JOONAS SIROLA

Early Postmenopausal Bone Loss

Population-Based Studies on Risk and Preventive Factors and Their Interactions

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium, Mediteknia building, University of Kuopio, on Saturday 13th of September 2003, at 12 noon

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University of Kuopio

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KUOPION YLIOPISTO
KUOPIO 2003
ISSN 1235-0303

ABSTRACT

Background: The transition to menopause has been proposed to be the most important determinant of bone loss in middle-aged women. However, some other factors related to lifestyle and nutrition may modify this loss. The aim of the present study was to investigate these risk and preventive factors and their interactions in early postmenopausal bone loss.

Subjects and methods: The study subjects were selected from the Osteoporosis Risk Factor and Prevention (OSTPRE) Study cohort (13 100 women born in 1932-41). It included 2025 randomly selected women measured with DXA absorptiometry (hip and spine) at baseline (1989-91), 5-year (1994-97) and 10-year follow-up (1999-2002). Postal inquiries querying risk and preventive factors for osteoporosis were sent to these women at baseline and at the two successive follow-ups. The rate of bone loss was studied between the baseline and five years as well as between five year and ten year measurements. The final number of study subjects ranged from 409 (Study I) to 954 (Study IV).

Results: Women in the early stage of menopausal transition were found to have the most accelerated bone loss. In later postmenopause, the bone loss continued at a significantly lower rate. There were differences in the pattern of the bone loss rate between spinal and femoral areas. With the exception of the menopausal transition itself, fatness and weight increase predicted significantly lower bone loss rate in early postmenopausal population in addition to hormone replacement therapy (HRT). Furthermore, weight loss related bone loss was prevented by HRT. These effects were more clearly observed in lumbar spine than in femoral neck.

In HRT users, nutritional calcium seemed to prevent bone loss whereas this was not observed in HRT non-users. Also, in women with low calcium intake HRT did not prevent bone loss. These effects were more readily seen in femoral neck than in lumbar spine. In addition, nutritional calcium intake did not predict bone loss in postmenopausal smokers whereas calcium intake positively predicted bone loss in non-smokers. However, there were no differences in the bone loss rate between smokers and non-smokers regardless of nutritional calcium intake status.

In early postmenopausal statin users, the bone loss rate was not different from non-users of statins and did not depend on normal or high blood cholesterol levels.

Conclusions: Menopausal transition is the most important determinant of bone loss in middle aged women. During menopausal transition, significant weight loss should be avoided although HRT may prevent the weight loss related bone loss. In addition, adequate nutritional calcium should be encouraged also for women on HRT. Furthermore, smoking may impair the bone protective effects of nutritional calcium. Finally, statins, as such, should not be considered as a therapeutic strategy for prevention of postmenopausal bone loss.

National Library of Medicine Classification: WE 250, WE 200

Medical Subject Headings: osteoporosis, postmenopausal; bone density; menopause; densitometry, X-ray; estrogen replacement therapy; calcium/therapeutic use; body weight; weight loss; smoking; hydroxymethylglutaric-CoA reductase inhibitors; femur neck; lumbar vertebrae; risk factors; questionnaires; epidemiologic studies; follow-up studies; female; Finland
Murphy once said:

“Beauty is skin deep; ugly goes right to the bone“
ACKNOWLEDGEMENTS

The present doctoral thesis study was carried out in Department of Surgery, Kuopio University Hospital and Research Institute of Public Health, University of Kuopio and was part of the Kuopio Osteoporosis Risk Factor and Prevention (OSTPRE) – study.

I owe my most sincere thanks to my principal supervisor Professor Heikki Kröger, M.D., for introducing me to the world of medical research and for getting me started. His work as a supervisor has certainly sharpened my enthusiasm to pursue future research attached to clinical work. His role in making this thesis possible is indispensable and without him this thesis would never have been completed on time.

My other supervisor Docent Risto Honkanen, M.D., has offered me his endless support in combating the challenges of epidemiological methodology. His encouraging attitude combined with his extensive experience in the field of epidemiological research has guaranteed the completion of this thesis. It has been an honour to be supervised by a true veteran of epidemiological research.

I truly appreciate the roles of Docent Markku Hakala, M.D., and Docent Markku Heliövaara, M.D., in reviewing my thesis. Your comments were essential and most helpful for completion of this work.

I am in gratitude to Docent Marjo Tuppurainen, M.D., for her advice during this study. Also, I sincerely thank Lorenzo Sandini, M.D., for all the support he provided, especially with regards statistics. You both have very unique personalities. I also appreciate the help of Professor Pekka Mäenpää, Ph.D.

The present work was built on well performed bone densitometry. For organising the DXA measurements and related comments during this study, I sincerely thank Docent Jukka Jurvelin, Ph.D. In addition, the skills of whole measurement staff were essential. Thank you.

It has been a privilege to work in a study where there has been so much extensive background work already done. For creating the OSTPRE study, and for the possibility to
participate in the present study, I am most grateful to Professor Esko Alhava, M.D., and Professor Seppo Saarikoski, M.D.

No study of this kind would have ever been completed without the skills of the OSTPRE secretary Seija Oinonen. I appreciate all the work you have done during my study.

Corrections in language were essential for completion of the present work. For improving the English of my dissertation I thank Ewen MacDonald. In addition, Richard Burton made essential corrections in the manuscripts.

I wish to thank all the co-researchers of OSTPRE study for their participation during this study.

Finally, I owe my deepest thanks to my wife, Helena, for her irreplaceable support and love during the years when I have been working on my thesis and the way you have balanced my life during the last years. I also express my gratitude to my family and friends for all their time and understanding.

This work has been supported financially by the Finnish Medical Society Duodecim, Research Foundation of Orion Corporation, The Fund for Doctoral Dissertation of University of Kuopio, Kuopio University Foundation, The Finnish Cultural Foundation of Northern Savo and the Academy of Finland.

Kuopio 3.9.2003,

Joonas Sirola
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>BMC</td>
<td>Bone mineral content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BMP-2</td>
<td>Bone morphogenic protein 2</td>
</tr>
<tr>
<td>BUA</td>
<td>Broadway ultrasound attenuation</td>
</tr>
<tr>
<td>CDA</td>
<td>Computed digital absorptiometry</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>DPA</td>
<td>Dual photon absorptiometry</td>
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<tr>
<td>DXA</td>
<td>Dual x-ray absorptiometry</td>
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<tr>
<td>DXR</td>
<td>Digital x-ray radiogrammetry</td>
</tr>
<tr>
<td>ERT</td>
<td>Estrogen replacement therapy</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>Hydroxymethylglutaryl coenzyme A</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>pDXA</td>
<td>Peripheral dual x-ray absorptiometry</td>
</tr>
<tr>
<td>pQCT</td>
<td>Peripheral quantitative computed tomography</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>QUS</td>
<td>Quantitative ultrasound</td>
</tr>
<tr>
<td>RA</td>
<td>Radiographic absorptiometry</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SERM</td>
<td>Selective estrogen receptor modulator</td>
</tr>
<tr>
<td>SOS</td>
<td>Speed of sound / ultrasound velocity</td>
</tr>
<tr>
<td>SPA</td>
<td>Single photon absorptiometry</td>
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<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
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1 INTRODUCTION

Postmenopausal osteoporosis was first described in 1940 but it was not until 1965 that the WHO and NIH conducted a worldwide epidemiological study giving recognition to the seriousness of the problem and in 1984 NIH publicised this disease (NIH 1984). No proper preventive methods were applied prior to the recognition of this “silent bone thief”. However, the consequences soon became apparent. Presently osteoporosis affects approximately 30% of the postmenopausal population and the number of hip fractures world-wide is expected to rise by over 200 percent by the year 2050 in the female population (Kanis et al. 1994, WHO 1994, Gullberg et al. 1997) leading to high morbidity and mortality (Lindsay 1992, Cooper and Melton III 1996).

Several studies have investigated risk and preventive factors for osteoporosis. However, most of these studies have been either cross-sectional or retrospective in design which makes them prone to biases and lack the possibility to draw valid conclusions about causality. Therefore, there is a need for long-term prospective population-based studies. Presently, there are few of these kinds of studies published in the literature.

Menopausal transition has been considered as the most important inevitable determinant of bone loss in middle-aged women (Hansen et al. 1991, Pouilles et al. 1993, Kröger et al. 1994, Ensrud et al. 1995). However, the detailed patterns of bone loss around menopause have not been adequately studied. In addition, there is much debate about other factors contributing to this loss. Role of only a few factors is well established, such as relationship of fatness and weight loss with high BMD and greater bone loss, respectively (Burger et al. 1998, Hannan et al. 2000). On the other hand, although calcium supplementation is generally known to protect against bone loss the role of nutritional calcium is not as fully understood (Cumming 1990, Feskanich et al. 1997, Heaney 1997, Kanis 1999). Furthermore, the role of life-style related risk factors, such as smoking, are not well recognized. Accordingly, the core of most important factors determining bone loss rate awaits resolution. In addition, interactions of calcium and body weight with HRT and smoking have been suggested but their contribution to postmenopausal bone loss rate
at the population level is not known (Nieves et al. 1998, Hermann 2000, Komulainen et al. 2000).

Hormone replacement therapy (HRT) has been demonstrated to be effective in the treatment and prevention of postmenopausal osteoporosis (Lindsay and Tohme 1990, Cauley et al. 1995, Schneider et al. 1997). Interestingly, some factors, such as genetic polymorphism, low body weight and calcium intake, have been suggested to modify its effect on bone metabolism and it has been found, that there is a variance in responsiveness to HRT between certain groups (Hassager et al. 1994, Nieves 1998, Komulainen et al. 2000, Salmen et al. 2000). Accordingly, it would be important to identify those risk factors for increased bone loss that could influence the magnitude of bone response to HRT in order to achieve effective prevention of postmenopausal osteoporosis in as many women as possible. These effects have not been studied prospectively at the population level.

In addition to HRT, the current management of osteoporosis includes other antiresorptive (osteoclast inhibiting) agents, most importantly bisphosphonates (Reginster et al. 2000). Although these have been found relatively effective, there is a need for an osteoblast stimulating drug. If developed, this kind of drug would revolutionise the treatment of osteoporosis. Promisingly, statins have been suggested to stimulate bone formation (Mundy et al. 1999). However, this has not been confirmed in all studies (van Staa et al. 2001, Reid et al. 2001). Furthermore, their effects at the population level have not been adequately estimated and, thus, their true effect has not been established.

The aim of the present study was to investigate the role of selected risk and preventive factors in early postmenopausal bone loss with special reference to interactive effects as well as examining whether statins could prevent postmenopausal bone loss.
2 REVIEW OF THE LITERATURE

2.1 Definition, classification and etiology of osteoporosis

Osteoporosis has been defined as a ‘systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk’ (Anon. 1993). This definition draws attention to processes that contribute to the maintenance of ideal bone quality. Accordingly, at the cellular level the maintenance of bone mass requires a balanced equilibrium between bone formation, i.e. osteoblast activity, and bone resorption, i.e. osteoclast activity (coupling). Factors interfering with this balance (uncoupling) are responsible for the subsequent deterioration of bone structure. The combination of catabolic bone tissue changes with sufficient trauma energy, such as encountered in falls, leads to fractures and makes the disease clinically relevant (established osteoporosis).

Osteoporosis is classified into primary and secondary osteoporosis. The secondary form is associated with several medical disorders, such as chronic diseases and some medications. Primary, or idiopathic, osteoporosis can be further divided into type I (postmenopausal osteoporosis) and type II (senile osteoporosis). Type I affects mainly trabecular bone and is associated with hormonal alterations at menopause (involutional osteoporosis), but is not typically manifest until 15-20 years after the menopausal transition (Melton and Riggs 1988). Type II affects both cortical and trabecular bone and is typical in both sexes over 75 years of age (Melton and Riggs 1988). Juvenile idiopathic osteoporosis and idiopathic osteoporosis in young adults represent rare forms of primary osteoporosis.

Osteoporosis has a multifactorial etiology. The peak bone mass achieved in early adulthood represents a starting point after which bone loss and development of the disease are determined by several factors related to gender, race and life-style (Figure 1).
Figure 1 The multifactorial etiology of osteoporosis after peak bone mass acquisition.

2.2 Epidemiology and epidemiological studies on osteoporosis

The prevalence of osteoporosis increases with advancing age and is greater among the female population. It has been estimated that 15% of the 50 year old women, 30% of the 70 year old women and 40% of the 80 year old women may have the disease and 54% of the postmenopausal population are at increased risk (Kanis et al. 1994, WHO 1994). This proportion is likely to represent a significantly greater number of women at risk in the next decade since the number of elderly individuals is on the increase (Cooper et al. 1992).
Low BMD is a strong predictor of an osteoporotic fracture (Seeley et al. 1991, Cummings et al. 1993, Kröger et al. 1995). The estimated lifetime risk for an osteoporotic fracture in a 50 year woman is about 40% (Melton III et al. 1992). Also, postmenopausal women with an osteoporotic fracture are at a many-fold risk for developing an additional fracture within the next year (Lindsay et al. 2001). However, there is a major geographical variation in the incidence of osteoporotic fractures (Kanis 1993, Lauderdale et al. 1998) and also postmenopausal bone loss rate may vary according to ethnicity (Luckey et al. 1996).

Osteoporosis is a major cause of mortality and morbidity in the elderly population (Lindsay 1992, Koval et al. 1998). Some studies have reported mortality rates of hospitalised hip fracture patients between 4%-11.5% and these figures have remained high several years after the fracture has happened (Nettleman et al. 1996, Levi 1996). As many as half of all hip fracture patients may not recover to their functional pre-fracture level during the first year (Koval et al. 1998). Additionally, pain and disability due to vertebral and wrist fractures further increases the burden of osteoporosis.

Osteoporosis is a substantial factor accounting for increased medical care costs (Lindsay 1992). In the USA, the estimated cost of fractures attributable to osteoporosis in 1995 was $13.8 billion (Ray et al. 1997). In Finland, health authorities expect to see a 38% increase in hip fracture incidence between 1988 and 2010 with a subsequent 71% increase in the hospital bed-days to treat these patients (Simonen 1988). It has been estimated that the costs due to hip fracture treatment will triple between 1990 and 2040 (Schneider and Guralnik 1990).

Prospective epidemiological studies on osteoporosis have two principal targets: the evaluation of the progress and outcomes of the disease. Firstly, by investigating the fractures (established osteoporosis) it is possible to prevent the disease becoming clinically relevant. In this scheme there are multiple secondary factors involved, most importantly falls, in addition to osteoporosis itself (Honkanen et al. 1983, Kannus et al. 2000). Secondly, the investigation on BMD, or more importantly BMD change, enables one to evaluate the factors that lead to the phase of manifest disease with increased
susceptibility for fractures. This view gives more specific insight to the primary etiology of osteoporosis.

There are very few population-based epidemiological studies on osteoporosis, especially those of prospective nature (Honkanen et al. 1991, Hofman et al. 1991, Hannan et al. 1992, Nguyen et al. 1993, Dargent-Molina et al. 1996, Mosekilde et al. 1999) (Table 1). A major part of current information on risk- and preventive factors is the product of cross-sectional or retrospective investigations. However, the advantages of prospective population-based study setting are indisputable. The possibility to follow BMD changes in a controlled cohort gives a more dynamic perspective of the factors influencing the progress of osteoporosis and more advanced control on confounding factors in comparison to cross-sectional or retrospective studies. Accordingly, it has been suggested that there is incongruence in the magnitude of BMD changes between cross-sectional and longitudinal data although variance between distinct skeletal sites may exist (Warming et al. 2002).

Controlled randomized trials form the basis for evidence-based and outcomes-based practise recommendations. Although no population-based cohort study could defeat the validity of these studies, these trials often lack the power of large population-based studies in predicting the economic impacts and detecting small effects especially with regards of habitual and nutritional factors. Accordingly, there is no surrogate for large prospective population-based studies.

2.3 Diagnosis of osteoporosis and techniques for bone mass measurements

2.3.1 Diagnosis

2.3.1.1 Diagnostic strategies

Osteoporosis is typically a silent disease with no appreciable symptoms. Instead, fractures usually raise the suspicion of osteoporosis. In particular, fractures of the spine, distal radius and proximal femur of the postmenopausal women should be alarm bells drawing attention to further procedures for screening osteoporosis, most importantly bone
Table 1 Population-based studies with BMD measurements on osteoporosis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Base population</th>
<th>Follow-up interval (Reference)</th>
<th>BMD measurements (size of measured population)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Danish Osteoporosis Study (DOPS)</strong></td>
<td>2016 Danish women aged 45-58 years</td>
<td>1990- (Mosekilde et al. 1999)</td>
<td>DXA: lumbar spine, hip region, forearm, total body (2016)</td>
</tr>
<tr>
<td><strong>Dubbo Osteoporosis Epidemiology Study</strong></td>
<td>1 789 Australian women and men aged &gt;60 years</td>
<td>1989-1992 (Nguyen et al. 1993)</td>
<td>DXA: lumbar spine and neck of femur (1789)</td>
</tr>
<tr>
<td><strong>Epidos</strong></td>
<td>7 575 French women aged &gt;75 years</td>
<td>1992- (Dargent-Molina et al. 1996)</td>
<td>DXA: neck of femur (7575)</td>
</tr>
<tr>
<td><strong>Framingham Osteoporosis Study</strong></td>
<td>1 164 American men and women aged &gt;68 years</td>
<td>1988- (Hannan et al. 1992)</td>
<td>DPA / DXA: lumbar spine and neck of femur SPA: ulnar/stal radius (1164)</td>
</tr>
<tr>
<td><strong>OSTPRE Study</strong></td>
<td>13 100 Finnish women aged 47-56 years</td>
<td>1989- (Horkanen et al. 1991)</td>
<td>DXA: lumbar spine and neck of femur (3222)</td>
</tr>
<tr>
<td><strong>Rotterdam Study</strong></td>
<td>9 161 Dutch men and women aged &gt;55 years</td>
<td>1990- (Hofman et al. 1991)</td>
<td>DXA: neck of femur (4308)</td>
</tr>
</tbody>
</table>
density measurements. However, as bone is lost throughout the skeleton, practically all fractures occurring in the elderly population can be considered to be osteoporotic to some extent due to low bone density (Seeley et al. 1991).

The treatment of established osteoporosis is expensive and may not result in a full functional recovery (Cooper and Melton III 1996). Hence, the trend in the present management of osteoporosis emphasises the role of pre-fracture recognition. Although present central measurement techniques are non-invasive and quite precise, the resources are limited and random densitometric screening of non-symptomatic population cannot be recommended (NHS 1992). Instead, directing patients for BMD measurements according to the individual profile of risk factors and possible symptoms aid in obtaining early diagnosis (phasing) and increases the cost-effectiveness of the process.

Attempts have been made to satisfactorily identify patients in most need for BMD measurements. For example, the National Osteoporosis Foundation (NOF) has formulated a set of indications for bone mineral density measurements (NOF 1998) (Table 2). These criteria may seem somewhat loose considering the current availability of bone densitometry and costs. Accordingly, some population-based studies on risk factors of osteoporosis have evaluated usefulness of certain risk scores to target BMD densitometry more specifically to those women at greatest risk (Michaelsson et al. 1996, Weinstein and Ullery 2000, Cadarette et al. 2001).

Table 2 Indications for bone mineral density measurements (NOF 1998)

- All postmenopausal women under age 65 who have one or more additional risk factor for osteoporosis (in addition to being postmenopausal and female)
- All women age 65 and older regardless of additional risk factors
- Postmenopausal women who present with fractures (to confirm diagnosis and determine disease activity)
- Women who are considering therapy for osteoporosis if BMD testing would facilitate the decision
- Women who have been on HRT/ERT for prolonged duration
2.3.1.2 Bone mass measurements

BMD has been recognised as the best single predictor of fracture risk (Sowers 1997). Due to the nature of osteoporosis, no indisputable cut-off points for BMD values can be set as a criterion for diagnosis. However, some arbitrary guidelines have been drawn to help in the practical diagnosis. The World Health Organisation expert committee has defined bone density value of -1 SD or higher as normal bone density, -1 to -2.5 SD as osteopenia (low bone mass) and -2.5 SD or lower as diagnostic of osteoporosis in comparison to young adults (WHO 1994). It should be remembered, however, that for each SD decline in BMD, the relative risk of fracture increases 1.5-3 fold (Melton 1991, Cummings et al. 1993, Kröger et al. 1995) though there may be a cut-off in the cumulative risk with regard to the lowest BMDs (Huopio et al. 2000). Furthermore, it has been demonstrated that the definition of osteoporosis, osteopenia or normal bone density on the basis of a single BMD measurement is highly dependent on the measurement technique and site of measurement (Greenspan et al. 1996, Faulkner et al. 1999). Thus, bone mass data should be accompanied by a clinical interpretation. In addition to being useful for purely diagnostic purposes bone mass measurements have a role in treatment monitoring (Miller et al. 1996).

2.3.1.3 Biochemical markers

Bone mass measurements are relatively static indicators of bone health and do not reflect the activity of remodelling cycle of bone tissue. For a more dynamic analysis of bone health, some markers of bone formation and resorption have been found to be useful in evaluation of bone remodelling (Delmas 1990). However, the poor accuracy and great inter- and intra-individual variation in the present methods limits the use of markers in the practical diagnosis (Jensen et al. 1997) and a diagnosis of osteoporosis cannot be set based on the serum or urine level of biochemical bone markers alone. Nevertheless, it has been shown that combining some bone markers with BMD measurements enhances the specificity of hip fracture prediction without any significant loss of sensitivity (Garnero et al. 1998). In addition to diagnostic purposes, bone markers have been suggested to be of
value in treatment monitoring (Greenspan et al. 1998, Garnero et al. 1999, Delmas et al. 2000).

2.3.2 Current techniques for measuring bone mass

In contrast to the previous use of isotopes and peripheral measuring techniques (Single photon absorptiometry (SPA), dual photon absorptiometry (DPA)), the current gold standard is a central measurement technique utilizing an x-ray source for diagnostic and study purposes. In addition, new peripheral methods with low radiation doses have been developed to ease clinical decision making.

**Dual X-ray absorptiometry (DXA)** is most widely used central measurement technique presently. It has been adopted as the golden standard for bone mass measurements (Appendix 1). DXA measures spine (L1 thru L4) and femoral (femoral neck, trochanter, Ward’s triangle) regions. The precision of central DXA is 1-2 % (CV) depending on the site of measurement (Genant et al. 1996, Grampp et al. 1997). The radiation dose is comparable with average daily background radiation, 1-10 uSv (Genant et al. 1993, Njeh et al. 1999). DXA can provide data either as bone mineral content (BMC, g/cm) or bone mineral density (BMD, g/cm²). Central DXA measurement is relatively expensive and hence its availability for clinical use restricted (Cummings et al. 2002).

**Peripheral DXA (pDXA)** is peripheral (calcaneus or distal radius) application of central DXA for measurement of bone density. The radiation dose is extremely low (about 0.1 uSv) and precision 1-2 % (CV) (Genant et al. 1996, Grampp et al. 1997). This method is predictive for fractures but does not predict hip or vertebral fractures as well as hip and vertebral DXA measurements (Siris et al. 2001, Cummings et al. 2002). However, peripheral tests are cheaper than central DXA of spine and hip and are thus more easily available for clinical use (Cummings et al. 2002).

**Peripheral quantitative computed tomography (pQCT)** has the advantage of discriminating the trabecular and cortical bone compartment and reporting the volumetric bone density (g/cm³). Measurements are performed either at the distal radius or tibia. The precision of this method is 1-2 % (CV), and the radiation dose is low, 1-3 uSv (Genant et al. 1996, Grampp et al. 1997).
Ultrasonography (QUS) is a flexible, radiation free and cheap measurement technique for peripheral bones (Gluer 1997). The technique is based on attenuation of sound in bone tissue. The correlation between QUS and DXA is not high and its correlation to BMD is low (Gluer et al. 1992, Kröger et al. 1995) but the recognition of fracture cases is comparable with DXA (Kröger et al. 1995, Hans et al. 1996). The precision varies between 2.5 \% (CV) for calcaneal BUA, 0.1-1 \% for calcaneal SOS and 1-2 \% for combined measurements (Genant et al. 1996, Grampp et al. 1997).

Radiographic absorptiometry (RA) uses conventional x-rays of the phalanges to assess bone density using an aluminium calibration wedge. RA has the advantage of its widespread availability. The radiation dose is about 10 uSv and precision is 1-2 \% (CV) (Genant et al. 1996, Grampp et al. 1997). One disadvantage of RA is that it measures a site distinct from typical osteoporotic fracture sites. However, prediction of fracture risk with new computed digital absorptiometry (CDA) techniques, which eliminates the need for conventional films, may be comparable to DXA (Bouxsein et al. 1997).

Digital X-ray radiogrammetry (DXR) is a new method that uses the dimensions of the metacarpals (metacarpal index, MCI) for determination of BMD. The precision of newest methods varies between 0.5-1 \% (CV) (Jørgensen et al. 2000). Although the radiation dose is low, this method seems to be only moderately correlated with central and appendicular DXA measurements (Adami et al. 1996).

One important facet of all BMD measurement techniques is quality assurance. Important quality assurance procedures are factors related to personnel, patient data, longitudinal monitoring, cross-calibration between different scanners and phantom data (Orwell and Oviatt 1991, Gluer et al. 1993). Those are ways in which the precision, accuracy and longitudinal comparisons of measurements can be improved.

It has been estimated that in 85 \% of the cases, measurements at different skeletal areas are in concordance with each other (Davis et al. 1994). Also, different measurement areas have been suggested to equally predict future fracture risk (Seeley et al. 1991, Cummings and Black 1995, Miller et al. 2002). The site for measurement and the choice of an appropriate measuring technique should be based on the clinical picture and on an appreciation of the strengths and limitations of each technique.
2.4 Review of risk- and preventive factors for postmenopausal bone loss

2.4.1 Genetics

Osteoporosis is likely to be a polygenic disease (Rizzoli et al. 2001). The heritability of peak bone mass has been clearly demonstrated. Daughters of osteoporotic women have been shown to have low BMD (Seeman et al. 1989, Ferrari et al. 1998) and twin studies have shown heritability of bone density ranging between 55%-82% (Danielson et al. 1999). In addition, adult daughters of postmenopausal women with decreased BMD have been found to have lower BMD than daughters of postmenopausal women with normal BMD (Barthe et al. 1998). In contrast, the heritability of the bone mass changes occurring at later life is not as apparent and is poorly understood. Monozygotic twins have been suggested to have a higher correlation of bone loss than dizygotic twins (Kelly et al. 1995). On the other hand, it has been claimed that the heritability of bone loss between mono- and dizygotic twins as assessed by bone biochemical markers is poor (Garnero et al. 1996). In addition, heritability of hormonal cycle and ovarian function in women may contribute to bone loss in later life (Sniieder et al. 1998) and estrogen receptor polymorphism may influence the rate of early postmenopausal bone loss (Salmen et al. 2000).

2.4.2 Age related bone loss

During pubertal maturation, the bone mineral mass more than doubles (Bonjour et al. 1991, Theintz et al. 1992). In the female population this occurs about two years earlier than in males (Theinz et al. 1992, Kröger et al. 1993). In adults, bone size varies little throughout life. However, through mechanisms of endosteal resorption, increased porosity and trabecular destruction, bone loss continues throughout life in both genders (Ensruud et al. 1995, Han et al. 1996). Accordingly, some bone loss has been suggested to occur at distinct skeletal sites well before menopause in women (Luckey et al. 1996, Slemenda et al. 1996). The suggested rates of annual BMD change in premenopausal women at lumbar spine have varied from a moderate loss (-1.5 to -0.79 %/year) to a slight gain (0.2 to 0.3
%/year) between different population-based estimates (Pouilles et al. 1993, Perrone et al. 1995, Tsunenari et al. 1995, Slemenda et al. 1996, Prior et al. 1998). In postmenopausal women, age related bone loss continues at age specific rate after the initial fastening during the menopausal transition (Hansen et al. 1995). The mechanism for this slow phase of bone loss in elderly women is connected to alterations on calcium and PTH homeostasis (Riggs et al. 2002).

2.4.3 Menopause

Female sex hormones play a vital role in bone health during female reproductive life and also in ageing women and men (Greendale et al. 1997, Riggs et al. 1998). Menopause related estrogen depletion has been observed to occur at a mean age of 51.3 years in western societies (Brambilla and McKinlay 1989) and causes typical symptoms, such as menstrual irregularities and hot flushes. Interestingly, it has been suggested that self-observed onset of menopause on a symptomatic basis may be a surprisingly sensitive measure of menopausal status (Dennerstein et al. 1993, Prior 1998).

At the beginning of menopause, the acute loss of the restraining effect of estrogen on receptors on the membranes of osteoblasts and osteoclasts leads to accelerated bone turnover, uncoupling bone formation from resorption (Ousler et al. 1996, Manolagas 1998, Riggs et al. 2002). The closer molecular mechanisms of estrogen depletion related bone loss have been linked to the overproduction of bone resorptive cytokines (Riggs et al. 2002). In addition, imbalance between calcium secretion and absorption following the estrogen depletion has been suggested to influence the accelerated bone loss rate (Adami et al. 1992).

It has been shown that menopausal transition is associated with both increased bone loss rate, reduced BMD and increased fracture incidence (Pouilles et al. 1993, Tuppurainen et al. 1993, Kröger et al. 1994, Pouilles et al. 1995, Prior et al. 1998, Ahlborg et al. 2001). The phase of the most accelerated bone loss is likely to take place at the very beginning of menopause (amenorrhea phase) after which the bone loss rate becomes progressively diminished for several years during the early postmenopause (Harris and Dawson-Hughes 1992, Pouilles et al. 1993, Pouilles et al. 1995, Prior et al. 1998). Some
differences have been observed in the pattern of menopausal bone loss between different skeletal sites which may be related to the different composition of these sites with respect to cortical and trabecular bone (Pouilles et al. 1995, Hansen et al. 1995, Young et al. 1996).

2.4.4 Body weight and weight changes

Body weight and weight changes are strongly linked to BMD changes in postmenopausal women regardless of body site. Weight and weight increase are associated with the maintenance of BMD and reduced bone loss whereas thinness and weight loss lead to low BMD and enhanced bone loss in early and later postmenopause (Brot et al. 1997, Yoshimura et al. 1998, Nguyen et al. 1998). In addition, high body weight is a strong independent predictor of lower postmenopausal fracture incidence (Huopio et al. 2000). These effects may partly be linked with fat tissue related estrogen production (Nelson and Bulun 2001) but also other mechanisms may contribute. Mechanical load as such is likely to lead to bone strengthening with mobility induced weight-bearing stress. In addition, hormones that regulate fat tissue metabolism, leptins, have been suggested to be closely related with bone metabolism (Yamauchi et al. 2001, Blain et al. 2002). The heavier population also has a higher nutritional intake and may thus consume more calcium and other bone preserving products. In addition, the differentiating role of muscles and fat in weight related bone mass changes remains unclear. It has been suggested, that in elderly women lean mass correlates with BMD irrespective of body site but that the association between muscle strength and BMD is site-specific (Blain et al. 2001) (see also next paragraph). However, part of the observed BMD changes related to weight alterations may be due to methodological difficulties encountered in the measurement techniques adopted to deal with body compositional factors, most importantly fat tissue (Bolotin 1998). Although the relationship between weight and bone metabolism is well recognized, the underlying pathophysiology is not yet perfectly understood.
2.4.5 Muscle strength and exercise

Muscle strength, impact and non-impact exercise as well as overall physical activity level are positively related with bone mass changes in postmenopausal women (Kröger et al. 1994, Nguyen et al. 1998, Wallace and Cumming 2000). Even short-term exercise above the anaerobic threshold level has been found to be effective in the prevention of bone loss after menopause (Hatori et al. 1993). Analogously immobilisation has been shown to accelerate bone loss (Kiratli 1996). In some reports, grip as well as quadriceps strength has been positively related to axial BMD in older women (Kritz-Silverstein and Barret-Connor 1994, Kröger et al. 1994, Blain et al. 2001). However, it has been observed that any improvement of bone density due to exercise or muscle strength is highly site specific and related to local skeletal strain (Kerr et al. 1996, Blain et al. 2001). Finally, estrogen and calcium have been suggested to potentiate the effects of exercise on postmenopausal bone (Prince et al. 1991, Kohrt et al. 1995). In addition to direct effects, exercise and muscular fitness preserve functional capacity and improve balance which might directly prevent fractures e.g. by reducing falls. However, due to lack of appropriate randomized trials no undisputable recommendations on exercise in prevention of fractures may be done at community level (Karlsson 2002).

2.4.6 Calcium and vitamin D

Calcium is absorbed from the gut through mechanisms of passive diffusion and active transportation. The recommended dietary calcium intake for adult women under 50 years old is 1000 mg / day and is elevated to 1500 mg /day in postmenopausal women over 50 years not on HRT and in all women over 65 years old (Goebel and Willhite 1998, NIH 1994). The Finnish recommendations are lower: 800 mg/day in women 60 years or older (Valtion ravitsemusneuvottelukunta 1998). However, studies have suggested that elderly women may ingest on average only 500 mg calcium /day (Barret-Connor 1989).

Calcium supplementation has been shown to be useful in the prevention and treatment of postmenopausal osteoporosis especially when combined with vitamin D (Cumming 1990, Kanis 1999). It has been shown that calcium supplementation is effective
at decreasing fracture incidence in osteoporotic women: the vertebral fracture incidence has been found to decrease from 58% to 28% and hip fracture risk by 25% to 70% with calcium supplementation in postmenopausal women (Recker et al. 1996, Cumming and Nevitt 1997). However, many of the studies exploring bone effects of calcium supplementation have been complicated by co-supplementation with vitamin D and thus the effects of additional calcium alone on fracture incidence are not consistent (Cumming and Nevitt 1997, Shea et al. 2002). Nonetheless, the beneficial effects of supplementation on BMD in elderly women have been demonstrated and the efficacy of supplementation in postmenopausal bone loss has been shown at both spinal and femoral sites (Reid et al. 1995, Shea et al. 2002). Interestingly, the effect of calcium supplementation on BMD has been found to be dependent on menopausal status, being stronger in later postmenopause (Dawson-Hughes et al. 1990). The positive role of dietary calcium in the prevention of postmenopausal bone loss, although recognised, has not been as well established and needs further investigation, especially at the population-based level (Cumming 1990, Cumming et al. 1993, Feskanich et al. 1997, Heaney 1997, Kanis 1999).

Vitamin D is produced both endogenously as a result of sunlight exposure and is present in the diet and is absorbed from the gut. The effects of vitamin D are mediated by its active metabolites which are responsible for maintaining serum levels of calcium and vitamin D by means of bone resorption and increased intestinal absorption (Reichel et al. 1989, Holick 1995). Vitamin D supplementation has been shown to reduce both bone loss and the risk of fractures in postmenopausal women (Chapuy et al. 1992, Dawson-Hughes et al. 1995, Ooms et al. 1995, Papadimitropoulos et al. 2002) especially when combined with calcium supplementation (Dawson-Hughes 1996, 1997). However, the protective effect of vitamin D in postmenopausal women has not been clearly shown in all studies (Lips et al. 1996, Komulainen et al. 1999) and needs further clarification especially with regards to differences between standard and hydroxylated vitamin D (Papadimitropoulos et al. 2002). The recommended daily vitamin D intake is 600-800 IU for elderly population (Byrne et al. 1995), in Finland 400 IU /day in women 60 years or older (Valtion ravitsemusneuvotelukunta 1998).
2.4.7 Smoking and alcohol intake

In postmenopausal women, smoking has been suggested to increase fracture risk and enhance bone loss especially at femoral sites (Law and Hackshaw 1997, Krall and Dawson-Hughes 1999, Bjarnason and Christiansen 2000, Hermann et al. 2000). This effect may partially be mediated through direct down-regulation of osteoblast function (Ferrara et al. 2000). Smokers may have lower circulating serum estrogen levels and also an earlier onset of menopause (Midg ette and Baron 1990). In addition, smoking has been suggested to counteract the protective effects of estrogen on bone in postmenopausal women (Kiel et al. 1992, Komulainen et al. 2000) and to impair intestinal calcium absorption through suppression of the PTH-calcitriol axis (Brot et al. 1999, Krall and Dawson-Hughes 1999, Rapuri et al. 2000a, Need et al. 2007). Part of the bone related adverse effects of smoking may be due to its weight suppressive effect (Hermann et al. 2000). Finally, postmenopausal bone loss attributable to smoking may be prevented by HRT (Bjarnason and Christiansen 2000).

The effects of alcohol on postmenopausal bone health are not well understood. Interestingly, small or moderate amounts of alcohol may increase postmenopausal BMD according to several studies (Laitinen and Välimäki 1993, Rapuri et al. 2000b, Turner et al. 2000) but excessive use of alcohol has been shown to reduce BMD and enhance bone loss (Hannan et al. 2000a, Turner et al. 2000). Alcohol intake increases the fracture incidence partly due to increased tendency to fall apart from any possible direct effects on bone (Honkanen et al. 1983, Hernandez-Avila et al. 1991, Laitinen and Välimäki 1993). However, alcohol may directly mediate its deleterious effects on bone through the inhibition of osteoblast proliferation (Klein and Carlos 1995) while the protective effect of moderate doses of alcohol may be related to favourable changes in PTH and estrogen levels (Rapuri et al. 2000b). Finally, it has been suggested that alcohol may predispose the individual to earlier menopause which could alter the patterns of postmenopausal bone loss (Torgerson et al. 1997).
2.4.8 Other nutritional factors

Coffee intake may enhance bone loss and increase fracture rates after menopausal transition (Barret-Connor et al. 1994, Harris and Dawson-Hughes 1994, Rapuri et al. 2001) although this has not been supported by other studies (Lloyd et al. 1997). Coffee induced bone loss may be related to the caffeine induced increased calcium excretion (Hasling et al. 1992) and thus any deleterious effect of coffee on bone may depend on the calcium intake status in postmenopausal women (Harris and Dawson-Hughes 1994, Barret-Connor et al. 1994).

Low dietary protein intake has been found to be related in most studies with both decreased BMD, increased bone loss and increased hip fracture incidence in postmenopausal women (Johnell et al. 1995, Michaelsson et al. 1995, Hannan et al. 2000b). These effects may be associated with an increase in the serum level of insulin-like growth factor 1 (IGF-I) (Schürch et al. 1998) and overproduction of some cytokines (Rizzoli et al. 2001). However, other studies have found no correlation between protein intake and bone metabolism (Rizzoli et al. 2001).

Some trace elements and vitamins have been claimed to relate with alterations in bone metabolism. As an example, magnesium deficiency may be associated with lower bone mass (Rude 2001) whereas fluoride is useful for prevention of osteoporosis (Dure-Smith et al. 1991). Vitamin K has recently become a focus of attention because of its possible protective effect on age-related bone loss (Booth 2001). In contrast, vitamin A may be associated with low BMD and increased susceptibility to fractures (Milstone and Leachman 2001). Overall, the role of these and other related factors are currently under investigation and remain to be resolved.

2.4.9 Parity, contraceptive use, and lactation

Nulliparity has been suggested to be a risk factor for postmenopausal osteoporosis whereas pregnancies may protect from low BMD after menopause (Nguyen et al. 1995, Tuppurainen et al. 1995a). Parity has also been found to protect against fractures later in life (Hoffman et al. 1993). However, these findings on BMD and fractures after
menopause have not been confirmed in all studies (Torgerson et al. 1996, Kojima et al. 2002) leaving the relationship unresolved.

Oral contraceptive use during late reproductive life may be associated with increased BMD and reduced fracture risk in postmenopausal women (Kritz-Silverstein and Barret-Connor 1993, Michaelsson et al. 1999, Cundy et al. 2002). However, this has not been demonstrated in all studies (Cooper et al. 1993, Tuppurainen et al. 1993, Kröger et al. 1994). Part of the bone protective effect of previous oral contraception use in postmenopausal women may be due to their higher bone density at the beginning of menopause (Corson et al. 1993).

Breastfeeding is associated with increased bone loss and enhanced bone turnover during the postpartum period (Kalkwarf et al. 1999, Polatti et al. 1999). Interestingly, previous lactation has been suggested to protect from low BMD in postmenopausal women (Berning et al. 1993). In pre- and perimenopausal women, the opposite effect has been observed (Ghannam et al. 1999). However, some studies have found no or only a negligible association of breastfeeding with bone metabolism in either pre-, peri- or postmenopausal women (Bauer et al. 1993, Melton et al. 1993, Tuppurainen et al. 1995a).

2.4.10 Secondary osteoporosis

Osteoporosis associated with medical disorders is defined as secondary osteoporosis. Many musculoskeletal, gastrointestinal, endocrinological, gynecological, renal and psychiatric disorders may contribute to bone loss. In addition, a number of medications have been found to increase bone loss: e.g. glucocorticoids, anticoagulants, non-thiazide diuretics, anticonvulsants, phosphate binding antacids and cytotoxic drugs. Thiazide-diuretics, however, may merely be considered as preventive agents for osteoporosis (Reid et al. 2000). A more detailed discussion of secondary osteoporosis falls beyond the scope of this text.
2.5 Preventive strategies and treatment of osteoporosis

2.5.1 Preventive strategies

Primary prevention of osteoporosis includes a risk factor assessment and educational resources to eliminate risk factors for bone loss rather than for fractures. Accordingly, primary prevention should be directed to the young population and concentrated primarily on peak bone mass acquisition but methods are also applicable for helping postmenopausal women to preserve bone mass. The main components of primary prevention are factors related to nutritional factors and exercise but also the avoidance of deleterious substances and habits discussed in the previous chapter. Accordingly, an adequate intake of calcium and vitamin D, regular exercise, moderate intake of alcohol and coffee together with cessation of smoking should be encouraged. In addition, fall prevention may be considered as primary prevention in elderly women.

Secondary prevention of osteoporosis concentrates on the prevention of fractures after an initial fracture and on the detection of low BMD. Compared to primary prevention, further protection by hormonal and/or non-hormonal products together with vitamin D and calcium supplements is also necessary.

There are two competing strategies in the prevention of osteoporosis. The high-risk strategy concentrates on screening of women applicable for preventive procedures based on the likelihood of their becoming osteoporotic. Attempts have been made to satisfactorily identify women at a high risk based on individual risk factors (Michaelsson et al. 1996, Weinstein and Ullery 2000, Cadarette et al. 2001), while unrestricted screening of women with bone density measurement is not recommended (NHS 1992). However, at present, there is no risk score accurate enough to be of any marked clinical value. Overall, the high-risk strategy may be considered meaningful while the true costs are unknown.

In contrast, the population-based strategy concentrates on prevention of osteoporosis in whole population irrespective of the accumulation of individual risk factors. It requires persistent changes in life-style and nutritional habits. Although it is
cheap and cost-effective, the true effect of changes in nutritional and lifestyle habits on bone loss and fractures are still unresolved and compliance of individuals is largely unknown.

Table 3 summarizes applicable prevention strategies in the female population at different ages.

Table 3  Prevention of osteoporosis in female population at different stages of their lifespan

<table>
<thead>
<tr>
<th>Age group</th>
<th>Target</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopause</td>
<td>Acquisition and maintenance of peak bone mass</td>
<td>- Calcium, vitamin D, exercise, etc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Primary prevention )</td>
</tr>
<tr>
<td>Perimenopause</td>
<td>Screening for bone mineral density</td>
<td>- Individual risk profile evaluation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Primary and secondary prevention )</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>Controlling bone mineral loss and consequences (fractures)</td>
<td>- Hormonal/non-hormonal pharmacologic protection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Fall prevention</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Primary and secondary prevention )</td>
</tr>
</tbody>
</table>

2.5.2  Treatment of postmenopausal osteoporosis

2.5.2.1  HRT and SERMs

Hormone replacement therapy includes both estrogen therapy (ERT) and combined estrogen-progesterone therapy given either continuously or sequentially. In addition to effective prevention of menopausal symptoms (hot flushes), HRT prevents postmenopausal bone loss and decreases the risk of fractures (Lindsay and Tohme 1990, Cauley et al. 1995, Schneider et al. 1997, Komulainen et al. 1998, NOF 1998). A recent meta-analysis showed a trend towards 34% reduction in vertebral and 13% reduction in
non-vertebral fracture incidence, although this was not statistically significant (Wells et al. 2002). The increase in BMD occurs during the first two years of treatment and is more pronounced at cancellous than cortical bone: 5.4% in lumbar spine and 2.5% in femoral neck after 1 year with a 1.5% increase at two years according to the meta-analysis of Wells et al. (Recker et al. 1977, Hillard et al. 1994, Komulainen et al. 1999, Wells et al. 2002). In addition, the effects of calcium and HRT have been suggested to positively interact and low calcium intake has been found to be a risk factor for no bone response to HRT (Davis et al. 1995, Mizunuma et al. 1996, Nieves et al. 1998, Honkanen et al. 2001). Also some other factors, such as smoking and body weight, may modify the effect of HRT on bone (Bjarnason and Christiansen 2000, Komulainen et al. 2000).

HRT may increase the risk of breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer 1997) but this finding has not been confirmed by all studies (Sellers et al. 1997). According to some estimates, the benefits of HRT outweigh the risk of breast cancer in many women (Col et al. 1997). However, the most recent data implies that risk of breast cancer as well as cardio-vascular incidents may be greater among HRT users (Rossouw et al. 2002). Hence, HRT cannot be considered as first-line therapy for all postmenopausal women without careful consideration of the individual risks and benefits.

The antiresorptive mechanisms by which HRT affects bone are likely to be multifactorial. Estrogen affects bone remodelling by both direct and indirect mechanisms (Ciocca and Roig 1995). Osteoblasts and osteoclasts have specific estrogen receptors on their cell membranes which probably mediate the direct effects of estrogen on bone (Ousler et al. 1996). In addition, estrogen has been proposed to mediate its bone protective effects through regulation of numerous cytokines and thus inhibit osteoclastogenesis (Pacifici 1996). Estrogen may increase intestinal calcium absorption by stimulation of vitamin D production (Gennari et al. 1990) and increase renal reabsorption of calcium (Gallagher 1996). It has been suggested that the improvement of bone density by long-term HRT reaches a plateau between 1-3 years from the beginning of HRT (Nielsen et al. 1994, Komulainen et al. 1999) and that after discontinuation of HRT, bone mass approaches the values found in non-replaced postmenopausal women (Lindsay et al. 1978, Christiansen et al. 1981, Tremollieres et al. 2001).
Selective Estrogen Receptor Modulators (raloxifene, tamoxifen) have been found to significantly increase BMD and reduce the risk of fractures whereas they have anti-estrogen effects on breast and uterus (Delmas et al. 1997, Cummings et al. 1999, Ettinger et al. 1999, Love et al. 1992). The increase in BMD has been suggested to be somewhat less than that observed with alendronate or HRT treatment (Delmas et al. 1997). SERMs exert their effects through modulation of the structure of estrogen receptors on cell membranes (Mitlak and Cohen 1997).

2.5.2.2 Bisphosphonates and calcitonin

Effects of bisphosphonates (alendronate, etidronate, risedronate, pamidronate) are mediated through inhibition of osteoclasts (Geddes et al. 1994). Bisphosphonates have been found to prevent postmenopausal bone loss and to reduce the incidence of postmenopausal fractures (Harris et al. 1993, Liberman et al. 1995, Black et al. 1996, Reginster et al. 2000). Generally, bisphosphonates are well tolerated and do not have the adverse effects of the hormonal products.

Calcitonin is secreted from the thyroid glands and its main biological function is to inhibit osteoclast function. The intranasal dose of salmon calcitonin is usually 200 μg daily. Calcitonin has been shown to increase BMD and prevent fractures though not quite as effectively as HRT or alendronate (Rico et al. 1995, Avioli 1998, Chesnut et al. 2000). In addition, calcitonin may have some added analgesic benefits which the other agents (bisphosphonates, HRT) do not (Gennari et al. 1991). Finally, there may be a positive interaction between calcitonin and calcium on bone mass (Nieves et al. 1998).

2.5.2.3 New experimental therapies

Parathyroid hormone (PTH) has been suggested to increase spinal, femoral and total body BMD and decrease both the vertebral and non-vertebral fracture rate (Lindsay et al. 1997, Neer et al. 2001). PTH mediates its effects through stimulation of osteoblasts (Rosen and Rackoff 2001). The dose, hPTH (1-34) 400-500 U/day, is given parenterally.
In an experimental animal study, widely used lipid lowering hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, have been shown to increase BMD by stimulating osteoblast activity (Mundy et al. 1999). Some observational studies have also found that statins may increase BMD (Chung et al. 2000, Edwards et al. 2000) and prevent fractures (Meier et al. 2000, Wang et al. 2000). However, this has not been confirmed in other observational studies (Reid et al. 2001, van Staa et al. 2001). The mechanism by which statins might affect bone metabolism is not known. Animal studies have suggested that statins may decrease the severity of steroid-induced osteonecrosis (Cui et al. 1997, Wang et al. 2000) and be associated with bone metabolism via inhibition of mevalonate synthesis analogously to the mechanism of action of bisphosphonates (Van Beek et al. 1999). Statins have also been claimed to stimulate the expression of bone morphogenetic protein 2 (BMP-2) (Mundy et al. 1999).
3 AIMS OF THE STUDY

The aim of the present study was to investigate the pattern of natural postmenopausal bone loss (Study I) in conjunction with environmental, habitual and nutritional factors (Study I-IV). Special emphasis was placed on the interactive effects between weight and HRT (Study II), HRT and nutritional calcium (Study III) as well as nutritional calcium and smoking (Study IV) in postmenopausal bone loss. In addition, the applicability of statin therapy in the prevention of early postmenopausal bone loss at population level was evaluated (Study V). The common denominator in all of the above-mentioned subjects was the long-term bone mineral density change in the early postmenopausal population.

The present study was conducted as a part of the prospective Kuopio Osteoporosis Risk Factor and Prevention (OSTPRE) Study
4 SUBJECTS AND METHODS

Osteoporosis Risk Factor and Prevention (OSTPRE) study is a population based 3-level prospective cohort study which investigates genetic and acquired factors associated with fractures, falls, bone mineral density (BMD) and bone loss in peri- and postmenopausal women (Honkanen et al. 1991). It was established in 1989 and is still ongoing (Figure 2).

4.1 The postal inquiries

The target population of the OSTPRE baseline questionnaire in 1989 was 14 220 women born in 1932-41 living in Kuopio district, Eastern Finland (Appendix 2). Name, address, social security number, place of birth and occupation were obtained from the National Population Register of Finland in April 1989. In all, addresses of 14 121 women were adequately recorded.

The baseline questionnaire included questions about previous sicknesses (chronic illnesses, fractures and their cause) and drug use (prescribed drugs, use of calcium and vitamin D supplements), gynecological history (date of last menstruation, menopausal symptoms, use of female sex hormone, number of pregnancies, parity, gynecological operations), nutritional calcium intake (from milk and cheese) and lifestyle (smoking, alcohol) habits, physical activity, as well as routine anthropometric information such as height and weight. In addition, willingness to participate in DXA densitometry as well as informed consent from the participants was requested.

The participants of baseline densitometry (n=3222) obtained additional inquiries at densitometry during 1990-91 and in the beginning of the year 1993. These questionnaires included more detailed information about deliveries, physical activity, consumption of alcohol and smoking, gynecological history (age at menarche, amenorrhea, breast-feeding, menstrual status, HRT use, gynecological operations) and incident fractures. This data was added to that obtained in the baseline questionnaires.
Similar questionnaires were sent to the 13 100 women who had responded at baseline at the five year follow-up in 1994 and again at the ten year follow-up in 1999. A response was obtained from 11 954 and 11 537 women at five years and ten years, respectively. The questionnaire information was partially updated with each participant at the time of bone densitometry.

In addition, the medical prescription records of The Social Insurance Institution, Finland (KELA) were used for validation of use of HRT and all other prescribed medications during 1996-2001 for the 13 100 women. These records included the number of pharmacy visits, name and number of medications delivered at each visit.

Figure 2 The OSTPRE study design.

4.2Bone mineral density measurements

The bone mineral densities of lumbar spine (L2-L4), left femoral neck, Ward’s triangle and trochanter major were determined using dual X-ray absorptiometry (DXA) (Lunar DPX, Madison, Wisconsin, USA). The measurements were carried out in Kuopio University Hospital by specially trained personnel. Quality standards were tested daily. The short term reproducibility of this method has been shown to be 0.9 % for lumbar spine
and 1.5 % for femoral neck BMD measurements (Kröger et al. 1992). The long-term reproducibility (CV) of the DXA instrument for BMD during the study period, as determined by regular phantom measurements, was 0.4 % (Komulainen et al. 1998).

Of the 13 100 respondents in 1989, 11 055 (84.4 %) were willing to undergo DXA densitometry (Figure 3). A densitometry sample of 3686 women (33.3 %) was selected for the measurements of which 3222 (87.4 %) women were actually undergoing baseline densitometry. Of these, the random sample included the 2025 women and the remaining 1197 women formed a non-random part. In all, 1873 women of the random part underwent the five year (1994-97) and 2832 women of the total densitometry sample came to the ten year (1999-2002) measurement. Serial valid measurements for lumbar spine and neck of femur were recorded for 1551 and additionally for Ward’s triangle and trochanter for 1548 women in baseline and five year measurements (random sample) and for 2129 women at ten year measurements (total densitometry sample). Accordingly, severe bone deformities, including spondyloarthritis (also osteofytes), scoliosis and severe compression fractures, were excluded by a systematic review of densitometry reprints by specially trained physicians.

The Lunar DPX scanner was changed to DPX-IQ between five and ten year densitometry (5/99). To reveal possible differences in the BMD results of these scanners, 90 women (age 60±8 years, body height 160.0±5.6cm and weight 69.4±12.8kg (Mean±SD)) were scanned on the same day with both instruments. In both femoral neck and lumbar spine BMD, a high linear correlation ($r_{\text{spine}}=0.990$; $r_{\text{neck}}=0.974$) was found between these devices and the best fit first order polynomial functions were calculated and used to correct results from DPX-IQ (Study V).
### 4.3 Selection of study populations

Figure 3 presents an overview of the selection of the populations used in the present study.

**Figure 3 Selection of the study populations**

<table>
<thead>
<tr>
<th>Frame/Sample</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>14 220</strong></td>
<td><strong>Target population</strong>: women aged 47-56 at baseline (1989)</td>
</tr>
<tr>
<td><strong>13 100</strong></td>
<td>Responded to undergo baseline inquiry in 1989</td>
</tr>
<tr>
<td><strong>11 055</strong></td>
<td>Willing to bone densitometry at baseline</td>
</tr>
<tr>
<td><strong>3222</strong></td>
<td>The stratified densitometry sample</td>
</tr>
<tr>
<td><strong>2025</strong></td>
<td><em>Random part</em> that actually underwent baseline densitometry during 1989-91</td>
</tr>
<tr>
<td><strong>1873 / 1551</strong></td>
<td>5-year densitometry in 1994-97/serial valid densitometry for lumbar spine and femoral neck. <em>Population for studies II, III and IV (Cf. Table 4)</em></td>
</tr>
<tr>
<td><strong>1548</strong></td>
<td>Additional valid densitometry for trochanter and Ward’s triangle. <em>Population for study I (Cf. Table 4)</em></td>
</tr>
<tr>
<td><strong>2832/2129</strong></td>
<td>10-year densitometry in 1999-2002/valid densitometry for lumbar spine and femoral neck (stratified sample).</td>
</tr>
<tr>
<td><strong>708</strong></td>
<td>Women measured in the 10-year follow-up till end of June 2000. <em>Population for study V (Cf. Table 4)</em></td>
</tr>
<tr>
<td><strong>620</strong></td>
<td>Valid densitometry for lumbar spine and femoral neck and confirmed use of statins</td>
</tr>
</tbody>
</table>
Study I analysed bone loss from baseline to the five year measurement at lumbar spine, femoral neck, Ward’s triangle and trochanter. For this study, hysterectomized women (for whom it was impossible to define the onset of menopause) and bilaterally ovariectomized women were excluded (n=445) of the 1548 women with valid measurements at all sites. Also, women with diseases or medications known to affect bone metabolism were excluded (n=435). Of the remaining 668 women, those with a history of estrogen replacement therapy before baseline or during the follow-up (n=259) were not included. Thus, the final study population consisted of 409 women aged 48-59 years. Of these women 13 were premenopausal, 116 perimenopausal, 172 early postmenopausal (under five years postmenopausal at baseline) and 108 late postmenopausal (over five years postmenopausal at baseline).

Study II and III analysed bone loss from baseline to the five year measurement at lumbar spine and femoral neck. For these studies hysterectomized women and bilaterally ovariectomized women were excluded (n=445) from the 1551 women. Of the remaining 1106 women, premenopausal women (n=152) were excluded. From these 954 peri- and postmenopausal women, 14 women who had used HRT only before baseline (but not during follow-up) were excluded. Thus, the final study population consisted of 940 women aged 48 to 59 years at baseline densitometry (Study II). These women were further divided into HRT non-users (n=547) and HRT users (n=393). Furthermore, weight losers (n=172) were analysed separately (97 HRT non-users, 75 HRT users) (Study II). In study III women with unclear HRT and/or calcium intake information were additionally excluded (n=3) and the final study population was 937 peri- and postmenopausal women (545 HRT non-users and 392 HRT users).

Study IV analysed bone loss from baseline to five year measurement at lumbar spine and femoral neck. For this study the following women were excluded from the 1551 with valid measurements: 1) hysterectomized women (for whom it was impossible to define the menopausal status) and bilaterally ovariectomized women (n=445), 2) premenopausal women (n=152). Accordingly, the final study population consisted of 954 women (beginning of menopause either before or during follow-up). Of these women 182 were smokers (ever smoked) and 772 non-smokers (never smoked).
Study V analysed bone loss from the five year to the ten year measurement at the lumbar spine and femoral neck. The study population consisted of 708 women who underwent the ten year densitometry by the end of June 2000. Women who did not have a valid BMD measurement value at either the 5-year or 10-year measurement or had unclear statin use information were excluded (n=88). Thus, the study population of this study consisted of 620 women aged 53-64 years. Of these women, 63 were occasional users and 55 continuous users of statins, 360 normocholesterolemic controls and 142 hypercholesterolemic controls. Sixty seven percent of the 118 statin users and 84 % of the 502 non-users were part of the original OSTPRE random densitometry sample.

Table 4 summarizes the division of the present study populations into subgroups.

4.4 Definitions and classifications of study variables

4.4.1 Menopausal status (Study I)

The beginning of menopause was defined as 12 months’ amenorrhea (WHO 1996). The beginning of amenorrhea was based on questions about the last natural periods in the inquiries. Women who reported themselves as premenopausal (no or < 12 months amenorrhea) in both baseline and 5-year follow-up were defined as premenopausal. Perimenopausal women reported a change from pre- to postmenopausal during the follow-up. Early- and late postmenopausal women were separated based on a duration of postmenopause of either under or over five years at baseline measurement, respectively.

4.4.2 Calcium intake (Studies III/III/IV)

The calcium intake of each participant was calculated according to self-reported ingestion of milk products at baseline and follow-up inquiry. Following questions were included: "How many deciliters of fluid milk products (milk, sour milk, yoghurt, etc.) do you consume daily ?" and "How many slices of cheese do you eat daily ?".
Table 4 Summary of the division of women into study groups.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population</th>
<th>Subgroups</th>
<th>Follow-up interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>409 pre- or postmenopausal women</td>
<td>Premenopausal women (n=13) Perimenopausal women (n=116) Early postmenopausal women (n=172) Late postmenopausal women (n=108)</td>
<td>Baseline to five year measurement</td>
</tr>
<tr>
<td>Study II</td>
<td>940 peri- or postmenopausal women</td>
<td>HRT users (n=393) HRT non-users (n=547) Weight losers (n=75) Weight losers (n=97)</td>
<td>Baseline to five year measurement</td>
</tr>
<tr>
<td>Study III</td>
<td>937 peri- or postmenopausal women</td>
<td>HRT users (ever HRT) (n=392) HRT non-users (never HRT) (n=545)</td>
<td>Baseline to five year measurement</td>
</tr>
<tr>
<td>Study IV</td>
<td>954 peri- or postmenopausal women</td>
<td>Smokers (ever smoked) (n=182) Non-smokers (never smoked) (n=772)</td>
<td>Baseline to five year measurement</td>
</tr>
<tr>
<td>Study V</td>
<td>620 pre- or postmenopausal women</td>
<td>Continuous statin use (n=55) Occasional statin use (n=63) No statin use (hypercholesterolaemia) (n=142) No statin use (normocholesterololaemia) (n=360)</td>
<td>Five year to ten year measurement</td>
</tr>
</tbody>
</table>
According to baseline validation in 76 women, self-reported dairy calcium intake and total nutritional calcium intake according to a 4-day food record correlated reasonably ($r=0.50$) (Lindstedt 1993). The amount of calcium was approximated to be 120 mg/dl for fluid milk products and 87 mg/slice for cheese. For each participant, mean calcium intake during follow-up was calculated ([calcium intake at baseline + calcium intake at 5 year]/2), used as categorical as well as continuous variable and reported as mg/day. For study III and IV the study population was divided into tertiles according to daily nutritional calcium intake as follows: 1) < 648.1 mg/day, 2) 648.1-927.0 mg/day, 3) > 927.0 mg/day. Similarly, baseline calcium intake was used and reported in mg/day (Studies I/II). The use of calcium supplements was recorded based on questions: "What prescribed medications do you use currently (name and dosage)?" and "Do you use any non-prescribed calcium or vitamin D product currently (including natural products)?".

4.4.3 Grip strength (Studies I/II)

Grip strength was measured at the time of densitometries with a hand-held dynamometer (Martin Vigorimeter, Germany) and recorded as the mean of 3 measurements.

4.4.4 Weight (Studies I/III)

The weight (kg) of each participant was measured at the time of baseline and five year densitometry. This was done with a calibrated scale in a controlled situation by trained personnel. Weight change (Study I/II) was calculated as follows: [Weight at five year follow-up - weight at baseline] and reported in kg as well as in % (Study II) of baseline weight.

4.4.5 HRT use (Studies II/III)

For the study II and III women were divided into two groups according to their use of HRT. The use of HRT was calculated based on the use of estrogen containing tablets and plasters during the follow-up taken for menopausal symptoms. HRT non-users had
not used estrogen therapy either before baseline or during follow-up, whereas *HRT* users had been on HRT continuously or occasionally during follow-up. Forty-five percent of HRT users had used HRT also prior to baseline. Information about the use of hormonal products was obtained from the questionnaires “Have you used any hormonal products and how long?”. Comparison between self-reported use of HRT and the national prescription records of The Social Insurance Institution, Finland (KELA) for the whole OSTPRE-cohort in 1996-2001 revealed that 97.8% of those who had received an estrogen drug prescription had actually reported HRT use in inquiries. On the other hand, in 25.5% of the self-reported non-users of HRT, some estrogen use (short-term, median 6.0 months) was recorded (Sandini et al. 2002:unpublished data). The most common form of HRT in our OSTPRE cohort was estrogen-progesterone combination products (56.2% of all HRT).

4.4.6 Smoking (Study IV)

The information on smoking habits was obtained with postal inquiries. Following questions were included in both baseline and follow-up inquiries: “Have you ever smoked (cigarettes, pipe, etc.)?”, “Do you smoke currently?” and “How much do You smoke (cigarettes/day) currently?”.

4.4.7 Statin use (Study V)

The use of statins as well as other drugs were obtained by postal inquiries (from five year to ten year follow-up) according to questions "What is your current medication and dosage?" and "How long have you been using this medication?". Information on deficient or unclear statin use (e.g. misspelled name of the drug, poor handwriting or missing information of exposure time) was clarified by phone inquiry. Also, the medical records of Finnish Social Insurance Institution were used as validation criteria for statin use. Statin users were divided into two groups on the basis of duration of the statin use: *continuous* (over 90% of the follow-up) and *occasional* (less than 90% of the follow-up) use.
4.4.8 Bone affecting diseases/medications (Studies I/II/III/IV)

Women were divided into two categories (yes, no) according to the presence/absence of bone affecting diseases or medications at baseline. Bone affecting diseases/medications have been described previously by Kröger et al. (Kröger et al. 1994). Diseases were: renal disease, liver disease, insulin-dependent diabetes, malignancies, rheumatoid arthritis, endocrine abnormalities (parathyroid/thyroid glands, adrenals), malabsorption (including lactose malabsorption), total/partial gastrectomy, postovariectomy status, premenopausal amenorrhea, alcoholism and long-term immobilisation. Medications were: corticosteroids, diuretics, cytotoxic drugs, anticonvulsive drugs, anabolic steroids, calcitonin, bisphosphonates, vitamin D.

4.5 Statistical methods

Statistical analyses were carried out with the Statistical Package for Social Sciences (SPSS) versions 9 and 10 for Windows. The annual BMD changes for each measurement site were calculated as a difference between the first (baseline or five years) and second (five years or ten years) measurements divided by follow-up time and reported as percentage of BMD at the first measurement.

Independent samples t-tests and chi-square tests were used to test differences between study groups with regards continuous and categorical baseline variables, respectively. Analysis of variance was used to test differences between multiple groups, and the Tukey or Least Significant Difference (LSD) post hoc tests to define the groups with differences (Studies I/III/IV). Analysis of covariance was used to adjust for putative confounders when appropriate.

The best fit cubic regression model (trinomial function) was used to describe the BMD changes related to years since menopause (Study I). Single and multiple regression models were used to observe linear trends between study-specific factors and annual BMD change. In study II, factors were selected for the multiple regression model based on their statistical significance in the univariate regression models (inclusion criteria: p≤0.2) and
additionally based on clinical relevance considered by the author. However, the same variables were forced into both models for both sites regardless of univariate significance $p<0.2$ or $p>0.2$ to achieve comparable analysis for each factor. In the multiple regression, all variables were entered simultaneously into the model.

In studies III and IV significances of interaction were obtained by entering the following variables into the same ANCOVA model (F-test) with both of the two separate components: [calcium intake x HRT use] (Study III), [calcium intake x smoking] (Study IV). These variables were categorical or continuous for interaction analyses in ANCOVA or regression models, respectively.
5 RESULTS

Table 5 shows the characteristics of the 1551 women with valid measurement results.

**Table 5** Characteristics of the 1551 women with valid measurement results.

### A. CONTINUOUS VARIABLES

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of follow-up (years)</td>
<td>5.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Baseline age (years)</td>
<td>53.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Baseline height (cm)</td>
<td>161.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Baseline weight (kg)</td>
<td>68.6</td>
<td>11.2</td>
</tr>
<tr>
<td>Mean weight change (BL→5 years)</td>
<td>3.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Baseline grip strength (kPA)</td>
<td>62.8</td>
<td>16.6</td>
</tr>
<tr>
<td>Mean calcium intake (mg/day)</td>
<td>789</td>
<td>316</td>
</tr>
<tr>
<td>Baseline lumbar BMD (g/cm²)</td>
<td>1.12</td>
<td>0.26</td>
</tr>
<tr>
<td>Baseline femoral BMD (g/cm²)</td>
<td>0.93</td>
<td>0.13</td>
</tr>
<tr>
<td>Annual lumbar BMD change (%) (BL→5 years)</td>
<td>-0.35</td>
<td>1.03</td>
</tr>
<tr>
<td>Annual femoral BMD change (%) (BL→5 years)</td>
<td>-0.51</td>
<td>0.91</td>
</tr>
</tbody>
</table>

### B. CATEGORICAL VARIABLES

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of any HRT during follow up (BL→5 years)</td>
<td>797</td>
<td>51.4</td>
</tr>
<tr>
<td>Any fracture before baseline</td>
<td>311</td>
<td>20.1</td>
</tr>
<tr>
<td>Wrist fracture before baseline</td>
<td>104</td>
<td>6.7</td>
</tr>
<tr>
<td>Alcohol over 1 drink (12 g) per week</td>
<td>579</td>
<td>37.3</td>
</tr>
<tr>
<td>Ever smoked (before 5 year measurement)</td>
<td>289</td>
<td>18.6</td>
</tr>
<tr>
<td>Bone affecting disease/medication at baseline</td>
<td>682</td>
<td>44.0</td>
</tr>
<tr>
<td>Calcium supplementation at baseline</td>
<td>478</td>
<td>30.8</td>
</tr>
<tr>
<td>Overall physical activity level*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>506</td>
<td>32.6</td>
</tr>
<tr>
<td>Moderate</td>
<td>537</td>
<td>34.6</td>
</tr>
<tr>
<td>High</td>
<td>474</td>
<td>30.6</td>
</tr>
<tr>
<td>Menopausal status at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>165</td>
<td>10.6</td>
</tr>
<tr>
<td>Perimenopausal</td>
<td>413</td>
<td>26.6</td>
</tr>
<tr>
<td>Early postmenopausal §</td>
<td>516</td>
<td>33.3</td>
</tr>
<tr>
<td>Late postmenopausal §§</td>
<td>457</td>
<td>29.5</td>
</tr>
</tbody>
</table>

*Combined physical activity at leisure and work, three-categorical variable.

§ < 5 years postmenopausal at baseline, §§ > 5 years postmenopausal at baseline
5.1 Menopausal transition and bone loss (Study 1)

In this study, peri- and late postmenopausal women seemed to have a longer duration of follow-up (5.9 years) in comparison to other groups (pre 5.8 and early post 5.7 years). In addition, the baseline BMDs seemed to be lower in early- and late postmenopausal women in comparison to pre- and perimenopausal (p<0.001) women.

Figure 4 shows the mean annual BMD change (%) for the study groups (n=409). The greatest bone loss rate at all sites was observed in perimenopausal women and it was significantly higher than in premenopausal women at all sites except femoral neck. Early postmenopausal women had a significantly lower bone loss rate than perimenopausal women whereas the mean annual bone loss in late postmenopausal women was found to be significantly lower than that of early postmenopausal women at the lumbar spine. Adjustment for age, BMI, baseline BMD and duration of follow-up did not change the results at any site.

The annual bone loss in peri- and postmenopausal women (n=396) was best described as a trinomial function of the duration of menopause (months) at all sites (Figure 5). Linear, logarithmic or binomial models did not improve the fit at any site. The pattern of bone loss in the trinomial model was found to differ between measurement sites: in lumbar spine, the decrease in bone loss reached a plateau in a later phase of postmenopause than in femoral areas. Due to the small number of women over 200 months postmenopausal (n=20, 5.1 % of the peri- and postmenopausal women) the cubic regression equations were found to be applicable for the prediction of bone loss rate only up to this limit (see Figure 5).
Figure 4 Annual BMD change (%) with 95% CI according to menopausal status and measurement site (n=409).

Significances between the groups:

<table>
<thead>
<tr>
<th>Groups in comparison</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perimenopausal vs. Premenopausal</td>
<td>p&lt;0.001 / p=NS / p&lt;0.02 / p&lt;0.01</td>
</tr>
<tr>
<td>Early postmenopausal</td>
<td>p&lt;0.001 / p&lt;0.02 / p&lt;0.001 / p&lt;0.001</td>
</tr>
<tr>
<td>Late postmenopausal</td>
<td>p&lt;0.001 / p&lt;0.05 / p&lt;0.001 / p&lt;0.001</td>
</tr>
<tr>
<td>Early postmenopausal vs. Late postmenopausal</td>
<td>p=0.04 / p=NS / p=NS / p=NS</td>
</tr>
</tbody>
</table>
Figure 5 Estimated mean annual BMD change (%) according to duration of menopause (at 5 year measurement): cubic regression analysis in peri- and postmenopausal women (n=396) (premenopausal women excluded).

Cubic regression equations:

<table>
<thead>
<tr>
<th>Method</th>
<th>Equation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>$AN = 7 \times 10^{-3} \times (DUR) + 4 \times 10^{-5} \times (DUR)^2 - 2 \times 10^{-7} \times (DUR)^3 - 1.42$</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>FN</td>
<td>$AN = 1.2 \times 10^{-2} \times (DUR) - 8 \times 10^{-5} \times (DUR)^2 - 1.9 \times 10^{-7} \times (DUR)^3 - 1.19$</td>
<td>$0.028$</td>
</tr>
<tr>
<td>WT</td>
<td>$AN = 2.2 \times 10^{-2} \times (DUR) - 1 \times 10^{-4} \times (DUR)^2 + 2.2 \times 10^{-7} \times (DUR)^3 - 1.68$</td>
<td>$0.004$</td>
</tr>
<tr>
<td>TR</td>
<td>$AN = 2.1 \times 10^{-2} \times (DUR) - 1 \times 10^{-3} \times (DUR)^2 + 2.2 \times 10^{-7} \times (DUR)^3 - 0.89$</td>
<td>$0.002$</td>
</tr>
</tbody>
</table>

ANN=annual BMD change (%); DUR=duration of menopause; p=significance in cubic regression analysis.

The arrow-block indicates the limit (in postmenopausal months) of the cubic regression equation in predicting the values of annual BMD change in postmenopausal women (= 200 months postmenopausal).
5.2 Risk factors for perimenopausal bone loss (Study II)

Table 6 presents the multiple regression analysis for the 940 perimenopausal women. Variables were selected based on significance in the univariate model. The factors tested in univariate model were (significance in univariate model LS/FN): baseline weight (p<0.05/p=0.220), weight change (from baseline to 5-year measurement) (p=0.061/p<0.001), baseline height (p=0.472/p=0.372), age (p<0.001/p<0.05), time since menopause (p<0.001/p<0.001), grip strength (p<0.05/p=0.072), calcium intake (p=0.083/p=0.074), coffee intake (p=0.463/p=0.296), alcohol intake (p=0.870/p=0.625), smoking (p=0.359/p=0.975), age at menarche (p=0.774/p=0.491), and parity (p=0.736/p=0.557).

In the multivariate model, recent menopause, no use of HRT and weight loss predicted greater bone loss at both lumbar spine and femoral neck. In contrast, higher age and low weight at baseline predicted increased bone loss only at lumbar spine and high grip strength lower bone loss only at femoral neck. The linear relationship between femoral neck bone loss and nutritional calcium intake was of borderline significance (p=0.053).

Multivariate models in Table 6 explained 26.5% of the BMD variation at lumbar spine (crude/adjusted $R^2=0.272/0.265$) and 13.6% of the variation at femoral neck (crude/adjusted $R^2=0.145/0.136$) of the bone mass changes.

5.3 Interaction between HRT and weight loss in postmenopausal bone loss (Study II)

In this part of Study II, HRT non-users were older, had a longer time since menopause, had a higher baseline weight, calcium intake, prevalence of wrist fractures and bone affecting co morbidity as well as lower grip strength and mean annual BMD change (p<0.05).
Table 6 Effects of selected factors on annual BMD change (%) according to multiple regression analysis in peri- and postmenopausal women (n=940).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>T ratio</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Lumbar spine (R²=0.265)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>-3.88</td>
<td>1.19</td>
<td>-3.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Months since menopause*</td>
<td>0.005</td>
<td>0.001</td>
<td>6.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HRT use**</td>
<td>1.25</td>
<td>0.09</td>
<td>14.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.02</td>
<td>0.003</td>
<td>6.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline age, years</td>
<td>0.06</td>
<td>0.01</td>
<td>4.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight change, kg</td>
<td>0.02</td>
<td>0.01</td>
<td>2.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height, cm</td>
<td>-0.01</td>
<td>0.01</td>
<td>-1.59</td>
<td>0.112</td>
</tr>
<tr>
<td>Calcium intake, mg/day</td>
<td>0.00001</td>
<td>0.0001</td>
<td>1.48</td>
<td>0.138</td>
</tr>
<tr>
<td>Grip strength, kPA</td>
<td>-0.001</td>
<td>0.002</td>
<td>-0.48</td>
<td>0.629</td>
</tr>
<tr>
<td>B. Femoral neck (R²=0.136)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>-0.47</td>
<td>1.09</td>
<td>-0.43</td>
<td>0.671</td>
</tr>
<tr>
<td>Months since menopause*</td>
<td>0.003</td>
<td>0.001</td>
<td>4.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HRT use**</td>
<td>0.68</td>
<td>0.08</td>
<td>8.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight change, kg</td>
<td>0.03</td>
<td>0.01</td>
<td>5.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grip strength, kPA</td>
<td>0.004</td>
<td>0.002</td>
<td>2.31</td>
<td>0.021</td>
</tr>
<tr>
<td>Calcium intake, mg/day</td>
<td>0.00002</td>
<td>0.0001</td>
<td>1.94</td>
<td>0.053</td>
</tr>
<tr>
<td>Height, cm</td>
<td>-0.01</td>
<td>0.01</td>
<td>-1.56</td>
<td>0.118</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.004</td>
<td>0.003</td>
<td>1.54</td>
<td>0.125</td>
</tr>
<tr>
<td>Baseline age, years</td>
<td>0.003</td>
<td>0.01</td>
<td>0.28</td>
<td>0.776</td>
</tr>
</tbody>
</table>

* at five-year (second) densitometry, ** percent of follow-up time
Figure 6 presents the effects of weight loss on mean annual BMD change (%) according to HRT use in a linear regression model. In all, of the studied 409 peri- and postmenopausal women a total of 172 (18.3 %) lost weight during follow up (mean 3.7 kg, -5.1 % of baseline weight).

In HRT never users, a negative linear trend was observed between weight loss and bone loss at both measurement sites. In HRT ever users, weight loss was not related with bone loss at either site although in femoral neck weight loss related bone loss was not entirely prevented by HRT in contrast to lumbar spine. Adjustment for age, weight, height, calcium intake, duration of menopause, baseline BMD and bone affecting diseases/medications (yes/no) did not change these results (Figure 6)

5.4 Interaction between HRT and nutritional calcium in the prevention of postmenopausal bone loss (Study III)

The study population and its characteristics were similar to those of Study II.

Figure 7 presents the effects of nutritional calcium intake on annual BMD change (%) according to estrogen repletion status in the analysis of variance in 937 peri- and postmenopausal women. In HRT never users, there were no differences between calcium intake tertiles. In HRT ever users, femoral neck bone loss was significantly lower in highest calcium intake tertile than in the first and the second tertile. In lumbar spine, a similar non-significant trend was observed. Figure 7 also presents the effects of HRT on bone loss according to nutritional calcium intake status (tertiles). At femoral neck, HRT users had a lower bone loss rate than HRT non-users in the third calcium tertile but there were no differences between HRT users and non-users in the other tertiles. At lumbar spine, the bone loss rate was lower in HRT users in all tertiles but the difference was greater in the second and the third tertile in comparison to the first tertile. In addition, at femoral neck, a significant interaction in ANCOVA was observed between HRT use and calcium intake. Adjustment for age, weight, baseline BMD, duration of menopause, bone affecting diseases/medications and use of calcium supplements (yes/no) did not change any of these results.
**Figure 6** Effects of weight change on BMD change according to HRT use status in peri- and postmenopausal weight losers (n=172). Linear regression model.

**Never HRT (n=97)**

- **Crude model** $p = 0.032$ (LS) $p = 0.011$ (FN)
- **Adjusted model** $p < 0.001$ (LS) $p = 0.026$ (FN)

**Ever HRT (n=75)**

- **Crude model** $p = 0.914$ (LS) $p = 0.236$ (FN)
- **Adjusted model** $p = 0.573$ (LS) $p = 0.166$ (FN)
Figure 7 Effects of nutritional calcium intake (mg/day) on mean annual BMD change according to HRT use in peri- and postmenopausal women (n=937). Analysis of variance.

A. Never HRT (n=545)

<table>
<thead>
<tr>
<th>Calcium intake tertiles *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st: ≤ 648.1, 2nd: 648.1-927.0, 3rd: &gt; 927.0</td>
</tr>
</tbody>
</table>

B. Ever HRT (n=392)

<table>
<thead>
<tr>
<th>Calcium intake tertiles *</th>
</tr>
</thead>
<tbody>
<tr>
<td>§ p&lt;0.05 in comparison to 3rd tertile / §§ p&lt;0.001 in comparison to 1st tertile</td>
</tr>
<tr>
<td>† p&lt;0.05 in comparison to corresponding tertile in ever HRT group / †† p&lt;0.001 in comparison to corresponding tertile in ever HRT group</td>
</tr>
</tbody>
</table>
In HRT users, high nutritional calcium intake significantly predicted lower femoral neck bone loss in the linear regression model (p<0.001). At lumbar spine, the mutual linearity was of borderline significance (crude p=0.063/adjusted p=0.065). In HRT non-users, no linear trend was observed. Adjustment did not change these results.

In a sub-analysis on continuous (duration of HRT >90 % of the follow-up period) HRT users only (n=95), no significant effect between nutritional calcium intake and bone loss rate was observed. In a sub-analysis on occasional users, the results remained similar with total population.

5.5 Interaction between smoking and nutritional calcium in the prevention of postmenopausal bone loss (Study IV)

The use of alcohol was found to be significantly higher in smokers than non-smokers (p<0.001). There were no differences in bone loss rate between smokers and non-smokers.

According to the linear regression model, high calcium intake prevented spinal bone loss in never smokers (p<0.02). At femoral neck, the similar linearity was of borderline significance (p=0.054) in the non-adjusted model but statistically significant after adjustment for age, weight, baseline BMD, duration of menopause, duration of estrogen therapy, bone affecting diseases and medications, use of calcium supplements and alcohol intake (p=0.020). In ever smokers, no statistically significant linear trend was observed. In a sub-analysis of only those women who reported smoking at both measurements, similar results were observed.

Figure 8 shows the effects of nutritional calcium on annual bone loss rate according to smoking status in an analysis of (co)variance. In never smokers, lumbar spine bone loss rate was significantly lower in the second and third tertile than in the first tertile. At femoral neck there were no differences between the tertiles in a crude model whereas in the adjusted model, the bone loss rate in the third tertile was lower than in the first tertile.

In lumbar spine, but not in femoral neck, a statistically significant interaction was observed between smoking and nutritional calcium intake. Furthermore, when the smoker
**Figure 8** Effect of nutritional calcium on the mean annual BMD change (%) according to smoking status. Analysis of variance. (n=946)

**NEVER SMOKERS (n=772)**

**EVER SMOKERS (n=182)**

**Calcium intake tertiles**

<table>
<thead>
<tr>
<th>Calcium intake tertiles*</th>
<th>Mean annual BMD change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td><img src="image1" alt="Graph" /></td>
</tr>
<tr>
<td>2nd</td>
<td><img src="image2" alt="Graph" /></td>
</tr>
<tr>
<td>3rd</td>
<td><img src="image3" alt="Graph" /></td>
</tr>
</tbody>
</table>

**Calcium intake tertiles**

<table>
<thead>
<tr>
<th>Calcium intake tertiles*</th>
<th>Mean annual BMD change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td><img src="image4" alt="Graph" /></td>
</tr>
<tr>
<td>2nd</td>
<td><img src="image5" alt="Graph" /></td>
</tr>
<tr>
<td>3rd</td>
<td><img src="image6" alt="Graph" /></td>
</tr>
</tbody>
</table>

**Significance of interaction (ANOVA)** (crude/adjusted): Lumbar spine p=0.033/0.913 Femoral neck p=0.761/0.452

*Ca intake tertiles (mg/day): 1st: ≤648.1, 2nd: 648.1-927.6, 3rd: > 927.0

§ p<0.05 in comparison to 1st tertile; §§ p<0.01 in comparison to 1st tertile; (§) p<0.05 in adjusted model only (see text).
group was added to the non-smoker group, the bone protective effect of nutritional calcium was no longer observed.

5.6 Effects of statins on postmenopausal bone loss (Study V)

In this study, continuous users of statins were older (p<0.05) and had a shorter duration of follow-up (p<0.001) than other groups. Similarly, all statin users had fewer concomitant illnesses and more medications than non-users (p<0.05).

Figure 9 shows the mean annual BMD changes according to the study groups in women with valid covariate information (n=607 vs. original n=620). In addition, annual BMD changes differing more than four SDs from the mean were considered as non-valid and were excluded from the analysis (n=2). The annual BMD changes at the spine or femur did not differ significantly between the statin users and non-users of statins regardless of the reported normal blood cholesterol level or hypercholesterolaemia. Adjustment for age, years since menopause, BMI, BMD at baseline, calcium intake, use of corticosteroids, estrogen therapy, duration of follow-up and statin use before the baseline did not change the results. Also, further adjustment for number of illnesses and number of prescribed drugs did not change the results.

In a comparison of the annual BMD changes between the different statin preparations (lovastatin, simvastatin, atorvastatin, pravastatin), no statistically significant differences were found. Analysis on women who did not use HRT during the follow-up indicated similar BMD changes than those of the total study population.

Table 7 summarizes the main results of the studies (1-V).
Figure 9 Lumbar spine and femoral neck mean annual BMD change (%) according to use of statins in analysis of variance (n = 607) (crude model).

Statin use during the follow-up

<table>
<thead>
<tr>
<th>Study group</th>
<th>N</th>
<th>Lumbar spine</th>
<th>Femoral neck</th>
</tr>
</thead>
<tbody>
<tr>
<td>No statin use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Chol -</td>
<td>355</td>
<td>0.47 (0.31, 0.63)</td>
<td>-0.33 (-0.47, -0.19)</td>
</tr>
<tr>
<td>2 Chol +</td>
<td>137</td>
<td>0.54 (0.31, 0.77)</td>
<td>-0.32 (-0.53, -0.11)</td>
</tr>
<tr>
<td>Statin use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Occasional</td>
<td>60</td>
<td>0.19 (-0.19, 0.47)</td>
<td>-0.54 (-0.84, -0.24)</td>
</tr>
<tr>
<td>4 Continuous</td>
<td>55</td>
<td>-0.20 (-1.04, 0.64)</td>
<td>-0.47 (-1.24, 0.30)</td>
</tr>
<tr>
<td>P value from ANOVA</td>
<td>(p = 0.134)</td>
<td>(p = 0.628)</td>
<td></td>
</tr>
</tbody>
</table>

* adjusted for age, years since menopause (continuous), BMI, BMD at baseline, calcium intake, use of corticosteroids (yes/no), estrogen therapy (no/occasional/continuous), duration of follow-up and statin use before the baseline (yes/no).

Chol +/- = reported/did not report high cholesterol level verified by a physician.
**Table 7** Summary of the main results from studies I-V

<table>
<thead>
<tr>
<th>Study</th>
<th>Aim(s) and duration of follow-up</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Investigate the patterns of postmenopausal bone loss and factors modifying this loss. Mean duration of follow-up 5.8 years</td>
<td>Bone loss most accelerated in early amenorrhea phase (perimenopausal women), rate of loss decreased in later menopause (postmenopausal women). Patterns of spinal and femoral bone loss differed from each other.</td>
</tr>
<tr>
<td>II</td>
<td>1. Risk factors for bone loss in peri- and postmenopausal population. 2. Does HRT prevent weight loss related postmenopausal bone loss? Mean duration of follow-up 5.8 years</td>
<td>1. Weight change had an inverse relationship to bone loss and it was the strongest predictor of bone loss with years since menopause (p&lt;0.001). 2. Weight loss did not predict bone loss in HRT users whereas in HRT non-users weight loss was associated with significant bone loss (p&lt;0.05). This effect was more clearly seen in lumbar spine.</td>
</tr>
<tr>
<td>III</td>
<td>Interaction of HRT and nutritional calcium in prevention of postmenopausal bone loss. Mean duration of follow-up 5.8 years</td>
<td>In HRT users, high calcium intake predicted lower femoral neck bone loss (p&lt;0.001) which was not seen in HRT non-users. Use of HRT did not predict lower femoral neck bone loss in women with low calcium intake.</td>
</tr>
<tr>
<td>IV</td>
<td>Interaction of nutritional calcium and smoking in postmenopausal bone loss. Mean duration of follow-up 5.8 years</td>
<td>High calcium intake predicted lower bone loss in non-smokers (p&lt;0.05) but not in smokers. This effect was more clearly seen in lumbar spine although femoral neck showed a similar kind of relationship.</td>
</tr>
<tr>
<td>V</td>
<td>Do statins prevent bone loss in early postmenopausal women? Mean duration of follow-up 4.4 years</td>
<td>No differences in bone loss detected between statin users and non-users of statins.</td>
</tr>
</tbody>
</table>
6 DISCUSSION

6.1 Study frame and methodology

The present study was performed with samples selected from the prospective Osteoporosis Risk Factor and Prevention (OSTPRE) study cohort, Kuopio, Finland. Information on risk factors of osteoporosis has been gathered via postal inquiries and three BMD measurements have been performed with the DEXA device at five year interval.

The OSTPRE study is unique because of its prospective population-based design with long-term follow-up. Also, the sample is large enough to permit the detection of even minor effects. The response rates to inquiries have been high (91%) (Tuppurainen et al. 1993) and validation of information in postal inquiries has been performed to some extent (Lindstedt 1993, Sandini et al. 2002: unpublished data). In addition, responses to postal inquiries have been partially checked at the time of bone densitometry and deficient or ambiguous information has been checked via the telephone during the coding phase.

In the present study, bone mass measurements were carried out with dual X-ray absorptiometry (Lunar DPX Madison, Wisconsin, USA and DPX-IQ model) which has been shown to be both an accurate and precise method (Cummings et al. 2002). Errors due to measurement technique were minimized by using specially trained personnel and by regular use of phantom measurements. Although the measurement device was changed during one study period (Cf. Study V), a comparison was performed and correlation functions used to eliminate possible interference from the change. The short- and long-term reproducibility of the device has been reported in previous studies (Kröger et al. 1992, Komulainen et al. 1998).

The study samples used were randomly selected. Thus, they were likely to be representative of the population. The only exception was study V in which 67% of statin users and 84% of controls belonged to the random sample and the rest belonged to the non-random part of the densitometry sample. This was due to the premature measurement of some statin users to increase the number of cases for study V. In addition, from the randomly selected samples, we either excluded women with diseases and medications
known to affect bone metabolism or treated the variables as confounders in multivariate models (Kröger et al. 1994). Thus, confounding due to these factors is unlikely.

The statistical analyses in the present study were performed with SPSS for Windows program. Regression, uni- and multivariate analysis of variance, post hoc, t-tests or chi-square test were used. Comprehensive adjustment for potential confounders was performed when appropriate. In multivariate regression models, the variables were selected based on their significance in the univariate model (p<0.2) and additionally based on clinical relevance, considered by the author, of each factor when appropriate. These procedures were intended to provide as unbiased results as possible.

However, there is always a possibility of bias in observational studies. The information on postal inquiries cannot be fully validated and some mistakes in both the answer as well as the coding/interpretation phase are possible. Also, the follow-up time in our study was long and varied between study subjects which may contribute to variation in bone loss rate over time. Moreover, the study populations (range 409-954 women) represented only a fraction of the original OSTPRE-cohort (n=13 100) and the random sample (n=2025). Lastly, we excluded women without a valid BMD measurement result (spondylarthrosis, scoliosis, etc.), women without information on the time of menopausal transition and in Study I HRT users. These exclusions may have resulted in a sample that does not accurately represent the total population. Furthermore, the bone loss in the present study was calculated as a difference between two successive measurements, masking the possible non-linear pattern of bone loss. In reality it is likely that bone loss is not truly linear. Accordingly, our study cannot accurately reflect the patterns of short-term BMD changes such as maximal bone loss rates. Finally, measurement errors cannot be entirely eliminated.

The division of women into different groups was quite straightforward in some parts of the present study. HRT users were divided into never or ever users regardless of the time of use and, similarly, smokers were either ever smokers or never smokers. This kind of straightforward categorisation masks possible differences between the effects of continuous and occasional exposures and thus the verification of possible dose-response relationships. Nevertheless, by this division, the group size increased and the need for
arbitrary cut off points was decreased. In the present study, supplemental analyses were performed, if the number of study subjects was sufficient, for different subgroups of women, i.e. heavy smokers and continuous HRT users, to evaluate the possible differences in bone response with respect to the amount or duration of exposure. In this way we were able, to some extent, to evaluate the possible underestimation of a true effect and dose response.

6.2 Determinants of postmenopausal bone loss

6.2.1 Menopausal transition

Menopausal transition is the most important and inevitable single factor in the evolution of postmenopausal osteoporosis (Hansen et al. 1991, Ensrud et al. 1995). The present study indicated that the most accelerated bone loss phase is manifest during the very first years of the menopausal transition. This effect in perimenopausal women has also been observed in previous reports (Harris and Dawson-Hughes 1992, Pouilles et al. 1993, Pouilles et al. 1995). In later postmenopause, the rate of bone loss has been observed to decline (Hansen et al. 1995, Pouilles et al. 1995, Young et al. 1996) as also demonstrated in our study. These alterations are attributable to the decline in estrogen production and imply that preventive HRT should be initiated as soon as possible, during the early amenorrhea phase.

In agreement with previous studies (Hansen et al. 1995, Luckey et al. 1996, Young et al. 1996), the present study also found differences in bone loss rate between different skeletal sites: the pattern of bone mass changes in lumbar spine was different from that in femoral measurement sites. These differences may be explained by the different bone composition of these structures. Lumbar spine consists mainly of trabecular bone whereas the cortical bone compartment is more noteworthy in femoral neck. Accordingly, as menopausal estrogen depletion leads to an intense level of bone remodelling, it is likely to have a greater impact on trabecular than cortical bone. Furthermore, also other factors, not related to menopausal transition itself, may modify the response to estrogen depletion
between spinal and femoral regions due to the structural differences between these bone sites.

Some differences between previous reports and the present study are worthy of consideration when evaluating the closer patterns of bone loss around menopausal transition. The determination of “postmenopause” itself has varied between different reports. Some studies have used 6 months and others, such as the present study, 12 months of amenorrhea as the cut-off point. Currently 12 months of amenorrhea is proposed to be used as the limit in epidemiological studies (WHO 1996). Furthermore, the menopausal transition has been determined either by detection of serum hormone levels or, as in the present study, by self-reports. Although hormonal detection may more accurately reflect the absolute change in estrogen levels, self-reports may be a surprisingly sensitive measure of corresponding endocrine status at the time of examination (Dennerstein et al. 1993, Prior 1998). However, in the present study, the category ‘perimenopausal’ was, in a sense, straightforward as it included all women with their menopausal transition during the follow-up, regardless of the actual time point of transition. Although this was the only way to avoid the use of artificial cut-off points, it has lead to underestimation of the fastest bone loss rate due to long follow-up interval (ca. 6 years) in the present study. The duration of the fast bone loss in early postmenopausal phase is, in fact, only 2-3 years (Pouilles et al. 1993, Pouilles et al. 1995). Thus, the present study was not ideal for investigating in detail the temporal patterns of bone loss. This would have entailed bone mass measurements at least at every one or two year intervals. In addition, the present study was not suitable for the estimation of the premenopausal bone loss rate due to the rather small number of premenopausal women in our study population.

The rate of bone loss attributable to natural menopausal transition itself in the present study was generally smaller than those previously reported. The fastest (perimenopausal) bone loss rate was observed in lumbar spine and Ward’s area, between -1 and -1.5 percent/year. In femoral neck, the fastest (perimenopausal) bone loss was under -1 percent/year and in trochanter under -0.5 percent/year. In previous studies, premenopausal bone loss rates of over -2 percent/year in spinal and over -1 percent/year in the femoral region have generally been reported (Harris and Dawson-Hughes 1992,
Pouilles et al. 1993, Pouilles et al. 1995, Prior et al. 1998, Ito et al. 1999). This might partly be due to differences in the populations between these studies. As an example, the present study population was somewhat overweight. In addition, the present study excluded a wide range of bone affecting co-morbidity and medications which might have distorted results in previous reports. Lastly, the straightforward classification of women into perimenopausal group, as discussed above, may contribute to the values for the bone loss rate found in the present study.

6.2.2 Contribution of other factors

As shown in the present study, ultimately there are only a few factors other than menopause that could alter the rate of postmenopausal bone loss. As also demonstrated in some previous studies, low body weight and weight loss seem to predict higher postmenopausal bone loss and vice versa (Burger et al. 1998, Hannan et al. 2000). Overall, this relationship between weight control and osteoporosis is problematic as large weight and a weight increase can protect from bone loss (Brot et al. 1997, Yoshimura et al. 1998, Nguyen et al. 1998) but, at the same time, overweight is a significant risk factor for increased mortality and morbidity especially in the postmenopausal age group (Srinivasan et al. 1996, Rea et al. 2001). Accordingly, a significant weight increase or overweight should not be encouraged despite its positive bone effects but, on the other hand, significant weight loss should not be recommended at the time of menopause, especially in women with normal body weight (BMI 20-25).

The present study observed that there may be differences between measurement areas which can impact on the postmenopausal risk factors for bone loss. This was observed most clearly in grip strength, which predicted only femoral neck BMD but lacked any predictive effect in lumbar spine. Accordingly, some risk factors for postmenopausal bone loss may be highly site specific as has actually been previously shown for muscular strength (Blain et al. 2001). Hence, the relationship between grip strength and femoral neck bone loss probably is due to better overall muscular fitness also in the femoral regions.
Overall, the small number of bone loss modifying factors, other than menopause itself, suggests that postmenopausal bone loss cannot be fully prevented simply by concentrating on lifestyle. Nevertheless, the previous population-based reports on significant effects of certain factors, such as smoking and nutritional calcium intake, on bone loss should not be completely discarded (Heaney 1997, Bjarnason and Christiansen 2000). Instead, the pathophysiological mechanisms underlying these effects and the reason for difficulties in repeating the significant relationship in other population-based studies should be closely investigated. Our study may provide some relevant information on this field.

6.3 Prevention and treatment of postmenopausal bone loss with HRT and nutritional calcium: contribution of interactive effects

6.3.1 The current role of HRT in treatment of postmenopausal osteoporosis

There is no question about the effectiveness of HRT in the prevention of postmenopausal bone loss and fractures (Lindsay and Tohme 1990, Cauley et al. 1995, Schneider et al. 1997. HRT increases 5.4 % lumbar spine and 2.5 % femoral neck BMD after first year of treatment with a 34 % reduction in vertebral and a 13 % reduction in non vertebral fracture incidence (Wells et al. 2002). Recently, there has been some debate about the safety of HRT. Most interestingly, and surprisingly, the Women’s Health Initiative (WHI) -study group published results of a long term HRT trial suggesting that long-term HRT may increase the risk of cardio-vascular incidents and breast cancer (Rossouw et al. 2002). As HRT has previously been considered as the first line therapy for postmenopausal osteoporosis, the need for new guidelines is obvious.

Presently, non-hormonal products (bisphosphonatcs, calcitonin) should be considered as first line therapy for postmenopausal osteoporosis and women on long-term HRT might benefit from a visit to a physician for an in-depth evaluation of their need for and the appropriateness of the therapy. Nevertheless, HRT is still indicated for menopausal symptoms and these postmenopausal women may use HRT instead of non-hormonal products also in the treatment of osteoporosis and climacteric symptoms. Also,
the long-term adverse effects of brief periods of HRT still remain to be clarified although
the positive effects of short-term HRT on bone are more or less inversed after the
cessation of therapy (Lindsay et al. 1978, Christiansen et al. 1981, Tremollieres et al.
2001). However, continuation of short-term HRT with e.g. bisphosphonates might indicate
hormonal therapy for symptomatic patients.

6.3.2 Interaction between HRT and body weight changes

Previous studies have observed that some women do not respond to HRT as
effectively as others due to life-style factors such as slenderness and low BMI
(Komulainen et al. 2000, Hassager et al. 1994). On the other hand, Bjarnason and
Christiansen have previously found that the deleterious impact on bone of low BMI
(measured at one time point) may be prevented by HRT (Bjarnason and Christiansen.
2000). To some extent, this interaction is not surprising, as body weight and weight loss /
increase have been found to be the most powerful determinants, as well as the use of HRT,
of postmenopausal bone loss rate as discussed previously.

In the present study, the interaction between HRT and body mass was determined
by analyzing weight loss instead of only one body weight measurement. This has not been
studied previously in a population-based study. The present study observed a significant
difference in bone loss between HRT users and non-users in significant weight losers (e.g.
-10 or -20 % of baseline weight). Accordingly, weight loss related bone loss was
effectively prevented by HRT, suggesting that weight losers might benefit from HRT
around menopause. In this way, an effective prevention of bone loss might be achieved in
all women regardless of their weight loss intentions. The effect was especially clear in
lumbar spine, whereas in the femoral neck it was more difficult to observe. In the present
study, the results were adjusted for baseline weight which should have eliminated the
possibility of bias due to differences in body mass at baseline. Interestingly, however, as
demonstrated in the present study, the bone preserving effect of HRT may relate to the
extent of weight loss as there were no differences in the bone loss rate between HRT users
and non-users in women who experienced no or only negligible weight loss. It may be that
differences in calcium intake, as well as alterations in physical activity level in weight
losers might additionally modify the HRT effect, as observed in previous studies (Honkanen et al. 2001, Prince et al. 1991, Kohrt et al. 1995). Muscular fitness has been found more site specific than lean mass in the prediction of postmenopausal bone loss (Blain et al. 2001). This might explain the more clear effect of the interaction in lumbar than femoral bone noted in the present study. Finally, as HRT has been criticised lately, it would be worthwhile to also study the efficacy of the newer antiresorptive agents (bisphosphonates, SERMs, etc.) on weight loss related bone loss in future studies.

Because of the observed interaction between HRT and weight change, a logical conclusion would be that the weight loss induced bone loss is mostly due to fat loss and related depletion in estrogen production (Nelson and Bulun 2001). Accordingly, the results with regards HRT seen in the present study would be attributable to the replacement of fat tissue related estrogen production by IIRT. However, the role of muscle loss as well as dietary factors in weight loss induced bone loss should be more closely investigated. As an example, exercise induced weight loss might have a less significant effect on bone loss in comparison to simple restriction in nutritional intake. On the other hand, overweight population is likely to ingest more calcium through ingestion of higher amounts of nutritional products which might further modify the weight related bone mineral changes. Another important point to consider is the possibility of pathologic weight loss e.g. due to malignancies that may directly affect bone metabolism. In the present study, the results were adjusted for known diseases affecting bone, making this kind of bias unlikely. In addition, the role of fat tissue regulating hormones, leptins, in bone metabolism remains unresolved but might provide an additional mechanism contributing to bone density in postmenopausal years (Blain et al. 2001, Yamauchi et al. 2001).

The role of methodological difficulties of measurement techniques to deal with body compositional factors, especially soft-tissue and fat, remains undetermined (Bolotin 1998, Bolotin et al. 2001). As the DXA technique uses the soft tissue density as its reference for bone density measurements, the unequal distribution of fat mass changes around and inside bones may result in significant errors in bone loss estimates (Bolotin et
al. 2001). This may additionally modify the effect of weight and its interaction with HRT on bone loss seen in the present study.

6.3.3 Interaction between HRT and nutritional calcium

The present study clearly showed that low nutritional calcium intake is a significant risk factor for no response to HRT whereas high nutritional calcium intake was found to potentiate the effects of HRT. This effect has also been observed with supplemental calcium in previous studies (Davis et al. 1995, Mizunuma et al. 1996, Nieves et al. 1998).

Interestingly, the present study suggested that the interaction between calcium and continuous HRT may not be associated with a similar kind of decline in bone loss compared to occasional HRT. This effect may be explained by the finding that improvement of BMD and the decrement of bone loss achieved with HRT plateaus between 1.5-3 years after initiation of the therapy (Nielsen et al. 1994, Komulainen et al. 1999) producing, in a sense, an “over-dosed” bone metabolic state. Accordingly, calcium, since it is a significantly weaker anti-resorptive agent, may hasten the acquisition of this plateau level but not necessarily improve the overall efficacy of HRT. On the other hand, calcium might also prevent or slow down the decline in BMD occurring after cessation of HRT (Lindsay et al. 1978, Christiansen et al. 1981, Tremollieres et al. 2001). However, the failure to see any calcium effect in continuous HRT users may also simply reflect lack of power due to inaccuracy in measurement of nutritional calcium intake and, thus, the rather small sample size. Lack of power is a well known problem with investigations on bone response to nutritional calcium in observational studies (Heaney 1997). In practice, the present study suggests that all patients on HRT should be advised to have adequate nutritional calcium intake.

The mechanisms by which HRT intensifies the bone protective effects of calcium, and vice versa, are not fully understood. The interaction may result from the additive effects of two antiresorptive agents whereas poor nutritional calcium intake might limit the efficacy of estrogen replacement to prevent bone loss. Estrogen also improves intestinal calcium absorption through stimulation of vitamin D production and the responsiveness of calcitonin secretion to calcium intake (Gennari et al. 1990). The vitamin D status of this
study population was not evaluated though the Finnish population is known to lack sunlight exposure related vitamin D production (Lamberg-Allardt et al. 2001). In the present study population, inadequate vitamin D levels might have impaired calcium absorption (Holick 1995). In addition, estrogen can increase the renal reabsorption of calcium (Gallagher 1996).

In the present study, the interactive effects of nutritional calcium and HRT were more readily observed in femoral than spinal bone which may be related to the different bone composition of these sites. Accordingly, the non-continuous nature of estrogen replacement in the present study population may have different effects on cortical and trabecular bone due to the non-steady bone metabolic state. As an example, the trabecular structure of lumbar vertebrae could be more labile in its response to non-continuous HRT. In addition, the greater weight-bearing stress at femoral neck may also contribute to this difference. Indeed, it has been suggested that HRT and exercise as well as calcium intake and exercise show positive interactions (Prince et al. 1991. Kohrt et al. 1995).

6.3.4 Interaction between nutritional calcium and smoking

The role of nutritional calcium in the prevention of postmenopausal osteoporosis and bone loss is complex. Although protective effects of calcium supplementation have been widely reported, no similar kind of relationship has been apparent with nutritional calcium (Cumming 1990, Cumming et al. 1993, Feskanich et al. 1997, Heaney 1997). Accordingly, there is a well established problem with evaluating the effect of nutritionally ingested calcium on bone loss because of the lack of power in observational studies (Heaney 1997). There may be several reasons for this finding. For example, it is impossible to obtain totally valid information on nutritionally ingested calcium and the amount of nutritionally ingested calcium varies significantly between populations. In addition, the use of calcium supplements may distort the true effect of nutritionally ingested calcium when the latter is used as a surrogate of total calcium intake, especially if supplementation is not taken into account.

The present study provided some additional clues for the poor reproducibility of significant positive results between nutritional calcium intake and bone loss in
postmenopausal women. Most interestingly, it was observed that in postmenopausal smokers, calcium may not prevent bone loss. This interaction also contributes to the knowledge of possible pathophysiology underlying the negative effects of smoking on postmenopausal bone loss (Law and Hackshaw 1997, Krall and Dawson-Hughes 1999, Bjarnason and Christiansen 2000, Hermann et al. 2000) although the present study could not confirm more significant bone loss rate in smokers. Also previous findings suggest that smoking significantly impairs the ability of the gut to absorb calcium through suppression of the PTH-calcitriol axis (Krall and Dawson-Hughes 1999, Rapuri et al. 2000b, Need et al. 2002) but its direct relationship with bone loss has not been established. Accordingly, the results of previous studies on negative bone effects of smoking may partially be determined by the amount of ingested calcium of the corresponding population (Krall and Dawson-Hughes 1999). On the other hand, the previously discussed interaction between HRT and nutritional calcium may additionally modify the bone related effects of nutritional calcium. Hence, the present study suggests that the poor correlation between nutritional calcium and postmenopausal bone loss in previous reports may reflect inadequate attention to the presence of heavy smokers and HRT users as potential confounders in the analyses. In future studies, vitamin D intake status should be more closely investigated in this interaction, since its role, especially in combination with calcium, is not perfectly understood. In addition, it must be recalled that in the present study population, the overall calcium intake was relatively high in comparison to some other populations which may allow a clearer detection of the impact of nutritional calcium on bone mineral status.

6.4 Statins for treatment of postmenopausal osteoporosis?

There is a clear need to discover osteoblast stimulating drug. The present treatment of osteoporosis (bisphosphonates, HRT) is based on inhibition of osteoclast function. In 1999 Mundy et al. reported that statins may increase osteoblast activity in rodents (Mundy et al. 1999). Subsequently, several studies have investigated the relationship between statins and BMD or fractures (Chung et al. 2000, Edwards et al. 2000, Meier et al.
In the present study, we found no evidence that statins would affect bone loss. This finding disagrees with many studies (Chung et al. 2000, Edwards et al. 2000, Meier et al. 2000, Wang et al. 2000) though some studies have also found no beneficial effect (Reid et al. 2001, van Staa et al. 2001).

These rather conflicting results imply that statins are still far from being accepted clinical therapy for the prevention of postmenopausal bone loss. The present study was not ideal for investigating the effects of statins due to the small number of statin users. Although this study may have missed a small effect of statins it is likely that it would have detected any major effect. Accordingly, the previous reports of positive effects of statins in bone loss in may have been biased due to their cross-sectional nature or the retrospective study design or the presence of selected study populations. In addition, it must be emphasized that positive effects in rodents and cellular cultures are not necessarily extrapolatable to humans. Statins may also have effects not related to bone itself, e.g. perhaps preventing the tendency to fall, which could explain the previous positive effects on fracture incidence. Finally, when considering the current data on the relationship between statin use and bone health, it is often the case that positive results are published prior to negative ones. Thus, there is a need for future research into this topic.

Although statins do not, as such, appear to be appropriate therapy for osteoporosis, the observed relationship with bone metabolism in some studies may provide new impetus for developing new osteoblast stimulating drugs. One of the most interesting findings has been that statins affect the same pathway as bisphosphonates which also are intimately related to cholesterol synthesis pathway. Based on the results of the present study, it was hypothesized that cholesterol might affect bone metabolism. Although the present study did not give any clear evidence for such an effect, some previous studies have noted this kind of relationship (Yamaguchi et al. 2002). It might be that statins directly affect bone metabolism but also modify their own bone effect through alteration of lipid levels thus explaining the highly differing results in observational studies and, at the same time, the rather clear effects demonstrated in cell culture systems (Mundy et al. 1999). Furthermore, the possible effects of statins on bone may be due interaction with other bone loss
determining factors, such as HRT, calcium intake and body weight. These should be investigated since in the present study many such additive effects with other factors were found to exist. In conclusion, the associations between statins, cholesterol levels and bone metabolism remain to be resolved and will require well performed, randomised, prospective trials with large populations.
7 CONCLUDING REMARKS AND CLINICAL RECOMMENDATIONS

1. Menopausal transition is the most important determinant of postmenopausal bone loss. Accordingly, preventive therapy should be initiated as early as possible, during the early amenorrhea phase.

2. Apart from menopausal transition itself, weight change is the most important determinant of postmenopausal bone loss rate. Significant weight loss should be avoided during the menopausal transition although the bone effects of exercise related weight loss remains to be resolved. However, in the case of obese patients, the bone loss during weight loss may be prevented by HRT.

3. Adequate intake of nutritional calcium should be recommended also for postmenopausal patients on HRT. It should be explained to the patient that a low calcium intake is a significant risk factor for no bone response to HRT.

4. Cessation of smoking should be encouraged because smoking may prevent the positive effect of calcium on postmenopausal bone loss.

5. Statins should not be recommended for the prevention or treatment of postmenopausal bone loss.
8 SUMMARY

The present study was conducted as a part of the prospective population-based OSTPRE–study. Baseline postal inquiries were sent in 1989 to all 14 220 women born in 1932-41 living in Kuopio Province. Follow-up inquiries were sent in 1994 and 1999 to respondents of the baseline inquiry. In addition, DXA absorptiometry of lumbar and femoral regions were performed at baseline, at the five year follow-up (1994-1997) and at the ten year follow-up (1999-2002) to a randomly selected sample of 2025 women at baseline. The actual study populations of the present study ranged from 409 to 954 women.

The present study confirmed that menopausal transition is the most important determinant of postmenopausal bone loss. The time of fastest bone loss was found to become manifest during the early amenorrhea phase and lumbar spine was observed to be mostly affected. The prevention of postmenopausal bone loss seemed to require methods other than those related to life-style and nutrition, low weight and weight loss were the most important determinants of increased postmenopausal bone loss rate whereas low grip strength predicted only increased femoral neck bone loss. Although the relationship between body weight and bone loss is problematic for public health promotion with regards cardiovascular morbidity, we observed that HRT was able to prevent the weight loss related bone loss. In this way equal prevention of bone loss may be achieved regardless of changes in body mass. Previously this interaction has not been prospectively studied in a large population sample.

HRT was observed to be an effective strategy in the prevention of postmenopausal bone loss. However, the efficacy of HRT was found to be highly dependent on the nutritional calcium intake. High nutritional calcium intake potentiated the bone protective effects of HRT whereas low calcium intake was found to be a significant risk factor for no bone response to HRT. These findings should be explained to patients on HRT. On the other hand, the efficacy of nutritional calcium in the prevention of postmenopausal bone loss was found to be modified by smoking, which seemed to inhibit the protective effects of nutritional calcium on bone. This effect is proposed to have contributed to the non-
significant effect of nutritional calcium on bone loss noted in previous observational studies. Overall, the present study was unique due to its ability to detect and elaborate these joint effects.

In the present study, no association between statins and the rate of bone loss was observed. It was concluded that the statins should not be considered as potential therapy for the treatment or prevention of bone loss. However, a closer investigation of the pathway through which statins mediate their effects, especially related to cholesterol metabolism, could be useful in the development of new osteoblast stimulating drugs.

In summary, the present population-based study confirmed the indisputable role of menopausal transition in postmenopausal bone loss. It is the first study to prospectively investigate the interactive effects between certain risk factors for postmenopausal bone loss and we also evaluated the role of a proposed new osteoblast stimulating drug in the prevention of postmenopausal bone loss. The results will aid in the identification of women at increased risk for osteoporosis and will help in the practical evaluation of treatment as well as preventive methods applicable for osteoporosis.
9 REFERENCES


Bouxsein ML, Michaeli DA, Plass DB, Schick DA, Melton ME. Precision and accuracy of computed digital absorptiometry for assessment of bone density of the hand. Osteoporos Int 1997;7:444-9


Col NF, Eckman MH, Karas RH, Pauker SG, Goldberg RJ, Ross EM, Orr RK, Wong JB. Patient-specific decisions about hormone replacement therapy in postmenopausal women. JAMA 1997;278:1140-1147


Cummings RG. Calcium intake and bone mass: a quantitative review of evidence. Calcif Tissue Int 1990;47:194-201


Dawson-Hughes B. Calcium and vitamin D nutritional needs of elderly women. J Nutr 1996;126:1165-1167


Delmas PD, Hardy P, Garnero P, Dain MP. Monitoring individual response to hormone replacement therapy with bone markers. Bone 2000;26:553-560


Garnero P, Dart C, Delmas PD. A model to monitor the efficacy of alendronate treatment in women with osteoporosis using a biochemical marker of bone turnover. Bone 1999;24:603-609


Genant HK, Faulkner KG, Gluer CC, Engelke K. Bone densitometry: current assessment. Osteoporosis 1993;3;91-97


Goebel SR, Willhite SL. Calcium intake in adolescence. U.S. Pharm 1998;23(10);50-5


Greenspan SL, Maitland-Ramsey L, Myers E. Classification of osteoporosis in the elderly is dependent on site-specific analysis. Calcif Tissue Int 1996;58:409-414


Hannan MT, Felson DT, Anderson JJ. Bone mineral density in elderly men and women: Results from the Framingham osteoporosis study. J Bone Miner Res 1992;7:547-553


Hansen MA, Overgaard K, Christiansen C. Spontaneous postmenopausal bone loss in different skeletal areas followed up for 15 years. J Bone Miner Res 1995;10:205-210

Harris S, Dawson-Hughes B. Rates of change in bone mineral density of spine, heel, femoral neck and radius in healthy postmenopausal women. Bone Miner 1992;17:87-95


Hassager C, Jensen SB, Christiansen D. Non responders to hormone replacement therapy for the prevention of postmenopausal bone loss: do they exist? Osteoporos Int 1994;4:36-41

Heaney RP. Nutrient effects: discrepancy between data from controlled trials and observational studies. Bone 1997; 21:469-471


Kalkwarf HJ, Specker BL, Ho M. Effects of calcium supplementation on calcium homeostasis and bone turnover in lactating women. J Clin Endocrinol Metab 1999;84:464-70


Kanis JA. The use of calcium in the management of osteoporosis. Bone 1999;24:279-90


Klein RF, Carlos AS. Inhibition of osteoblastic cell proliferation and ornithine decarboxylase activity by ethanol. Endocrinology 1995;136(8):3406-11


Komulainen M, Kröger H, Tuppurainen MT, Heikkilänen A-M, Alhava E, Honkanen R, Jurvelin J, Saarikoski S. Prevention of femoral and lumbar bone loss with hormone replacement therapy and


Kritz-Silverstein D, Barret-Connor E. Grip-strength and bone mineral density in older women. J Bone Miner Res 1994;9:45-51


Lamberg-Allardt C, Outila T, Kärkkäinen M et al. Vitamin D deficiency and bone health in Northern Europe-could this be a concern in other parts of Europe? J Bone Miner Res 2001;16:2066-2073.


Manolagas SC. The role of IL-6 type cytokines and their receptors in bone. Ann New York Academy Sci 1998;840:194-204


Miller PD, Bonnick SL, Rosen CJ. Consensus of an international panel on the clinical utility of bone mass measurements in the detection of low bone mass in the adult population. Calcif Tissue Int 1996;58:207-214


Pouilles JM, Tremolieres F, Ribot C. The effects of menopause on longitudinal bone loss from the spine. Calcif Tissue Int 1993;52:340-343


Rapuri PB, Gallagher JC, Kinyamu HK, Ryschon KL. Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes. Am J Clin Nutr 2001;74(5):694-700


Schneider EL, Guralnik JM. The ageing of America: Impact on health care costs. JAMA 1990;263:2335-2350


Torgerson DJ, Thomas RE, Campbell MK, Reid DM. Alcohol consumption and age of maternal menopause are associated with menopause onset. Maturitas 1997;26:21-25


