Muscles, Estrogen, and Bone

BY

EVA LJUNGGREN RIBOM

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Abstract

Sweden has one of the highest incidences of osteoporotic fractures in the world. A more sedentary lifestyle is one of several proposed reasons for the increase in osteoporosis seen in the developed countries. The aim of this thesis was primarily to study the influence of muscle strength, and body composition, on bone mineral density, BMD, in young adults. The second aim was to evaluate the possible influence of estrogen on muscle strength in women.

A population-based study of 113 subjects (53 men and 60 women) aged 22-85 showed associations for premenopausal, but not postmenopausal women, between isometric quadriceps muscle strength and BMD in the total body, lumbar spine, and femoral neck. In men there was only an association between muscle strength and BMD in the total body. Another population-based study of 125 randomly selected young adults (64 women and 61 men) showed that total body BMD, TBMD, is influenced by isokinetic knee flexion and extension strength in women but not in men where body composition influenced TBMD. In 159 randomly selected young adult women (20-39 years) knee flexion and extension strength influenced not only TBMD but also total hip BMD, and heel BMD. However, lean body mass and body weight were better predictors for BMD at these skeletal sites. An extension of this study involving 335 women again demonstrated that lean body mass is the best predictor of BMD. This study also showed that Uppsala women aged 20-39 years have a BMD that is approximately 0.1-1.2 SD (2-12 %) above international/national references. In addition marked variations in BMD T-scores between various skeletal sites were noted.

In Conclusion: The association between muscle strength and BMD is evident in women in their early twenties but with age lean body mass and body weight becomes better predictors for BMD. In men lean body mass and body composition but not muscle strength predicted BMD. Hormone replacement therapy does not influence muscle strength and there is no association between allelic variations in the estrogen receptor alpha and muscle strength in women.

Keywords: Muscle strength, Bone mineral density, Ultrasound, Body composition, Hormone replacement therapy, Estrogen receptor alpha

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List of Publications

The thesis is based on the following manuscripts, which will be referred to by their roman numerals:

I. Ribom E, Olofsson H, Piehl-Aulin K, Mallmin H, Ljunghall S.
   Correlations between isometric quadriceps muscle strength and bone mineral density.

II. Ribom E L, Piehl-Aulin K, Ljunghall S, Ljunggren Ö, Naéssen T.
    Six months of hormone replacement therapy does not influence muscle strength in postmenopausal women.

III. Grundberg E, Ribom E L, Brändström H, Ljunggren Ö, Mallmin H, Kindmark A.
     Allelic variation in the gene for estrogen receptor alpha does not correlate with muscle strength or body composition in young adult Swedish women.
     Submitted for Publication.

IV. Ribom E L, Ljunggren Ö, Piehl-Aulin K, Bratteby L-E, Samuelson G, Mallmin H.
    Muscle strength correlates with total body bone mineral density in young women but not in men.

V. Ribom E L, Ljunggren Ö, Mallmin H
    Influence of muscle strength and body composition on bone mineral density in young adult Swedish women, a population-based study.
    Submitted for Publication.

VI. Ribom E L, Ljunggren Ö, Mallmin H
    Manuscript.
## Abbreviations

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<th>Description</th>
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<tr>
<td>BMC</td>
<td>Bone Mineral Content</td>
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<td>BMD</td>
<td>Bone Mineral Density</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BUA</td>
<td>Broadband Ultrasound Attenuation</td>
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<td>BW</td>
<td>Body weight</td>
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<tr>
<td>DXA</td>
<td>Dual-Energy X-ray Absorptiometry</td>
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<td>ERα</td>
<td>Estrogen receptor alpha</td>
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<tr>
<td>FM</td>
<td>Fat mass</td>
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<td>FNBMD</td>
<td>Femoral neck BMD</td>
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<tr>
<td>HBMD</td>
<td>Heel BMD</td>
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<tr>
<td>HRT</td>
<td>Hormone Replacement Therapy</td>
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<tr>
<td>ISCD</td>
<td>International Society for Clinical Densitometry</td>
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<tr>
<td>LBM</td>
<td>Lean body mass</td>
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<tr>
<td>LSBMD</td>
<td>Lumbar spine BMD</td>
</tr>
<tr>
<td>MESr</td>
<td>Minimum Effective Strain for remodeling</td>
</tr>
<tr>
<td>MESm</td>
<td>Minimum Effective Strain for modeling</td>
</tr>
<tr>
<td>MyHC</td>
<td>Myosin Heavy Chain</td>
</tr>
<tr>
<td>QUI</td>
<td>Quantitative Ultrasound Index</td>
</tr>
<tr>
<td>QUS</td>
<td>Quantitative Ultra Sound</td>
</tr>
<tr>
<td>SI</td>
<td>Stiffness index</td>
</tr>
<tr>
<td>SOS</td>
<td>Speed of Sound</td>
</tr>
<tr>
<td>TBMD</td>
<td>Total body BMD</td>
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<tr>
<td>THBMD</td>
<td>Total hip BMD</td>
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</table>
Introduction

Osteoporosis

Osteoporosis is defined as: A systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk (NIH, 2001). After the age of 50, half of the female and one third of the male population will be affected by a fracture related to osteoporosis (Cooper et al., 1992; Gullberg et al., 1997; Melton, 1993). Various forms of osteoporosis are defined based on the underlying pathogenetic mechanisms; Primary osteoporosis including postmenopausal, and age-dependent osteoporosis and secondary osteoporosis, where osteoporosis is a consequence of a disease or induced by pharmaceuticals, e.g. by cortisone treatment. Idiopathic osteoporosis is osteoporosis in a young adult without any apparent secondary cause (Mellström, 2002).

The risk of fragility fractures increases progressively with bone loss. It is estimated that the risk for hip fracture increases by a factor 2.6-3.6 times for each standard deviation decline in femoral bone mineral density (Cummings et al., 1993; Ekman et al., 2001). A WHO consensus conference in 1994 suggested the following definition of postmenopausal osteoporosis in white females based on bone densitometry measurements (WHO, 1994)

1. Normal bone density: A normal BMD and BMC when the measured value is within 1 standard deviation (SD) of the mean value of young Caucasian adult females.
2. Osteopenia: A value of BMD and BMC of less than 1 SD but more than 2.5 SD below the mean value of young Caucasian adult females.
3. Osteoporosis: A BMD and BMC more than 2.5 SD below the mean value of young Caucasian adult females.
4. Manifest osteoporosis: A value of BMD or BMC of more than 2.5 SD below the mean value of young Caucasian adult females, and the presence of at least one fragility fracture.

Peak BMD has been proposed to be an important determinant for the future risk of osteoporosis, and a 5% increase in peak BMD is followed by a 40%
decrease in osteoporosis risk (Bachrach, 2000). Peak BMD is reached sometime around the age of 18 to 30 depending on anatomical site. A cross-sectional study has shown that the proximal femur reaches its peak earlier than the lumbar spine and distal forearm. (Zanchetta et al., 1995). Thereafter a steady state is entered. There is an age dependent decrease in BMD. The loss is accelerated during the first 5-10 postmenopausal years in women, but both genders loose BMD with increasing age (Löfman et al., 1997; Wishart et al., 1995). Bone mineral density is determined largely by genetic factors, hormonal status, medication, environmental and lifestyle factors such as, smoking, nutrition, physical activity, muscle strength, and body composition (Mellström, 2002). The potential for each of these factors to affect final peak BMD is uncertain. Physical activity appears to have the greatest influence on BMD when applied during the years when rapid skeletal mineral acquisition occurs, i.e. before puberty (Bass et al., 1998; Kannus et al., 1995; McKay et al., 2000; Morris et al., 1997) (fig 1).

It must be emphasized that osteoporosis is to be regarded as a risk factor for fracture, and that fractures per se cause morbidity and also increased mortality rates. It is not only the amount of BMD that contributes to fracture risk. Reduced balance, sight and muscle strength are factors known to influence the occurrence of falls, which can lead to a fracture. Although certain risk factors such as age, gender, heredity, earlier fracture and body height are more or less impossible to influence, there are several other risk factors such as smoking, physical inactivity, inadequate nutrition, low body weight, early menopause, amenorrhea, impaired vision, impaired health, secondary osteoporosis, and factors associated with falls that can be influenced by different interventions (Cummings et al., 1995).
Bone and Bone Metabolism

The human body comprises of two types of bone: flat bones (scapula, scull bones, mandible, and pelvis) and long bones (femur, tibia, and humerus etc). Long bones show wider parts, epiphyses, a cylindrical part, diaphysis, and between these two parts a development zone, metaphysis. When studying a longitudinal section of a long bone, the epiphysis and metaphysis mainly have trabecular or cancellous bone whereas the diaphysis has more cortical bone. Trabecular and cortical bone are made up of the same cells and matrix elements, but there are functional and structural differences. The trabecular bone serves mainly a metabolic function and the cortical bone a mechanical and protective function. The structural difference is mainly quantitative where approximately 20% of the trabecular bone is calcified, whereas 80-90% of the compact bone is calcified, leading to the functional differences (Kanis, 1994).

The osteoblast is the cell that is responsible for the production of collagen and other bone proteins, and the subsequent mineralization of the matrix. Once bone matrix is mineralized some of the osteoblasts are retained on the bone surface and termed “resting” osteoblasts or bone-lining cells. Osteocytes are osteoblasts which have been trapped within the bone matrix during the process of bone formation. The osteocytes are interconnected to osteoblasts and other osteocytes by fine intercellular projections running within the canaliculi. This makes signaling from the interior of bone to the surface possible. It has been suggested that the osteocytes are responsible for sensing mechanical strain in bone due to their strategic position (Lanyon, 1993).

The osteoclast is the other major cell type found in bone. This is a large multinucleated cell. It degrades mineralized bone by attaching to a bone surface and secreting acids and lysosomal enzymes into the space between its apical surface and the mineralized bone surface.

Bone modeling is a process that occurs primarily during the growing years. The process alters the shape, length, size, and mass of bone in response to growth hormones and mechanical loading factors, primarily by cortical bone apposition. Subtle
increases in a bone’s cross-sectional geometry will contribute heavily to its structural properties. Body weight has been identified as a determinant of bone mineral density, but some controversy exists whether it has independent effects or if it is lean mass and fat mass that are of importance (Baron, 1999).

Bone is throughout life a metabolically active structure. The annual bone turnover of the skeleton is 5-10% and new bone replaces old and damaged bone. This is called remodeling. In trabecular bone remodeling takes place on the bone surfaces, and in the cortical bone it takes place on surfaces surrounding channels centered on blood vessels in Haversian systems. The remodeling cycle begins with an activation phase when the osteoclasts are activated and attached to a locus on the bone surface. During the resorption phase osteoclasts excavate erosion cavities to a depth of 40-60 µm over 4-12 days. After resorption the osteoblasts are attracted to the eroded surface and synthesize an osteoid matrix. Matrix synthesis is rapid during the initiation of the formation phase. A few days after the onset of matrix formation by osteoblasts, the newly formed osteoid undergoes mineralization. The whole remodeling cycle takes between 100 and 200 days after which a resting phase occurs (Kanis, 1994).

Muscle Strength

The skeletal muscles make it possible for the individual to move the body and to lift heavy burdens. This is done under the control of the nervous system which makes the muscle contract and thereby increasing tension. To be able to increase this tension skeletal muscle tissue is constructed in a specific manner. The muscle fibres constitute the main part of the muscle mass. There are two main muscle fibres with...
different innervations and metabolic qualities. There is a type I fiber, containing slow twitch myosin heavy chain (MyHC), which contracts slowly and is resistant to fatigue. The type II fibres have subgroups IIA, IIB, IIC and have fast motor-units. The subgroups contain different fast isoforms of MyHC, contract quickly and, depending on the subtype, show various degrees of resistance to fatigue (Saltin and Gollnick, 1983).

The muscles and tendons insert to the skeleton and the transformation of the tension from the contraction into force is thereby possible. When the muscle exerts tension as it is shortening it is called a concentric contraction, when the muscle lengthens during contraction it is called eccentric contraction. If the muscle tension does not achieve any movement this is called static or isometric contraction. To measure muscle strength the resistance put on a muscle can either be dynamic, i.e. isokinetic, which allows the muscle to work either in a concentric or an eccentric way, or it, can be static which gives an isometric contraction. Isokinetic resistance is dependent on a chosen angular velocity. For instance knee flexion from 0 to 90 degrees at 90 degrees/second means that the resistance given is exactly high enough for it to take the subject one second to bend the knee. With increasing angular velocities the peak torque occurs later in the range of motion. This might be a problem when testing weak muscle groups, since the limb may pass the optimal joint position for optimal muscular performance (Kannus and Beynnon, 1993). If the angular velocity is 0 degrees/second the contraction is isometric. The results from an isokinetic muscle measurement is a peak torque, expressed in Newton meters (Nm), which has been shown to be a highly reproducible variable in previous studies of muscle strength (Blain et al., 2001; Kannus, 1994).

The maximal muscle strength decreases with age primarily due to loss of muscle mass and partly by reduced activities. There is an approximate decrease in muscle strength during the adult life of 30-40%. However, there is little loss in muscle strength before the age of 45 but this is followed by a decrease of 25% to the age of 65 (Grimby, 1986; Grimby and Saltin, 1983). There seems to be a decline in the number of muscle fibres and fibres’ cross-sectional area with age (Grimby
and Saltin, 1983) although there are conflicting results regarding the effect of ageing on the composition of type I and type II muscle fibres (reviewed in Aoyagi and Shepard, 1992). In the elderly groups a reduction in fibre size in the fast type II fibres has been reported. The levels of both aerobic and anaerobic enzyme levels in muscle tissue in the elderly are consistent with those of younger individuals in terms of their activity per unit muscle weight (Aoyagi and Shepard, 1992).

The Influence of Estrogen on Bone, Fractures and Muscle Strength

Estrogens inhibit osteoclasts and stimulate osteoblasts and the bone loss seen at the time of menopause is therefore a direct consequence of the withdrawal of endogenous estrogens. Hormone replacement therapy, HRT, decreases or eliminates the postmenopausal bone loss. The BMD can be preserved even in advanced age with HRT if the level of serum-estradiol is kept at a sufficient level. Women with established osteoporosis also have a beneficial effect of HRT on BMD and fracture risk. The reduced fracture risk seen with HRT is not fully understood, because the effect is evident even though the effect on BMD is sparse (Naessén, 2002). However, the positive effect of HRT on BMD and fracture risk disappears when the substitution is withdrawn (Christiansen et al., 1981; Marcus and Watts, 2000; Michaelsson et al., 1998).

The influence of estrogen on muscle strength has been debated. Some studies have shown an increase (Heikkinen et al., 1997; Skelton et al., 1999) others a preservation (Cauley et al., 1987; Greeves et al., 1999; Philips et al., 1993), and yet others have shown no effect of HRT on muscle strength (Armstrong et al., 1996; Bassey et al., 1996; Brown et al., 1997; Kritz-Silverstein and Barrett-Connor, 1994; Preisinger et al., 1995; Seeley et al., 1995; Taaffe et al., 1995) (Table 1.). A review claims that the exact effect of estrogen on skeletal muscle strength remains uncertain (Meeuwsen et al., 2000).
Physical Activity

Physical activity can be described as the opposite of physical inactivity. This means that anything but being an idler can be considered as physical activity. However, the frequency, duration, and intensity of the activity are of importance. Frequency represents how many times per day/week/month, duration for how long, and intensity how intensively the exercise is performed. During the 1990’s, younger people (15 years-old) have reported a more sedentary lifestyle with reduced training and more television watching and computer games. Approximately 20 % of the Swedish population is sufficiently active from a health point of view (Boström, 2001). Physical fitness can be improved during the whole life span. Physical activity in different forms is known to be beneficial for several physical functions; heart, lungs, colon, glucose homeostasis, and bone mineral density etc (Blair et al., 2001). Physical activity should preferentially put strong, fast dynamic strain to the skeleton in different directions in order to be beneficial to the skeleton and BMD (Frost, 2001; Lanyon, 1996). Examples

Table 1. Studies showing different effects of hormone replacement therapy on muscle strength.

<table>
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<tr>
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<th>Improves muscle strength</th>
<th>Preserves muscle strength</th>
<th>No influence on muscle strength</th>
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<td>Cauley JA et al, 1987</td>
<td>Chron Dis</td>
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<td>Kritz-Silverstein D et al, 1994</td>
<td>JBMR</td>
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<td>X</td>
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<tr>
<td>Preisinger E et al, 1995</td>
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<td>Seeley DG et al, 1995</td>
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<tr>
<td>Taaffe DR et al, 1995</td>
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<td>Brown J et al, 1997</td>
<td>Gerontol</td>
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The Influence of Muscle Strength and Physical Activity on Bone Mineral Density

Bone is adaptive and responds to the load-related strain, or deformation. This means that if the habitual loading increases, bone will adapt with an increase in BMD; whereas, when loading decreases BMD is reduced. This is stated in Wolff’s law. The mechanostat theory by Frost (Frost, 1992; Grimston, 1993), states that two thresholds or set points exists in a given bone; minimum strain for remodeling (MES$_r$), and the minimum effective strain for modeling (MES$_m$). In normal mechanical usage, the typical peak strains generated in bone will fall between the boundaries defined MES$_r$ and MES$_m$. When the strain is under MES, this will impair the properties of the bone until a new equilibrium has been reached. If the strain achieves a sufficient load the bone properties will change and a stronger bone will be achieved to prevent fatigue damage (Burr, 1993) (fig 3).

Athletes have higher BMD than sedentary controls, with the largest effect seen when physical activity includes load-bearing activities such as strength training or gymnastics with mechanical loading in different directions. The effect on BMD of endurance training such as running, cycling, cross-country skiing, and swimming is somewhat more contradictory. It is possible that the MES$_m$ of such events is not of such a magnitude that it will results in gain in BMD. Endurance trained athletes also often have low body weight and some also are hypogonadic, which might
explain the lack of skeletal response to endurance training (Nordström and Lorentzon, 2002).

There are several reports indicating a positive effect of physical activity on the growing skeleton (Bass et al., 1998; Blimkie et al., 1996; Cooper et al., 1995; Kannus et al., 1995; McKay et al., 2000; Morris et al., 1997). One of these studies reported a 2-4 time side difference in BMC between the dominant and non-dominant arm in young female tennis players if the training started before menarche as compared to after menarche (Kannus et al., 1995) (fig 1). The effect of physical activity on BMD diminishes with age and after puberty merely helps to maintain bone mass (Blimkie et al., 1996). Some studies show that if the young athlete changes his or her lifestyle and becomes sedentary the positive effect of the physical activity on the skeleton disappears after some decades. This has been shown for football players and weightlifters (Karlsson et al., 1996; Karlsson et al., 2000). Although there are numerous reports on muscle strength, physical activity and BMD the majority of these have not been performed on population based materials. There are four randomized controlled studies investigating the effect of physical activity interventions on prepubertal children (6-10 years) with a follow-up period of 7-10 months. The interventions in these were jumping, aerobics, weightlifting and school-gymnastics and the results showed positive correlations between the interventions and BMD (Bradney et al., 1998; Fuchs et al., 2001; McKay et al., 2000; Morris et al., 1997).

With age a lot of physical functions are reduced i.e. balance, visual function, muscle strength etc. One of the main advantages of persistent high physical activity in the elderly is that intact muscle strength and balance diminish the fear of falling and as a consequence thereby the falls themselves decrease. Muscle strength can increase up to 170% even in 70-80 year olds (Lexell et al., 1995). Bone mineral density can also be improved by high-intensity strength training in postmenopausal women but the major effects are seen on muscle strength, muscle mass, and balance (Nelson et al., 1994).
**Bone Densitometry and Quantitative Ultrasound**

Bone mineral density (g/cm$^2$) and BMC (g/cm) is measured by dual energy x-ray absorptiometry, DXA. This technique operates with an X-ray source using two energy peaks. The patient is examined in a supine position. A pencil or a fan beam is used to scan the patient. A pencil beam makes the scanning time longer but there is no major problem with image magnification. The radiation is reported to be 1-5 µSv and is considered low-risk for the patient. The accuracy error of DXA is reported to be 5-8% (Genant, 1996). In a clinical setting the precision error is approximately 1-2 % for repeated measurements.

Bone mineral density can be presented either as g/cm$^2$ or as T-scores i.e. comparison in SD to a sex-specific reference population of young adults (20-30, -40, -50 year-olds). Z-score is a SD comparison to a weight-adjusted sex- and age-specific reference population. The skeletal sites most often measured are the spine (L2-L4), various regions of the hip, heel, distal radius and total body. When the total body is measured, body composition is also attained including fat and lean body mass. Total body measurements provide data for body composition i.e. LBM and FM. Different T-scores in the same individual depends on skeletal sites, the equipment and reference material pose a problem in the clinic. The lowest value of the different skeletal sites is proposed by International Society for Clinical Densitometry (ISCD) to be used for diagnosis. More knowledge about the correlations of bone density at various regions is needed in order to eventually develop a Golden standard in clinical densitometry.

**Figure 4.** DXA-measurement of proximal femur bilaterally
In order to be able to compare DXA measurements for the total hip and lumbar spine derived from different manufacturers the standardized BMD value is used. This value is based on an equation compensating for differences between measurements from the Lunar, Hologic and Norland DXA equipments (Genant, 1996; Hanson, 1997). Standard BMD is expressed as mg/cm².

Ultrasound examines velocity, attenuation or reflection of ultrasound in peripheral bone. The results are presented as Broadband ultrasound absorptiometry (BUA), speed of sound (SOS) and Stiffness index (Achilles Express, Lunar) or Quantitative Ultrasound index (Sahara, Hologic). The advantage for QUS is that the equipment uses a non ionizing technique, is portable and the information attained provides information on the structural organization of bone in addition to BMD (Bouxsein and Radloff, 1997).

The ISCD has reached the following consensus regarding peripheral devices. “Peripheral devices are useful for assessment of fracture risk. However until device-specific cut-points are established it can not be applied in clinical practice to identify patients with osteopenia or osteoporosis”.

**Aims of the Study**

The thesis focuses on two main areas:

- The influence of muscle strength, and body composition on bone mineral density in men and women.
- The influence of estrogen on muscle strength in women.

The specific aims of this thesis were to investigate:

1) The influence of muscle strength, and body composition, on bone mineral density in Swedish adults. (paper I, IV, V, VI)

2) Whether estrogen status, in postmenopausal women, affects muscle strength (paper II)

3) The possible influence of genetic variations in the estrogen receptor alpha gene on muscle strength and lean body mass (paper III)

4) Bone mineral density values at different skeletal sites in young Swedish women compared with
international reference values (paper VI).

Subjects and Methods

Subjects

This thesis is based on different samples of subjects.

Paper I; a population-based randomly selected sample of 113 subjects, 60 women and 53 men (22-85 years), from the population register. Paper IV; population-based randomly selected adolescents from the Uppsala-region who previously had participated in a longitudinal study at 15 and 17 years of age were invited by mail when they were 21 years old and contacted by telephone. From the original 209 subjects a total number of 125 subjects, 64 women and 61 men, took part in the study. The participant rate was 60 %.

Paper II; postmenopausal women were invited by the local newspaper to take part in a randomized placebo controlled study on HRT. Forty women entered this study.

Paper III, V and VI; a population-based randomly selected sample of 685 subjects, aged 20-39 years, was recruited from the population register of Uppsala, 342 fulfilled the studies and a total of 335 were used in the analysis (Paper VI). A sub-group of 159 subjects took part in muscle measurements and of these 153 donated blood for genetic analyses (Paper V, III). The participant rate in these studies was 50 %.

Bone Densitometry and Quantitative Ultrasound

Paper I, IV; DXA was performed to measure TBMD (Paper I-IV), LSBMD (paper I), and FNBMD (paper I) and expressed as BMD (g/cm²) and content BMC, (g/cm). DXA measurements of the total body also measured LBM (g) and FM (g). The DXA used was a DPX-L™, Lunar Co., Madison WI, USA (Mazess, 1995). The long term precision error for a spine phantom expressed as the coefficient of variation was less than 1% for L2-L4 BMD during the study. Quantitative Ultrasound was used in Paper I, V and VI. An Achilles™, Lunar Co., Madison, WE, USA was used in Paper I.

Paper V and VI; TBMD, LSBMD, and bilateral THBMD were measured using a DPX-IQ, Lunar CO, Madison
WI, USA. The precision error for a spine phantom expressed as the coefficient of variation was less than 0.5% for the L2-L4 BMD during the study period. BMD in the heel (Paper V, VI) was measured by a PIXI Lunar CO, Madison WI, USA. Ultrasound of the heel was performed with an Achilles Express Lunar CO, Madison WI, USA, and a Sahara Hologic Inc, Bedford MA, USA.

In paper I left FNBMD and Stiffness index were used in the analysis. In paper V left THBMD, HBMD, Stiffness index, and QUI were used in the analysis, since it is common to use the non-dominant side.

**Body Composition**

Height and weight were assessed at a clinical examination by Harpender stadiometer (Holtain Ltd., Crosswell, Wales, U.K.) and digital scale (EKS International AB, Sweden) prior to the DXA scanning. Body mass index was calculated by the formula (body weight) / (body height)^2. Lean body mass and FM was measured by DXA and presented as g (see Bone densitometry and Quantitative Ultrasound).

**Muscle Strength**

In this thesis we studied muscle strength using handgrip and knee flexion and extension. These muscle groups were chosen due to their close relations to clinical relevant fracture sights such as the hip and distal forearm.

Handgrip strength was measured by a JAMAR hydraulic hand dynamometer (5030J1), Jackson, MI, USA, and the results were expressed in kilograms. The measurements were made with the subjects sitting and with the arm in a comfortable position (Spijkerman et al., 1991). Both hands were tested in all studies and the best result for each hand was used in the analysis.

Isometric quadriceps muscle strength (expressed in kilograms) was measured using a muscle-force-testing chair (Rodby Elektronik AB, Sweden). The measurement was made in a sitting position with the hips fixed to the seat and the ankle in a nonelastic band connected to a strain-gauge device. Isokinetic knee-flexion and extension strength was measured by a Cybex II dynamometer (Lumex Corp., Bay Shore, N.Y.) with a modified lever arm (Aero Technical Corp., Stockholm,
devised by Knutsson and Litton) and attached to an examination table (Alfex). The subjects were strapped in a sitting position with adjustable belts around the thigh and chest. The axis of the lever arm of the dynamometer was adjusted according to the axis of the knee-joint. The range of motion measured was between full extension (zero degrees) and 90 degrees of knee flexion and back to full extension. The angular velocity used was 90 degrees/second. The best result of three consecutive maximal isometric contractions in the right leg was used.

In paper IV the mean value of the left and right side was used in the analysis. In paper V left knee flexion, extension and handgrip strength values were used in the analysis.

Physical Activity

The level of physical activity was recorded by three different questionnaires. The first, used in paper I, contained parts from the MEDOS Study Questionnaire and was used to assess physical activity during childhood, youth and adulthood (Dequeker et al., 1991). The second, used in paper III, V, VI, included questions regarding present and previous physical activity, but also things like age of menarche, pregnancies and number of children, diseases, fractures, medications, food intake etc. The subjects were asked to indicate which form of activity or sports they performed, the duration and frequency per month and how many months per year they performed exercise (Pettersson et al., 2000). The type of activity was classified as low and medium/high strain, where low strain included walking, bicycling, cross-country skiing and swimming and the other activities were considered as medium/high strain. The third questionnaire, used in paper II, measured physical activity habits by a classification system of physical activity composed of a six-grade scale. This classification system has previously been used and evaluated in elderly (Mattisson-Nilo et al., 1990). In the six-grade scale number one indicates almost no physical activity at all and number six extraneous or very extraneous physical activities regularly several times a week, during which physical exertion is great (Study IV).
Detection of Gene Polymorphisms

Paper V; Genomic DNA from each individual was extracted from leukocytes from 3 ml EDTA blood using a Wizard Genomic DNA purification kit (Promega, Madison, WI, USA), typically yielding approximately 50-75 µg of genomic DNA/sample. The polymorphism analyzed in the gene for the ERα is situated in the promoter region and constitutes a TA-repeat. The TA-repeat was amplified by polymerase chain reaction (PCR), using fluorescently labeled forward primers (Thermo Hybaid, Interactive Division, Ulm, Germany) and unlabelled reverse primers. The PCR reactions were run on an ABI-877 Integrated Thermal cycles PCR robot (Applied Biosystems, Warrington, UK) using 0.1 µg genomic DNA, Ampli-Taq Gold kits and standard reagents. The amplification procedure was carried out according to the following protocol: denaturation for 10 min at 95 °C, 36 cycles of denaturation for 30 s at 95 °C, a touchdown annealing at 57-59 °C for 30 s and extension at 72 °C for 1 min and finally an extension step at 72 °C for 10 min.

The gene fragments were separated by electrophoresis on a 6% polyacrylamide gel using a 96-well ABI 377 automated sequencer Gel data and allele sizes were analyzed using the software programs Genescan Analysis 3.1 and Genotyper 2.0 (All Applied Biosystems, Warrington, UK).

Statistics

The Statview SE+Graphics software package (Abacus Concepts, Berkeley, CA, USA and Statistica 6.0, Statsoft Scandinavia AB, was used for statistical analysis. Shapiro-Wilk’s test were used for normality testing. Simple, stepwise and multivariate regression was used to assess the coefficient of determination (r²). Students paired and unpaired t-test was used to assess differences in muscle strength on an individual basis and between the group means. Significance level used was p<0.05.
Results

Influence of Muscle Strength and Body Composition on Bone Mineral Density

Three population-based randomly selected samples, with different age-distributions, were gathered to study the influence of muscle strength on BMD in Swedish adults (I, IV, V).

Isometric quadriceps muscle strength was correlated to TBMD, LSBMD, and FNBMD in premenopausal women and to TBMD in men. No association was seen in postmenopausal women (Paper I, Table 1). In stepwise regression knee flexion and extension were the only predictors ($R^2=0.27$) for TBMD in young women whereas in men LBM, FM, weight, and height predicted ($R^2=0.43$) TBMD (Paper IV, Table 2). This indicates that muscle strength at weight bearing sites is related to TBMD in women whereas body composition is related to TBMD in men. The impact of lower limb strength on TBMD was only found in young women indicating a gender difference.

Study V was an investigation of similar design as study IV measuring BMD in several skeletal sites and an extended age-group of women aged 20-39 years. In this cohort as well muscle strength was a good predictor of BMD at different skeletal sites with the exception of LSBMD, although, LBM and BW had higher coefficient of determination (Paper IV, Table 2). In multivariate analysis including LBM or weight, the effect of muscle strength diminished (Paper IV, Table 3). Body weight in this sample was higher compared to study IV, $p=0.012$. However, not only were the body weight and BMI higher, the participants also were 10 years older (Table 2). In paper VI LBM was again was the best predictor of BMD of all body composition variables used.

Influence of Estrogen on Muscle Strength

Paper II; A placebo-controlled double blind longitudinal study was performed to study the effect of six-months of HRT on muscle strength in knee flexion, extension, handgrip, and physical activity. Forty volunteers were gathered and divided into two groups, one receiving placebo treatment and the other HRT.
Handgrip strength in the right hand, increased significantly in both groups (HRT p<0.001 and placebo p<0.01) and in the left hand in the HRT group (p<0.01). However, there were no differences in muscle strength between the two groups. There was no significant change in isokinetic knee flexion or extension after six months in either of the groups. The estimated physical activity increased slightly in the placebo group, but there were no significant differences compared to the women who received HRT. Our data show that six months of HRT does not influence muscle strength in postmenopausal women.

**Table 2.** Comparison between muscle strength and body composition in women aged 21 years and 20-39 years.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women 21 years</th>
<th>Women 20-39 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.2 (9.3)</td>
<td>66.1 (10.7) **</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.8 (6.5)</td>
<td>167.9 (5.7)</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>22.1 (2.8)</td>
<td>23.4 (3.4) **</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>13.8 (7.8)</td>
<td>21.0 (7.7) ***</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>45.7 (4.6) ***</td>
<td>41.8 (4.7)</td>
</tr>
<tr>
<td>Knee flexion (Nm)</td>
<td>61.9 (17.2)</td>
<td>60.3 (12.0)</td>
</tr>
<tr>
<td>Knee extension (Nm)</td>
<td>114.6 (24.8)</td>
<td>118.2 (19.8)</td>
</tr>
<tr>
<td>Handgrip (Kg)</td>
<td>32.5 (4.1) ***</td>
<td>32.5 (5.1)</td>
</tr>
</tbody>
</table>

**  p<0.01  
***  p<0.001

Handgrip strength in the right hand, increased significantly in both groups (HRT p<0.001 and placebo p<0.01) and in the left hand in the HRT group (p<0.01). However, there were no differences in muscle strength between the two groups. There was no significant change in isokinetic knee flexion or extension after six months in either of the groups. The estimated physical activity increased slightly in the placebo group, but there were no significant differences compared to the women who received HRT. Our data show that six months of HRT does not influence muscle strength in postmenopausal women.

In Paper III the allelic variation in the gene for ERα, was studied in relation to muscle strength in knee flexion and extension, handgrip, and body composition. This study showed no associations between allelic variations in the gene for ERα and muscle strength or body composition in young adult Swedish women.

**Bone Mineral Density in Young Adult Swedish Women**

All mean BMD values, expressed as T-scores, at all skeletal sites measured were significantly higher than the reference delivered by the manufacturers, but differed between different skeletal sites. T-score for the total body 1.16 was (1.07-1.25), lumbar spine 0.32 (0.20-0.44), total hip 0.59 (0.48-0.70), heel BMD 0.11 (0.00-0.22). When comparing the BMD values with the NHANES-III reference for total hip
For Stiffness index and QUI the T-score values were approximately the same as for the manufacturer derived references (-0.06 and -0.08) (Figure 5). Using this cohort as its own reference the numbers of subjects diagnosed with osteoporosis on the hip or spine did not differ whereas the numbers of osteopenic subjects was significantly increased.

The correlations for BMD and QUS measures varied between r=0.30 (LSBMD and QUI) and r=0.92 (total hip and FNBMD).

### Table 3. Influence of allelic variations in the gene for Estrogen receptor alpha on muscle strength and body composition.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ERα genotype</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ee n=47</td>
<td>eE n=73</td>
</tr>
<tr>
<td>Grip strength (Kg)</td>
<td>32.6±4.8</td>
<td>33.8±4.9</td>
</tr>
<tr>
<td>Quadriceps (Nm)</td>
<td>115.9±18.0</td>
<td>117.4±21.6</td>
</tr>
<tr>
<td>Hamstrings (Nm)</td>
<td>56.5±10.9</td>
<td>58.3±12.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.6±15.4</td>
<td>66.0±10.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.1±5.6</td>
<td>168.0±5.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2±5.3</td>
<td>23.3±3.2</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>21.5±7.1</td>
<td>20.7±7.4</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>41.2±4.1</td>
<td>41.9±4.8</td>
</tr>
</tbody>
</table>

BMD the values were 0.5 SD higher (p<0.001). For Stiffness index and QUI the T-score values were approximately the same as for the manufacturer derived references (-0.06 and -0.08) (Figure 5). Using this cohort as its own reference the numbers of subjects diagnosed with osteoporosis on the hip or spine did not differ whereas the numbers of osteopenic subjects was significantly increased.

Data in the literature suggests an important role for physical activity in order to prevent fragility fractures. However, it has been difficult to clearly demonstrate this in an evidence based manner (SBU, 2003a). This in contrast to the results from large clinical trials with bone specific drugs that convincingly have shown fracture reduction (SBU, 2003b). It is therefore

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**General Discussion**

Data in the literature suggests an important role for physical activity in order to prevent fragility fractures. However, it has been difficult to clearly demonstrate this in an evidence based manner (SBU, 2003a). This in contrast to the results from large clinical trials with bone specific drugs that convincingly have shown fracture reduction (SBU, 2003b). It is therefore
important to further investigate the precise role of physical activity and muscle strength on BMD. Not only to define a basis for primary prevention in the society but also to establish an unquestionable role for non-drug intervention in the treatment of manifest osteoporosis. This thesis focuses on the relation between muscle strength and BMD, and on the role of estrogen as a putative regulator of muscle strength. The data gathered during the project also provides descriptive data on BMD and body composition in a sample of young adult Swedish women.

Muscle Strength and Bone Mineral Density

The results from the studies in paper I, IV, and V show a correlation between isometric quadriceps muscle strength and TBMD, LSBMD, and FNBMD in pre-menopausal but not in post-menopausal women. In 21 year old women there was an association between isokinetic knee muscle strength and TBMD. This association was not seen in 21 year old men. When investigating women in their 20-30ths it became evident that in this age group LBM and BW are better predictors for BMD at all skeletal sites, as compared

Figure 5. T-score at different anatomical sites compared to manufacturers reference population and NHANES.
to muscle strength. We therefore conclude that in young women at, and probably below, the age of 21 BMD is correlated to muscle strength while for 20-39 year old women it is body composition rather than muscle strength that predicts BMD. One explanation for this difference might be that the body weight was higher in the older group, and that the younger subjects therefore might compensate the difference in body weight with muscle strength, putting a higher strain to the skeleton (Rubin and Lanyon, 1984). This could also explain the difference between the 21-year-old men and women. The boys were stronger, had higher BMD, and were heavier than the women, and perhaps the maximal skeletal response to strain already had been reached by the influence of body weight. In support of this view it has been shown that there is a correlation between muscle strength and BMD in more sedentary youngster than highly active sportsmen/women (Nordström et al., 1996; Sandström et al., 2000). The consequence of this reasoning is that physical activity which puts the skeleton under strain without loss in body weight would give the optimal bone gaining effect. All-round training like school gymnastics could fill this criterion if performed regularly several times a week. This would also give the opportunity for young people to learn that physical activity should be part of the daily routine, which might result in a less sedentary lifestyle in the long run.

Osteoporosis often begins after menopause and the patients are obviously in different physical shape. Some are very physically active and some are not. For very physically fit women with osteoporosis the effect of exercise could not be expected to achieve very much benefit for the skeleton, but recommendations regarding the connection between low body weight and osteoporosis might be suitable. Perhaps changing the exercise from endurance training to strength training could give an increase in lean body mass. This would be beneficial for the skeleton in two ways, by increasing the strain put on the skeleton and by increasing lean body weight. To the untrained, sedentary living women, the effect of physical activity might be considerable. This assumption lead to a postulated training regime focusing on strength, balance, and to a lesser extent
endurance in order to help the patient avoid falls and thereby fractures.

**Estrogen and Muscle Strength**

Hormone replacement therapy increases BMD in postmenopausal women, but the data concerning effects on muscle strength is more conflicting. Data in paper II show no influence of HRT on muscle strength measured as handgrip, isokinetic knee flexion and extension. The suggestion that HRT does not influence muscle strength is supported by several studies (Armstrong et al., 1996; Bemben and Langdon, 2002; Brown et al., 1997; Kritz-Silverstein and Barrett-Connor, 1994; Preisinger et al., 1995; Seeley et al., 1995; Taaffe et al., 1995). Other studies, however, have shown an increase or preservation of the muscle strength by HRT (Greeves et al., 1999; Heikkinen et al., 1997; Philips et al., 1993; Skelton et al., 1999). Both studies indicating an increase in muscle strength and one showing a preservation of muscle strength were placebo-controlled trials, with duration between 9-24 months. Two studies indication no effect were also placebo-controlled trials, with duration of 11 months. In the Cochrane database only the studies by Heikkinen, Skelton and Armstrong fulfilled the criterion to be evidence-based. These studies had 78-116 subjects and duration between 11-24 months. Our study included 40 women and the duration was 6 months. Our data add to the view in the literature that estrogen replacement does not enhance muscle strength in postmenopausal women.

Association studies between allelic variations in candidate genes and various diseases is a rapidly expanding field of research. Surprisingly, very little has been done concerning the genetic background of muscle strength and lean body mass. There are conflicting data regarding the presence of estrogen receptors in muscle tissue (Meeuwsen et al., 2000). Therefore, again, this is a line of investigation that is important to pursue in order to eventually gain reliable knowledge. Our study, paper III, showed no correlation between allelic variations in the estrogen receptor alpha and muscle strength. This is supported by similar findings that baseline, or 5-year grip strength, values were not influence by estrogen receptor alpha genotype in early postmenopausal women (Salmén
et al., 2002). Studies using well defined clinical cohorts, with respect to body composition, will eventually discover the genetic influence on muscle strength. Presumably, this will be a rapidly expanding field of research, and future findings will enable us to identify the polygenic influence on muscle strength, and perhaps also genetic aspects on the response to training.

**Bone Mineral Density in Swedish Women**

In paper VI the T-score values were significantly higher for Swedish women than the references at all skeletal sites. This was true also for the two other cohorts gathered in this thesis, indicating that Swedish women living in the Uppsala region have a higher BMD as compared to the reference values used. Regarding previous reports in the literature these cohorts present a higher BMD also compared to other Swedish cohorts (Karlsson et al., 1993; Löfman et al., 1997). The use of the BMD material gathered from women age 20-39 as the basis for a Swedish t-score would lead to an increase in postmenopausal women diagnosed as having osteoporosis. It is beyond the scope of this thesis to discuss the clinical routine in the treatment of osteoporosis. Still, the data presented might be used in a further discussion regarding diagnostic procedures, whether we should use reference materials that reflect the ethnical region or comply with the present concept of international standards, based primarily on reference materials from USA.

The incidence of osteoporotic fractures in Swedish women is one of the highest in the world. The question however, after considering the data in this thesis, is how the BMD values in old Swedish women compare with data from countries with fewer fractures? Perhaps Swedish women, contrary to the present believe, do not have lower BMD than women from other countries. Maybe they fracture at a higher BMD value than other. If future studies confirm our data that Swedish women does not have more osteoporosis, rather less. This would imply that other risk factors, such as risk of falling, cause the relatively high incidence of fractures in elderly Swedish women. This is of importance since these might be risk factors that
are suitable to counteract with physical activity and fall prevention.

**Summary and Conclusion**

1. Isokinetic muscle strength correlates with bone mineral density in 21-year old women, but not in men where lean body mass and body composition correlate with bone mineral density. Isometric quadriceps muscle strength correlates to BMD in premenopausal women but not in postmenopausal women. In women aged 20-39, LBM has the highest coefficient of determination of bone mineral density.

2. HRT in postmenopausal women does not have an effect on muscle strength as compared with placebo-treatment. Furthermore, there is no relationship between polymorphisms in the gene for the estrogen receptor alpha and muscle strength at the age of peak bone mass.

2. BMD T-score values are dependent on the skeletal site that is measured. Women living in Uppsala have higher BMD values as compared to the manufacturers’ reference populations. In the total hip this difference was +0.5 SD compared to NHANES.
<table>
<thead>
<tr>
<th>Paper I</th>
<th>Cross-sectional study</th>
<th>Population-based Subjects: n=113 60 ♀ and 53 ♂ 22-85 years</th>
<th>Methods: Paper I</th>
<th>Results: Paper I Isometric quadriceps strength correlates to TBMD in both ♀ and ♂ and to LBBMD in ♂. Isometric quadriceps muscle strength correlates to TBMD, LBBMD, FNBMD in pre- but not postmenopausal ♀. Isometric quadriceps strength correlates to ultrasound heel in both ♀ and ♂ aged 41-60 years.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper II</td>
<td>Longitudinal study Volunteers Subjects: n=40 Postmenopausal ♀ 60-78 years</td>
<td>Methods: Paper II Isokinetic knee flexion and extension muscle strength Handgrip strength Physical activity scoring</td>
<td>Results: Paper II Six months of hormone replacement therapy does not influence muscle strength in postmenopausal ♀</td>
<td></td>
</tr>
<tr>
<td>Paper III, V, VI</td>
<td>Cross-sectional studies Population-based Subjects: n=153 (P III) Subjects: n=159 (P V) Subjects: n=335 (P VI) Premenopausal ♀ 20-39 years</td>
<td>Methods: Paper III, V, VI DXA total body, lumbar spine, total hip, heel Ultrasound heel Isokinetic knee flexion and extension muscle strength (PIII, V) Handgrip strength (PIII, V) PCR (P III)</td>
<td>Results: Paper III Allelic variations in the human ERa gene do not correlate with muscle strength or body composition in young adult Swedish ♀. Results: Paper V In young adult Swedish ♀ the influence of muscle strength on BMD almost disappears when including LBM or BW in the same analysis. Results: Paper VI This cohort of Swedish women has a higher t-score BMD at all measured skeletal site, but SI and QUI were somewhat lower than reference values. Correlations between T-score BMD at different skeletal sites differ (R=0.3-0.92). When mean value from this cohort was used as t-score, the number of osteopenic ♀ was doubled, but not osteoporotic ♂, which remained constant.</td>
<td></td>
</tr>
<tr>
<td>Paper IV</td>
<td>Cross-sectional study Population-based Subjects: n=125 64 ♀ and 61 ♂ 21 years</td>
<td>Methods: Paper IV DXA total body Isokinetic knee flexion and extension muscle strength Handgrip strength</td>
<td>Results: Paper IV Muscle strength at weight-bearing sites is related to TBMD in ♀, whereas body composition is related to TBMD in ♂.</td>
<td></td>
</tr>
</tbody>
</table>

**Thesis at a Glance**
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Illustration: “Spökskelett” by Johan Ljunggren
References


Heikkinen, J., Kyllönen, E., Kurttila-Matemo, E., Wëlén-Rosenqvist, G.,


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine*. (Prior to October, 1985, the series was published under the title “Abstracts of Uppsala Dissertations from the Faculty of Medicine”.)