004, Kelley & Kelley 2004), while results from high-impact exercise interventions have been more consistent, possibly indicating higher osteogenic potential (Bassey & Ramsdale 1994, Friedlander et al. 1995, Heinonen et al. 1996, Bassey et al. 1998, Shibata et al. 2003a, Kato et al. 2006) (Table 2). This is also supported by recent findings from athletes, suggesting that loading modalities with high impacts or impacts from atypical loading directions are most efficient (Nikander et al. 2005). More promisingly, high-impact exercise, even with low repetitions, is efficient, as Kato et al. reported that 10 maximal vertical jumps three times per week increased femoral neck BMD by 3.8% and lumbar spine BMD by 1.8% during 6 months of training in premenopausal women (Kato et al. 2006).

Changes in the skeleton are inevitable during ageing. An old and more fragile skeleton cannot endure as much as a younger one and its abilities to adapt are reduced. Older people tend to show only minor gains in bone mass in response to
exercise interventions intended to build bone mass or slow down its loss (Karlsson 2004). Low-impact exercise, such as non-strenuous calisthenics or walking, seems to produce no benefits, and only higher impact or more strenuous activities appear to be effective in slowing down the rate of bone loss in older adults (Kerr et al. 1996, Heinonen et al. 1998, Uusi-Rasi et al. 1999, Korpelainen et al. 2006b). Several meta-analyses, including the Cochrane collaboration report, have reported variable and inconsistent results, most probably due to different search and selection criteria (Bérard et al. 1997, Kelley 1998a, Kelley 1998b, Wolff et al. 1999, Wallace & Cumming 2000, Bonaiuti et al. 2002). Nevertheless, several studies suggest that loading such as jumping, strength-training exercises, or a combination of these can slightly increase or conserve hip bone mass in postmenopausal or elderly women (Kohrt et al. 1995, Bassey & Ramsdale 1995, Going et al. 2003, Kemmler et al. 2004a, Kemmler et al. 2005, Engelke et al. 2006, Korpelainen et al. 2006b). Prevention methods focusing on other risk factors of osteoporotic fractures, such as prevention of falls, may thus be more recommendable in elderly people (Kannus et al. 2005).

2.7.2 Bone geometry

In addition to bone mineral accrual, also structural changes have been observed. During childhood, exercise may enhance bone strength by altering the bone geometry. Exercise-induced increases found in cortices as the periosteal enlargement have provided wider bone dimensions and increased cortical thickness as well as enhanced section modulus and bone strength indices at the loaded bone sites (Bradney et al. 1998, Haapasalo et al. 2000, Heinonen et al. 2000, Petit et al. 2002, Kontulainen et al. 2002, McKay et al. 2005, Macdonald et al. 2007). It has been suggested that during the growth period, loading produces actual structural changes through periosteal expansion, while additional bone acquired after skeletal maturity is deposited along the existing structures (Forwood & Burr 1993, Kannus et al. 1995, Haapasalo et al. 2000). During childhood most of the BMD changes are due to increased bone size, not actual increment of volumetric BMD (Haapasalo et al. 2000, Seeman 2001).
Table 2. Randomised controlled trials of exercise interventions in premenopausal women.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Sample size (n)*</th>
<th>Age (year)</th>
<th>Time (months)</th>
<th>Exercise</th>
<th>% change in BMD (adjusted per year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Snow-Harter et al. 1992)</td>
<td>W: 12 R: 10 C: 8 (total 52)</td>
<td>20 8</td>
<td>Weight lifting: 3 sets of 14 exercises at 65-85% of 1-RM</td>
<td>3 times/wk. Running: 4-10 miles at 70-80% HRmax, ≥3 times/wk.</td>
<td>LS W: 1.8 R: 2.0 C: -1.2 FN W: 0.6 R: 2.1 C: -0.8</td>
</tr>
<tr>
<td>(Bassey &amp; Ramsdale 1994)</td>
<td>E: 14 C: 13 (total NA)</td>
<td>32 6</td>
<td>High-impact exercise:</td>
<td>1h/week +50 jumps/day at home (GRF &gt;2 BW).</td>
<td>LS E: 1.0 C: 1.5 FN E: 2.4 C: -1.8</td>
</tr>
<tr>
<td>(Friedlander et al. 1995)</td>
<td>E: 32 C: 31 (total 127)</td>
<td>28 24</td>
<td>High-impact aerobics 1h at 70-85% HRmax + weight training 3 times/wk.</td>
<td></td>
<td>LS E: 0.7 C: 0.1 FN E: 0.3 C: -1.0</td>
</tr>
<tr>
<td>(Lohman et al. 1995)</td>
<td>E: 22/59 C: 34/47 (total 127)</td>
<td>34 18</td>
<td>Strength training: 1hr, 3 times/wk, 12 exercises at 70-80% of 1-RM.</td>
<td></td>
<td>LS E: 1.0 C: -0.2 FN E: -0.6 C: -0.3</td>
</tr>
<tr>
<td>(Heinonen et al. 1996)</td>
<td>E: 45/49 C: 39/49 (total 127)</td>
<td>39 18</td>
<td>Progressive High-impact exercises: aerobics or steps 3 times/wk, 60 min (GRF 5.6 BW).</td>
<td></td>
<td>LS E: 1.5 C: 0.5 FN E: 1.1 C: 0.4</td>
</tr>
<tr>
<td>(Sinaki et al. 1996)</td>
<td>E: 37/60 C: 42/60 (total 127)</td>
<td>36 36</td>
<td>Non-strenuous weight lifting exercise 30 minutes 3 times per week (1 supervised).</td>
<td></td>
<td>LS E: 0.2 C: 0.1 FN E: 0.1 C: 0.0</td>
</tr>
<tr>
<td>(Bassey et al. 1998)</td>
<td>E: 30 C: 25 (total 61)</td>
<td>38 6</td>
<td>50 vertical jumps on 6 days/wk of mean height 8.5 cm (GRF 3 BW).</td>
<td></td>
<td>LS E: 2.1 C: 2.1 FN E: 4.1 C: 0.8</td>
</tr>
<tr>
<td>(Shibata et al. 2003a)</td>
<td>E: 11/15 C: 17/26 (total 61)</td>
<td>39 35</td>
<td>E: 10 maximum vertical jumps at home. All: 10,000 walking steps.</td>
<td></td>
<td>LS E: 2.0 C: 0.0 FN E: -1.0 C: -1.0</td>
</tr>
<tr>
<td>(Kato et al. 2006)</td>
<td>E: 18/21 C: 18/21 (total 61)</td>
<td>21 21</td>
<td>10 maximum vertical jumps 3 times/wk (GRF 4.8 BW).</td>
<td></td>
<td>LS E: 4.8 C: 1.2 FN E: 5.3 C: -2.3</td>
</tr>
</tbody>
</table>

* Sample size is defined as completed/participated subjects per group if applicable.
E= exercise group, C= control group, W= weight-lifting group, R= running group, LS= lumbar spine, FN= femoral neck, BMD= bone mineral density, 1-RM= one repetition maximum, HRmax= age predicted heart rate maximum, GRF= ground reaction force, BW= bodyweight, NA= not applicable.
Site-specific geometrical adaptation has also been observed in adult athletes, particularly in sports with high-impact loading. Triple jumpers have been found to have thicker and wider bone cortices resulting in weight-bearing bones that are 20-30% stronger compared to controls (Heinonen et al. 2001). Similar bone geometry changes resulting in more favourable bone geometry have also been found in racquet sport players when comparing their playing arm to the non-dominant arm (Ashizawa et al. 1999, Kontulainen et al. 2002). The ability of bone to enhance its structure has been found to persist to some extent, as Adami et al. (Adami et al. 1999) found that upper extremity strength training was beneficial for ultradistal radius bone geometry by redistributing the bone even though it did not increase BMD in postmenopausal women. These geometrical changes may have clinical relevance as they may offset bone fragility caused by age and menopausal-related endocortical bone resorption.

### 2.7.3 Bone turnover

Structural and geometrical adaptations in bone require activation of the remodelling cycle. Intense exercise has been found to have acute effects on bone turnover and calcium homeostasis, even though the results have been quite inconsistent. A single bout of strength training has been found to decrease both formation and resorption (Ashizawa et al. 1998) while endurance training has been found to increase bone turnover (Wallace et al. 2000, Maimoun et al. 2006), stimulate bone resorption (Welsh et al. 1997), or have no effect on bone turnover (Maimoun et al. 2005). Most of the previous reports concerning exercise effects on long-term bone turnover are from cross-sectional studies on athletes. The results are as inconsistent as were the results from acute bone turnover responses. Some studies have shown increased turnover (Karlsson et al. 2003), some have indicated increased formation (Creighton et al. 2001) and others have reported increased resorption (O’Kane et al. 2006).

There are only a few controlled prospective trials on the long-term effect of impact exercise on bone metabolism. Shibata et al. (Shibata et al. 2003b) found a significant increase in bone alkaline phosphatase level indicating increased bone formation in Japanese premenopausal women after six months of impact training. There was no change in BMD at any measurement site or in the level of osteocalcin (OC), PTH or in type I collagen cross-linked N-telopeptides (NTx). Kemmler et al. (Kemmler et al. 2004a) found no significant long-term changes in specific bone formation or resorption markers after a complex resistance and
impact training period in early postmenopausal women. However, 8 weeks of aerobic training have been reported to lead to reduced bone resorption activity, whereas anaerobic training has been reported to accelerate bone turnover (Woitge et al. 1998a). There are probably several reasons behind these confusing results. First, the exercises used have had varying types, intensities and durations. Furthermore, the subjects have not been uniform as to age, sex and activity background, and measurement protocols and times have varied as well. However, exercise has great potential to affect bone turnover as it has been found to alter calcitropic hormones as well (Zittermann et al. 2002, Kemmler et al. 2003, Maimoun et al. 2005, Maimoun et al. 2006).

2.8 Prevention of diseases with exercise

Regular physical activity has long been regarded as an important component of a healthy lifestyle. Despite this evidence and the apparent public acceptance of the importance of physical activity, deaths associated with a sedentary lifestyle are increasing (Mokdad et al. 2004). Epidemiologic research has demonstrated a protective correlation of varying strength between physical activity and risk for several chronic diseases, including coronary heart disease, hypertension, non-insulin-dependent diabetes mellitus, osteoporosis, colon cancer, anxiety and depression (Fentem 1994, Pate et al. 1995). Low level of physical activity is associated with markedly increased all-cause mortality rates (Paffenbarger, Jr. et al. 1986, Blair et al. 1989) and a midlife increase in physical activity is associated with a decreased risk of mortality. It has been estimated that 17% of the total deaths per year in the United States are attributable to a lack of regular physical activity or poor diet (Mokdad et al. 2004). It is thus crucial to promote physical activity. As cardiovascular diseases (CVD) are the leading cause of death in women (Peden et al. 2002), they were chosen as a secondary target of this trial and are reviewed here briefly along with osteoporosis

2.8.1 Osteoporosis and fragility fractures

Osteoporosis is a complex disorder of bone tissue with multifactorial origin characterised by compromised bone strength, predisposing to an increased risk of fracture (NIH 2000). Exercise, as reviewed above, has beneficial effects on bone strength, but the evidence showing that it directly prevents fractures is sparse (Karlsson 2004). In large prospective studies of older women, moderate or high
levels of leisure time physical activity have been associated with an overall 28-36 percent reduction in hip fractures (Cummings et al. 1995, Gregg et al. 1998, Hoidrup et al. 2001). Exercise for more than 2 hours per day was shown to reduce hip fracture risk by 53%, and less than 2 hours of activity per day reduced the risk by 25% as compared to sedentary individuals in the baseline measurements of a prospective cohort study (Gregg et al. 1998). Moreover, walking at least 4 hours per week was associated with 41% lower risk of hip fracture compared with walking less than an hour per week (Feskanich et al. 2002). There is still lack of randomised prospective trials with fractures as an end-point variable, even though there is a recent study that reported a significantly decreased number of fall-related fractures in postmenopausal women with osteopenia during 30 months of impact, balancing and strengthening exercise (Korpelainen et al. 2006a).

The potential of exercise in the prevention of osteoporosis and fragility fractures is based on its ability to affect both major risk factors of fractures: decreased bone strength and falls (Kohrt et al. 2004). However, we still do not know the actual dose-response relationship of the osteogenic effects of exercise (Karlsson 2004, Kohrt et al. 2004). Another big question is the cessation of exercise. We have some data on the persistence of acquired bone benefits (Kontulainen et al. 2001), but data against persistence of benefits exist as well (Winters & Snow 2000). Altogether, physical activity and exercise alone probably is not efficient enough in preventing osteoporosis and fractures. In addition to exercise, a number of other preventive strategies need to be considered to achieve clinically significant results. These strategies include, but are not limited to, adequate nutrition, carefully considered medication, reduction of caffeine intake, cessation of smoking, treating impaired vision, and minimising environmental hazards (Cummings et al. 1995). All in all, in terms of prevention of fractures, prevention of falls is essential (Kannus et al. 2005).

2.8.2 Cardiovascular diseases

Cardiovascular diseases (CVD) are an interesting object for bone-targeted training regimens as there have been suggestions that osteoporosis and CVD may share some aetiologic factors (Whitney et al. 2004). CVDs are the leading cause of death in women (Peden et al. 2002). The worldwide number of cardiovascular deaths is expected to be nearly doubled from 13.1 million in 1990 to 24.8 million in 2020 (Poulter 2003). Lipid and lipoprotein levels, blood pressure, cardiorespiratory fitness, obesity, diabetes and smoking are the main modifiable
risk factors of cardiovascular disease (Poulter 2003). A sedentary lifestyle is also an independent risk factor for cardiovascular, whereas a high amount of habitual physical activity can reduce the risks (Paffenbarger, Jr. et al. 1986, Blair et al. 1989). Physical activity is likely to be protective through a combination of effects on other recognised risk factors, on metabolic and regulatory processes, on the profile of cholesterol and blood lipid concentrations and clotting factors, possibly on arterial blood pressure, cardiorespiratory fitness, endothelial function, glucose metabolism, visceral fat and through its role in weight reduction (Fentem 1994, Kemi et al. 2004).

Stepping and jumping exercises that have been found to be osteogenic may have an additional positive effect on cardiorespiratory fitness and serum lipid levels as well (Olson et al. 1991, Uusi-Rasi et al. 2003, Kemmler et al. 2004a). Especially high-intensity exercise may be beneficial in reducing the risks of CVD (Kraus et al. 2002). If high-intensity osteogenic exercise reduces the risks of CVD, it will provide an excellent opportunity to “kill two birds with one stone”. This is important as it is unlikely that people will do several different kinds of exercises to prevent different diseases (Marcus 1998)
3 Purpose of the study

The primary objective of the present study was to investigate the effect of exercise-induced impact loading on bone mineral density (BMD), geometry and metabolism in healthy premenopausal women with the intention of assessing the intensity and amount of impact loading required to gain adaptive effects. The secondary objective was to evaluate the effect of a bone-targeted exercise training programme on extra-skeletal risk factors of osteoporotic fractures, physical performance and main risk factors of cardiovascular diseases. The specific aims were:

1. To evaluate the effects of a 12-month supervised high-impact exercise on BMD (I), bone geometry (III) and bone metabolism (IV) in healthy 35- to 40-year-old women.
2. To study the effects of bone-targeted impact exercise training on muscle strength, balance, cardiorespiratory fitness and serum lipid profiles in healthy 35- to 40-year-old women (V).
3. To study whether the intensity and amount of exercise-induced impact loading is associated with 12-month changes in BMD (II), bone geometry (III), bone metabolism (IV), and physical performance and serum lipid profiles (V).
4 Subjects and methods

This study was carried out during the years 2001-2006 at the Department of Medical Technology and the Department of Physiology, University of Oulu, and the Department of Sports Medicine, Oulu Deaconess Institute. The approval of the local Ethics Committee and written informed consent from all subjects was obtained before the study. The principles of the Declaration of Helsinki were followed.

4.1 Subjects

The study design was a population-based randomised controlled exercise intervention trial (Figure 6). All data within this thesis were derived from this exercise intervention. The study population consisted of a random sample of Finnish women from a cohort of 5,161 women aged 35 to 40 years residing in the city of Oulu, Finland, in March 2002. The names, addresses and date of birth of the women in the cohort were obtained from the National Population Register of Finland. To detect a 3% (with expected 5.4% standard deviation) difference between the exercise and control groups in BMD, with 5% significance level and power of 80%, 120 participants were needed with an equal dropout rate of ten subjects per group. The participants were contacted in random order, and to get 120 participants, 287 women were contacted. Of these, 125 women were unwilling to participate, and 42 women were excluded. The exclusion criteria were cardiovascular, musculoskeletal, respiratory, or other chronic diseases that might limit training and testing; diseases or medication affecting the bone; pregnancy and breastfeeding; and regular current or previous participation in impact-type exercises and long-distance running more than three times a week. The subjects were randomly assigned to an exercise group (n=60) or a non-exercise control group (n=60) using computer-generated random numbers.

4.2 Measurements

The measurements and the variables reported in the original studies are listed in Table 3. The precision of measurements is reported as the coefficient of variation when applicable. More detailed information of the measurement protocols is given in the original reports.
Fig. 6. Study protocol.
Table 3. Variables measured in the original studies and the methods used.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Studies</th>
<th>Methods/references</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>I-V</td>
<td>Standard wall mounted tape measure</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>I-V</td>
<td>Mechanical scale (Seca, Vogel&amp;Halke)</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>I-V</td>
<td>mass (kg) / height (m)</td>
<td></td>
</tr>
<tr>
<td>Body fat %</td>
<td>I-V</td>
<td>Bio-impedance measurement, Bodystat</td>
<td></td>
</tr>
<tr>
<td>Lean mass %</td>
<td>I-V</td>
<td>1500 (Bodystat Ltd.) (Ghosh et al. 1997)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>IV</td>
<td>Standard tape measure</td>
<td></td>
</tr>
<tr>
<td>Hip circumference</td>
<td>IV</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td><strong>DXA bone measurements (BMD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine total</td>
<td>I, II</td>
<td>Hologic Delphi QDR (Hologic, Inc.)</td>
<td></td>
</tr>
<tr>
<td>L1, L2, L3, L4</td>
<td>I, II</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Hip total</td>
<td>I, II</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>I, II</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Trochanter</td>
<td>I, II</td>
<td>*</td>
<td></td>
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<tr>
<td>Ward’s triangle</td>
<td>I, II</td>
<td>*</td>
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<td>Radius</td>
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<td>Osteometer DTX 200</td>
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<tr>
<td>Ulna</td>
<td>I</td>
<td>(Osteometer Meditech)</td>
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<tr>
<td>Distal radius</td>
<td>I, II</td>
<td>*</td>
<td></td>
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<tr>
<td>Ultradistal radius</td>
<td>I, II</td>
<td>*</td>
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<tr>
<td><strong>Calcaneal QUS measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broadband ultrasound attenuation</td>
<td>I, II</td>
<td>Hologic Sahara (Hologic, Inc.)</td>
<td></td>
</tr>
<tr>
<td>Sound of speed</td>
<td>I, II</td>
<td>*</td>
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<tr>
<td><strong>QCT mid-femur measurements</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bone circumference</td>
<td>III</td>
<td>Siemens Somatom Emotion (Siemens GmbH) (BonAlyse Oy)</td>
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<tr>
<td>Cortical CSA</td>
<td>III</td>
<td>with GEANIE 2.1 software</td>
<td>0.3</td>
</tr>
<tr>
<td>Cortical attenuation</td>
<td>III</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Cortical wall thickness</td>
<td>III</td>
<td>(Jämsä et al. 2004)</td>
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<tr>
<td>Maximum CSMI</td>
<td>III</td>
<td>*</td>
<td>0.3</td>
</tr>
<tr>
<td>Minimum CSMI</td>
<td>III</td>
<td>*</td>
<td>0.2</td>
</tr>
<tr>
<td>Muscle CSA</td>
<td>III, IV</td>
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<tr>
<td>Fat tissue CSA</td>
<td>III, IV</td>
<td>*</td>
<td>0.7</td>
</tr>
</tbody>
</table>

CV=Coefficient of variation, for the QCT measurements as root-mean-square coefficient of variation of duplicate measurements and for the laboratory measurements as intra-assay/interassay coefficient of variation. DXA=dual energy x-ray absorptiometer, BMD=bone mineral density, QUS=quantitative ultrasound, QCT=quantitative computer tomography, CSA=cross sectional area, CSMI= cross sectional moment of inertia.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Studies</th>
<th>Methods/references</th>
<th>CV (%)</th>
</tr>
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<tr>
<td><strong>QCT proximal tibia measurements</strong></td>
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<tr>
<td>Bone circumference</td>
<td>III</td>
<td>Siemens Somatom Emotion (Siemens GmbH) with GEANIE 2.1 software</td>
<td>0.5</td>
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<tr>
<td>Cortical CSA</td>
<td>III</td>
<td>(BonAlyse Oy)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cortical attenuation</td>
<td>III</td>
<td>(Jämsä et al. 2004)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cortical wall thickness</td>
<td>III</td>
<td>&quot;</td>
<td>0.5</td>
</tr>
<tr>
<td>Maximum CSMI</td>
<td>III</td>
<td>&quot;</td>
<td>1.5</td>
</tr>
<tr>
<td>Minimum CSMI</td>
<td>III</td>
<td>&quot;</td>
<td>0.9</td>
</tr>
<tr>
<td>Muscle CSA</td>
<td>III, IV</td>
<td>&quot;</td>
<td>0.8</td>
</tr>
<tr>
<td>Fat tissue CSA</td>
<td>III, IV</td>
<td>&quot;</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>QCT distal tibia measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular attenuation</td>
<td>III</td>
<td>(Jämsä et al. 2004)</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Physical performance measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal oxygen uptake</td>
<td>IV</td>
<td>Medikro 919 (Medikro Oy) (Oja 1973)</td>
<td></td>
</tr>
<tr>
<td>Maximal isometric leg force</td>
<td>IV</td>
<td>Strain-gauge dynamometer (Digitest Oy)</td>
<td></td>
</tr>
<tr>
<td>Maximal isometric grip strength</td>
<td>IV</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Explosive leg strength: static jump</td>
<td>IV</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Explosive leg strength: CM jump</td>
<td>IV</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td><strong>Lipid and glucose metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>IV</td>
<td>Konelab (Thermo Electron Oy)</td>
<td>0.9</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>IV</td>
<td>&quot;</td>
<td>0.9</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>IV</td>
<td>&quot;</td>
<td>1.0</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>IV</td>
<td>(Friedewald et al. 1972)</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>IV</td>
<td>Konelab (Thermo Electron Oy)</td>
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<tr>
<td>Insulin</td>
<td>IV</td>
<td>Enzyme-linked immunosorbent assay</td>
<td>&lt;7.5</td>
</tr>
<tr>
<td><strong>Bone metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PINP</td>
<td>V</td>
<td>Radioimmunoassay (Orion Diagnostica) (Melkko et al. 1996)</td>
<td>4.1 / 4.8</td>
</tr>
<tr>
<td>TRACP5b</td>
<td>V</td>
<td>Immunoassay (Halleen et al. 2000)</td>
<td>6.8 / 8.5</td>
</tr>
<tr>
<td>Uncoupling Index</td>
<td>V</td>
<td>Calculated as $Z_{PINP} - Z_{TRACP5b}$ (Eastell et al. 1993)</td>
<td></td>
</tr>
<tr>
<td>Intact parathyroid hormone</td>
<td>V</td>
<td>Enzyme-linked immunosorbent assay</td>
<td>4.9 / 4.5</td>
</tr>
<tr>
<td>25OHD</td>
<td>V</td>
<td>Radioimmunoassay</td>
<td>4.7 / 5.3</td>
</tr>
</tbody>
</table>

CSA = cross sectional area, CSMI = cross sectional moment of inertia, CM = countermovement, HDL = high-density lipoprotein, LDL = low-density lipoprotein, PINP = procollagen type I aminoterminal propeptide, TRACP5b = tartrate-resistant acid phosphatase 5b, 25OHD = serum 25-hydroxyvitamin D, Z = Z-score
4.2.1 Questionnaires

A self-administered health questionnaire was mailed to all women contacted. Information was asked about weight history and height, occupational activity history (Jaglal et al. 1995), current and past leisure time physical activity (Greendale et al. 1995) and medical factors, fractures beyond the age of 15, menarcheal age, menstrual status, parity, months of breast-feeding, current and previous use of hormones, current and previous dietary factors including intake of calcium and vitamin D (Tuppurainen et al. 1995), current and past smoking and consumption of alcohol, and possible vitamin or mineral supplementation.

4.2.2 Bone measurements

Assessment of bone status was performed with each item of equipment at the baseline and at 12 months by the same experienced operators unaware of the women’s trial status. Dual-energy X-ray absorptiometry (DXA) was used to measure areal bone mineral density (BMD) at the lumbar spine (L1–L4) and the left proximal femur (Figure 7). The scanner was calibrated daily by bone phantoms for quality assurance, and no evidence of machine drift appeared during this study. The CV of the measurement in the laboratory has previously been found to be 0.5% (Korpelainen et al. 2006b).

![Fig. 7. Measuring of proximal femur BMD and calcaneal QUS.](image)

Areal BMD was also measured from the upper extremities with peripheral DXA. The distal radius was defined as the 24-mm long section of bone immediately proximal to the reference line where the separation between radius and ulna is 8 mm. It consists of 87% cortical bone and 13% of trabecular bone. The ultradistal
measurement site was the area distal to the 8-mm reference line, and contains 45% cortical and 55% trabecular bone (Patel et al. 1998). Calcaneal speed of sound (SOS) and broadband ultrasound attenuation (BUA) were evaluated with quantitative ultrasound (QUS). The precision range of calcaneal QUS measurements has previously been found to be 0.1% to 5% (Fogelman & Blake 2000).

Bone structural characteristics of femur and tibia of both sides were assessed with a spiral QCT scanner and the mean of the sides was used in calculations (Figure 8). Three cross-sectional scans were made, one at mid-femur (50% of the estimated bone length from the distal endplate of the femur), one at proximal tibia (67% from the distal endplate of the tibia) and one at distal tibia (5%). The scan lines were adjusted using the scout view of the scanner software. Subject positioning was standardised and calibration quality assurance was performed daily according to the manufacturers’ recommendations.

Fig. 8. Measuring of lower limbs on QCT and measurement sites.

4.2.3 Laboratory assessments

Venous blood samples were collected using a standardised procedure from all the subjects at baseline, at 6 months and at 12 months. After an overnight fast and rest, a blood sample was drawn at 08:00–11:00 a.m. Serum was separated by centrifugation and stored at -70°C. All samples were analysed at the end of the study.

Bone formation was assessed by using serum intact aminoterminal propeptide of type I procollagen (Melkko et al. 1996). Type 5 tartrate-resistant acid phosphatase (TRACP5b) was measured as marker of bone resorption by using in-
house immunoassays (Halleen et al. 2000). Serum 25-hydroxyvitamin D and intact parathyroid hormone immunoassays were used to assess changes in calcitropic hormones. An Uncoupling Index (UI) was calculated to assess the relative balance of the formation and resorption processes of bone remodelling as suggested by Eastell et al. (Eastell et al. 1993). The UI was calculated as $UI = Z_{PINP} - Z_{TRACP5b}$, where $Z_{PINP}$ is the $Z$-score of bone formation and $Z_{TRACP5b}$ is the $Z$-score of bone resorption in this study. A positive UI indicates unbalanced bone remodelling in favour of formation, and negative UI indicates remodelling in favour of resorption.

Fasting values of serum insulin, glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured to define the effects of exercise on glucose metabolism and lipoprotein profiles. Low-density lipoprotein cholesterol (LDL-C) was calculated by using Friedewald’s equation (Friedewald et al. 1972).

### 4.2.4 Anthropometrics and physical performance measurements

Anthropometrics were measured during the physical performance testing at baseline and at 12 months. The weight of the subjects was measured with a standard mechanical scale, and body height was measured with a wall mounted tape measure. Body mass index was calculated. Percentage of fat and lean mass was assessed in supine position with bioimpedance equipment (Ghosh et al. 1997).

Maximal oxygen uptake ($VO_2\text{max}$) was determined directly using a step protocol consisting of 3 minutes of progressive uphill walking to a voluntary maximum on a treadmill. The test protocol was modified from that of Pennsylvania State University (Oja 1973). Maximum isometric leg extensor strength was assessed bilaterally with a computerised strain-gauge dynamometer in the seated position, the knee set at 108°. Grip strength was measured with a hand dynamometer. In all strength measurements, the best result of the three consecutive recordings was used in the final analysis. The explosive force of the leg extensor muscles was measured during a counter-movement (CM) jump and static jump on a contact mat connected to a digital timer ($\pm 0.001$ s).
4.2.5 Continuous measurement of impact loading

Impact loading was measured during the study with the indirect accelerometric method. Vertical acceleration peaks were recorded with a one-dimensional accelerometer-based human body movement monitor (Figure 9) (Newtest Ltd., Oulu, Finland). It was designed for long-term monitoring of physical activity, recording the histogram of daily number of impact peaks, classified according to peak acceleration, to describe the intensity of exercise.

![Figure 9. An accelerometer-based body movement monitor was attached tightly to the right iliac crest with a belt (Jämsä et al. 2006).](image)

For individual quantification of daily physical activity, all subjects in the training intervention study were asked to carry the monitor on their waist daily, during all waking hours, i.e. from morning to evening, for 12 months. The monitor gathered data at a sampling rate of 400 samples per second, filtered, pre-analysed and classified according to peak acceleration in 24-h periods. However, the time labels for each acceleration peak were not saved. The classified data were transferred onto a server computer approximately every second week. The number of daily impacts was analysed at 33 acceleration levels from 0.3 to 9.9 g as given by the activity monitor, 0 g corresponding to standing (acceleration of gravity subtracted, resulting in a 33-level histogram of impacts according to their peak acceleration value (Figure 10). The individual daily number of impacts at each acceleration level was calculated for the analysis.
The reliability of the accelerometer-based method was tested previously using a three-dimensional prototype (Vihriälä et al. 2003). The average peak amplitude error of the device was less than 2%. A preliminary study was performed with 10 women (age 20–58 years, BMI 19.1-29.7 kg/m²) to estimate typical vertical acceleration levels attained in different exercise patterns using the prototype (Hietala & Ylönen 2003). Peak values were predominantly obtained immediately after heel contact. The average peak accelerations in different exercise patterns are shown in Figure 11. The reproducibility error, given as the root-mean-square coefficient of variation (CVRMS), was 4.0%. The peak acceleration values had a high correlation (R = 0.989; n = 572 recordings) with the values obtained simultaneously using a standard optical motion analysis system (Vihriälä et al. 2004), showing that the method measures reliably local acceleration at the hip. The acceleration values also correlated significantly with the GRF (r = 0.735 for the peak acceleration values, r = 0.937 for the area under the acceleration peaks; n = 462 recordings), measured with a force plate (Kistler 9287A with a Kistler 9865C charge amplifier, Kistler Instrumente AG, Switzerland), when the acceleration values were multiplied by body weight.
4.3 Exercise training program

The training sessions were carried out three times a week for 12 months between May 2002 and May 2003. All training sessions were supervised by a physiotherapist and were done to the accompaniment of music. The training regimen was based upon a pilot study and previous literature. Each workout lasted 60 min, including a 10-min warm-up, a 40-min high-impact training session, and a 10-min cooling down and stretching period. The warm-up period included walking and running on the spot, with and without arm movements and knee bends. The high-impact period included step patterns, stamping, jumping, running, and walking. After 3 months of training, one-step bench (height 10 cm) (Reebok UK, Lancaster, UK) was used to enhance the impact effect and after 6 months, two or three benches were used (Figure 12). The cooldown mainly consisted of stretching. The programmes were modified bimonthly and the programme became gradually more demanding during the intervention, including higher jumps and drops. Additionally, the participants were asked to train daily at home for 10 min following a specially designed programme, which consisted of
patterns of exercise similar to those in the supervised sessions. The home programme was also modified seasonally. The women in the control group were asked to continue their normal daily life and to maintain their current physical activity during the 12 months.

4.4 Statistical methods

The null hypothesis of the data analysis was that the impact exercise training would not affect bone mineral density (I), bone geometry (III), bone metabolism (IV) and physical performance and risk factors of cardiovascular disease (V), and thus there would be no differences in outcome variables between the exercise and control group during the intervention and measured impact loading would not be associated with changes in outcome variables (II-V). The alternative hypothesis was that exercise would have an effect on outcome variables and the intensity and amount of measured impact loading would be associated with these beneficial changes.

The data were analysed using the SPSS statistical package (SPSS 11.5 or 12.0 for Windows, SPSS Inc. Chicago, Illinois, USA). The results are reported as mean and standard deviation (SD) or 95% confidence interval (95% CI). All participants, including the subjects who discontinued exercise, were invited for follow-up measurements. All subjects with both baseline and follow-up data were included in the analysis according to their original group assignment. Distributions of outcome variables were tested for normality. Independent samples t-test and chi-square tests were used to assess the differences between the study subjects and women who did not participate in the study.

The individual average number of daily accelerations was normalised by the mean values of the controls and analysed on five acceleration levels to describe exercise volumes at different loading levels: 0.3-1.0 g (e.g. walking), 1.1-2.4 g (e.g. stepping), 2.5-3.8 g (e.g. jogging), 3.9-5.3 g (e.g. running, jumping), and 5.4-9.2 g (e.g. jumping). Multiple stepwise regression analysis was used to quantify the association between the intensity of exercise and change in endpoint variables and to determine confounding factors used in subsequent analyses in studies II-V. The variables used in the stepwise regression analyses are listed in detail in the original studies. Correlation coefficients (Pearson’s) were used to study the association between the relative number of acceleration peaks at each level and the change in endpoint variables. Partial correlation was used to control for the influence of covariates on the correlation coefficients, if necessary in studies II-V.
Independent samples t-test (or Mann-Whitney’s U-test) and analysis of covariance (ANCOVA) was used to compare the groups with respect to changes from baseline in outcome variables between the study groups as well as the differences between the study subjects and drop-outs. Paired samples t-test (or Wilcoxon Signed-Rank test) and repeated measures ANCOVA was used to analyse percent change in outcome variables from baseline within the groups. Sub-group analyses were performed with the repeated measures ANCOVA in quartiles of the measured number of impacts at different loading levels within the pooled groups and in quartiles according to the number of exercise sessions attended within the exercise group. Quartiles were derived separately for each acceleration level. In all tests, $p<0.05$ was considered to be statistically significant.
5 Results

5.1 Effects of high-impact exercise training

All 120 (100%) subjects and 70 (42%) excluded or unwilling women returned the baseline questionnaire. The baseline characteristics of the non-participants did not differ significantly from the study group (Table 4). Thirty-nine women (65%) in the training group and 41 women (68%) in the control group completed the study, representing a dropout frequency of 33.3%. The reasons for withdrawal are presented in Figure 6. For women completing the study, the average compliance defined as exercise sessions attended was 0.9 times per week in supervised sessions and 2.2 in home sessions. The training programme was well tolerated by all participants, and none developed stress-related or other injuries needing medical attention. During the study, the participants consulted an attending physician three times for the following reasons: mild ankle distortion (one), tibial contusion (one), and unspecified stomach pain (one). According to the endpoint questionnaires, 6 participants from the control group estimated that they were physically less active, 7 that they were more active, and 28 reported being equally active compared with baseline.

5.1.1 Bone mineral density (I)

Baseline variable values with absolute exercise effects on measured variables are presented in the original reports. During the 12 months of high-impact exercise intervention, bone mineral acquisition was significantly greater in the exercise group than in the control group at most of the proximal femur sites (Figure 12). The changes within the exercise group were significant in every variable in the proximal femur. In the lumbar area, L1 BMD increased more in the exercise group than in the control group (2.2% vs. -0.4%; p=0.002), and the change was also significant within the exercise group. There were no significant changes in the L2–L4 region between or within the groups. Calcaneal BUA increased in the exercise group (7.3%) and decreased in the control group (-0.6%) (p=0.015). The changes between or within the groups were not significant in non-weight-bearing sites in the distal forearm.
### Table 4. Characteristics of the subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n=60)</th>
<th>Exercise group (n=60)</th>
<th>Non-participants (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Questionnaire</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age; years</td>
<td>38.5 (1.6)</td>
<td>38.1 (1.7)</td>
<td>37.8 (1.8)</td>
</tr>
<tr>
<td>Calcium intake; mg/day</td>
<td>1099 (511)</td>
<td>1099 (657)</td>
<td>1126 (451.1)</td>
</tr>
<tr>
<td>Menarcheal age; years</td>
<td>12.6 (1.5)</td>
<td>12.8 (1.4)</td>
<td>12.9 (1.1)</td>
</tr>
<tr>
<td>Exercise; times/week</td>
<td>3.8 (4.1)</td>
<td>3.2 (2.6)</td>
<td>3.5 (2.0)</td>
</tr>
<tr>
<td>Exercise time (one period); minutes</td>
<td>52.6 (21.7)</td>
<td>49.4 (21.7)</td>
<td>49.6 (20.5)</td>
</tr>
<tr>
<td>Number of children</td>
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<td>1.8 (1.3)</td>
<td>1.8 (1.4)</td>
</tr>
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<td>Smokers; %</td>
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<td>18.6</td>
<td>20.0</td>
</tr>
<tr>
<td>Alcohol &gt; 1 drink/week; %</td>
<td>26.7</td>
<td>22.0</td>
<td>24.3</td>
</tr>
<tr>
<td>Any fracture beyond the age of 15; %</td>
<td>15.0</td>
<td>15.3</td>
<td>18.6</td>
</tr>
<tr>
<td>Any use of hormone medication (&gt;1 year); %</td>
<td>63.8</td>
<td>72.9</td>
<td>84.3</td>
</tr>
<tr>
<td>Height; cm</td>
<td>164.6 (6.0)</td>
<td>162.8 (5.8)</td>
<td></td>
</tr>
<tr>
<td>Weight; kg</td>
<td>69.4 (12.3)</td>
<td>68.0 (12.6)</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25.7 (4.6)</td>
<td>25.6 (4.4)</td>
<td></td>
</tr>
<tr>
<td>Percentage body fat</td>
<td>31.2 (6.7)</td>
<td>30.3 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Percentage lean body mass</td>
<td>68.8 (6.7)</td>
<td>69.7 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Waist; cm</td>
<td>82.3 (11.6)</td>
<td>81.1 (10.3)</td>
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<tr>
<td>Hip; cm</td>
<td>100.8 (8.0)</td>
<td>100.5 (8.6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD), unless otherwise stated.

**Fig. 12.** Change in proximal femur BMD during the study. The error bars represent 95% confidence intervals; p values are for differences in changes between the groups over the study period. *p <0.05, **p <0.01, ***p <0.001, for the change within the group. FN=femoral neck, TR=trochanteric region, HT= hip total, WT=Ward’s triangle.
5.1.2 Bone geometry (III)

The exercise group showed a significant 0.2% (95% CI: 0.01 to 0.35%; p=0.033) gain in mean bone circumference at mid-femur compared to the control group during the one-year study period (Figure 13). At the proximal tibia, the cortical attenuation decreased significantly in both groups, and minimum cortical CSMI in the exercise group. None of the treatment effects were significant at either proximal or distal sites of tibia. Sub-group analyses of the exercise group, which were performed in quartiles according to the number of exercise sessions attended, showed significant differences between the most and least compliant participants. At the proximal tibia, subjects in the most compliant quartile (>66 exercise sessions during the 12 months) showed a 1.2% (95% CI: 0.2 to 2.2; p=0.03) gain in bone circumference and a 0.5% (95% CI: 0.0 to 0.9; p=0.04) gain in cortical CSA compared to the subjects in the lowest quartile (<19 sessions). There was also a 2.5% (95% CI: 0.0 to 5.2; p=0.05) gain in maximum CSMI in favour of the most compliant subjects. Cortical attenuation in the proximal tibia did not change in the least compliant quartile, but decreased significantly in all other three quartiles. The decrement was -0.8% (95% CI: -0.2 to -1.4) compared to the most compliant quartile.

Fig. 13. Changes in mid-femur bone geometry and proximal tibia bone geometry over the 12-month study period. P values are for differences in changes between the groups over the study period. *p<0.05, **p<0.01, ***p<0.001 for the change within the group. CSA= cross-sectional area, CSMI= cross sectional moment of inertia.
5.1.3 Bone turnover (IV)

Statistically significant differences were found in the change of Uncoupling Index (0.32 vs. -0.28 p= 0.03) and PTH (-11.2 vs. -2.2 pg/mL; p= 0.03) between the exercise group and control group, respectively (Figure 14). Most of the PTH change within the exercise group occurred during the first six months of the study. The levels of serum 25OHD were highest at 6 months in the beginning of December. However, no significant differences were found in independent bone formation and resorption markers between the groups.

Fig. 14. Bone metabolism and calcitropic hormone changes during the study and the significance of the difference between the groups in the repeated-measures ANOVA. Bars represent 95% confidence intervals (N=76). 25OHD = serum 25-hydroxyvitamin D (1ng/mL = 2.5nmol/L), PTH= intact parathyroid hormone, PINP= procollagen type I amino-terminal propeptide, TRACP5b= tartrate-resistant acid phosphatase 5b, UI= Uncoupling Index.
5.1.4 Anthropometrics (I, III, V)

During the trial, there was a mean weight loss of 0.7 kg in the exercise group, while the control group gained weight 0.9 kg (p=0.09). The exercise group demonstrated a significant decrease compared to the control group in waist circumference (-1.1 vs. 0.9 cm; p=0.05) and hip circumference (-1.0 vs. 1.1 cm; p=0.04). In addition, exercise training led to increases in the muscle cross-sectional area (CSA) in mid-thigh (0.9 vs. 0.2 cm²; p=0.15) and calf (0.9 vs. -0.5 cm²; p=0.02) compared to the control group. In the exercise group, those who participated in more than 66 exercise sessions (highest quartile) during the 12 months showed increases in thigh (3.6 vs.-2.4; p=0.001) and calf muscle CSA (2.3 vs. -1.0; p<0.001) compared to the subjects in the lowest quartile of exercise sessions attended (<20 sessions).

5.1.5 Cardiorespiratory fitness and muscle strength (V)

The maximal oxygen uptake increased significantly more in the exercise group than in the control group (6.2 vs. 3.1 ml/kg/min; p=0.008) (Figure 15). The women in the exercise group also improved their countermovement (2.3 vs. -0.3 cm; p<0.001) and static jump (1.4 vs. -0.3 cm; p=0.004) heights significantly more compared to the controls. Both exercise and control groups improved their leg strength significantly (mean improvement 351 N, p<0.001 and 289 N, p<0.001, respectively), but there was no difference in the observed changes between the groups (p=0.69).

5.1.6 Blood lipids (V)

The exercise group demonstrated a statistically significant reduction in LDL-C concentration (-0.2 mmol/l; p=0.02) during the 12 months, but no significant differences between the groups was found. Glucose concentrations increased significantly in both exercise and control groups, while no changes appeared in serum insulin levels.
Fig. 15. Relative change during the 12 months in cardiorespiratory fitness and dynamic strength. P values are for differences in changes between the groups over the study period.*p <0.05, **p <0.01, ***p <0.001, for the change within the group.

5.2 Significance of loading amount and intensity

The distribution of the daily average number of acceleration peaks at each acceleration level is shown in Figure 16. The number of accelerations at the lowest level was similar in the study groups. At higher acceleration levels, however, the daily average numbers in the exercise group compared with the controls were approximately twofold within 1.1–2.4 g (650.3 vs. 393.1), twofold within 2.5–3.8 g (141.5 vs. 60.0), fourfold within 3.9–5.3 g (52.8 vs. 13.3), and nearly sixfold within 5.4–9.2 g (25.2 vs. 4.5).

5.2.1 Bone mineral density (II)

The BMD change was related to the physical activity data, especially at the hip. The relative daily number of accelerations at the levels of 3.9 g or more correlated significantly and positively with the 12-month BMD change at the femoral neck, trochanter, and Ward’s triangle (Figure 17). The L1 BMD change also correlated positively with the activity level of 5.4 g or more (p<0.05). Calcaneal SOS, in contrast, correlated positively with the activity level of 1.1–2.4 g (p<0.05). Acceleration level was not associated with the change in BMD at the distal radius.
or L2–L4. Each significant correlation was accompanied with a significant increase in BMD in the highest exercise quartile.

5.2.2 Bone geometry (III)

In the pooled groups, the relative daily count of impacts correlated significantly and positively with the change at the mid-femur in the cortical attenuation and maximum cortical CSMI at acceleration levels of 1.1 g or more (p<0.05-p<0.01), bone circumference at 2.5 g or more (p<0.05-p<0.01), and cortical thickness at 3.9 to 5.3 g (p<0.05). There was also a 0.7% (95% CI: 0.0 to 1.3; p=0.048) gain in maximum CSMI in favour of subjects in the highest quartile of the total number of impacts. At the proximal tibia, the number of impacts at the very low acceleration level of 0.3 to 1.0 g correlated positively with changes in bone circumference and maximal cortical CSMI (p<0.05), and negatively with cortical thickness (p<0.05). The change in the relationship between the maximum and minimum CSMI in mid-femur was positively associated with the number of impacts at 1.1 to 5.3 g (p<0.05-p<0.01). This change was significantly higher in the subjects with the highest number of impacts (0.4%) compared with the lowest (-0.8%) (p=0.038) in the quartiles of the total number of impacts.

The relative number of impacts at levels of 1.1 g or more was the most significant independent predictor of change in bone circumference at the mid-femur, accounting for 20% of the variance in the changes. Measured impact loading, weight change and muscle CSA change together accounted for 51% of
the variation in cortical attenuation at levels exceeding 1.1 g. Accelerations exceeding 2.5 g were statistically significant predictors of cortical thickness change at the mid-femur. Additionally, the number of impacts at the level of 2.5 g or more, together with weight change, was a predictor of maximum cortical CSMI change explaining 25% of the change. Change in muscle CSA was the only significant predictor of the changes in cortical CSA explaining 18% of the change. Low impacts at an acceleration level of less than 1.0 g were the only significant independent predictors of bone circumference, cortical thickness and maximum cortical CSMI at the proximal tibia.

**Fig. 17.** Number of impacts at different acceleration levels were significantly associated with changes in bone mineral density, bone geometry and bone turnover. CSMI = cross-sectional moment of inertia.

### 5.2.3 Bone turnover (IV)

In pooled groups, the most active subjects (the highest quartile) at each impact intensity level exceeding 2.5 g had significantly greater increases in UI compared to the subjects in the lower quartiles (Figure 18). There was a significant decrease in the serum PTH in the third quartile at acceleration levels of 2.5 to 5.3 g. There were no significant differences in the change of PINP or TRAP5b between the quartiles. Changes in bone metabolism were also significantly associated with the
measured number of impacts. Impacts exceeding 2.5 g were associated with changes in UI (p<0.05-0.001) in pooled groups. In the multiple regression analyses, the number of impacts above 5.4 g accounted for 31% of the change in UI and for 14% of the change in PTH. The number of impacts exceeding 2.5 g explained 10% of the variance in TRACP5b.

Fig. 18. Mean 12-month change in UI and PTH in quartiles of impact loading. The quartiles are defined by the number of daily impacts at each acceleration level in pooled groups (N=64). Error bars represent 95% confidence intervals. g=Acceleration of gravity (9.81 m/s$^2$), UI= Uncoupling Index, PTH= intact parathyroid hormone. *p<0.05, **p <0.01, ***p <0.001

5.2.4 Anthropometrics (V)

The changes in the mid-thigh and calf muscle CSA were positively associated with the measured number of impacts exceeding 1.1 g (p<0.05-0.001). In regression analyses, the measured number of impacts explained 19% of the change in the mid-thigh muscle CSA and 13% of the change in the proximal calf muscle CSA, being the most important predictor of the changes. Results of the sub-group analyses are presented in Figure 19.
5.2.5 Cardiorespiratory fitness and muscle strength (V)

In pooled groups, the changes in the VO$_2$max and CM jumping height were positively associated with the measured number of impacts exceeding 3.9 g (p<0.05-0.001). The measured number of impacts accounted for 29% of the variation in the CM jump height change and for 12% of the variation in the VO$_2$max change. Additionally, low initial values in VO$_2$max and static jump height predicted greater improvements in response within the exercise group.

5.2.6 Blood lipids (V)

In efficacy analyses, total cholesterol and LDL-C decreased significantly more in the subjects in the highest activity quartile compared to the lowest activity quartile of measured exercise volumes at every intensity level exceeding 1.1 g (Figure 20). The difference in the changes was -0.5 mmol/l (95% CI -0.8 to -0.2 mmol/l; p=0.005) in LDL-C concentrations. The measured number of impacts was the only significant determinant of the change in LDL-C explaining 19% of the change.
Fig. 20. Mean 12-month change in serum lipids in quartiles of impact loading. (N=65).
Error bars represent 95% confidence interval. *p <0.05, **p <0.01, ***p <0.001.
6 Discussion

The potential of mechanical loading as an adaptive stimulus to promote skeletal integrity has been acknowledged since the late 1800s (Wolff 1892). However, the adaptive effects of exercise on bone have only become the focus of increasing interest over the past few decades due to the alarmingly rising consequences of osteoporosis. While it has been well accepted that exercise, especially impact exercise, leads to beneficial bone adaptations, the specific type, intensity, frequency, and duration of loading that best enhance bone structural integrity are still largely unknown. Optimal loading patterns that produce clinically significant benefits in bone have not been clearly defined to date. This is mainly due to the lack of easily adaptable methods for quantifying exercise intensity during the interventions in terms of bone-loading forces, as stated by the recent American College of Sports Medicine Position Stand (Kohrt et al. 2004). The main objective of this thesis was to investigate the effect of exercise-induced impact loading on bones with the intention of assessing the intensity and amount of impact loading required to gain adaptive bone effects.

6.1 Bone response to impact exercise

Childhood and adolescence have been suggested as being the optimal time to improve bone strength by maximising peak bone mass (MacKelvie et al. 2002). Starting age has been found to significantly affect bone response to exercise (Kannus et al. 1995), and higher bone responses have been achieved with exercise intervention during childhood compared to adulthood (Hind & Burrows 2007). However, it is still unclear whether these benefits are maintained into and throughout adulthood due to missing follow-up studies lasting several decades (MacKelvie et al. 2002, Hind & Burrows 2007). It is also difficult to identify individuals at risk of osteoporosis during childhood and adolescence, and therefore older people may be a more practical target for specific prevention programmes, even though all kinds of exercise should be promoted among children and adolescents. We conducted this impact exercise intervention study in healthy premenopausal women as exercise has been found to be a feasible and safe method to successfully improve BMD in premenopausal women (Heinonen et al. 1996). With age, subjects with increased risk of osteoporosis also become more apparent than in childhood. Previous results from exercise intervention studies are more consistent in premenopausal women than in postmenopausal
women who have been suggested to have the ability to mostly conserve BMD (Bonaiuti et al. 2002). The feasibility of strenuous exercise needed for osteogenic effects is also more uncertain in elderly subjects. Thus, prevention methods focusing on other risk factors of osteoporotic fractures such as prevention of falls and injury-site protection along with bone strength have been recommended for elderly people (Kohrt et al. 2004, Kannus et al. 2005, Korpelainen et al. 2006a).

Several high-impact intervention trials have been successful in improving bone mass (Wallace & Cumming 2000), whereas resistance training has been shown to be most effective for muscle strength and mass (Lohman et al. 1995). Impact exercise was thus adopted as the exercise of choice in this study. Our impact-training regimen with progressive increase in impact intensities proved to be safe and efficient in healthy premenopausal women by increasing bone mass in weight-bearing bones while there were no reported stress-related injuries and only few appointments with physician. The most distinct effects in BMD were found in the proximal femur. The exercise group demonstrated a significant 1.1% gain in femoral neck BMD, while there was 0.5% decrease in the control group, which is found to be physiological for women in this age group (Russo et al. 2003). These changes are in agreement with previous high-impact exercise interventions and the change magnitudes represented a good average when compared to previous studies (Table 2) (Bassey & Ramsdale 1994, Friedlander et al. 1995, Heinonen et al. 1996, Bassey et al. 1998, Shibata et al. 2003a, Kato et al. 2006).

The greatest exercise effects in magnitude were found in calcaneal bone, consistent with the theory that impact loading attenuates progressively in ankle, knee and hip joints (Bauer et al. 2001, Stansfield et al. 2003). Despite this, a minor effect was also found in the lumbar spine. Impact exercise seemed to strengthen the lumbar vertebra L1 by a 2.6% increase in BMD, but did not have any effect on the total lumbar BMD or the lumbar vertebrae L2 to L4. Impact exercise interventions have previously indicated benefits of exercise effects in lumbar BMD of up to 3.6% per year (Kato et al. 2006). However, few data exist on the difference in the sensitivity of the lumbar vertebrae to impact loading, since previous studies have only reported the combined BMD values for L1-L4 or L2-L4. The BMD measurement of the vertebrae one by one is less reliable than the combined values from a set of vertebrae, which has to be considered. However, the biomechanical loading varies between the vertebrae, which might partly explain the difference. The cross-sectional area of L1 is smaller than that of L2-L4, which generates higher loading stresses. In the baseline, BMD was also lower in L1 than in the lower spine, and impact loading may therefore have had a
more positive effect on this site with lower BMD (Leichter et al. 1989, Winters-Stone & Snow 2003). The effects of impact loading especially for L1 may be of clinical importance since the number of atraumatic fractures is the greatest in L1 due to the transition area (Th11-L1) between the low-mobility thoracic region, making the highly mobile lumbar area susceptible to injury (Davis et al. 1999, Takata et al. 2000, Takata & Yasui 2002, Ferguson 2003). However, these findings require confirmation, and further studies are needed to clarify the effects of impact loading on other vertebrae in the transition area.

Dual energy x-ray absorptiometry is currently the most widely used and viable method to assess bone properties in clinical practice, even though it has been acknowledged as having several shortcomings due to its sources of error and planar nature (Bolotin et al. 2001, Bolotin et al. 2003, Engelke & Gluer 2006). The mechanical behaviour of bone not only depends on its material properties, but largely also on the spatial distribution of bone material within the structure. Bone strength can be significantly influenced by geometric factors such as external diameter and cortical thickness (Turner 2002). Nevertheless, most of the previous exercise intervention studies have studied only the effects on bone mass, which may lead to an underestimation of the effects of exercise on bone strength (Järvinen et al. 1999, Kontulainen et al. 2002). This points out the importance of our finding that impact exercise appears also to improve bone geometry by increasing periosteal circumference. In a previous exercise intervention study, strength training was found to lead to redistribution of bone mass without affecting BMD in postmenopausal subjects (Adami et al. 1999), indicating that bones adjust their apparent structural rigidity, not only BMD, to the changes that occur in their loading environment. Redistribution of bone tissue away from the central axis allows the bone to resist bending and torsional loads better. Even though the scale of these bone depositions was small, they may lead to significant improvements in bone strength since new bone formation tends to localise on bone surfaces where the mechanical strains are greatest (Wolff 1892, Turner & Robling 2005, Beck et al. 2006). However, the QCT measurements in our study also have potential limitations along with the relatively small treatment effect, such as the segmentation of cortical bone and the use of only single slice measurements. In addition, repositioning at the follow-up is critical. Unfortunately, we were not able to measure geometric changes in the proximal femur since the techniques available did not allow us to achieve high enough precision with reasonable radiation exposure. In addition, we were not able to use hip structural analysis to assess geometry and strength indices with DXA. This
significantly hampers the generalisation of these results to hip fracture prevention, as hip fracture risk has been found to be significantly affected by site-specific geometric changes (Mayhew et al. 2005, El Kaissi et al. 2005).

All bone adaptations require activation of the remodelling cycle to take place. In this study, we found activation in bone turnover, resulting in altered metabolism favouring bone formation. This positive change was found in the Uncoupling Index (UI) (Eastell et al. 1993), describing the balance between bone formation and resorption. There were no significant changes in the independent biochemical bone formation and resorption markers, PINP and TRACP5b. This may partly be caused by the nature of bone biochemical markers, as they reflect bone metabolism changes of the whole skeleton (Nishizawa et al. 2001), while the exercise in our study mainly affected weight-bearing bones. However, both markers showed an increasing trend, complying with the theory that bone formation is closely coupled with resorption (Harris & Heaney 1969). Regardless of this coupling, our results suggest that the balance between formation and resorption rates may still be affected by exercise-induced mechanical loading. However, there is no evidence of how reversible this change is and how long it will possibly take to restore the levels back to baseline level. Relating these results to previous studies is difficult as the existing results have been quite inconsistent and as study settings and timing of measurements vary greatly. Cross-sectional athlete studies have shown increased turnover (Karlsson et al. 2003), increased formation (Creighton et al. 2001) or increased resorption (O’Kane et al. 2006). The few prospective intervention studies that have been conducted in women have not found changes in bone turnover or reported any measures of bone turnover balance (Shibata et al. 2003b, Kemmler et al. 2004a). For the future, UI may provide an interesting method to assess the effects of physical activity on bones as it combines elements of bone formation and bone resorption indicating turnover balance more specifically.

We also observed changes in serum PTH levels during the 12-month study period. Previous experimental studies have suggested that mechanical loading and PTH have an interacting effect on bone turnover (Ma et al. 1999b) and that this interaction may occur during mechanotransduction (Bakker et al. 2003). In clinical studies, a short bout of exercise has been found to stimulate transient PTH secretion (Maimoun et al. 2005, Maimoun et al. 2006). A low basal level of serum PTH was associated with high BMD in active endurance runners (Brahm et al. 1997), while a high basal level was related to low forearm BMD (Lamberg-Allardt et al. 2001). In this study, a significant decrease in serum basal PTH levels
was found in the exercise group compared to the control group, while bone turnover was slightly increased. It is possible that a decreased basal PTH level results in higher osteogenic effects after a single exercise session by enabling a greater difference between the basal PTH level and transient exercise-induced PTH peaks. This may alter bone turnover balance in favour of bone formation, suggesting that exercise-induced long-term alterations in bone metabolism may partly be mediated or modified by PTH.

Osteogenic response may also be mediated or modulated by other endocrine and paracrine factors. Especially oestrogen has been suggested to be a potential modulator (Turner 1991, Lanyon & Skerry 2001, Joldersma et al. 2001, Frost 2003) even though recent findings have indicated that the effects of loading and oestrogen in bone are independent of each other (Pajamäki 2007). Unfortunately, oestrogen levels were not measured in our study, and no additional information on this topic was thus revealed by our study.

6.2 Significance of impact intensity

Although we did find significant alterations in bone mineral density, geometry and metabolism, these data do not bring us any closer to the answer of optimal osteogenic exercise and does not provide any breakthroughs in the field of osteoporosis prevention. Designing efficient training programmes would most likely foster the prevention of osteoporosis in individuals who are already active without reaching passive subjects at the highest risk (Marcus 1998, Nelson & Bouxsein 2001). On population level, competition between recreational activities has become more and more rigorous, suggesting that the significance of specified training programmes in the prevention of osteoporosis would probably be minimal (Nelson & Bouxsein 2001). However, determination of quantified intensity thresholds for efficient exercise would give us tools to promote osteogenic exercise patterns to be included in everyday routines and personal activities and thus increase the possibility to affect public health as well.

The difficulty of quantifying exercise in terms of bone loading has made determination of optimal osteogenic exercise during clinical interventions unattainable until now. Accelerations are an indirect surrogate of bone strains that are suggested to be related to impact load forces during exercise (Servais et al. 1984, Janz et al. 2003). They are easily measurable with accelerometers, even though previous accelerometer-based devices have not been suitable for measuring actual impact loadings, as they have been designed to measure aspects
such as energy expenditure, physical activity or gait (Wolff 1892, Mathie et al. 2004). Thus, a novel accelerometer-based measurement device was designed and constructed to measure impact loading in the long term, and an impact exercise intervention was organised to determine the characteristics of optimal osteogenic exercise.

Our study showed a significant dose- and intensity-dependent relationship between bone adaptations and measured impact loading. The number of impacts exceeding 3.9 g was associated with BMD changes at the proximal femur. Changes in calcaneal bone were found at the much lower intensity level of 1.1 to 2.4 g. Geometric changes in cortical CSMI and changes in the ratio between maximum and minimum CSMI were related with impacts exceeding 1.1 g in mid-femur, indicating effects on the cross-sectional shape, presumably due to bone mineral deposition and reshaping of bone. BMD changes discovered at vertebra L1 were only associated with impacts exceeding 5.4 g, which may be due to the exercise programme being mainly targeted at lower limbs. Thus additional back-specific exercise may be more efficient for the spine. Bone turnover balance was also substantially associated with loading exceeding 2.5 g. It seems that the effect of osteogenic loading varies significantly between different bone sites, as has been previously suggested in an experimental study (Hsieh et al. 2001). Alternatively, it may be speculated that the different types of adaptive responses may require different loading stimuli, as preferential adaptation in geometry over density may provide a more optimised structure to prevailing loading conditions, compared to the modest increases in volumetric BMD (Currey 2003, Welch et al. 2004). Nevertheless, this suggestion needs further research. There may also be other than endogenous reasons for the variable responses. Differences between the hip and other sites may be caused by site-specificity of our measurement device attached next to the iliac crest, as the human body is not rigid and local accelerations in different body parts during exercise can vary substantially in terms of magnitude and direction (Derrick 2004). External measurements are also unable to quantify the evident attenuation of loading forces in joints (Bauer et al. 2001, Stansfield et al. 2003). In addition, joint reaction forces may be a significant determinant of osteogenic effects, as the rapid dynamic muscle contractions during high-impact exercise may create rapidly rising force profiles that may be able to create significant strains and strain rates (Bassey et al. 1997).

Our acceleration values become more interesting when they are compared to our preliminary data, which show that vertical accelerations exceeding 4 g are reached during normal physical activities including vertical jumping and fast
running. Also proximal femur changes were related to a relatively small number of those high impacts, although the total average number of measured impacts was over 9,000 per day. The mean number of impacts exceeding the level of 4 g was 80 per day in exercisers and 20 in controls. The difference that separates BMD gainers from losers is thus approximately 60 per day, and even the upper 95% confidence interval limit of the exercise group was less than 100 daily impacts. In previous studies, preclinical data have shown that 36 and 1,800 consecutive loadings cycles per day have been equally effective in increasing bone mass (Rubin & Lanyon 1984). More recent animal studies have suggested that even 5 jumps per day on 5 days per week with progressive jumping height increase bone mass and improve bone geometry during eight weeks of training in mice (Umemo et al. 1997). In clinical settings, Bassey and colleagues (Bassey & Ramsdale 1994, Bassey et al. 1998) have found in two previous trials that 50 vertical jumps per day are effective in increasing femoral neck BMD in premenopausal healthy women. Kato and colleagues (Kato et al. 2006) even surpass these results as they recently reported that 10 maximum vertical jumps 3 times per week improved femoral neck BMD nearly 4% during 6 months of training in women in their early twenties. Together with our current findings these results indicate that high impacts are effective at relatively minor doses. These doses are well tolerated by premenopausal women. As feasibility, safety and efficacy may change significantly in other age groups, these results should be confirmed before applying them into more generalised practice.

Bone gains in the studies of Bassey et al. (Bassey & Ramsdale 1994, Bassey et al. 1998) and Kato et al. (Kato et al. 2006) exceed the osteogenic improvements found in the present study and the study of Heinonen and colleagues (Heinonen et al. 1996). Interestingly, there was also a slight difference in training programmes between these studies, as the former included merely high impacts while the latter included a combination of low and high impacts during the supervised training sessions. This gives rise to the question: Do low impacts reduce the osteogenic effect? Animal studies have shown that osteogenic response saturates after a few loading cycles and are restored after a period of no loading (Robling et al. 2001, Umemo et al. 2002, Robling et al. 2002a, Robling et al. 2002b). It has been suggested that bone loses more than 95% of its mechanosensitivity after the first 20 loading cycles, and that most of it is restored after 4 to 8 hours and almost all is restored after a rest period of 24 hours (Turner et al. 2003). In our study, the physical activity monitor recorded the daily number of impacts but it was not able to differentiate the rest periods or to constantly
control the time of wearing the monitors. In future exercise regimens targeted to achieve an osteogenic effect, timing and length of loading periods should also be carefully considered, as currently available results indicate that 50 to 100 high impacts alone dosed daily or even divided into two bouts per day would be better than long training sessions two to three times per week. A long-term progressive increase in strain intensities is also essential to minimise the risk of injuries.

Training divided into several sessions may also be more feasible as it does not require any specific equipment or premises and can be individually executed during normal everyday routines, thus being possibly more adoptable in community level. This would be important, as the compliance and drop out rate in our study was higher than expected. Higher compliance with exercise sessions might have resulted in clearer exercise-induced effects on bone geometry. However, the diversity in individual physical activities enabled us to evaluate the relationship between actual impact loading levels and bone changes. The drop-out rate was considerably high albeit average compared to other exercise interventions in this age group (Table 2). The drop-outs in the exercise group were predominantly unrelated to the study, whereas incompliancy with accelerometer measurements was the major drop-out reason in the control group. This is most probably due to the continuous measurement protocol. It has recently been suggested that 3 to 5 days of monitoring is required to reliably estimate habitual physical activity with an accelerometer among adults (Trost et al. 2005). However, the time needed during exercise interventions is presumably a week or more, and the measurements should be repeated often enough if the exercise program is progressive. Furthermore, habitual physical activity varies between the four seasons (Bergstralh et al. 1990), and all these issues have to be considered when planning measurements.

Future studies should also consider effects of atypical loading, as we were only able to measure vertical acceleration. This is necessary since Nikander et al. (Nikander et al. 2005) recently reported that loading from atypical directions, so-called odd impacts, is equally effective as high impacts in improving femoral neck strength in women athletes. Also importantly, the bone is anisotropic material and loading resistance varies significantly according to direction (Currey 2001). Forces that fracture the hip during a fall most likely arise from other than the vertical direction (Greenspan et al. 1998, Parkkari et al. 1999). Thus, the effects of loading directions during exercise should be observed by using three-axial accelerometers in future studies. Strain rate, frequency and rest periods should also be quantified along with impact magnitude since they are an integral
part of osteogenic stimulus (Turner et al. 2003). In addition, one important issue in future studies is trying to identify individuals who are most likely to gain exercise-induced bone benefits. In our study the baseline BMD values were inversely associated with the BMD change at the proximal femur, indicating that exercise is most effective for people with low BMD, i.e. persons with an increased risk of osteoporotic hip fracture. Further research is also needed to study the impact of genetics for osteogenic responsiveness. There are a few studies that have indicated that oestrogen receptor $\alpha$ gene and vitamin D receptor may alter bone osteogenic response (Tsuritani et al. 1998, Lee et al. 2003, Lee & Lanyon 2004, Suuriniemi et al. 2004) but also controversial results exist (Järvinen et al. 1998, Järvinen et al. 2003a). Quantification of loading environment would probably enable more specific clinical studies to detect the genetic background of osteogenic responsiveness.

Regardless of the above noted limitations in our study and the points needing further investigation, we are now a step closer to defining the characteristics of osteogenic exercise. We can currently recommend 50 to 100 high-impact jumps daily, perhaps divided into two or three bouts, for healthy premenopausal women. However, the starting level and progressive nature of training always call for individual consideration to ensure the efficacy and safety of exercise.

6.3 Potential of impact exercise to promote cardiovascular health

Osteoporosis and fragility fractures are not the only major public health problem. The burden of cardiovascular diseases is even greater than osteoporosis and fragility fractures, especially as cardiovascular diseases (CVD) are the leading cause of death in women (Peden et al. 2002). A sedentary lifestyle is the main factor behind both diseases, and exercise and increased physical activity are potential prevention methods for CVD as well. Our exercise regimen initially designed to load weight-bearing bones demonstrated positive effects on some main risk factors of CVD as well as on the extra-skeletal risk factors of osteoporotic fractures and physical performance. The most distinct improvements were observed in the explosive power properties and muscle mass of the lower limbs and oxygen uptake. In addition, improvements in cardiorespiratory fitness, waist and hip circumference and blood lipid levels were found, implying a reduced risk of cardiovascular diseases.

A previous high-impact exercise intervention was also efficient in improving muscular performance, dynamic balance and oxygen uptake in healthy
premenopausal women (Heinonen et al. 1996). The 12-month treatment effect of 13% in lower limb plyometric force found here is higher than reported previously (Heinonen et al. 1996). This may be due to slight differences in the exercise training programmes. We found no treatment effect in isometric leg muscle strength, which is in line with the study of Heinonen et al (Heinonen et al. 1996), even though definite improvements were found in both groups in our study. Most of the subjects were unaccustomed to testing procedures, and regardless of careful instructions, the increases found in isometric leg strength may partly be explained by the learning effect, even though muscle CSA was clearly improved by exercise training. These changes in muscle power and CSA signify the importance of muscle forces during high-impact exercise that may also be able to affect osteogenic response.

Exercise improved cardiorespiratory fitness as well, and had a 12% treatment effect on maximal oxygen uptake, which is consistent with previous osteoporosis-prevention exercise interventions (Heinonen et al. 1996, Uusi-Rasi et al. 2003, Kemmler et al. 2004a). Previously, maximal oxygen uptake has been found to be the strongest predictor of CVD-related mortality in the Finnish population (Laukkanen et al. 2001). Blood lipid, especially LDL-C, concentrations are also major determinants of vascular events (Poulter 2003). A drug-induced decrease of 1 mmol/L in the LDL-C concentration has been reported to lead to a 25% reduction in the relative risk of CVD events (Heart Protection Study Collaborative 2002). In our study, the twelve-month exercise period induced a difference of 0.5 mmol/L in the change in LDL-C concentration between the most active and least active subjects. The difference is clinically significant and demonstrates the potential of exercise in the reduction of CVD risk as well. Importantly, a recent study has indicated that this positive effect increases with continued training (Kemmler et al. 2007). Analogous to bone changes, high initial serum lipid concentrations and low physical capacity levels independently predicted better response to exercise, indicating that exercise is most effective for those at greatest risk.

Similarly to bone improvements, a dose-response relationship between measured impact loading and physical fitness and lipid changes was discovered. Improvements were found in quite a wide intensity range, including even light exercise types such as slow aerobic stepping when the number of repetitions was high. Walking and other very low-intensity exercise was not associated with changes, while the greatest exercise effects between the most active and least active subjects were found at moderate to high intensity levels of exercise. The
amount of impacts in the most active subjects was 1,500 per day exceeding 1 g
being equivalent to 1.5 km of jogging or to 15 minutes of step-aerobics, for
example. Our findings are complementary to the previous studies which have
suggested that the amount of exercise is more important than intensity for
improving lipoprotein profiles and insulin sensitivity (Kraus et al. 2002, Houmard
et al. 2004). However, increases in either the intensity or the amount of exercise
may yield additional benefits in terms of cardiorespiratory fitness (Duscha et al.
2005).

Our study suggests that impact exercise may also be recommended for the
prevention of CVD as it seems to be strenuous enough for the cardiovascular
system. This is crucial as the Western society remains fundamentally sedentary.
Exercise that provides multiple benefits has better prospects since it is unlikely
that people will do one sort of exercise to benefit the heart, another to increase
muscle strength, and yet another to fortify bones. (Marcus 1998)
7 Conclusion

This series of studies demonstrated that 12 months of regular impact exercise-induced mechanical loading leads to favourable adaptive changes in the weight-bearing bones of premenopausal women. Impact exercise stimulates bone turnover leading to beneficial alterations in bone metabolism in favour of bone formation. This activation of bone adaptation results in beneficial bone mass changes by increasing bone mineral density in weight-bearing bones, especially in the clinically significant femoral neck and trochanteric regions. The observed geometric changes demonstrate adaptation of bone structure via bone mineral deposition and reshaping to ensure optimal bone strength.

Bone adaptations were shown to have a dose- and intensity-dependent relationship with measured impact loading. Especially BMD changes at the femoral neck and trochanteric regions were threshold-dependent, signifying the importance of high impacts exceeding the acceleration level of 4 g as an osteogenic stimulus. The number of impacts needed to achieve this stimulation appeared to be 60 per day, comparable to the same number of daily jumps. In addition, the study showed that subjects with low baseline BMD have the relatively highest gains in BMD, indicating that impact exercise is the most efficient for people with low BMD, i.e. for individuals with the highest risk of fragility fractures.

Changes in bone metabolism appeared to be similarly related with high impacts exceeding the level of 4 g, while changes in bone geometry have a dose- and intensity-dependent relationship at a wider intensity range from 1g onwards, the quantity being equivalent to e.g. 1.5 km of jogging or 15 minutes of step-aerobics. The necessary impacts were acquired here during normal exercise training that proved to be safe in healthy premenopausal women, thus being readily applicable in general use.

The bone-targeted exercise programme also had favourable effects on physical performance and overall health. It enhanced dynamic muscle forces and cardiorespiratory fitness and decreased waist and hip circumference with a moderate amount of exercise at a wide intensity range, thus implying a reduced risk of cardiovascular diseases. In addition, impact exercise also affected serum lipids positively by decreasing low-density lipoprotein levels with high doses of exercise.

These results signify the importance of the type and intensity of exercise as an osteogenic stimulus. Determination of quantified intensity thresholds for
exercise provides tools to promote osteogenic exercise patterns to be included in everyday routines and personal activities, thus increasing the possibility to obtain population-level benefits. If done on a regular basis, impact exercise may be an efficient and safe way of preventing osteoporosis. In addition to the prevention of osteoporosis, impact exercise can be recommended for the prevention of cardiovascular diseases.
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BONE ADAPTATION TO IMPACT LOADING—SIGNIFICANCE OF LOADING INTENSITY

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