Prediction of changes in bone mineral in early postmenopausal women

Ph.D. thesis

by

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Aarhus University Hospital
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1. INTRODUCTION

1.1) Setting the stage

Osteoporosis is an important disease in western cultures as it is frequent (1) and as it is associated with an increased mortality after fractures (2). Osteoporosis has been defined as “a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk” (1) i.e. a condition with low bone biomechanical competence leading to an increased risk of fracture even with minute load on the bone (3). On the other hand even strong bones may fracture under sufficient load (4). The fracture of the bone is thus the end-point of interest from a clinical point of view, but the reduced bone biomechanical competence is the substrate (environment) in which the low-energy (fragility) fractures occur.

Numerous factors, such as bone mineral content, non-mineralised matrix, and architecture are associated with bone strength (5-29).

Among these, the calcium content/density (bone mineral content/density - BMC/BMD) of the skeleton is of particular interest, as it seems to explain a large percentage of the bone biomechanical competence (30). Furthermore, the bone mineral content can be readily measured through the use of various scanning techniques (Dual energy X-ray absorption [DEXA], single photon absorption [SPA], dual photon absorptiometry [DPA], and quantitative computed tomography [QCT]) (31-37). In accordance with the in vitro studies of fracture threshold versus bone mineral (30), several clinical studies have demonstrated that bone mineral content/density is a significant predictor of fractures in vivo (15,38-41), the fracture risk increasing with decreasing bone mineral content/density.

However, two problems are associated with the use of measurements of actual bone mineral through scanning:

1) Scanners are not universally available in the clinical setting and the economic cost of scanning may limit its availability

2) bone mineral content/density changes with age (42-45) and a normal content in one part of the skeleton at one time point may not necessarily predict a normal content later in life in other parts of the skeleton (46-49) (fig. 1 shows the reconstructed course in women with menopause at 50 years of age)

It may thus be of interest to have simple, easily available, fast and cheap methods to estimate bone mineral in the clinical setting. Such methods could be used for assessing fracture risk and to identify individuals at high risk of low bone mineral density, who would then be candidates for measurement of bone mineral through scanning (50). Furthermore, such methods may be used for predicting the rate of bone loss and thus estimate the risk of developing low bone mineral despite a normal bone mineral at the actual measurement point. The rationale being that with extensive knowledge of bone turnover and heritable risk factors a stratification into groups with different risks
of having low bone mineral may be possible, and perhaps with increasing knowledge of a number of risk factors a secure prediction may become possible.

A particular time period of interest in predicting bone density and bone loss is the time period around menopause in women where an accelerated loss of bone takes place due to the loss of endogenous oestrogen production (1). For this purpose, the Danish Osteoporosis Prevention Study (DOPS) offers a large cohort of peri- and recent postmenopausal women.

Fig. 1
Bone mineral density throughout life in women - reconstructed from cross-sectional studies (42). From a low level in adolescence, a peak bone density is reached around the age of 30 years, and from menopause (around the age of 50 years) an accelerated bone loss takes place followed by a more gradual bone loss. The solid lines represent cut-off values at 1 standard-deviation and 2.5 standard-deviations below the mean BMD of a 30 year old woman (the cut-off value for osteopenia and osteoporosis respectively (1) - see section 2.1 for a definition of T-score). A large proportion will develop osteopenia or osteoporosis.
1.2) The Danish Osteoporosis Prevention Study (DOPS)

The Danish Osteoporosis Prevention Study is a comprehensive cohort study designed to evaluate the preventive effect of oestrogen supplementation (hormonal replacement therapy - HRT) on osteoporotic fractures through 20 years of follow-up among 2016 postmenopausal women (51,52). It is designed as a partly randomised pragmatic multicentre cohort study with the possibility for participants to chose or refuse HRT (comprehensive cohort study) (fig. 2).

Fig. 2

A) Inclusion of subjects in the Danish Osteoporosis Prevention Study

B) Distribution by centre of the included participants
Among the inhabitants in four Danish communities, the participants were recruited by a short questionnaire concerning menopausal status mailed to a random sample of 45 to 58 year old healthy women drawn from the population in each area (fig. 2B). Women returning the questionnaires, willing to participate and fulfilling the inclusion criteria were invited to receive further information and clinical examination. Inclusion criteria were: 1) Women with intact uterus aged 45 to 58 years and 3 to 24 months past last menstrual bleeding or experiencing perimenopausal symptoms (including irregular menstruations) - the latter combined with elevated serum follicular stimulating hormone (FSH), and 2) Hysterectomised women aged 45 to 52 years and having elevated FSH. Exclusion criteria were: 1) Metabolic bone disease, including osteoporosis defined as non-traumatic vertebral fractures on X-ray; 2) Current oestrogen use or oestrogen use within the past three months; 3) Current or past treatment with glucocorticoids > 6 months; 4) Current or past malignancy; 5) Newly diagnosed or uncontrolled chronic disease; or 6) Alcohol or drug addiction.

Of the 47,720 who were contacted, 49% responded (23,500), of these 15% (3,500) fulfilled the inclusion criteria, and among these little more than half accepted to participate (2,016).

After written informed consent (Helsinki II) the women were allocated to four treatment groups between November 1990 and March 1993. (1): Randomised to receive HRT (502 women); (2): Randomised not to receive HRT (504 women), (3): Received HRT by personal choice (221 women) and (4): No HRT by personal choice (789 women). Group 1 and 2 were block-randomised in groups of 10 by the envelope method. The study was not blinded neither to investigator nor to participant as the first line HRT used in this study induced menstruations in women with intact uterus. The study was partly randomised (groups 1 and 2) to be able to compare a stringently performed randomised study to the situation in daily clinical practice (the non randomised). HRT was provided free of charge.

First-line HRT were: 1) Sequential oral oestrogen and progestogen in a 28 day cycle: one tablet containing 2 mg oestradiol for the first 12 days, one tablet containing 2 mg oestradiol and 1 mg norethisterone acetate for the next 10 days followed by one tablet containing 1 mg oestadiol for 6 days (Trisequens™, Novo Nordisk, Denmark) in women with intact uterus and 2) oral continuous oestadiol 2 mg per day (Estrofem™, Novo Nordisk, Denmark) in hysterectomised women.

The study had 88% power to detect a 40% reduction in the rate of all osteoporotic fractures after 20 years. It had a 76% power for detecting a 40% increase in life-time breast cancer risk. The study was approved by the ethics committee (#1990/1821).

Upon inclusion a number of life-style variables were recorded, blood and urine samples were drawn, and bone mineral was measured. At follow-up after 6 month, 1 year, 2 years, 3 years and five years bone mineral was re-measured, and blood & urine samples were repeated.

The DOPS is thus well suited for studying longitudinal changes in bone mineral as well as cross-sectional bone mineral in a group of peri- and recent postmenopausal women (see section 2.5 for a discussion of the definition menopause and perimenopause).

In this thesis the Aarhus centre will be used to study the changes in bone mineral and to develop mathematical models to predict actual bone mineral as well as changes in bone mineral in different parts of the skeleton in both HRT treated and HRT untreated subjects.
1.3) Aims of the thesis

1. To study the rate of bone loss in postmenopausal women not receiving hormonal replacement therapy (HRT) and factors of significance to this loss in different regions of the skeleton.
2. If possible to propose an easy-to-use and cost-effective algorithm suitable to identify women at risk of low actual bone mineral density or of high postmenopausal bone loss with low cost. The algorithm will be developed using one of the DOPS centres and evaluated on the population in one of the others.
3. To study factors of importance to gain in bone mineral in women taking HRT.

2) Bone mineral density and the concept of osteoporosis

2.1) Background and definition of osteoporosis

As stated in section 1, the BMC and BMD explains a large percentage of the biomechanical competence of the skeleton (“strength”, ability to withstand external load) - the coefficients of determination between BMD and fracture threshold being 0.60 to 0.89 (30). However, as can be seen from fig. 1, BMD changes throughout life in women. Fig. 1 shows a concept of the development in spinal bone mineral density in women from early age to the age of 85 years (42). After accretion of a peak bone mass around the age of 30 years a gradual loss continues until the more abrupt loss around the menopause and a more gradual loss later in life (53).

However, fig. 1 illustrates the problem of defining one general normal range for BMD (42) for all age groups. The idea of a “normal range” is based on one of two concepts, namely 1) what is representative of the population (i.e. a state characterising most of the subjects in the population), or 2) what is the optimal state (i.e. what is “good” or “best” for the individual subject). As most subjects loses bone mineral from the age of 30 years and onwards, most elderly subjects will sooner or later fall below the normal range of a 30-year old individual (the optimal point at the curve) but still be well within what is characteristic for most of the population in the actual age group. From this point of view two measures of actual BMD has been developed (1):

1) The T-score. This is a measure of the deviation of the actual BMD from the mean BMD of a 30 year old female, i.e. the deviation from the peak bone mineral.
   \[ T\text{-score} = \frac{\text{actual BMD} - \text{mean BMD at 30 years}}{\text{standard deviation of BMD in a 30 year old}} \]

2) The Z-score. This is a measure of the deviation of the actual BMD from the mean BMD in a population of the same age, i.e. an age-adjusted normal range.
   \[ Z\text{-score} = \frac{\text{actual BMD} - \text{mean BMD of the same age group}}{\text{standard deviation of BMD in actual age group}} \]

Both these scores assume Gaussian distribution of the bone mineral density.

Based on these concepts a working group associated to WHO (1) has defined osteoporosis in women as: “a condition in which the measured bone mineral content or density is below 2.5 standard deviations from the mean value in young healthy women (30 years of age - also named - 2.5 SD in T-score)”. No definition was established for men (1).

This definition does not require the presence of low-energy fractures but only the presence of a risk factor (substrate) for the occurrence of these fractures namely a reduced bone strength in this case measured by bone mineral.
It may thus be suggested to subdivide osteoporosis into two groups: 

**asymptomatic osteoporosis** (BMC or BMD below -2.5 SD of T score but as of yet no low-energy fractures), i.e. osteoporosis according to the WHO definition, and

**symptomatic osteoporosis** (severe osteoporosis: BMC or BMD below -2.5 SD of T score and present or previous low-energy fracture).

Osteopenia (1) is defined as a state of reduced bone mineral content but not to a degree as mentioned above (between 1 and 2.5 standard deviations below the mean value in young individuals) in the same way as osteoporosis.

The concepts of symptomatic and asymptomatic osteoporosis may - along with osteopenia - be useful in preventive terms (see below).

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### 2.2) From low bone mineral to low energy fractures

Fractures are the clinical endpoint of interest in prevention. Low bone mineral is the substrate for low energy fractures, the likelihood of bone fracturing at low external load will increase with decreasing bone strength. Many factors besides actual bone mineral, namely the type and geometry of external load as well as muscular strength may play a large role in fracture occurrence (30) making the fracture a stochastic process in which a given trauma with high probability will fracture the bone. However, the probability will decline with increasing bone strength (bone biomechanical competence). Contrary, the likelihood that a low impact will fracture the bone is low, but yet by chance a low energy fracture may occur in a bone with e.g. a T-score above -2.5.

Low energy trauma refers to falls on the same level (54) resulting especially in forearm fractures (Colles fractures), hip fractures and vertebral fractures (1). Fractures without apparent trauma may also occur and usually result in vertebral fractures being noticed only upon the occurrence of kyphosis, and occasionally femoral neck fractures without falls.

Although not all such fractures may be attributable to osteoporosis, a high proportion is attributable to osteoporosis (55,56).

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### 2.3) Prevention

With the knowledge that low bone mineral is linked to low bone biomechanical competence and that fractures are the clinical end-point of interest, the following categories of osteoporosis prevention can be defined (57):

1) **Primary prevention:** Preventing the loss of bone mineral (and thus fractures) in the general population. This would be the case if e.g. a general recommendation of changes of life-style or implementation of oestrogen prophylaxis in all postmenopausal women was issued irrespective of their bone mineral.

2) **Secondary prevention:** Preventing further loss of bone mineral in subjects with low bone mineral. I.e. treating otherwise asymptomatic individuals who upon screening or by chance had been shown to have low bone mineral.

3) **Tertiary prevention:** Preventing recurrence of low-energy fractures in individuals with low bone biomechanical competence who have already suffered at least one low-energy fracture. This would be the case in e.g. a subject with an osteoporotic hip fracture.

Secondary prevention would be reserved for individuals with osteopenia or asymptomatic osteoporosis and tertiary prevention would be reserved for patients with symptomatic osteoporosis (58).
Following the expected spontaneous development in skeletal calcium content presented in fig. 1, more than half of all women would sooner or later develop osteoporosis (symptomatic or asymptomatic - dark grey area) or would develop osteopenia (light grey area). The subsequent interaction between low bone mass and an increased tendency to falls with increasing age (29,59) thus produces the high number of fractures with advancing age. The WHO report estimated the lifetime risk of proximal femur, vertebral or distal forearm in a 50-year old to be 39.7 % (95 % CI: 38.7 - 40.6 %) (1). In accordance with fig. 1 it has been estimated, that 70 % of American women would have osteoporosis by the age of 80 (1).

Focusing on the early osteoporotic fractures (the subtype of Colles fractures and vertebral fractures occurring in women under the age of 70 years (60)) women with normal BMC/BMD could develop symptomatic osteoporosis following a rapid loss of especially trabecular bone (48) leading to Colles fractures, vertebral crush fractures and fractures of the ankle (48).

Such “fast losers” would be suited for secondary prevention (i.e. prevention despite the presence of a normal BMC/BMD at the time of measurement). In this scenario, “fast bone loss” would be the selection criterion and not low bone mineral.

This thesis has its main objectives at trying to identify subjects at high risk of low bone mineral and at high risk of losing bone mineral. Its main focus is thus in connection with secondary prevention.

2.4) Bone remodelling and bone turnover
Bone remodelling is the process by which bone tissue (mineral and non-mineral) is renewed and degraded. The basis for this is the bone remodelling unit, involving the complex interaction between bone degrading osteoclasts and bone forming osteoblasts (53). This turnover of bone is mirrored in components in the blood and urine representing degradation products of the collagen matrix of the bone (the non-mineral matrix) and of calcium itself.

By tradition these markers have been grouped into those linked to bone formation (the making of mineralised bone):
- Serum alkaline phosphatase
- Serum osteocalcin (BGP)
- Procollagen type I extension peptides (P1CP, P1NP)

and those linked to bone resorption (bone degradation):
- Fasting urinary calcium /representing the balance between resorption and formation)
- Fasting urinary hydroxyproline
- Urinary pyridinolines
- Serum 1CTP

These biochemical markers may thus be used as indices of bone turnover (61-63) - see section 3.1.2 and other predictors (table 1 and 2).

2.5) Menopause
Menopause is defined as the cessation of menstruations in women and the associated climacteric syndrome of endocrine, somatic, and psychic changes occurring around menopause (64) due to the cessation of endogenous oestrogen production. A recent definition is that menopause can be diagnosed after 12 month of amenorrhoea resulting from the permanent cessation of ovarian function (65). The term perimenopause is not firmly defined (66), but refers to the period a several years before (65) and after menopause. The definition of menopause by cessation of menstruations is hampered by the fact, that a large percentage of women in the actual age category are
hysterectomised. In the DOPS study FSH measurements were used in hysterectomised women to define menopause. The participants in DOPS were perimenopausal at inclusion as a period of 3 month past last menstruation may still involve irregular menstruations - i.e. the longer the time span since the last menstrual bleeding, the larger the probability of menopause becomes (65). In this context the period of only three month was an advantage in allowing the study of BMD changes secondary to the early changes in endogenous oestrogen production. A detailed discussion of the interaction of oestrogen and bone can be found in section 3.1.2.

2.6) Bone mineral and bone loss in different age groups

2.6.1) Childhood and infancy
An increase in both BMC (growth of the skeleton) and BMD is seen during adolescence (67) (fig. 1). Details of this will not be discussed in this thesis.

2.6.2) Peak bone mass
Fig. 1 shows a model for the changes in bone mineral density. After a gain (accretion) early in life a peak value is assumed to be reached around the age of 30 years. Of significance to this is among others heritage (68), ethnic group (69-72), oestradiol levels (73), physical activity (74), and calcium intake in infancy and childhood (45,74). Further details will not be discussed in this thesis.

2.6.3) Premenopause
Controversy exists as to the changes after peak bone mass is reached. Nilas et al. (46) found a low loss before the menopause in the forearm and insignificant changes in the spine in women aged 29-45 years. This corresponded with the findings of Recker et al. (44) who found insignificant changes in the forearm and spine (<1 %/yr.) before the menopause in women aged 46 years or more who were still menstruating. On the other hand Riggs et al. (47) found low rates of loss in the forearm but a high loss rate in the spine before the menopause in women aged 20 years or more, who had been menstruating within the last 6 month or had serum oestradiol within normal range (47,48). Block et al. (75) using QCT of spine in a cross-sectional design found a stable BMD in the age group 20 - 50 years and a significant decline hereafter. Citron et al. (76) reported that in oestrogen and calcium-replete premenopausal women aged 38-41 years, spinal trabecular bone density measured by QCT declined significantly (0.86 %/yr.), while midradius BMD remained stable over a four year follow-up period. In the cross-sectional studies a cohort effect may be present (see section 2.10).

2.6.4) Perimenopause and postmenopause
A general agreement on an accelerated bone loss during and the first years after the menopause followed by a more gradual loss hereafter seems to exist (43,46,47,77-85). Whereas in males a more gradual bone loss has been observed (86) after the age of 30 years.
In the perimenopausal period the loss rates in the forearm has been estimated to be 1.3 %/year in distal forearm and 1.1 %/year in proximal radius in 495 Japanese-American women aged 45-81 years (BMC by SPA) (87). In the mid-radius the loss of BMD by SPA after the menopause was 1.15 ±2.75 %/year in 139 women aged 20-88 years (47). However, Falch et al. (66) in 73 Norwegian
women observed a loss rate by DPA in the forearm of 2.8±1.31 %/year to 3.47±1.30 %/year for BMC. Other studies have yielded intermediate results (88,89). In the spine the loss in BMD was estimated to be 0.97±3.08 %/year (47) and 2.60±3.54 %/year for BMC (46) by DPA. Another study have found somewhat higher loss rates in the spine by DPA (88) of 4.2 %/year. Clements et al. (90) estimated the bone loss to be 2.41 %/yr. (95 % CI: loss 3.55 to gain 1.27 %/yr.) of spine BMD by QCT and 0.78 %/yr. (loss 1.73 to gain 0.18 %/yr.) of radius BMD by SPA in women aged 45 to 60 years. A summary of loss rates can be found in table 2 - however, several of the studies in this sample used relatively broad age ranges (91,92), included a mixture of pre- and postmenopausal women (93-96) - some of the latter receiving HRT (94), studied women several years after menopause (97-102) or women age 63 years or more (103-105).

2.6.5) Older age
A continuing significant bone loss has been observed in women 65 years or more (103,106-108). Even a positive effect of older age on bone mineral has been suggested from simulations of based on cubic models of the development with age of bone mineral - this is the reason for the isolated finding of a positive effect of age in one study in table 1 (109). In the group of elderly subjects concurrent disease (110) and factors such as the increasing use of medications (e.g. thiazides) and deficient nutrition becomes of increasing significance (111). Furthermore, low bone mineral may contribute to mortality (57) and thus induce a collection bias among the surviving elderly subjects. Details will not be discussed in this thesis.

2.7) Treatment and compliance with treatment
It is well-known that oestrogen supplementation preserves bone mineral after the menopause (112) and that bone loss resumes shortly after cessation of oestrogen intake (112-120). The preventive potential of oestrogen on osteoporotic fractures has mainly been evaluated in retrospective studies (121-123), historical follow-up studies (124) and one smaller prospective study (125). A meta-analysis concluded that oestrogen treatment probably could reduce the risk of hip fracture by 25 % (126). Compliance with oestrogens has also been variable (127-135) and could be a major obstacle to the use of oestrogens as osteoporosis prevention (136-141). Oestrogen treatment and compliance with treatment are thus significant predictors of bone mineral and bone loss after menopause, and knowledge of BMD status is a predictor of initiating preventive measures, e.g. start HRT (142).

2.8) Prediction of bone mass and bone loss
2.8.1) Cross-sectional bone mineral
Cross-sectional BMD represents the integral results of prior skeletal processes as well as actual remodelling. From the discussion in section 2.6 it must be concluded, that different factors affect bone mineral at different time periods in life. Table 1 presents an overview of studies on the association of a number of factors with cross-sectional bone mineral in women who were peri- or postmenopausal. From table 1 it can be seen, that many factors have been associated with cross-
sectional bone mineral (BMC and/or BMD) in peri- and postmenopausal women, some of the studies also covering age groups above 65 years (107,109). Most of the studies presented in table 1 only found moderate correlations explaining around 20-30 % of the total variation despite the inclusion of a large study group and many independent variables (50,107). Many of the studies in table 1 have been developed on small populations or on populations with a broad age range.

2.8.2) Bone loss rates (longitudinal change)

2.8.2.1) Untreated subjects

Bone loss represents an ongoing process with changes in bone mineral, that can be transient remodelling or permanent loss of bone e.g. following menopause (1). Previous studies have claimed, that women with a high bone loss rate could be readily identified using biochemical variables and life-style variables (143-148). However these studies have found different variables to be linked to the rate of bone loss (143-147) (see also table 2), and the accuracy of the prediction has not always been high (143,147). Although loss rates of bone over several years have been shown to correlate to actual point bone turnover assessed by skeletal uptake of bisphosphonate (149) correlations to biochemical markers of bone turnover have been weak (143-147). Based on the group with high bone loss the existence of a specific group of “fast losers” has been proposed (150,151). This concept could be interpreted in two ways:

1) The existence of a specific group separated from the main group of postmenopausal women (fig. 3a) - adapted from Christiansen et al. (151)

2) A group of women with a continuously high bone loss who just represents the extreme of the continuum of loss rates (fig. 3b)

In connection with the latter it should be remembered, that it has been shown, that the rates of bone loss may differ from one period to another in the same individual (152).

Fig 3:

A) Distribution of loss rates in fast losers (the curve on the left) and normal/slow losers (the curve on the right) - the concept of a bimodal distribution. The X-axis is the loss rate per year and the ordinate is number. Modified from Christiansen et al. (151).
B) Unimodal distribution of bone loss (i.e. no clear distinction between fast and slow losers). A cut point has been defined and the black part of the curve is the fast losers. The X-axis is the loss rate per year, the ordinate is number of subjects.

Hui et al. (153) and Keen et al. (154) found a normally distributed rate of bone loss in postmenopausal women supporting the view in fig. 3b, while Ross et al. (150) found a smaller group of “fast losers” (i.e. a bimodal distribution as in fig 3a), but this group was mainly due to overt health problems such as physical impairment. However, the findings of Hui et al. (153) does not preclude the existence of fast-losers e.g. if the combination of fast-losers and normal persons may add up to a Gaussian distribution (i.e. the statistical phenomenon that two independent Gaussian distributions combined will yield a new Gaussian distribution). The identification of component distributions in a “mixture” distribution of several subgroups may be difficult (155). In this thesis the identification of subgroups will be made through the use of multiple linear regression (see section 3.5).

To overcome the problems of identifying a subgroup as suggested in fig. 3a, several studies have adopted a simple cut point separating between “fast”, “slow”, and even “no” losers (148). However, it has been subject to debate whether a specific group of “fast-losers” (as defined by fig. 1 3a) do in fact exist (151).

Reginster et al. (148) in a recent study defined fast losers as those who lost more than 10% of spine BMD over 3 years, slow losers as those who lost from 3.4 to 10 % of spine BMD over 3 years, and no losers as those whose loss was lower than the minimal change detectable with a 1% level of confidence (< 3.4 % over 3 years). The 10 % loss over 3 years equals a 3.5 % loss per year, while the 3.4 % equals an annual loss of 1.1 %. Christiansen et al. (143) and other subsequent studies (156,157) identified fast losers as those with an annual loss of forearm BMC of more than 3% per year. Falch et al. (146) defined fast losers as those who lost more than 2.8 % of forearm BMC per year in Norwegian women and 1.4 % per year in Dutch women.

Lyritis et al. (158) found, that by applying the predictive model by Christiansen et al. (143) it was possible to predict and reduce a high turnover by administration of calcitonin.

Using a discriminant analysis based on a measurement of lumbar BMD and two measurements of biochemical bone markers with an interval of six month - instead of a multiple regression method and several biochemical measurements as proposed by Christiansen et al. (143), Reginster et al.
reported, that 100 % of postmenopausal “fast bone losers” could be identified with an overall specificity of 76 %.

That changes in bone mineral assessed by scanning may be of interest even in patients with osteoporosis and vertebral fractures undergoing treatment was recently also shown by Riggs et al. (159), who found a relation between magnitude of the loss rate for bone mineral in the lumbar spine and the rate of fractures independently of the actual BMD in women treated with fluoride. This effect of the loss rates was seen as a marker of the effect on bone turnover and thus also on bone biomechanical competence as a result of drug therapy (159).

Several problems associated with the concept of fast losers exist. Hui et al. (152) reported, that the rates of bone loss in two subsequent periods were poorly correlated over time, indicating that the intra-individual rate of bone was variable in time. He at al. (160), and Ross et al. (150) found that by extending the observation period, the standard deviation of the loss rate estimate declined. This is the natural consequence of the fact, that by extending the observation period the change in the dependent parameter will be larger and the significance of the measurement error declines.

Davis et al. (161) and Clements et al. (90) found considerable variation in the loss rates between different skeletal sites. In the proximal and distal part of the radius the absolute loss declined with age as did the percentage lost per year (161). In the calcaneus the loss rates remained stable from the age of 60 and onwards, while it was high in women in their fifties (161). It was also shown by Davis et al. (161), that longitudinal measurements resulted in loss rates different from those anticipated from cross-sectional studies whose results were then extrapolated to longitudinal results. In this thesis these difficulties will be dealt with through the use of specific statistical techniques (e.g. repeated measures ANOVA, see section 3.5)

Furthermore, the calculation of loss rates used in the identification of fast-losers may be subject to bias due to the methods used for calculating these rates. Many of the studies have used linear regression to compute the annual change and has then computed the % change by dividing this slope with the initial measured bone mineral value.

It is well known that a statistical bias will be introduced by including the initial measurement in the calculation of a loss rate in % (initial value was included in the regression in (143-147)) as demonstrated by Davis et al. (162). This means, that women with higher initial BMC would be found to have higher loss of BMC than women with low BMC due to methodological problems.

By including initial values the potential effect of regression towards the mean must be considered (163).

Besides, the methods used to calculate rates of bone loss have not been evaluated in detail - i.e. it has not been evaluated, whether these methods to assess bone loss rates were valid and reproducible. Furthermore, the same cohort that was used to develop the model of bone loss has also been used for evaluation of the model (143), i.e. no external validation was performed.

Also, one of the methods to predict bone loss (164) has been subject to criticism due to changes in e.g. biochemical markers over a short time interval (165). However, the criticism was founded on observations stemming from a few individuals (165). The criticism of the predictive methods has in part been founded on the great intra- and inter-individual variability in bone biochemical markers (166,167).

Despite these difficulties and controversies the concept of describing an “osteoporosis profile” to predict future risk of osteoporosis has been introduced on a commercial basis (164).
2.8.2.2) Oestrogen treated subjects

Oestrogens has been recommended for primary (112), secondary and tertiary prevention (125). Christiansen and Rødbroe (168) showed, that there was no difference between the response to oestrogen supplementation in “fast” and “slow” losers.

While non-responders to oestrogen therapy probably does not exist (89) several factors seems to modify the response to oestrogens (112,115). A dose dependent response has been demonstrated (168-170). As it has been subject to debate whether the addition of progesterone and the type of progesterone was of significance to the gain in bone after HRT treatment (112,171,172), only subjects who adhered to the same treatment schedule were analysed in this thesis - see section 1.2.

The PEPI trial (112) found, that among HRT treated subjects the gain in lumbar spine and femoral neck BMD was higher in older (55-64 years of age) than in younger (45-54 years of age). Hysterectomised women and women with recent or prior HRT use had a smaller gain in BMD than those with intact uterus or those with no recent or prior HRT use. Furthermore, those with a low initial lumbar spine BMD seemed to have a higher gain than those with a high initial lumbar spine BMD.

Bjarnason et al. (173) found, that bone gain among tibolone treated subjects was correlated positively to baseline urinary Crosslaps™. In the study of Bjarnason et al. (173) inverse correlations were also reported between bone gain the average of the change in both serum osteocalcin and Crosslaps™ averaged over the two year study period. A study by Riis et al. (174) showed inverse relations between changes in BMC and changes in bone markers during HRT (17β-oestradiol and progesterone) illustrating that individuals with the largest decrease in bone turnover (and therefore in remodelling space) also showed the greatest increase in bone mass. However, these authors (174) did not evaluate relations between initial turnover and BMC gain.

Conclusion 1

Many factors have been associated with bone loss and several mathematical models have been applied. It is thus necessary to study many factors and to implement several study methods to clarify associations as these may be weak. The methods used to calculate loss rates must be validated.

2.8.3) The concept of calculating loss rates

In this thesis the emphasis will be put on the accelerated bone loss in recent postmenopause. However, to estimate loss rates a conception of the time course of the BMD must be present, e.g. is the loss of equal magnitude at all time points or does it vary with time (e.g. is the change per time unit greater in early postmenopause than in late). These variations may be expressed as e.g. linear or exponential decline in BMD (fig. 4).
The velocity of the loss (loss rate) can be described in two simple ways, calculated at an individual level (i.e. for each individual separately) by the method of least squares:

1) As a steady decline in BMD:
   \[ BMD(t) = a \cdot t + b \]
   Were \( t \) is an arbitrary time point and \( a \) and \( b \) are constants (\( b > 0 \)).
   \( a \) is the loss rate, which is constant in time (equivalent to a 0-order kinetic model in pharmacology), and \( b \) is the initial BMD.

2) As a constant percentage of bone being lost every year:
   \[ \frac{dBMD(t)}{dt} = a \cdot BMD(t) \]
   \[ BMD(t) = b \cdot \text{EXP}(a \cdot t) \]
   Where \( dBMD(t) \) is the change in BMD in the infinitesimal time interval \( dt \) and \( a \) and \( b \) are constants (\( b > 0 \)). \( \text{EXP} \) is the exponential function.
   \( a \) is the loss rate and \( b \) is the initial BMD.
   This is equivalent to a 1. order kinetic model in pharmacology (first-order process).

In several of the studies mentioned in table 2 the annual loss in % has been calculated by taking the ratio between the calculated linear change (\( a \) in model 1) and the measured initial value and not the calculated initial value (\( b \) in model 1): e.g. first the slope \( a \) has been calculated from a series of
BMD measurements. Then the change per year in % has been expressed as $a/BMD_0$, where BMD$0$ is the initial BMD, a value already used to calculate the slope $a$ (11,76,91,143,145,147).

In model 2 the % change in BMD per year is readily available from the model using the initial BMD only once (162). By using model 2 the potential bias from using the initial BMD is thus avoided.

In this thesis only the 4 time points: 1 year, 2 years, 3 years, and 5 years will be used to calculate % loss rates for the two models to avoid the potential bias of the initial value. In model 2, the actual measured BMD values have been transformed by logarithmation and the coefficient $b$ has been found by transformation taking the antilogarithm.

### 2.9) Significance of bone loss

From the discussion above it can be concluded, that there are two principal mechanisms for having low bone mineral and thus increased fracture risk after menopause:

1) A low peak bone mass, i.e. the starting point before the bone loss is low

2) A high and sustained bone loss over time, i.e. even a subject with a high initial BMD would eventually develop low bone mineral due to a large loss

These two mechanisms may of course be combined (fig. 5) (156,175)
Developing a model to predict loss rates would thus be an attractive goal as it could help select subjects who - despite a normal point BMD - would be at risk of developing low BMD due to a high loss rate later in life (148). The period around and after menopause is thus of particular interest, as it according to present theory represents a time period of high bone loss that may contribute particularly to the development of low bone mass.

2.10) Discussion of the concepts presented above

Fig. 1 is the basis for most of the discussion above and for many of the concepts presented. However, it is based on cross-sectional studies which makes cohort effects likely (176). By cohort effects is meant effects occurring in one or more birth cohorts, but not in others. As stated above, many factors are associated with cross-sectional BMD. If e.g. the older birth cohorts were systematically subject to deficiency of calcium in infancy and deprivation of e.g. vitamin D through lack of sunshine exposure, it would be natural, that their BMD would be lower than in younger birth cohorts without this being an effect of age itself. On the other hand, the younger birth cohorts may have led a more sedentary lifestyle and thus have lower bone mineral due to lower physical activity. Fig. 1 is thus not based on a longitudinal follow-up. Therefore, it is necessary to supplement cross-sectional studies with longitudinal studies (177). The study cohort in this thesis offers an excellent opportunity for a longitudinal study and thus to study bone loss around the menopause in detail. It should also be remembered, that calcium content expressed as BMC or BMD only explains a part of the compressive strength of the bone (30,178,179). Furthermore the loss in bone mineral density with age only explains part of the increasing fracture risk with age (1). It should also be noted, that although e.g. the COLIA1 gene (table 1) was not associated with BMD, it seemed associated with fracture risk (180). The fact that e.g. only 20 % of the total variation may be explained by the included independent variables (50) may not directly translate into the conclusion that other - as of yet not disclosed - variables (such as e.g. heritable factors) may explain the remaining say 80 %. It may be the case, that bone mineral is the integrated stochastic result of many interacting factors that may not be described in detail.

Table 1 summarises some of the factors that influence cross-sectional BMD/BMC measurements. In the analysis of loss rates around the menopause factors that are no longer active can be omitted (e.g. prior breast-feeding) - see section 3 for further discussion. Table 2 shows, that most studies on loss rates around the menopause have been conducted on SPA measurements of the forearm. However BMD measurements in the forearm may not correlate well with BMD in other parts of the skeleton e.g. the spine (1) - an observation also done in the DOPS study (181). Furthermore, Riggs et al. (47) found no correlation between loss rates in the forearm and the spine in 139 pre- and postmenopausal women, while Riis and Christiansen (182) found significant though low correlation coefficients between loss rates in radius and spine (r = 0.27 to 0.40). It may thus be necessary to study the skeletal regions independently.

Most of the relations presented in table 2 (except (146)) have also been tested on the same sample used to develop the relation. Among the cross-sectional studies, only the study by Bauer et al. (107) used a validation procedure: the model was developed on one half of the 9704 women aged 65 years or older and then tested on the other half. Another problem is, that the agreement between measured and predicted value only has been evaluated in one study (144) using correlation coefficients and not taking into account e.g. differences in agreement in subjects with high or low values that may be disclosed as suggested by Bland and Altman (183).
By studying table 2 it can be seen, that the correlation coefficients were rather low, and that many different variables seems to be linked to the loss rate. In some of the studies the number of patients was limited and not all independent variables have been entered in all studies. The statistical approaches have mainly been limited to linear regression of loss rates. In some studies (143) only the unadjusted correlation coefficients have been used, and it is well known, that these tend to grow towards 1 if more variables are included (so-called “inflation” (184)). To solve some of this “inflation” problem corrected correlation coefficients may be used. Section 3.4 will deal with the techniques applied in this thesis to solve some of the problems mentioned above.
In the context of loss rates and cross-sectional measurements of BMD it should be borne in mind, that cross-sectional measurements are an integral measure of the sum of previous formative and resorptive processes, while the loss rates reflects current processes.

2.11) Research questions
Loss of bone implies, that future bone mass can only be estimated with uncertainty (185) besides the uncertainty involved in the actual measurement that may be the base of the estimation.
From the above mentioned correlations and from the commentary by Blumsohn and Eastell (186) the following problems can be formulated:
1) Can cross-sectional bone mineral be predicted from simple clinical and biochemical observations ?
2) Is the perimenopausal bone loss of such a magnitude, that it significantly contributes to later development of osteoporosis in relation to already achieved bone mass and subsequent bone loss with age ?
3) How is the longitudinal course of bone loss ?
   Does it follow a linear trend or perhaps an exponential decay or is the decay a random process that does not follow any particular course in time ?
4) Is there a group of fast losers at all and is this group identifiable ?
5) Is it possible to correlate the magnitude of the bone loss to any specific markers, be they biochemical or other ?
6) Instead of a correlation to loss rates is there a correlation between repeated measures of absolute bone mineral and certain time dependent measures ?
Although problems do exist as to the validity of BMD with varying body sizes (187), in this study BMD will be used as the parameter studied as the changes in weight must be anticipated to be small in most participants.
Table 1
Factors of importance to bone mass (BMC or BMD) in peri- and postmenopausal women in cross sectional studies (may not apply to all skeletal regions). The correlations are presented, so that they are related to the presence of the specified factor or increasing value of the specified factor unless otherwise stated (e.g. most of the correlations with age were negative meaning that BMD/BMC decreased with increasing age).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Positive effect</th>
<th>No effect</th>
<th>Negative effect</th>
</tr>
</thead>
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<td>Weight</td>
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<td>BMI</td>
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<td>Height</td>
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<td>(188)</td>
<td>(198)</td>
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<td>Waist hip ratio</td>
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<td>(107)</td>
<td>(212)</td>
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<tr>
<td>Fat mass</td>
<td>(196,204,214)</td>
<td>(197)</td>
<td>(199,214)</td>
</tr>
<tr>
<td>Lean body mass</td>
<td>(217)(196)*</td>
<td>(199,214)</td>
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</tr>
<tr>
<td>Fair complexion (blond hair + skin)</td>
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<td></td>
<td>(212)</td>
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<td><strong>Biochemical parameters</strong></td>
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<tr>
<td>Alkaline phosphatase</td>
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<td>(7,99,204,218,219)</td>
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<td>Serum osteocalcin</td>
<td>(199)</td>
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<tr>
<td>S-calcium</td>
<td></td>
<td></td>
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<td>S-oestradiol and oestrogens</td>
<td>(204,220-225)</td>
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<td>S-testosterone+androgens</td>
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<tr>
<td>PTH</td>
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<td>(93,199,201)</td>
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<tr>
<td>Tubular re-absorption of phosphate</td>
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<td>(199)</td>
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<tr>
<td>U-OH/creatinine</td>
<td>(99,201)</td>
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<td>U-Ca/creatinine</td>
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<td>Urine cross-links</td>
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<tr>
<td>S-1,25-OH vit. D</td>
<td>(93,227)</td>
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<td>S-25-OH vit. D</td>
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<td>IGF-I</td>
<td>(228,229)</td>
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<td><strong>Life-style</strong></td>
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<td>Smoking</td>
<td>(19,27,208)</td>
<td>(11,107,188,199,209,210,212,230)</td>
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<td>Coffee intake</td>
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<td>Alcohol consumption</td>
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<td>Calcium intake</td>
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<td>(76,98,188,200,206)</td>
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<td>Milk intake</td>
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<td><strong>Reproductive history etc.</strong></td>
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<td>Surgical menopause</td>
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<td>Menopause age</td>
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<tr>
<td>Length of reproductive period</td>
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<td>Years since menopause</td>
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<td>Menopause (vs. premenopause)</td>
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<td>Menarche age</td>
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<td>Ever pregnant</td>
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<td>Parity</td>
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<td>(197,205,217)</td>
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<td>Breast feeding</td>
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<td>(233)</td>
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<td><strong>Medications</strong></td>
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<td>Oestrogen use (incl. oral contraceptives)</td>
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<td>Steroid use</td>
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<td>Thiazide use</td>
<td>(107,109,237)</td>
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<td><strong>Load related factors</strong></td>
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<td>Physical activity</td>
<td>(13,27,109,188,197,208,213,217,238)</td>
<td>(107,200)</td>
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<td>Grip strength</td>
<td>(107,109,208)</td>
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<td><strong>Hereditary factors</strong></td>
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<td>Family history of fractures/osteoporosis</td>
<td>(107,188,209,210,212,239,240)</td>
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<td>Vitamin D receptor alleles (BB vs. bb genotype)</td>
<td>(241-244)</td>
<td>(100,245)</td>
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<td>TGFβ</td>
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<td>COLIA1 gene (ss genotype)</td>
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<td>(250,251)</td>
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<td>Oestrogen receptor</td>
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<td>Twin sibling BMD</td>
<td>(68,253-255)</td>
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<td><strong>Concurrent diseases</strong></td>
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<td>Gastric surgery</td>
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<td>Osteoarthritis</td>
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<td>NIDDM</td>
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* correlation with total body BMD, regional correlation coefficient negative
<table>
<thead>
<tr>
<th>Measure of bone mineral</th>
<th>Study group</th>
<th>Study variables</th>
<th>Method</th>
<th>Conclusions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC, forearm, SPA</td>
<td>178 postmenopausal women (3 month - 36 month past last menses), age 45-54 years, 6 - 36 month past natural menopause.</td>
<td>U-Ca (spot-urine), U-OH (spot-urine), S-alkaline phosphatase, S-oestrone/oestradiol, fat mass (fat mass calculated by a specific formula)</td>
<td>multiple linear regression (all variables included/4 variables included)</td>
<td>Loss rate: from +2 to -8%/yr., most around 2%/yr.</td>
<td>(143)</td>
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<td></td>
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<td>BMC/yr. = - 0.008<em>U-Ca - 0.273</em>U-OH - 0.007<em>S-ALP + 6.796</em>Fat mass + 3.195</td>
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<td>56% positive predictive value, 92% negative predictive value (using the 4 variables)</td>
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<tr>
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<td>70 healthy postmenopausal women, 45 - 54 years (6 - 36 month past last menses)</td>
<td>U-Ca/creatinine ratio, U-OHP, S-alkaline phosphatase (S-ALP), P-BGP, fat mass (calculated by a specific formula)</td>
<td>multiple linear regression (manually stepwise using uncorrected r value)</td>
<td>Loss rate: from 0 to -6%/yr., most around 2%/yr.</td>
<td>(145)</td>
</tr>
<tr>
<td></td>
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<td>BMC/yr. = - 0.088<em>BGP - 0.004</em>S-ALP - 0.002<em>U-Ca/cr - 0.192</em>U-OH+2.684</td>
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<td>75% positive predictive value, 81% negative predictive value</td>
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<td>121 postmenopausal women (same as (143)) followed for 12 years</td>
<td>Reproductive history, calcium intake, vitamin D intake, caffeine consumption, alcohol consumption, smoking, physical activity, height, weight</td>
<td>t-test for 2 samples</td>
<td>No correlations with initial loss rate, alcohol consumption was associated with lower bone loss after 12 years</td>
<td>(11)</td>
</tr>
</tbody>
</table>
### Measure of Bone Mineral

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Study Variables</th>
<th>Method</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>73 Premenopausal White Women 47 Years at Inclusion Followed for 10 Years Were Used to Develop the Model. The Model Was Then Validated on 86 Perimenopausal Women Age 49 - 57</td>
<td>Smoking, Menarche Age, Menopause Age, Family History of Fractures, Reproductive History, Height, Weight, Daily Intake of Calcium, Protein, Vitamin D and Energy, Isometric Strength, Exercise Capacity and Knee Extension, S-Ca, S-P, S-Alkaline Phosphatase, S-Creatinine, S-Androstendione, S-Oestrone, S-Prolactin, U-Ca/Creatinine Ratio, TmPO₄/GFR</td>
<td>Multiple Linear Regression (Separately for Smokers and Non-Smokers)</td>
<td>Non Smokers: BMC/yr. = -0.078<em>Weight - 1.8</em>TmPO₄/GFR + 11.8 ( r² = 0.54 ) in Norwegian Study. BMC/yr. = -0.056<em>Weight - 1.8</em>TmPO₄/GFR + 7.0 in Dutch Study. In Smokers the Smoking Alone Was a Risk Factor</td>
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</table>

| 154 Postmenopausal Women Age 45 - 54 Years (Same as (143)) Followed for 12 Years (Not Using HRT) | U-Ca/Creatinine, U-OH/Creatinine, S-Alkaline Phosphatase | Observed BMC After 12 Years Compared to Predicted Value Using Initial BMC and Loss Rate in the First 2 Years | Loss Rate 1.9 ± 1.9 %/yr. in the First 2 Years and 1.7 ± 0.7 %/yr. Over 12 Years. Good Correlation Between Observed and Expected BMC After 12 Years (r = 0.9). |

| 9704 White Women 65 Years or Older Upon Inclusion. 231 Had Repeat Calcaneal Measurements and 218 with Repeated Hip Measurements Who Did Not Use HRT at Baseline | Age, Weight, Sex Hormone Binding Globulin (SHBG), Serum 25-OH Vitamin D, 1-25-(OH2) Vitamin D, Serum Calcium, Serum PTH, Serum Oestradiol | Linear Regression, With Adjustments for Covariates Separately or With All Covariates in the Model at the Same Time. Associations Were Adjusted for Age and Weight. | High SHBG and Low Serum Oestrone Associated with High Loss Rates in Both Calcaneus and Hip. Low Serum 25-OH Vitamin D Associated with Higher Hip But Not Calcaneal Loss |

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**Notes:**
- BMC of right forearm (DPA) at 1 and 8 cm site calculated bone loss in %/yr. over 6 years by linear regression expressed using the last pre-menstrual year and 6 years ahead. Reference value was the average of the 2 last pre-menstrual years.
- BMC of forearm (SPA). Prediction made from the results from the first two years (same as (143)).
- Hip BMD and calcaneal BMD. Calcaneal BMD measured at inclusion and after 5.7 years by SPA, hip BMD 2 years after inclusion and again after 3.5 years by DEXA, annual % change.
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<td>BMC in non-dominant forearm by SPA (corrected for fat and bone width). Loss rate calculated by linear regression (periods of 1 - 4 years) expressed as absolute values or % of initial value</td>
<td>68 healthy women age 50 - 76 (median 60) years</td>
<td>Spot urine pyridinoline and desoxypyridinoline, U-oestradiol and metabolites, height, weight, BMI, years from menopause, age</td>
<td>Multiple stepwise linear regression</td>
<td>Within the first 5 years after menopause: BMC(dist radius)/yr. = - 1.051*U-oestradiol - 0.042 * U-Pyr + 0.162 * BMI - 1.658 ($r^2 = 0.58$)</td>
<td>(91)</td>
</tr>
<tr>
<td>BMD by DEXA in spine (L2 - L4) and proximal femur. Absolute rates of loss by linear regression over 2 years</td>
<td>122 postmenopausal women (mean 58.1±5.0 years, 9.2±5.3 years since menopause) followed for 2 years allocated to placebo or calcium 1 g/day</td>
<td>weight, rate of weight loss, fat mass, rate of loss of fat mass, lean body mass and rate of loss of lean body mass, S-Ca, S-P, S-creatine, S-iPTH, S-25-OH-D, S-alkaline phosphatase, U-OH/creatinine, U-Ca/creatine, renal tubular re-absorption of calcium and phosphate, S-Oestrone, S-Oestradiol, S-Androstendione, S-DHAS, S-Testosterone, S-SHBG, S-IGF-I, dietary intake (calcium, phosphate, energy, sodium), physical activity</td>
<td>Multiple linear regression (stepwise)</td>
<td>Body: BMD/yr. = 4.1<em>Treated - 54</em>BMD + 0.5<em>Fat + 2.8</em>Fat-change + 12<em>tubular Calcium resorption + 6.9 Lumbar spine: BMD/yr. = 6.9</em>Treated 2.4<em>Fat-change - 6.6 Femoral neck: BMD/yr. = - 63</em>BMD + 0.43<em>Fat + 40 Ward's triangle: BMD/yr. = 8.6</em>Treated - 59<em>BMD + 21</em>tubular Calcium resorption - 16 Trochanter: BMD/yr. = - 39<em>BMD + 0.47</em>Fat + 3.3<em>Fat-change + 8.8</em>lean-change + 0.23*SHBG - 9</td>
<td>(97)</td>
</tr>
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<tr>
<td>BMC of forearm by SPA</td>
<td>37 postmenopausal women (age 45 - 53 years, menopause 3 to 36 month previously)</td>
<td>U-pyridinoline (U-Pyr), U-desoxypyridinoline (U-dPyr), P-BGP, U-OH</td>
<td>Multiple linear regression (with U-Pyr and U-dPyr included respectively)</td>
<td>r = 0.75 for U-Pyr + U-OH + BGP, r = 0.77 for U-dPyr + U-OH + BGP Bone loss around 2-3 % per year, not stated exactly</td>
<td>(147)</td>
</tr>
<tr>
<td>BMD of forearm by SPA</td>
<td>51 postmenopausal women, age 58 - 64 years, 3-10 years postmenopausal, followed for 10 years with annual measurements</td>
<td>BMI, S-Oestradiol, years since menopause, age, calcium intake</td>
<td>Stepwise multiple linear regression</td>
<td>Loss rate 0.7±0.8 %/year in those who ingested &lt;800 mg calcium/day, 1.3± 0.8 %/year with &gt;1350 mg/day. Only BMI was negatively related to loss rate.</td>
<td>(98)</td>
</tr>
<tr>
<td>BMC by SPA, difference before and after 9 month</td>
<td>522 postmenopausal women</td>
<td>Initial BMC, age, years from menopause, weight, age, alkaline phosphatase, U-OH/creatinine, U-Ca/creatinine, serum osteodiol</td>
<td>Univariate and multivariate regression analysis</td>
<td>Loss not stated, probably around 1.7 %/yr. (S-13 in paper)</td>
<td>(99)</td>
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<tr>
<td>BMD of spine (L1 - L4), femur (neck, trochanter, Ward’s triangle and total hip), distal radius and whole body by DEXA measured at baseline and after 2 years. Loss rate in %/yr.</td>
<td>268 untreated postmenopausal women 1 - 26 years past menopause, age 50 - 70 years</td>
<td>Loss rate, age, years from menopause, BMI, dietary calcium intake, serum 25-OH vit. D, baseline BMD, serum calcium, serum phosphate, serum creatinine, serum PTH, vitamin D receptor genotype (VDR)</td>
<td>ANOVA</td>
<td>Loss rates 0.01 to 0.26 %/yr. in spine, 1.02 to 1.57 in femoral neck, and 0.38 to 0.70 in distal radius No significant effect of VDR genotype in the whole material. In a subgroup of 128 women less than 10 years postmenopausal (mean age 56±3 years, the loss rate in the spine but not in other regions were associated with VDR</td>
<td>(100)</td>
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<td>BMC of forearm by SPA. Loss rate determined by linear regression of 9 measurements over 2 years in % of initial value</td>
<td>35 randomly selected women age 45 - 55 years, 6 to 36 month past last menstruation</td>
<td>CrossLaps (degradation product of type collagen)</td>
<td>Correlation</td>
<td>Loss rate correlated negatively with CrossLaps ($r = -0.61$). Loss around 2-3 %/yr., not stated exactly</td>
<td>(256)</td>
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<td>BMD by DEXA of hip (total, neck, Ward’s triangle, trochanter, intertrochanter) and of spine (L1 - L4). Loss rate determined as value at 1 year in % of baseline value</td>
<td>85 women (mean 77 years, range 66 - 93 years)</td>
<td>Height, weight, BMI and age</td>
<td>Linear correlation</td>
<td>Loss in longitudinal study was $0.95 \pm 2.88$ %/yr. in total hip with a gain of $0.94 \pm 3.34$ %/yr. in spine BMD</td>
<td>Negative correlation between age and change in total hip BMD ($r = -0.22$), positive correlation between change in spine BMD and height ($r = 0.25$) and between change in intertrochanter BMD and BMI ($r = 0.24$)</td>
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<tr>
<td>BMD of radius by SPA. Loss rate as 5 year value in % of baseline value</td>
<td>217 white women age 22 - 54 years</td>
<td>Age, oestrogen status (still menstruating or receiving oestrogen treatment vs. none of these), parity, age at first pregnancy, weight, PTH, S-1,25-OH vit. D, bone specific alkaline phosphatase, BGP</td>
<td>Stepwise multiple linear regression</td>
<td>Annual loss 1.1 %</td>
<td>Values at 5 year closely linked to baseline values ($r^2 = 0.64 - 71$ % with positive, 59 % with negative oestrogen status). Baseline BMD weakly negatively associated with loss rate in positive oestrogen status ($r^2 = 0.06$) but not with negative oestrogen status. All other correlations were weak.</td>
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<td>BMD of lumbar spine (L2-L4) by DPA. Loss rate as value after 1 year in % of baseline value</td>
<td>249 postmenopausal women without radiographic abnormalities on spine X-rays (mean age 61±5 years, 12.9 ±6.3 years past menopause, 44 of these with radiographic abnormalities (age 58±5, time since menopause 10.2±19.8 years)</td>
<td>Age, presence of radiological abnormalities on X-ray</td>
<td>Direct comparison of the two groups Loss rate in spine 0.11±0.51 %/yr. with radiographic abnormalities, but 0.97±0.26 %/yr. without. In the forearm, loss rates were similar: 0.41±0.65 %/yr. with and 0.39±0.36 %/yr. without abnormalities</td>
<td>Significant increase in the number of radiographic abnormalities with age (osteophytes, aorta-calcification etc.). Loss rates were less in those with radiological abnormalities due to influence from these extraosseous calcifications.</td>
<td>(101)</td>
</tr>
<tr>
<td>BMD of forearm (SPA), same as (143) - %/year, initial value as reference</td>
<td>Initially 315 healthy postmenopausal women, after 15 years 182 women were re-examined</td>
<td>Number of fractures, absolute BMD after 15 years, height, weight, Urinary Crosslaps, S-BGP</td>
<td>Direct comparisons between the group of fast bone losers (&gt;3%/yr.) and the group of normal bone losers</td>
<td>After 15 years: Height did not differ, weight was lower among fast losers while Crosslaps and BGP were higher. Fracture rates were higher among fast losers.</td>
<td>(156)</td>
</tr>
<tr>
<td>Total body calcium by neutron activation and BMC of distal radius by SPA. Loss rates by linear regression. There was a mean of 3.09 measurements in each subject, i.e. 2 years of follow-up (102)</td>
<td>44 women with repeated annual measurements of BMC and total body calcium (age 45 - 55 years)</td>
<td>Age, weight, height, FSH, LH, PTH, serum oestradiol, serum oestriol, serum prolactin, serum progesterone, serum 25-OH vit. D, serum 1,25-OH vit. D, Urinary hydroxyproline, dietary calcium intake, ratio urinary calcium/dietary calcium and serum T4 (thyroxin)</td>
<td>Bivariate Pearson correlation. Loss rate in forearm 0.92 %/yr., and in total body 0.45 %/yr.</td>
<td>Correlations for distal radius: T4 (r=-0.32), PTH (r=-0.46). Correlations for total body calcium: T4 (r = 0.34), FSH (r = -0.32), dietary calcium (r = 0.39), % calcium absorbed (r = 0.48) and serum 25-OH vit. D (r = 0.30)</td>
<td>(219)</td>
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<td>BMD of lumbar spine (at least 2 vertebrae between L1 and L4), femoral neck by DEXA measured by 2 different machines, values being compared after using a correction formula. BMD was measured every 6 month for 2 years. Loss rates were calculated by linear regression in % of baseline value</td>
<td>17 premenopausal women not receiving hormones, 40 postmenopausal women not receiving hormones and 24 postmenopausal women receiving hormones</td>
<td>Age, time from menopause, weight, height, BMI, serum BGP, serum P1CP, serum 1CTP serum alkaline phosphatase, BAP, serum tartrate resistant alkaline phosphatase, urinary OH/creatinine ratio, urinary pyridinoline/creatinine ratio, urine desoxypyridinoline/creatinine ratio, urinary calcium/creatinine ratio</td>
<td>Stepwise multiple linear regression</td>
<td>Loss rate in untreated postmenopausal women 1.53 %/yr. in spine and 1.4 %/yr. in femoral neck</td>
<td>(94)</td>
</tr>
<tr>
<td>BMD of lumbar spine (L2 - L4) by DEXA. Loss rate as difference between BMD at 2 years and baseline in %/yr. of baseline value</td>
<td>209 Japanese women age 35+ years from a rural area. Divided into: 1) premenopausal - those who were still menstruating during follow-up, 2) perimenopausal: those who entered menopause during follow-up, and 3) postmenopausal</td>
<td>age, age at menopause, height, weight, BMI, hysterectomy or not, serum calcium, serum phosphorous, serum iPTH, serum BGP, bone specific alkaline phosphatase, urinary OH/creatinine ratio, urinary pyridinoline/creatinine ratio, urinary desoxypyridinoline/creatinine ratio</td>
<td>Stepwise multiple linear regression</td>
<td>Loss rate 2.4±0.54 %/yr. in perimenopausal women, and 0.85±0.21 % in the whole group of postmenopausal women</td>
<td>(95)</td>
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<td>Same as (146)</td>
<td>72 postmenopausal women, aged 63-65</td>
<td>Vitamin D receptor (VDR) genotype associated with loss rate and fractures</td>
<td>Bivariate comparison. Loss rate 0.08-1.2%/yr. in spine (L2-L4), 0.2-1.2%/yr. in femoral neck and 0.8 - 1.5%/yr. in ultradistal forearm</td>
<td>No correlations between VDR and loss rates or fractures</td>
<td>(105)</td>
</tr>
<tr>
<td>BMD of lumbar spine by DEXA, loss rate as value at 2 years in % of initial value</td>
<td>86 women (age 50 - 81 years) with forearm fractures and 297 controls of similar age</td>
<td>Age, height, years from menopause, use of HRT or not, fracture status (forearm fracture or not)</td>
<td>t-test for 2 samples, the effect of age was examined by multiple linear regression</td>
<td>Higher loss rate in lumbar spine in women with fractures (-0.59%/year) than in women without fractures (0%/year, p=0.02). In controls age (r=0.14, p=0.02) and time since menopause (r=0.12, p=0.05) were related to loss rates. In the cases time since fracture was related to loss rate (r = 0.24, p=0.03).</td>
<td>(92)</td>
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<tr>
<td>BMC of forearm by SPA, loss rate by linear regression of 8 points measured over 2 years</td>
<td>62 healthy women from 3 month to 3 years past last menstrual bleeding</td>
<td>Urinary excretion of 99mTe-bisphosphonate, postmenopausal age</td>
<td>Bivariate correlation Loss rate around 2 - 3%/yr., not stated exactly</td>
<td>Loss rate not related to postmenopausal age (r = -0.19, p&gt;0.05). Loss rate related to urinary bisphosphonate (r=-0.36, p&lt;0.005)</td>
<td>(157)</td>
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<tr>
<td>BMD of lumbar spine (L2 - L4) by DPA, change over 3 years</td>
<td>92 healthy white women from 6 to 36 month past last menstrual bleeding. The women were a control group in trial of nasal calcitonin. All were supplemented with 500 mg calcium per day</td>
<td>Height, weight, BMI, serum calcium, serum phosphorous, serum alkaline phosphatase, bone specific alkaline phosphatase, serum oestrone (E1), serum oestadiol (E2), serum calcitonin, iPTH, serum vitamin D, serum androstenedione, serum testosterone, urine calcium, urine hydroxyproline and changes within the first 6 month in these.</td>
<td>Discriminant analysis. Fast losers defined as those who lost more than 10 % over 3 years, and this measured value was “golden standard” for testing</td>
<td>DF1= -1.39+0.155<em>d6-P+129</em>d6-U-Ca/crea-0.0815<em>d6-E1+0.0916</em>d6-BMD DF2= 1.45-0.0561<em>d6-P+37.9</em>d6-Ca/crea+0.0444<em>d6-E1+0.0508</em>d6-BMD 100 % accuracy in predicting fast losers No correlations with initial parameters</td>
<td>(148)</td>
</tr>
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<td>BMD of lumbar spine and femoral neck by DPA, BMD of non-dominant forearm by SPA, loss calculated as change over 2 years (1 year in subjects without 2 year scans)</td>
<td>320 women with low calcium intake participating in a calcium supplementation trial age 40-70 years and more than 6 month past last menstruation</td>
<td>Intake of calcium, caffeine, physical capacity, serum calcium, serum phosphorous, serum alkaline phosphatase, PTH, urinary calcium (24 h), smoking, age, time since menopause, BMI The primary aim was to compare smokers to non-smokers</td>
<td>Stepwise multiple linear regression model Loss in non-smokers 0.971±2.16 in spine, 0.155± 2.842 in femoral neck vs. 1.305± 2.186 and 0.576±2.9 in smokers Only in forearm was the loss rate significantly higher in current smokers than in current non-smokers</td>
<td>(257) compare with(258)</td>
<td></td>
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<tr>
<td>BMD of lumbar spine and femoral neck by DPA, follow up scans at a median of 3.0 years (all had at least 2 scans), change calculated as linear slope</td>
<td>19 dizygotic and 21 monozogotic (5 postmenopausal, 13 premenopausal, and 3 male) twin pairs</td>
<td>dizygotic or monozygotic type</td>
<td>Covariance analysis Loss 0.02-0.06 %/yr. in spine and 0.29-0.84 %/yr. in femoral neck ( many premenopausal) Higher correlations of bone loss in mono- than in dizygotic twins (in lumbar spine 0.92 vs. 0.54, in femoral neck 0.15 vs. 0.05).</td>
<td>(96)</td>
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<td>Whole body BMC by DEXA (corrected for shift from Hologic 1000/w to Hologic 2000/w scanner), bone loss as difference over 2 years in % per year of initial value</td>
<td>433 white women (DOPS Copenhagen centre), mean age 50.9±2.9 years, 3 - 24 month past last menstrual bleeding</td>
<td>baseline weight, age, height, smoking (pack-years), reproductive period (years), family history of fractures, working capacity, weight changes</td>
<td>Stepwise backward multiple linear regression method</td>
<td>Loss 0.9 %/yr.</td>
<td>(209)</td>
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<td>BMD of radius by SPA calculated by linear regression of annual measurements over 8 years in %</td>
<td>50 early postmenopausal women, 58 - 64 years, 3 to 10 years postmenopausal</td>
<td>Calcium intake, BMI, age, years since menopause</td>
<td>Multiple regression analysis, all parameters entered</td>
<td>Loss rate 1.6±1.5 %/yr.</td>
<td>Only BMI correlated significantly positively with loss rate, 2p &lt; 0.001 (loss less in obese).</td>
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<tr>
<td>BMC of non-dominant radius by SPA every year for 8 years. Loss rate determined by linear regression in % of initial value</td>
<td>154 healthy peri-menopausal women age 47-56 years who were initially premenopausal, but became postmenopausal during the study period.</td>
<td>Age, years from menopause, mean BMI over time, dietary intake of calcium, dietary intake of phosphorous, dietary intake of protein</td>
<td>Multiple linear regression</td>
<td>Loss rate 1.3-1.9 %/yr.</td>
<td>Only BMI correlated positively to bone loss rate (loss smaller in obese)</td>
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<td>BMD of spine and femoral neck by DEXA, loss calculated as loss over 4 years</td>
<td>138 healthy peri- and postmenopausal women age 45 - 65 years. After 4 years 76 had not received HRT and 31 had received HRT</td>
<td>Treatment group, age, weight, PTH, serum oestradiol, serum alkaline phosphatase, serum calcium, serum phosphate</td>
<td>Partial correlation coefficients, adjusted for height and weight</td>
<td>Loss rate 0.39 %/yr. in spine and 0.51 %/yr. in femoral neck in untreated</td>
<td>(260)</td>
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<tr>
<td>BMD (L2-L4) of lumbar spine and hip by DEXA, each measurement in duplicate. Measurements made at baseline and after 36 month, loss in % of baseline.</td>
<td>126 women age 45-64 years, from 1 to 10 years past natural or surgical menopause on placebo as part of the PEPI trial</td>
<td>Age, ethnicity (white vs. others), smoking, alcohol intake, calcium intake, physical activity, BMI, initial BMD, hysterectomy, prior hormone use, recent hormone use.</td>
<td>Repeated measures ANOVA</td>
<td>Placebo users: Higher age associated with smaller loss Low BMI associated with higher loss. Smoking associated with higher loss in hip but not spine. High calcium intake associated with smaller loss in spine but not hip. Prior and recent hormone use associated with higher loss</td>
<td>(112)</td>
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Peter Vestergaard  
PhD Thesis: Prediction of changes in bone mineral in postmenopausal women  

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<td>Same as (118). BMD of lumbar spine and proximal femur by DEXA. BMD of forearm by SPA. Follow-up period 18 years, loss rate within the first two years by linear regression as in (143) for the forearm, loss from year 12 to 18 in spine and hip was calculated as year 18 in % of year 12, loss over the entire period in forearm as year 18 in % of year 0.</td>
<td>136 women aged 45-54 years, 6 to 36 month past last natural menstrual bleeding.</td>
<td>Age, weight, height, years since menopause, vitamin D receptor genotype</td>
<td>Multiple linear regression</td>
<td>No significant relations between vitamin D receptor genotypes and loss rates. Loss rate over 18 years from 23.3 to 26.9 % in forearm, over 6 years from 1.0 to 3.9 % in spine and 3.1 to 5.3 % in hip.</td>
<td>(245)</td>
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<td>BMD of lumbar spine and total body by DEXA measured 3 times at 6 month intervals, change expressed in %/year</td>
<td>205 non-smoking postmenopausal women, age 61±5 years, 13±7 years postmenopausal</td>
<td>Caffeine intake, calcium intake</td>
<td>Covariance analysis, loss (%/yr.) adjusted for years since menopause, BMI, physical activity, baseline BMD</td>
<td>Among women consuming more than the median of 744 mg calcium/day - coffee intake did not affect loss rates. Among those consuming less than 744 mg/day, those consuming more than 450 mg caffeine/day had a higher bone loss in the lumbar spine (1.36±2.70 %/yr.) than those consuming less (0.26± 2.74 and 0.70±2.70 %/yr.)</td>
<td>(12)</td>
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<td>BMD of whole body and femur (neck, Ward, trochanter) by DEXA. BMD at baseline and after 1 year. Loss as absolute difference between 1 year and baseline</td>
<td>50 caucasian women at least 3 years postmenopausal and &lt; 65 years of age (mean 60.0±3.7 years, time since menopause 11.5±6.1 years)</td>
<td>Initial BMD, fat tissue mass, lean tissue mass.</td>
<td>Multiple linear regression</td>
<td>Initial fat tissue mass and lean tissue mass not correlated to change in BMD. Change in weight and change in fat tissue mass were positively related to change in BMD (i.e. a gain in fat mass was related to a gain in BMD and vice versa) adjusted for initial BMD</td>
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<td>BMD of lumbar spine (L2-L4) by DEXA at baseline and after 1 year, loss expressed in % of baseline</td>
<td>96 Japanese women age 45-78 years, 1-29 years since menopause</td>
<td>Vitamin D receptor genotype</td>
<td>Fisher’s protected least-significant difference</td>
<td>Rate of bone loss greater in Bb genotype (-4.5±1.0 %/yr. &lt;10 yrs from menopause, -2.6±0.4 %/yr. ≥10 yrs from menopause) than in bb genotype (-0.77±0.44 %/yr. &lt;10 yrs from menopause, -0.03±0.17 %/yr. ≥10 yrs from menopause)</td>
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<td>BMD of lumbar spine and femoral neck by DPA at inclusion, after 1, 2, and 4 years. Loss rate by linear regression, intercept of regression line used to calculate % loss</td>
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<td>Multiple stepwise regression</td>
<td>No correlations between loss rates and biochemical markers or hormones despite ample power (80%) to detect a correlation of 0.5 between bone loss and any independent variable. Loss rate 1.41±0.18 %/yr. (mean±SEM) in the spine and 0.86±0.22 %/yr. at the femoral neck</td>
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3) Material and methods
In and exclusion criteria are shown in section 1.2. The material on which the models were
developed was the 595 participants in the Aarhus centre. The testing was done against the Odense
Centre.
A number of independent variables were studied to see, if the use of any combination of these could
be used to predict actual BMD and loss of BMD over time (the dependent variables).

3.1) Independent variables
A number of variables influence bone loss. In this section only variables that - from a biological
point of view - were likely to influence bone turnover were considered. Variables that may have
acted at earlier time-points or that were likely to appear at time-points after the menopause were not
considered.
Some variables may be intercorrelated - a phenomenon sought highlighted and thus dealt with in the
statistical analysis by using collinearity diagnostics in the stepwise multiple linear regression.

3.1.1) Physiological variables
Age:
Age was an important factor for BMC/BMD in almost all cross-sectional studies on BMC/BMD
(table 1).
Age has also been included in several studies on longitudinal loss rates of BMD (91-95,98-
100,104,108,209,260) (table 2). However, only few studies report significant correlations with age
(92,104). Peel et al (92) reported a positive correlation between age and loss rate in lumbar spine,
meaning that the loss was more positive (i.e. smaller) with increasing age. In contrast Greenspan et
al. (104) found a negative correlation between loss rate in the total hip and age meaning, that the
loss rate was higher (more negative) with increasing age in women age 65 years or more. However,
Greenspan et al. (104) could not find significant correlations with age at other sites. None of the
other studies (91,93-95,98-100,108,209,260) reported age effect on loss rates. Per se age is probably
not a factor of importance but a proxy-variable to degenerative processes as BMC tends to decline
by age and as the rate, with which this decline takes place, seems to be different at different points
in time (e.g. the proposed increased loss around menopause).
In this perspective it seems reasonable to include actual age, as the rate of loss may change in the
narrow span of time around the menopause.

Age at menopause and years from menopause:
Menopause is defined in section 2.5 as the permanent cessation of ovarian function (64,65), often
being diagnosed after 12 month from the last menstrual bleeding (65). Age at menopause is thus the
age at which menstrual bleedings stop. Years from menopause is the time from last menstrual
bleeding until present age.
To a certain degree these variables will be linked to age (high age at menopause will also imply a
higher actual age). However, these variables may also reflect biological age more than actual age by
birth-year.
A problem with these variables is, that they may be difficult to determine exactly: the probability
that menopause has occurred increases with the time span from last menstrual bleeding - i.e. a
woman around 50 years of age one month past last menses may in fact be postmenopausal, but the probability is lower than in a woman of the same age 12 month past last menses. As the occurrence of symptoms may also differ among women (263) - both in severity and in types of symptoms - occurrence of symptoms may increase the probability of postmenopausality. Furthermore, the hallmark of irregular menses is not assessable in women, who have undergone hysterectomy and may further mask the occurrence of menopause. In this thesis time since menopause will only be studied in the subgroup of women with intact uterus. Menopausal age was only recorded in one study (146), but was not included in the final equation (table 2). As the menopausal age (absolute value) is related to the actual age, it was not included in this thesis.

Body mass index (BMI):
BMI is calculated from height and weight and is thus correlated with these variables. In the present analyses only BMI will be considered to avoid this interrelation.

Cross-sectional BMC is naturally associated with height and weight but also to BMI (table 1).

BMI is closely associated with body-fat mass (5) and it is readily accessible. Fat mass seemed related to loss rates in (143) and (97) but was eliminated after introduction of BGP in the equations in (145), as an increase in correlation coefficient from 0.61 to 0.76 was found (11,145). It is however not clear in these studies (table 2), if it was subject to statistical testing, whether this observed change in correlation coefficient ($r$) from 0.61 (11) to 0.76 (145) was significant. However, it should be borne in mind that body size - which is related to BMI - influences the measurements of BMD, which may be underestimated in small individuals (187).

Weight has been correlated to BMD in several cross-sectional studies (table 1), however measurement errors in BMD may be linked to body composition (264) especially to changes in weight and thus also changes in body composition. This may be part of the explanation for correlations found between longitudinal changes in weight and changes in BMD (209,214). However, Felson et al. (215) reported, that prior weight change was a strong predictor for actual BMD - relation not explained by changes in body composition.

Waist-hip ratio:
This ratio is a marker of fat mass and of fat-distribution (apple-shape with abdominal fat in males and pear-shape with predominantly hip deposits of fat in females). The ratio does not seem to have been subject to consideration for loss rates (table 2), but has been considered in cross-sectional studies (table 1) (210,212).

Fat is a hormonally active tissue and may influence bone through metabolites with oestrogen activity, it thus seems reasonable to consider both BMI and waist-hip ratio as both are markers of fatty tissue and as the waist-hip ratio may reflect a “male” (abdominal) or “female” (hip) fat distribution and possibly also a difference in hormonal activity. The waist-hip ratio will thus be included in this thesis.

Hereditry:
In a single cross sectional study (212) the phenotype (fair complexion) was linked to the occurrence of osteoporosis.
This study cohort is very homogenous concerning background (Danish women from a limited geographical region). Contrasts in phenotype are thus limited and phenotype is omitted. Genetic markers are the subject of a separate study under the DOPS protocol (180). Riggs et al. (265) hypothesised, that two distinct types of osteoporosis exists: type I predominantly seen before the age of 65 (fractures of spine, wrist and ankle) and type II seen late in life (fractures of femoral neck and vertebrae). The fractures in type I may be a result of both low peak bone mass and rapid postmenopausal bone loss. Theoretically, Maternal fractures may thus be a marker of an inherited disposition to rapid bone loss.

Furthermore, family history of fractures were linked to cross-sectional bone mineral in several studies (107,188,212,239) (Table 1) and also to fracture risk (54). In a follow-up study, Falch et al. (146) studied the effect of family history of fracture on bone loss, but no significant effect was present. Diaz et al. (266) found a relation between a maternal history of hip fracture and presence of vertebral deformity especially in men. Low bone mass has also been reported in relatives of osteoporotic patients (267,268).

In this thesis maternal and paternal fracture history were included as follows: a maternal fracture history was consisted present if on inclusion the participant reported the presence of a prior hip or forearm fracture in their mothers, and conversely a paternal fracture history was the presence of a hip or forearm fracture in their father. Among male monozygotic (n=42 pairs) and dizygotic (n=38 pairs) twins (age 44-55 years), a longitudinal study (269) of radial bone mass for a period of 16 years showed, that the loss was primarily linked to environmental and not to inheritance. Flicker et al. (253) estimated, that 75 % of residual variation in BMD of lumbar spine and femoral neck was attributable to genetic factors in elderly females (60 -89 years of age). Krall et al. (270) estimated, that 46 - 62 % of BMD variation was attributable to heredity. Despite this seemingly high percentage, information on family fractures has only had moderate predictive power (50).

Genetic markers (as VDR, TGFβ, COLIA1, oestrogen receptor genotypes etc.) are not subject to study in this thesis as they 1) are not readily available, and 2) are costly to determine. Furthermore, it may be anticipated, that any effect of the genetic markers will be reflected in biochemical markers and in family history of fracture.

Prior fractures:
Prior fractures may be a result of low bone mass. In this group of women who were close to their peak bone mass, prior fractures may be linked to low peak bone mass, and this parameter was thus included in the equations. Fractures in childhood (<15 years) were excluded, as they occurs before accretion of peak bone mass, and as they may be linked to other factors than bone mass, especially as a dramtical increase in fracture risk is seen around menarche in both boys and girls (271). Gilfillan et al. (200) reported, that forearm BMD was lower in women with a prior fracture after the age of 25. Furthermore, girls with forearm fractures (age 3 - 15 years) tended to have lower BMD of lumbar spine, forearm, femur, and total body than girls without fractures (272), a feature also seen in women older than 50 years of age (40,273,274). As prior fractures are unlikely to affect actual loss rate - especially if they have occurred many years prior, they were not included in the analysis of loss rates. As mentioned above under heredity, a prior fracture may also be a marker of genetic markers.

Disease processes and other skeletal changes:
Presence of diseases to the skeleton (fractures, osteomyelitis, osteoarthritis (191) etc.) may along with other factors such as scoliosis and osteophytes alter the results of the scannings especially of
the spine. Patients with obvious disease processes in the region of interest were excluded from the analyses. Patients with arteriosclerosis were not excluded from the analysis (see section 4.1).

3.1.2) Biochemical measurements

These measurements constitute a wide range of possible predictors, several having been tested against loss rates for bone (table 2). However, the complex interaction has not been studied in detail. It is generally accepted, that rates of bone changes during remodelling depend mainly on bone turnover and balance per remodelling cycle (see section 2.4). Variations in these variables are reflected in serum levels and renal excretions of specific biochemical bone markers, which may reflect bone resorption (urinary excretions of hydroxyproline, and pyridinolines, and serum 1CTP) or bone formation (serum alkaline phosphatase, serum osteocalcin etc.). Furthermore, bone mineral loss may lead to secondary changes in calcium homeostasis as reflected by serum calcium levels or fasting renal calcium excretions. Finally changes in hormones and calcium homeostasis may accelerate or reduce bone loss. The following biochemical measurements were included in the mathematical modelling process:

3.1.2.1) Biochemical markers of bone turnover

3.1.2.1.1) Resorptive bone markers

Fasting second void urinary hydroxyproline/creatinine ratio (U-OHP, μmol/mmol):
Measured as U-OH/creatinine ratio on a fasting morning spot urine (second void on a diet low on collagen/gelatine) by standard laboratory procedures (Hypronosticon®, Organon Teknika, Holland, Klinisk Biokemisk afd., Århus Amtssygehus).
Urinary hydroxyproline (U-OHP) has mostly been studied by methods not involving stepwise regression (143,145,147). In studies involving stepwise procedures (97,154) it has not been a significant predictor. A problem with hydroxyproline is, that many other sources than bone matrix contributes to the excretion, and that measurement must take place under dietary precautions.

Fasting second void urinary pyridinolines/creatinine ratio (U-PYR, nmol/mmol):
Urinary pyridinolines: Measured in spot urine as pyridinoline/creatinine ratio and desoxypyridinoline/creatinine ratio by HPLC (275,276) with inter-assay CV <11.3 % and intra-assay CV < 7.6 %.
These degradation products of collagen (62,276-279) have been subject to interest as they perhaps represent better markers of bone turnover. Mole et al. (91) and Uebelhart et al. (147) studied U-pyridinolines and found, that they correlated with loss rate in the forearm. Ubelhart et al. (147) used the mean of several measurements to overcome the variability of these markers (280). Fledelius et al. (262) found, that urinary desoxypyridinoline, but not free pyridinoline, correlated with bone loss rate in postmenopausal women. However, Keen at al. (154) found no correlation between baseline pyridinolines and loss rate over a period of 4 years in spine or femoral neck BMD in a large study group.
The pyridinolines are included in the analysis, as some studies have found correlations with loss rates. However as the purpose of this thesis is to derive a simple measure bone turnover, it was considered impractical to measure a parameter more than once. Both urine pyridinoline and desoxypyridinoline are included in this thesis.
Serum carboxyterminal peptide of procollagen type 1 (1CPT, µg/l):  

1CPT (264) and was measured by RIA (Orion Diagnostica, Espoo, Finland), intra-assay CV < 6.2 %, intra-assay CV < 8 %. The variable 1CPT was considered by (94) to predict loss rates in spine and femur - however the correlation was weak (r=0.62 and 0.56 respectively). Because it seems correlated to bone loss (94), and as it has not been studied in detail, it is included in the study.

3.1.2.1.2) Formative bone markers

Serum bone specific isoenzyme of alkaline phosphatase (BAP, U/l):  

Measured by lectin precipitation (281) as the bone specific part of total alkaline phosphatase. Intra-assay CV 8%, inter-assay CV 25%.  

This variable or total alkaline phosphatase is included in several studies (76,97,146,154) but only in some equations in table 2 (143,145). In this thesis it will be included.

Serum osteocalcin (BGP, Bone Gla protein, ng/ml):  

Measured by RIA (281) (Intra-assay CV 5%, Inter-assay CV 10%).  

This variable is included in several equations in table 2 (145,147). It will thus be included in this thesis.

Serum markers of collagen type I:  

These are represented by P1CP (C-terminal propeptide of type 1 collagen, µg/l) and P1NP (N-terminal propeptide of type 1 collagen, µg/l) (275,282-286). P1CP and P1NP are markers of formation (264). P1NP was measured by RIA (Orion Diagnostica, Espoo, Finland), inter-assay CV < 13.7 % and intra-assay CV < 8.2 %. P1CP was measured by RIA (Orion Diagnostica, Espoo, Finland), intra-assay CV < 3.2 %, inter-assay CV < 6.6 %.

The ability of P1CP to predict loss rates in spine and femur was studied by (94). P1CP was not found to be a significant predictor, but the equations included 1CPT (94) (see above, section 3.1.2.1.1).

Thus only some of these variables have been studied and not in detail.

3.1.2.2) Calcium homeostasis

Albumin adjusted serum Calcium (S-Ca, mmol/l):  

Albumin adjusted S-Ca was measured by standard laboratory methods (Klinisk Biokemisk afd., Århus Amtssygehus).

This variable has been included in several studies but has not been found to correlate to bone loss rate (76,97,146) - possibly due to the fact that it is strictly regulated, and that small fluctuations may only reflect were temporary changes in bone calcium. In this context it is included merely for comparison with other studies.

Fasting second void urinary calcium/creatinine ratio (U-Ca, mmol/mmol):  

Measured as U-Ca/creatinine ratio on a fasting morning spot urine by standard laboratory methods (Klinisk Biokemisk afd., Århus Amtssygehus).

In studies using stepwise regression analysis this analysis has not been found to correlate to loss rates (97,146,154). The variable has only been included as a predictor variable in studies using non-stepwise procedures (143,145), and it has not been subject to evaluation in these studies, whether the correlation was statistically or clinically significant. U-Calcium/creatinine may therefore not
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... seem to be a reliable marker of bone turnover in the long run. However, as earlier studies have not stratified for potential confounders such as calcium intake, this variable is included for convenience in this analysis.

Serum intact 1-84 PTH (PTH, pmol/l):
Serum intact parathyroid hormone was measured by DPC Immulite (chemilucens) with an inter-assay CV of 11% and an intra-assay CV of 6%.

Serum PTH has not been studied in detail (table 2). It has been shown, that serum PTH was related to both histomorphometric activation frequency and bone resorption rate in type I osteoporosis (287). In the longitudinal studies, PTH was included in (260) as a predictor, but was not found to be significant, however, some of the subjects had received HRT treatment. PTH was also studied in (257), but as part of a calcium supplement trial in women with low calcium intake. It was not found to be a predictor. Reginster et al. (148) included PTH among the predictors, but did not find it to be a significant contributor. Among postmenopausal Japanese women (95) PTH was also not found to be a significant predictor variable. Also PTH was not included in (93), but many of these women were premenopausal. Garnero et al. (100) also found no correlation between bone loss and PTH. When adjusting for tubular calcium re-absorption Reid et al. (97) found no correlation between bone loss and PTH. Stone et al. (103) found no significant predictive value of PTH. Total body calcium change was found to correlate to PTH, but only in bivariate correlations (219).

In this thesis PTH is only included to supplement the other variables and to study the complex interactions.

3.1.2.3) Hormones
Serum Oestradiol (S-Oestradiol, nmol/l):
Measured by AutoDelfia assay (Wallac OY). Intra-assay CV 5.2%, Inter-assay CV 8.5%. Oestradiol is of great significance to bone turnover in women (21,288,289) as also seen by the bone loss induced after oophorectomy in premenopausal women (31,207,290,291). The effect of oestrogens also seems present even many years after menopause (223).

S-Oestradiol and S-Oestrone were included in an alternative equation in (143). However this equation (BMC/yr. = -0.008 * U-Ca - 0.275 * U-OH + 0.006 * S-AP + 0.015 * S-Oestrone - 0.033 * S-Oestradiol + 5.822 * fat mass + 1.856) did not give higher predictive value than the one presented in table 2 using sensitivity and specificity.

S-Oestradiol was evaluated by bivariate analysis in (76) and was not found to correlate with loss rates. Falch et al. (146) did not find an effect of S-Oestrone on loss rates. Mole et al. (91) studied urinary metabolites of oestradiol and found higher loss rates in the distal radius with increasing U-oestradiol. Reid et al. (97) found no effect of base-line measurements of S-Oestradiol on loss rates of lumbar spine BMD.

Keen at al. (154) in large study group found no correlations between serum oestradiol, serum oestrone, serum oestrone sulphate and loss rate of BMD. Falch et al. (146) and Reid et al. (97) both used stepwise analysis, while Christiansen et al. (143) used a model, in which all variables were forced into the equation, which may explain some of the difference.

Insulin like growth factors:
Serum IGF-I (μg/l) and IGF-II (μg/l) were measured by the Institute of Experimental Clinical Research, Aarhus Kommunehospital, Denmark as previously described by Frystyk et al. (292).
Intra-assay CVs were less than 5%, and inter-assay CVs were less than 10% respectively. Cross-reactivity was less than 0.0002% in heterologous assays with recombinant human (rh) proinsulin and rh insulin cross-reacting less than 0.01% in the IGF-I assay. Serum IGFBP-3 (μg/l) was measured by an immunoradiometric assay (DSL-6600 Active™, Diagnostic Systems Laboratories Inc., Texas) with intra- and inter-assay CVs of less than 4% and 2% respectively. Details can be found in (293).

IGF-I, IGF-II and IGFBP-3 are newcomers and have not been studied in detail in the context of bone loss rates. The bone loss around menopause in women is linked to the loss of endogenous oestrogen production. Furthermore, an acute increase in circulating IGF-I and IGF-II has also been induced in premenopausal women, who were made oestrogen deficient through administration of GnRH agonists (294). The interaction between oestrogen and GH is complex (295), and decreases in GH during oestrogen administration may be seen (295). Oestrogen is also a complex mediator of the IGF-I synthesis in the liver and may inhibit IGF-I synthesis (296).

Nasu et al. (228) and Sugimoto et al. (229) found a positive correlation between forearm BMD and IGF-I in cross-sectional studies (Table 1). Nasu et al. (228) found no correlation with IGF binding protein 2 and 3 (IGFBP-2 and 3), while Sugimoto et al. (229) found a positive correlation with IGFBP-3. Furthermore Nasu et al. (228) found a decline in IGF-I one year after menopause and an increase in bone turnover markers. Sugimoto et al. (229) also found significantly lower values of IGF-I and IGFBP-3 in patients with osteoporotic spinal fractures than in control subjects. In ovariectomised and oestrogen treated rats a correlation between circulating IGF-I and cortical bone was found (297). Furthermore, low IGF-I has been found in men with idiopathic osteoporosis (298). In children a correlation of IGF-I with BMD has also been shown (299).

IGF-I, IGF-II, and IGFBP-3 are for these reasons included in the mathematical model.

3.1.3) Dietary and life-style variables
These are variables that reflect the external environment to the bones (e.g. intake of calcium, exposure to potential toxic substances such as smoking or alcohol).

Dietary habits:
The relation between intake of calcium and bone loss rate has been studied by several authors (11,76,97,146) and has not been found to influence loss rates except in connection with calcium supplements (97). However, only some of these studies (97,146) have linked calcium intake to calcium excretion, and the basic calcium intake has also been variable and was not stated in all studies. For comparative reasons dietary calcium is therefore included.

A number of other dietary habits have also been considered in relation to osteoporosis (300). In this context only intake of vitamin D will be considered.

These intakes of calcium, phosphorus and vitamin D were all recorded by a clinical dietician by a diet record and calculated as daily intake weighted for seasonal differences. CV was 15% for calcium intake and 28% for vitamin D intake computed from a repeated sample (n=15).

Life-style factors:
It has been shown in two studies in perimenopausal women (146,257), that smoking significantly affected bone loss, so smoking is included in this analysis (dichotomised: smoking vs. no smoking).

A study in men and women aged 65+ years also showed a negative effect of smoking on bone mineral loss rates (258).
The intake of caffeine has been shown to be related to osteoporosis (8,12,14,16,231,301) especially of the femoral neck (16), but the effects of caffeine may apparently be offset by intake of calcium (231). In this analysis caffeine intake is expressed as standard cups of brewed coffee per day upon inclusion.

As tea also contains caffeine and has diuretic properties, it is also considered in the models and expressed as standard cups of tea per day at inclusion.

Alcohol consumption has also been considered a factor of importance to bone loss (11), so the intake of alcohol was expressed as a dichotomised variable: daily intake of alcohol vs. no daily intake of alcohol.

Physical activity and physical capacity (grip strength, work capacity on bicycle ergometer) was included in several cross-sectional studies (table 1) and in some longitudinal studies (11,97,146,209,257), but did not come out significant in any of the longitudinal studies. In this study the total weekly physical activity at inclusion (hours per week spent on jogging, gymnastics, cycling, swimming, standing/walking on the job) was included for convenience. Interventional studies have also demonstrated a smaller bone loss in groups assigned to physical exercise and calcium supplements than in control groups (302,303). Ross et al. (150) found, that the distribution of loss rates was skewed partly due to physical impairment. However, only a minor fraction (approx. 10 %) of women with high loss rates (> 2 SD below the mean) had physical impairment.

As the sun and maybe to a certain degree sun beds (“solarium”, “tanning booth”) provides vitamin D, the use of sunbathing and also sunbeds was included in the analysis. This inclusion was both a variable for sunlight exposure and thus vitamin D but perhaps also for a certain life-style (a proxy-variable).

Other life-style variables such as birth history (total number of pregnancies or deliveries) were not included in the longitudinal study, as it is unlikely, that pregnancies many years earlier may affect present rate of bone loss. However, pregnancies may very well have affected cross-sectional bone mineral which represents the sum of previous events (table 1) and is thus included in the cross-sectional analysis.

Medications:
Thiazide diuretics modifies calcium excretion and Wasnich et al. showed, that they modified loss rates in men (304). It therefore seems reasonable to exclude users of thiazides in the analysis of factors of significance to bone loss.

Furthermore women commencing on systemic oestrogens in the study-period are excluded from the analysis.

### 3.2) Dependent variables

In this thesis BMD of the spine, femoral neck, and ultradistal forearm are chosen as the dependent variables, as these seems the best predictors of fracture risk in the regions of particular interest to osteoporosis, namely the femoral neck, the vertebrae, and the wrist (39).

#### 3.2.1) BMD of spine, hip and forearm

Bone mineral was measured in 3 regions using Hologic™ QDR scanners:

1) Spine: The total BMD of L2 - L4 measured by AP projection (Software version V4.55). CV 1.5% (181). The spine consists mainly of trabecular bone (>70 %) (47).
2) The neck-box of the femur (software version V4.55). CV 2.1 % (181). This region consists of approximately 75 % trabecular bone (43).

3) The ultradistal part of the forearm (25 mm distal part of radius and ulna starting at the radius end-plate, Software version V5.54). CV 1.9 % (181). This region consists of approximately 60 % or more trabecular bone (43,305).

On inclusion a Hologic™ 1000 W was used but later replaced by a Hologic™ 2000 W QDR scanner. However, despite efforts to correct between the Hologic 1000/w and 2000/w scanner (306), it remained questionable whether the scannings could be compared. Data from the manufacturer (Hologic Inc., Waltham MA) denotes, that the same normal material is used to calculate T and Z-scores in both the 1000, 2000 and the latest 4500 QDR scanner, suggesting direct comparability of scannings.

In this thesis calculations are thus only made on the basis of Hologic 2000/w scannings, i.e. the 1, 2, 3 and 5 year values.

The precision was established by repeated scans of a phantom circulating between the 4 centres and by daily quality controls. Long-term precision was high (changes below 0.2%/year) (181).

3.3) Use of hormonal therapy or no hormonal therapy

3.3.1) Participants initially on HRT

These participants were either randomised to HRT (n=160) or on HRT by personal choice (n=40, fig. 2A). In DOPS a pragmatic approach was used to mimic the conditions in general practice - i.e. the patients were first offered one treatment schedule, and if this schedule was not acceptable, several other alternatives were available, if a permanent discontinuation was not warranted. Further details on the methods can be found in (51). Around the menopause and in the postmenopausal state hormonal therapy is thought of as a type of replacement therapy that replaces the oestrogens lacking due to the physiological cessation of production of sex steroids in the ovaries.

First line study drugs are described in section 1.2. If a change of HRT type for reasons not requiring permanent discontinuation was requested and the woman accepted, the following HRT types were available. In women with intact uterus: (1) Transdermal continuous oestradiol patch (25, 50 or 100 μg/day) plus oral medroxy-progesterone 10 mg for the first 10 days of a calendar month (Estraderm™, Ciba Geigy, Denmark, combined with Perlutex™ tablets, Leo, Denmark); (2) Transdermal continuous oestradiol 50 μg/day with norethisterone 0.25 mg during the last 14 days of a 28 days cycle (Estracomb™ patch, Ciba Geigy, Denmark); (3) In women with intact uterus and insufficient control of climacteric symptoms tablets with increased oestradiol content were available (Trisequens Forte™, Novo Nordisk, Denmark: 4 mg oestradiol for 12 days, 4 mg oestradiol and 1 mg norethisterone acetate for 10 days followed by 1 mg oestradiol for 6 days); (4) Substitution of norethisterone by medroxy-progesterone: 2 mg oestradiol for 12 days, 2 mg oestradiol and 5 mg medroxy-progesterone for 10 days followed by a 6 day pause (Klimaxil™, Leo, Denmark); or (5) 2 mg oestradiol and 1 mg norethisterone per day continuously (Kliogest™, Novo Nordisk, Denmark). This latter preparation differed by not inducing menstruation beyond 6 month in most women (307).

It should be noted that preparations (2) and (5) first became available after the initiation of the study.

In hysterectomised women, transdermal continuous oestradiol (Estraderm™, described above) or tablets with continuous 4 mg oestradiol per day (Estrofem Forte™, Novo Nordisk, Denmark) were available.
3.3.2) Participants initially on no HRT

As stated in section 1.2 there were two groups of women not receiving therapy: one group randomised to no treatment (n=200 at the Aarhus Centre) and one group who did not choose to receive therapy (n=395 at the Aarhus centre).

3.4) statistical methods

3.4.1) Regression by the method of least squares

The method of least squares is a statistical method for fitting a series of pair-wise measurements of dependent (y) and independent measurements (time, t), by which the parameters of the best fit line are calculated.

As the exact course of the bone loss was unknown, two different mathematical functions were fitted in this thesis:

- The linear loss \( BMD(t) = a \times t + b \)
- The exponential loss \( BMD(t) = b \times \exp(a \times t) \) (see 2.3).

The latter equation is calculated on the log-transformed values. These were fitted for each individual using the measured BMD values at each time point 1, 2, 3 and 5 years.

Bias from inclusion of the initial value is excluded as described in section 2.3.

These fitted values using several time points are compared with the absolute bone loss (i.e. the difference between the final and the initial value).

The linear model has been applied in several previous studies (see table 2 and the discussion in 2.2 above).

3.4.2) The Bland-Altman method

This method was proposed by Bland and Altman (183) for determining agreement between two methods of measurement, when correlation coefficients (see below) were not sufficient. This is especially the case, when the difference between the two methods is dependent on the magnitude of the measurements (183). This method is used to compare the changes in BMD from baseline to the five year value.

3.4.3) Coefficient of determination

A coefficient of determination ("correlation coefficient") can be calculated that estimates the fraction of the variation explained by the specific equation used in 3.5.1.

This coefficient can vary between -1 and +1. If the value is 0, the equation does not explain any of the variation. This does however not mean, that the variables are uncorrelated, but they do not correlate in the way described by the equation.

If the value is +1, there is a perfect match, and if it is -1, there is also a perfect match, however in the opposite direction (instead of increasing y with x, y decreases with x).

3.4.4) Multiple linear regression

This is a joint label used to describe statistical procedures employed to evaluate relations between one or more independent variables (x1, x2, x3...xn) and one dependent variable (y) (184). It describes the linear relationship:

\[ y = a1 \times x1 + a2 \times x2 + a3 \times x3 \ldots an \times xn + K \]
Where \( K \) is a constant.

As in the simple linear equation: \( y = a \times x + b \), the linear goodness of fit can be described by a coefficient of determination (“correlation coefficient”). However, as stated in a previous section, this coefficient tends to increase the more variables that are added (so-called “inflation”). This problem may partially be dealt with by correcting for the number of variables in the so-called corrected coefficient (184).

Furthermore, statistical procedures exist to select among a group of variables those variables that yields the highest determination coefficient. The level of selection may be changed, so that more or less variables were included.

The selection procedure may be a forward procedure, where only those variables with a certain level of probability for their determination coefficient are included.

The procedure may also be backwards, where all variables are initially included in the equation followed by a subsequent elimination of those variables, whose level of determination are below a certain level of significance.

A combination of these two procedures (stepwise analysis) will be applied in the present analysis (184). This method first uses a forward procedure (inclusion of independent variables whose probability of association is below e.g. 0.05) followed by a backward elimination of variables no longer significant (e.g. their probability of association is now > 0.10 after inclusion of the other variables due to intercorrelation) until a final equation is reached. In this thesis the inclusion probabilities have been set at: \( \text{Pin} = 0.05 \), and the exclusion probabilities during the elimination procedure has been set at: \( \text{Pout} = 0.10 \) (184).

As stated earlier it is also possible to force variables into the equation, this procedure will however only be used in limited ways in the present analysis to avoid analyser-bias - i.e. the selection by the analyser of certain variables who are then “forced” into the equations. By including all variables it is possible to get an overview of all probabilities, however an observer effect with preferences for certain variables is possible.

Furthermore, it should be noticed, that by using the stepwise procedure it is not possible to eliminate bias but only to limit it to a certain degree.

The multiple regression equation describes the variation of the dependent variable, but it is possible that different equations may describe the variation equally well, e.g.:

\[
y = a_1 \times x_1 + a_2 \times x_2 + \ldots + a_n \times x_n + K_1 = b_1 \times z_1 + b_2 \times z_2 + \ldots + b_n \times z_n + K_2
\]

One way in which bias was limited was the analysis of collinearity applied in this thesis. By collinearity is meant the phenomenon, that several independent variables are highly correlated and thus provide similar information (in contrast to interaction). In this thesis diagnostics will be performed to detect the presence of collinearity and assess its potential degradation of estimated parameters (184).

The prerequisites for this analysis is, that the residuals are normally distributed.

### 3.4.5 Logistic regression

This is a statistical procedure to establish correlations between a binomial effect variable and a set of independent variables (308). In contrast to the linear regression where the dependent variable is continuous in the logistic regression the dependent variable is dichotomous. Furthermore the logistic regression uses a multiplicative model \( (y = a_1 x_1 a_2 x_2 a_3 x_3 \ldots a_n x_n) \) as its basis for the relationship between the independent variables where the multiple regression uses an additive model (see above).
In contrast to the linear model the logistic function allows for the estimation of risks associated with a parameter.

3.4.6) Discriminant analysis
This is a statistical model for determining a set of predictor variables (independent variables) that best determines the group membership of a dependent variable. In contrast to the logistic function the dependent variable can assume more than two values (308). However, in this thesis the dependent variable is binomial (see section 5 and 6). The discriminant function has been claimed by Reginster et al. (148) to give a high degree of certainty in predicting bone loss rates. However, the conditions who have to be met by the variables are rather strict for the discriminant function (155) and considerably more so than for the logistic regression (155). A testing for normality of the included variables has been performed (table 4, section 4).

3.4.7) Repeated measures ANOVA
This is a statistical method for analysing the interactions between sets of repeated measurements in the same subject as with the repeated BMD measurements in this study (309).

3.4.8) ROC curves
The receiver operating characteristics are functions, that allow the comparison of sensitivity (ability of a give test to correctly identify subjects with a given characteristic, i.e. those with a given illness) and specificity (ability of a given test to correctly identify those without a given characteristic, i.e. those without a given disease). ROC curves can thus be used to determine the optimal “cut-point” (cut-off point) for a given test, i.e. the point that with the greatest sensitivity and specificity separates between those with e.g. a disease and those without. In this thesis the ROC curves are used to separate between those with low BMD and those with high BMD. The cut-points tested were T-score -1.5, -1.0, -0.5, 0.0 and +0.5 (310).

3.4.9) Conditions having to be fulfilled by the models
In all models a thorough testing for e.g. Gaussian distribution of the variables has been performed (see section 4), and the variables has been entered as logarithmated variables if logarithmation was required to achieved normality of the distribution. The testing for normality has been performed using normal QQ-plots both logarithmated and unlogarithmated to judge if the distribution was Gaussian or could be made Gaussian through logarithmation.

4) Results I: Postmenopausal bone loss and the effect of HRT
All presentations in this section are based on the 595 participants included at the Aarhus Centre (see section 1.2) that supplied the participants used to develop the predictive models.

4.1) Baseline characteristics
Table 3 gives baseline characteristics of the participants at the Aarhus Centre. Type of distribution refers to whether or not the distribution of the continuous variables was Gaussian. A total of 154 fractures was sustained by 128 participants prior to inclusion but after the age of 15 years, 35 of the 154 fractures being forearm fractures.
Table 3: Baseline characteristics (median and range) of participants from the Aarhus Centre, n= 595

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Median and range</th>
<th>Type of distribution*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>595</td>
<td>50 (43-58)</td>
<td>normal</td>
</tr>
<tr>
<td>Weekly physical activity (hours)</td>
<td>593</td>
<td>19 (0-61)</td>
<td>normal</td>
</tr>
<tr>
<td>Hysterectomy: Yes/No</td>
<td>103/492</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Daily alcohol intake (g/day)</td>
<td>589</td>
<td>8.3 (0-148)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Daily alcohol use: Yes/No</td>
<td>86/509</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Current smoker: Yes/Not</td>
<td>244/350</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sunbathing: Never/Sometimes/Regularly</td>
<td>57/228/309</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Use of sun bed: Never/Sometimes/Regularly</td>
<td>427/110/42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maternal history of fractures: Yes/No</td>
<td>118/477</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paternal history of fractures: Yes/No</td>
<td>14/581</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total number of pregnancies</td>
<td>556</td>
<td>2.0 (1-9)</td>
<td>log-normal**</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>595</td>
<td>65.8 (43.1-123.5)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>594</td>
<td>165 (146-185)</td>
<td>normal</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>594</td>
<td>24.3 (16.8-43.4)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Waist-hip ratio (cm/cm)</td>
<td>587</td>
<td>0.778 (0.563-1.531)</td>
<td>log-normal (approx)</td>
</tr>
<tr>
<td>Total daily energy intake (kJ/day)</td>
<td>589</td>
<td>7380 (2677-23677)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Daily intake of vitamin D in food (µg/day)</td>
<td>589</td>
<td>2.5 (0.2-18.3)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Daily calcium intake (mg/day)</td>
<td>589</td>
<td>877 (166-3825)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Daily coffee intake (standard cups/day)</td>
<td>595</td>
<td>5.0 (0-45)</td>
<td>log-normal**</td>
</tr>
<tr>
<td>Daily tea intake (standard cups/day)</td>
<td>594</td>
<td>1 (0-20)</td>
<td>log-normal**</td>
</tr>
<tr>
<td>Serum albumin (mmol/l)</td>
<td>593</td>
<td>670 (550-773)</td>
<td>normal</td>
</tr>
<tr>
<td>Serum albumin adjusted calcium (µmol/l)</td>
<td>590</td>
<td>2.3 (2.05-2.6)</td>
<td>normal</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>591</td>
<td>72 (48-108)</td>
<td>normal</td>
</tr>
<tr>
<td>Serum oestradiol (nmol/l)</td>
<td>588</td>
<td>0.065 (0.006-2.09)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum BGP (ng/ml)</td>
<td>591</td>
<td>17.5 (5.3-43.8)</td>
<td>normal</td>
</tr>
<tr>
<td>Serum 1CTP (µg/l)</td>
<td>294</td>
<td>2.7 (0-7.5)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum P1CP (µg/l)</td>
<td>294</td>
<td>132.5 (39-391)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum P1NP (µg/l)</td>
<td>294</td>
<td>54.1 (6.2-134.8)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum PTH (pmol/l)</td>
<td>587</td>
<td>3.7 (0.7-13.1)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum BAP (U/l)</td>
<td>590</td>
<td>73.5 (16-246)</td>
<td>log-normal</td>
</tr>
<tr>
<td>U-Ca (mmol/l)/creatinine (mmol/l)</td>
<td>583</td>
<td>0.269 (0-2.4)</td>
<td>log-normal</td>
</tr>
<tr>
<td>U-PYR (nmol/l)/creatinine (mmol/l)</td>
<td>289</td>
<td>68.5 (4.0-423.1)</td>
<td>log-normal</td>
</tr>
<tr>
<td>U-dPYR (nmol/l)/creatinine (mmol/l)</td>
<td>289</td>
<td>23.9 (1.2-151.9)</td>
<td>log-normal</td>
</tr>
<tr>
<td>U-OHP (µmol/l)/creatinine (mmol/l)</td>
<td>581</td>
<td>20.1 (1.9-85.7)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum IGF-I (µg/l)</td>
<td>585</td>
<td>155 (64-364)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum IGF-II (µg/l)</td>
<td>585</td>
<td>918 (553-1544)</td>
<td>log-normal</td>
</tr>
<tr>
<td>Serum IGFBP-3 (µg/l)</td>
<td>589</td>
<td>3588 (2151-6641)</td>
<td>log-normal</td>
</tr>
</tbody>
</table>

* Tested by visual examination of normal probability plots. ** Discrete variables, the underlying distribution was a Poisson distribution.
In some participants there were missing values, 1CTP, P1CP, P1NP, U-dPYR, and U-PYR were only measured in participants, who did not receive any type of hormonal replacement during the 5 year follow-up period (see next sections).

### 4.2) Changes in HRT with time

As stated the DOPS was a pragmatic study with the possibility, that the participants could change type of HRT, and that the untreated had the opportunity of starting HRT without being excluded from the study. To cope with this the next tables gives the number of women stratified by whether or not they adhered to their initial group allocation.

Table 4, 5, and 6 shows the characteristics of the participants stratified by the group, they were allocated to (randomised to HRT/no HRT or HRT/no HRT by own choice), and whether or not they started HRT, ended HRT or continued in their original group. Table 4 shows, that those who chose not to receive HRT were older than the other groups. Table 5 shows, that prior hysterectomy was more frequent in those, who continued on HRT and less abundant among those, who stopped HRT. Table 6 shows, that those who ended HRT and those who continued without HRT during the study period were generally older and heavier than the other participants. Participants not on HRT at baseline, who started HRT during the study, tended to have higher serum oestradiol at baseline than the other participants.

**Table 4: Distribution of treatment groups at baseline (median and range)**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Total (595)</th>
<th>Hysterectomised (103)</th>
<th>Intact uterus (492)</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Serum E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomised to HRT (group 1)</td>
<td>160</td>
<td>20</td>
<td>131</td>
<td>50 (45-57)</td>
<td>24.6</td>
<td>0.062</td>
</tr>
<tr>
<td>Randomised to no HRT (group 2)</td>
<td>158</td>
<td>37</td>
<td>122</td>
<td>49 (45-57)</td>
<td>24.0</td>
<td>0.083</td>
</tr>
<tr>
<td>HRT by own choice (group 3)</td>
<td>40</td>
<td>3</td>
<td>32</td>
<td>49 (46-55)</td>
<td>22.5</td>
<td>0.066</td>
</tr>
<tr>
<td>No HRT by own choice (group 4)</td>
<td>237</td>
<td>30</td>
<td>207</td>
<td>50 (45-58)</td>
<td>24.3</td>
<td>0.059</td>
</tr>
<tr>
<td>Kruskal-Wallis test (2p)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.14</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 5: Changes in use of HRT or not from baseline to the five year visit (of the initial 595 participants, 525 attended the five year visit).

<table>
<thead>
<tr>
<th>Status</th>
<th>Hysterectomised</th>
<th>Intact uterus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continued with original treatment schedule among treated (groups 1 and 3)</td>
<td>20</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>Continued without HRT in non treated (groups 2 and 4)</td>
<td>37</td>
<td>252</td>
<td>289</td>
</tr>
<tr>
<td>Ended HRT among treated (groups 1 and 3)</td>
<td>7</td>
<td>49</td>
<td>56</td>
</tr>
<tr>
<td>Started HRT among untreated (groups 2 and 4)</td>
<td>19</td>
<td>41</td>
<td>60</td>
</tr>
<tr>
<td>Changed type of HRT in treated but stayed on treatment (groups 1 and 3)</td>
<td>3</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>439</td>
<td>525</td>
</tr>
</tbody>
</table>

\( \chi^2 = 16.7, p = 0.002 \)
Table 6: Characteristics of the participants stratified by use of treatment or not (median and range):

<table>
<thead>
<tr>
<th>Status</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Serum E2</th>
<th>E2: Oestradiol (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continued with original treatment schedule among treated</td>
<td>49 (45-56)</td>
<td>23.6 (17.1-39.2)</td>
<td>0.051</td>
<td>(0.006-1.010)</td>
</tr>
<tr>
<td>(groups 1 and 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continued without HRT in non treated (groups 2 and 4)</td>
<td>50 (45-58)</td>
<td>24.5 (17.4-42.7)</td>
<td>0.057</td>
<td>(0.008-2.090)</td>
</tr>
<tr>
<td>Ended HRT among treated (groups 1 and 3)</td>
<td>51 (45-55)</td>
<td>25.3 (16.8-35.6)</td>
<td>0.175</td>
<td>(0.022-1.010)</td>
</tr>
<tr>
<td>Started HRT among untreated (groups 2 and 4)</td>
<td>49 (45-55)</td>
<td>22.5 (17.9-37.3)</td>
<td>0.185</td>
<td>(0.018-1.520)</td>
</tr>
<tr>
<td>Changed type of HRT in treated but stayed on treatment (groups 1 and 3)</td>
<td>49.5 (45-57)</td>
<td>23.2 (18.7-31.8)</td>
<td>0.050</td>
<td>(0.012-0.786)</td>
</tr>
<tr>
<td>Kruskal-Wallis test (2p)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion 2:
Time dependent changes in HRT must be included in analyses of rates of change and must be taken into account upon comparison of treatment groups.

4.3) Methodological problems: influence of degenerative changes and ectopic calcifications

As the scanners determine BMC and BMD by using X-ray techniques they are potentially vulnerable to ectopic calcifications in the region of interest. In the age group in question the lumbar spine is particularly of interest as both calcifications of the lumbar vertebrae (spondylosis - defined as osteophytes or other ectopic bony formations on the vertebrae visible on the X-ray) and of the abdominal aorta are frequent (101). This section will study the effects of spondylosis (311) and aortic calcification on lumbar spine scannings.

From table 2 it can be seen that only one study (101) addressed the issue of radiographic abnormalities upon loss rates.

Table 7 shows the bone mineral content and density stratified by occurrence or not on lateral spine X-rays of spondylosis.

Table 7: The effects of spondylosis and arteriosclerosis in the spine (L2-L4) upon bone mineral in the spine

<table>
<thead>
<tr>
<th>Condition</th>
<th>Age (years)</th>
<th>Total BMC (g)</th>
<th>Total BMD (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spondylosis</td>
<td>50.9±2.9 (n= 75)</td>
<td>50.74±9.28 (n= 75)</td>
<td>1.09±0.16 (n= 75)</td>
</tr>
<tr>
<td>No spondylosis</td>
<td>49.9±2.9 (n=492)</td>
<td>46.97±8.06 (n=489)</td>
<td>1.02±0.13 (n=489)</td>
</tr>
<tr>
<td>Arteriosclerosis</td>
<td>50.0±2.8 (n= 48)</td>
<td>45.86±8.09 (n= 47)</td>
<td>1.01±0.15 (n= 47)</td>
</tr>
<tr>
<td>No Arteriosclerosis</td>
<td>50.0±2.9 (n=519)</td>
<td>47.62±8.33 (n=517)</td>
<td>1.03±0.14 (n=517)</td>
</tr>
</tbody>
</table>

It appears, that women with spondylosis were slightly older than women without (2p<0.01) but had higher BMC (2p<0.01) and BMD values (2p<0.01). Moreover, a multiple regression at baseline with age, body mass index, arteriosclerosis of the abdominal aorta (binomial) and spondylosis (binomial) as independent variables revealed that age was negatively associated with BMD (2p<0.0001, coefficient -0.0095), while aortic sclerosis was unassociated with BMD (2p=0.31).
BMI (2p<0.0001, coefficient 0.0081) and spondylosis (2p<0.0001, coefficient 0.0764) were both positively associated with total spine BMD. Subjects with or without arteriosclerosis of the abdominal aorta did not differ in age (2p=0.99), BMC (2p=0.17) or BMD (2p=0.27) values. Figure 6 shows an example of how spondylosis may affect the course of spine BMD in one subject. In this example taken from a participant who had not received any medication i.e. no HRT shows a marked increase in apparent bone density due to newly formed osteophytes.

**Figure 6**

A) Example of the influence of spondylosis upon changes in BMD in the spine in one participant:

![Image of BMD over time](image)

B) Example of an X-ray with spondylotic changes

**Conclusion 3:**

Patients with radiographic signs of lumbar spondylosis on spine X-rays must be excluded from the analysis of lumbar spine loss-rates. Patients with spondylosis on spine X-rays are thus excluded.
from the following analyses (those with spondylosis on the baseline X-ray from the cross-sectional analysis, and those with spondylosis on either baseline or five year X-ray from the longitudinal study). Patients with calcifications of the aorta does not need to be excluded from this analysis.

4.4) Distribution and magnitude of bone loss in untreated women

4.4.1) Separating fast and slow bone loss

The absolute decreases in BMD over the 5 years were normally distributed at all three measuring sites as judged from histograms (fig. 7-9). Equal results were found using normal probability plots and for the decreases from year 1 to 5 (data not shown). In none of the figures a separate group of “fast losers” could be identified with certainty as a bimodal distribution.

Figure 7

Histogram of distribution of absolute bone loss over the 5 years in the lumbar spine shown with superimposed normal curve. n=226
Figure 8
Histogram of distribution of absolute bone loss over the 5 years in the femoral neck shown with superimposed normal curve. n=289

Figure 9
Histogram of distribution of absolute bone loss over the 5 years in the ultradistal forearm shown with superimposed normal curve. n=230

Conclusion 4:
No specific group of fast losers is apparent. It is thus necessary to determine a cut-point in the continuous distribution (see fig. 3 B above and section 5.2 on ROC analysis).

Table 8 and 9 shows the loss rates in the spine, femoral neck and ultradistal forearm calculated as absolute loss, linear loss and exponential loss over the entire five year period (from 0 to 5 years, table 8) and in the interval from year 1 to year 5 (table 9). Both the linear loss and the percent loss tended to be smaller in the period from 1 to 5 years than in the entire 5 year period at all three measurement sites.
Table 8: Change in BMD in women who did not receive any form of HRT during the five years (0 to 5 years)

<table>
<thead>
<tr>
<th>Site</th>
<th>Absolute change in BMD over 5 years (g/cm²)*</th>
<th>Annual change in BMD by linear regression (all 5 time points) (g/cm²/yr.)</th>
<th>Annual % change in BMD by exponential model (all 5 time points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine (L2-L4)</td>
<td>-0.059±0.051 (n=231)</td>
<td>-0.012±0.010 (n=184)</td>
<td>1.224±1.060 (n=184)</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>-0.052±0.051 (n=289)</td>
<td>-0.011±0.010 (n=232)</td>
<td>1.389±1.229 (n=232)</td>
</tr>
<tr>
<td>Ultradistal forearm</td>
<td>-0.032±0.022 (n=286)</td>
<td>-0.006±0.005 (n=230)</td>
<td>1.764±1.286 (n=230)</td>
</tr>
</tbody>
</table>

* Note that this included the shift between the Hologic 1000/w scanner applied at inclusion and the Hologic 2000/w scanner applied from year 1 and onwards.

The percent loss over the five years amounted to 8.4±5.8 % in the ultradistal forearm, 6.4±6.2 % in the femoral neck, and 5.8±5.1 % in the lumbar spine.

Table 9: Change in BMD in women who did not receive any form of HRT from year 1 to year 5

<table>
<thead>
<tr>
<th>Site</th>
<th>Absolute change in BMD from year 1 to year 5 (g/cm²)</th>
<th>Annual change in BMD by linear regression (4 time points) (g/cm²/yr.)</th>
<th>Annual % change in BMD by exponential model (4 time points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine (L2-L4)</td>
<td>-0.044±0.046 (n=216)</td>
<td>-0.011±0.011 (n=184)</td>
<td>1.105±1.123 (n=184)</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>-0.038±0.043 (n=272)</td>
<td>-0.009±0.010 (n=232)</td>
<td>1.240±1.355 (n=232)</td>
</tr>
<tr>
<td>Ultradistal forearm</td>
<td>-0.023±0.023 (n=270)</td>
<td>-0.006±0.006 (n=230)</td>
<td>1.603±1.526 (n=230)</td>
</tr>
</tbody>
</table>

Table 10 is an extension of table 8 and 9 stratifying the absolute change in bone density by periods.

From table 11 it can be seen that there was a declining trend in the loss of bone density with time.

Table 10: Absolute differences (g/cm²) at each site in the different time periods:

<table>
<thead>
<tr>
<th>Site</th>
<th>From baseline to 1 year*</th>
<th>From 1 to 2 years</th>
<th>From 2 to 3 years</th>
<th>From 3 to 5 years (divided by 2)</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine (L2-L4)</td>
<td>-0.013±0.031</td>
<td>-0.018±0.028</td>
<td>-0.012±0.030</td>
<td>-0.007±0.016</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>(n=226)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>-0.017±0.036</td>
<td>-0.011±0.032</td>
<td>-0.015±0.031</td>
<td>-0.006±0.016</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(n=232)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultradistal forearm</td>
<td>-0.010±0.014</td>
<td>-0.007±0.014</td>
<td>-0.006±0.015</td>
<td>-0.005±0.007</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(n=230)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Note that this included the shift between the Hologic 1000/w scanner applied at inclusion and the Hologic 2000/w scanner applied from year 1 and onwards. There was no difference between hysterectomised women and women with intact uteri.

** Page test for trend

4.4.2) Individual variability in bone loss

Fig. 10 shows examples of different patterns of change in spine BMD in untreated subjects without spondylosis on X-ray. Both in a subject with a small gain in BMD, a subject with a relatively stable BMD and a subject with a steady loss of BMD. Although two of the subjects have almost identical initial BMD, their BMD after 5 years differs greatly.
Fig 10: Spine BMD changes in three subjects.

Table 11 shows a grouped view of the changes from baseline to year five in participants, who did not receive any form of HRT. The spine BMD of the patients was stratified into quartiles at the baseline (first quartile: BMD≤0.91, second quartile: 0.91<BMD≤0.998, third quartile 0.998<BMD≤1.086, fourth quartile BMD>1.086 g/cm²) and at the five year visit (first quartile: BMD≤0.859, second quartile: 0.859<BMD≤0.934, third quartile: 0.934< BMD≤1.02, fourth quartile: BMD>1.02 g/cm²).

Table 11: changes in BMD from baseline to the five year value in quartiles

<table>
<thead>
<tr>
<th>Quartile</th>
<th>1. five year</th>
<th>2. five year</th>
<th>3. five year</th>
<th>4. five year</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. baseline</td>
<td>43</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>2. baseline</td>
<td>14</td>
<td>30</td>
<td>13</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>3. baseline</td>
<td>1</td>
<td>11</td>
<td>35</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>4. baseline</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>47</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>55</td>
<td>60</td>
<td>58</td>
<td>231</td>
</tr>
</tbody>
</table>

From table 11 it can be seen, that only approximately 50 % of individuals stayed within the original quartile (at the lower and upper quartiles the participants cannot move up/down due to the stratification), approximately 20-25 % increased and another approximately 20-25 % decreased one or more quartiles.

**Conclusion 5:**
Change in BMD differs greatly from one individual to the next, subjects with high initial BMD may decline to a lower level of BMD within a short period of time.
4.5) Influence of time since menopause on change in BMD in the untreated

This section deals with the influence of time since menopause on the spontaneous changes in BMD in the different skeletal sites. According to section 2.6.3 controversies exist as to the change in BMD immediately before menopause. In this cohort, the women were perimenopausal, and the time since menopause could influence their change in BMD due to the changes in oestrogen levels around menopause. As menopause could only be determined with certainty in women with intact uterus, this section is limited to this group (fig. 11-13).

Fig. 11: Changes in spine BMD over 5 years (absolute change) stratified by time since menopause.

![Graph showing changes in spine BMD over 5 years stratified by time since menopause.]

Time since menopause

Fig. 12: Change in femoral neck BMD over 5 years (absolute change) stratified by time since menopause.

![Graph showing changes in femoral neck BMD over 5 years stratified by time since menopause.]

Time since menopause
Conclusion 6:
The skeletal sites differ as to the change in BMD with time. In the lumbar spine, the highest loss was seen in women less than one year postmenopausal, while the loss rate declined with increasing time since menopause. In the femoral neck women more than 18 months postmenopausal had a smaller loss than women less than 18 months postmenopausal, while time since menopause had no apparent effect in the ultradistal forearm.
The skeletal sites must thus be analysed separately.

4.6) Influence of treatment or no treatment on change in BMD with time
Fig. 14-16 shows the absolute BMD values at all three sites stratified by whether the participants were compliant with the treatment schedule or not (table 8).
Figure 14
Change in lumbar spine BMD (L2-L4). Error bars represent SEM.
Figure 15
Change in femoral neck BMD. Error bars represent SEM.
Fig. 14-16 shows, that the change in BMD differed greatly determined by their treatment status and whether or not the participants were compliant with the treatment. In the untreated there was a loss at all three sites during the entire observation period, a loss that tended to diminish with time (cf. table 10), while the treated who were compliant with their first line HRT gained BMD in the lumbar spine until a plateau was reached from year 3 to year 5.

**Conclusion 7:**
Untreated participants lost BMD with time, but the loss diminished with time. Treated participants gained BMD or maintained their BMD. Those changing HRT type, stopping HRT or initiating HRT had intermediate patterns, varying over time.
4.7) Influence of BMD level on change in BMD

This section deals with the problem, whether the level of BMD would influence the change in BMD, i.e. if untreated subjects with a low BMD were less likely to lose bone than those with high BMD (e.g. in the form of a negative feed-back mechanism), or if those with low initial BMD would gain more BMD during HRT than those with a high BMD.

Figure 17 and 18 shows Bland-Altman plots of the baseline BMD values versus the five year values in treated (the same HRT without change) and untreated (no HRT at all). The differences were calculated as the five year BMD minus the initial BMD and the mean value as the mean of the baseline and the five year BMD. None of the figures shows trends towards systematic deviations, i.e. the differences seemed of the same magnitude with both high and low mean BMD. From fig. 17 and 18 it can be seen, that the difference in BMD tends to be the same at both high and low mean BMD, indicating that the change was not a simple percentage of initial BMD, i.e. those with a high initial BMD e.g. lost the same amount of BMD as those with a low BMD among the untreated. This follows from the argumentation, that the change in BMD was small compared to the absolute BMD. Therefore if \( \Delta \text{BMD} = \text{BMD}_5 - \text{BMD}_0 \), and \( \text{BMD}_5 \approx \text{BMD}_0 \) then the following approximation will apply: \( \Delta \text{BMD}/(\text{BMD}_0 + \text{BMD}_5) \approx \Delta \text{BMD}/(2 * \text{BMD}_0) \). A constant ratio could also be anticipated in this presentation form as \( \Delta \text{BMD} \) was small compared to \( \text{BMD}_0 \) and \( \text{BMD}_5 \).

Figure 17

Changes in lumbar spine BMD from baseline to the five year value (BMD5 - BMD0, g/cm²/5 years) in untreated subjects.
Figure 18
Changes in lumbar spine BMD from baseline to the five year value (BMD5 - BMD0, g/cm²/5 years) in the treated subjects.

The picture was essentially the same in the femoral neck and ultradistal forearm (data not shown).

Figure 19 and 20 shows the relative changes in BMD, i.e. an expression of the percent change in BMD.

Figure 19
Relative change in BMD from baseline (bmdtot) to the five year value (bmdtot5) in the spine in the untreated
Figure 20
Relative change in BMD from baseline (bmdtot) to the five year value (bmdtot5) in the lumbar spine in the treated subjects

\[
\ln(bmdtot * bmdtot5), 20, 10, 0, -10
\]
\[
\ln(bmdtot5 / bmdtot), 20, 10, 0, -10
\]

The picture was essentially the same in the femoral neck and ultradistal forearm (data not shown). From fig. 17 to 20 it can be deducted, that there was no difference between the absolute and the relative change in describing the difference between the baseline and the five year value, i.e. the loss or effect of HRT was the same for high as for low mean BMD values.

Conclusion 8:
The absolute and % change in BMD with time was the same in those with high as in those with low BMD - i.e. no trend towards e.g. a smaller loss with low BMD in untreated or a higher gain with HRT in those with low BMD.

4.8) Accuracy of estimates and which model to choose
This section deals with the comparison of loss rates calculated by linear regression (the slope of the straight line shown in fig. 4) with the absolute loss of BMD over the five years (BMD5 - BMD0). Hereby making it possible to determine whether it is sufficient with two measurements during the five year observation period, or whether a more accurate estimate is achieved by using all measurements. It is also attempted to determine, whether the linear model or the exponential model presented in fig. 4 is the better for describing the shape of the bone loss over time. From fig. 14-16 and table 10 above it can be deducted, that the change from 3 to 5 years in the spine and femoral neck and to a lesser degree in the forearm seemed to be smaller among the untreated than the changes in each of the preceding time periods. There was a close agreement between the absolute loss from baseline to 5 years, and the loss calculated by linear regression (fig. 21-23). By using the results from section 4.5 (the Bland-Altman plots) it can also be deducted, that no systematic deviations were present dependent upon whether the BMD was high or low.
Figure 21

A) Relationship between absolute loss (BMD5 - BMD0, g/cm²/5 years) and rate of bone loss calculated by linear regression (slope, a, converted to the 5 year value: g/cm²/5 years) in the lumbar spine (L2-L4) in the untreated. n=226

B) Bland-Altman plot of the difference (absolute difference over 5 years - linear slope over 5 years) vs. the mean (absolute difference over 5 years + linear slope over 5 years)/2. Note that the deviations (the abs. dif - regression) were mostly less than 20% of the mean.
Figure 22

A) Relationship between absolute loss (BMD5 - BMD0, g/cm²/5 years) and rate of bone loss calculated by linear regression (slope, a, converted to the 5 year value: g/cm²/5 years) in the femoral neck in the untreated. n=232

B) Bland-Altman plot of the difference (absolute difference over 5 years - linear slope over 5 years) vs. the mean (absolute difference over 5 years + linear slope over 5 years)/2 in the femoral neck
Figure 23

A) Relationship between absolute loss (BMD5 - BMD0, g/cm²/5 years) and rate of bone loss calculated by linear regression (slope, a, converted to the 5 year value: g/cm²/5 years) in the ultradistal forearm in the untreated. n=230

B) Bland-Altman plot of the difference (absolute difference over 5 years - linear slope over 5 years) vs. the mean (absolute difference over 5 years + linear slope over 5 years)/2 in the ultradistal forearm.

Except for a few outliers which were identified as a result of defective scans in the forearm, there were no trends towards systematic deviations.

Results were the same for the 1 to 5 year observation period (data not shown).

Table 12 shows a comparison of the determination coefficients for the exponential and the linear model spanning different time periods. With the five year time span the exponential model tends to give little higher determination coefficients at all sites and thus a better fit of the curve than the linear function. The shorter the time span, the more the two models are alike. It should be noted, that as the determination coefficients were derived estimates, they cannot be subject to testing by
statistical methods. In the individual subject no difference between the exponential and linear model could be found (Fisher’s z-transformation).

Table 12: Squared determination coefficients in the different regions with the linear and the exponential method in the untreated for the change in BMD with time (median and range).

<table>
<thead>
<tr>
<th>Time period</th>
<th>Site</th>
<th>Exponential model</th>
<th>Linear model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire 5 years*</td>
<td>Lumbar spine (n=184)</td>
<td>0.693 (0.001 - 0.993)</td>
<td>0.689 (0.001 - 0.994)</td>
</tr>
<tr>
<td></td>
<td>Femoral neck (n=232)</td>
<td>0.657 (0 - 0.997)</td>
<td>0.648 (0 - 0.998)</td>
</tr>
<tr>
<td></td>
<td>Ultradistal forearm (n=230)</td>
<td>0.776 (0 - 0.995)</td>
<td>0.771 (0 - 0.998)</td>
</tr>
<tr>
<td>From 1 to 5 years</td>
<td>Lumbar spine (n=184)</td>
<td>0.718 (0.000-0.999)</td>
<td>0.718 (0.000-0.999)</td>
</tr>
<tr>
<td></td>
<td>Femoral neck (n=232)</td>
<td>0.663 (0.003-0.997)</td>
<td>0.657 (0.004-0.999)</td>
</tr>
<tr>
<td></td>
<td>Ultradistal forearm (n=230)</td>
<td>0.818 (0.000-0.999)</td>
<td>0.819 (0.000-0.998)</td>
</tr>
<tr>
<td>From 1 to 3 years</td>
<td>Lumbar spine (n=188)</td>
<td>0.775 (0.000-1.000)</td>
<td>0.774 (0.000-1.000)</td>
</tr>
<tr>
<td></td>
<td>Femoral neck (n=235)</td>
<td>0.705 (0.000-1.000)</td>
<td>0.701 (0.000-1-000)</td>
</tr>
<tr>
<td></td>
<td>Ultradistal forearm (n=235)</td>
<td>0.800 (0.001-1.000)</td>
<td>0.799 (0.001-1.000)</td>
</tr>
</tbody>
</table>

* Includes the initial 1000/w scannings

Among the HRT treated the pattern of gain or loss varied from skeletal site to skeletal site (fig. 14-16). In this case the absolute change in BMD must be considered as good an estimate as any estimate based on intermediate values, especially in subjects changing between treatment or no treatment (or vice versa - fig. 14-16).

**Conclusion 9:**
The exponential model had a little higher coefficient of determination than the linear model over the entire 5 years, while the two models yielded almost equal fit with shorter time intervals, this being the result of the fact, that bone loss rate diminished from year 3 to year 5 in untreated subjects. There was a high degree of agreement between a linear loss rate calculated from baseline to year 5 (using 5 time points: 0, 1, 2, 3 and 5 years) and the absolute bone loss from baseline to year 5 (using two time points: baseline and year 5). I.e. it must be concluded, that with a time span of five years two time points gives the same precision as five time points, the latter also having a higher cost due to the more scans.

The difference between the exponential and linear model is small over five years, the absolute loss can therefore be used to replace the exponential model thus saving scans in untreated subjects.
5) Results II: Factors of importance to cross sectional BMD

5.1) Included variables:
The predictor variables included were: weekly physical activity (hours), age (years), current daily alcohol intake or not (1 vs. 0), current smoking or not (1 vs. 0), scanner type (1000/w vs. 2000/w), serum osteocalcin (ng/ml), serum BAP (U/l), urine hydroxyproline/creatinine ratio, urine calcium/creatinine ratio, BMI, waist-hip ratio, calcium intake, vitamin D intake, serum albumin adjusted calcium, serum iPPTH, serum oestriadiol, serum IGF-I, serum IGF-II, serum IGFBP-3, paternal fracture history (1 vs. 0), maternal fracture history (1 vs. 0), prior fracture history (1 vs. 0), coffee intake, tea intake, use of sun bed (never, occasionally, regularly), sunbathing (never, occasionally, regularly), and total number of live births. A separate analysis also including years from menopause was performed in subjects with intact uterus.

5.2) ROC-curves
In this section the agreement between observed BMD (expressed as T-scores) and expected BMD from the different predictive models is investigated. From the measured BMD values the participants were stratified e.g. into those with T-score above -1 versus those with T-score below -1. The sensitivity is then defined as those who did in fact upon measurement have low T-score (e.g. below -1) out of all those who from the model were predicted to have low BMD (e.g. below -1 in T-score). The sensitivity was defined as those, who did in fact have a high T-score (e.g. above -1) of those predicted by the model to have a high T-score (e.g. above -1). The T-scores were calculated from a Danish normal material designed for the actual scanner (Data from Kim Brixen, MD PhD. These normal values were in close accordance with the reference values from the Hologic Company (312)).

By choosing several cut points it was possible to identify the one, that gave the most profitable ratio between sensitivity and specificity, i.e. was able correctly to identify the largest percentage with low BMD without misclassifying too many with high BMD as having low BMD. This ROC analysis was only possible for those models (the logistic and discriminant function), who used dichotomisation of the outcome variable.

In the lumbar spine the optimal cut-point (the “shoulder of the curve”, i.e. the point at the greatest distance from an imaginary line from 0 to 100) was -0.5.

Fig. 24 shows the ROC curve for the lumbar spine with the logistic regression.
Fig. 24: ROC curve for the lumbar spine (logistic regression)

In the discriminant function for the lumbar spine an optimal cut point could not be determined with certainty (data not shown).
In the femoral neck the optimal cut point lay at a T-score of -1 (data not shown).

5.3) Spine (L2-L4)
The predictor variables were identified using multiple stepwise linear regression, logistic regression (stepwise forward method with likelihood ratio), and discriminant analysis (stepwise method). A T-score of -0.5 which - based on the ROC analysis - was the optimal cut point in the logistic regression equalled a BMD of 1.0254 g/cm².

Table 13 shows those variables identified as significant predictors in the spine. The logistic function had a higher sensitivity (ability to correctly identify those with low BMD) than the discriminant function but at the cost of a lower specificity (ability to correctly identify those with a high BMD).
Table 13: predictor variables in the lumbar spine, only significant variables shown. In the equations below a negative sign in the multiple regression and discriminant function refers to a decline in predicted BMD. In the logistic regression an OR of less than 1 refers to a decline in BMD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Expression</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple regression, n=484, $r^2 =0.191$</td>
<td>Regression coefficient</td>
<td></td>
</tr>
<tr>
<td>Serum Osteocalcin (ng/ml)</td>
<td>$-28.7 \times 10^{-4} \pm 10.1 \times 10^{-4}$</td>
<td>0.0047</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>$50.8 \times 10^{-4} \pm 13.9 \times 10^{-4}$</td>
<td>0.0003</td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>$3.5 \times 10^{-4} \pm 1.1 \times 10^{-4}$</td>
<td>0.0025</td>
</tr>
<tr>
<td>Serum BAP (U/l)</td>
<td>$-14.8 \times 10^{-4} \pm 2.3 \times 10^{-4}$</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Logistic regression:

- Sensitivity: 73.4 %, specificity: 66.5 %
- OR:
  - Age (years) 0.9081 (0.0119)
  - Serum Osteocalcin (ng/ml) 0.9603 (0.0369)
  - BMI (kg/m²) 1.0894 (0.0013)
  - Serum BAP (U/l) 0.9812 (<0.0001)

Discriminant function:

- Sensitivity: 67.5 %, specificity: 69.4 %
- Function coefficients:
  - Age (years) -0.3528 (<0.0001)
  - Serum Osteocalcin (ng/ml) -0.3198 (<0.0001)
  - BMI (kg/m²) 0.4203 (<0.0001)
  - Serum BAP (U/l) -0.5914 (<0.0001)

In the multiple regression, inclusion of all variables yielded a $r^2$ of 0.187. Adding the term “time since menopause” among women with intact uterus decreased the $r^2$ value to 0.183 and replaced serum osteocalcin and IGF-I by age and time since menopause.

Changing the cut point to a T-score of -1 (The WHO criterion of osteopenia (1), in this case a cut point of 0.9612 g/cm²) changed the sensitivity and specificity of the logistic function to 38.9 % and 88.3 % respectively. The included variables changed to: weekly physical activity, BMI, serum IGF-I, BAP, use of sun bed, and U-OHP.

In the discriminant function a cut point of -1 in T-score meant a sensitivity of 64.9 % and a specificity of 70.0 % with the same included predictors as the logistic function except for use of sun bed which was not included.

### 5.4) Femoral neck

Predictor variables were identified as in the spine. A T-score of -1 which was the optimal cut point in the ROC-curve analysis in the logistic regression equalled a BMD of 0.7863 g/cm². There was little difference between T-scores of -1.5, -1, and -0.5, with a very small advantage at -1. In the discriminant function an optimal cut point could not be determined with certainty.
Table 14: predictor variables in the femoral neck. Only significant variables shown. In the equations below a negative sign in the multiple regression and discriminant function refers to a decline in predicted BMD. In the logistic regression an OR of less than 1 refers to a decline in BMD.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Expression</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple regression n=595, $r^2 = 0.2204$</td>
<td>Regression coefficients</td>
<td>0.0091</td>
</tr>
<tr>
<td>Age (years)</td>
<td>- $42.1 \times 10^{-4} \pm 16.1 \times 10^{-4}$</td>
<td>0.0002</td>
</tr>
<tr>
<td>Serum Osteocalcin (BGP - ng/ml)</td>
<td>- $31.2 \times 10^{-4} \pm 8.4 \times 10^{-4}$</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>$96.9 \times 10^{-4} \pm 10.8 \times 10^{-4}$</td>
<td>0.0497</td>
</tr>
<tr>
<td>Vitamin D intake (µg/day)</td>
<td>$40.7 \times 10^{-4} \pm 20.7 \times 10^{-4}$</td>
<td>0.0097</td>
</tr>
<tr>
<td>Serum BAP (U/l)</td>
<td>- $4.8 \times 10^{-4} \pm 1.9 \times 10^{-4}$</td>
<td>0.0464</td>
</tr>
<tr>
<td>Smoker (1) or not (0)</td>
<td>- $185.6 \times 10^{-4} \pm 93.0 \times 10^{-4}$</td>
<td></td>
</tr>
</tbody>
</table>

| Logistic regression: Sensitivity: 63.7 %, specificity: 66.3 % | OR |
| Age (years) | 0.9184 | 0.0138 |
| Serum Osteocalcin (BGP - ng/ml) | 0.9630 | 0.0311 |
| BMI (kg/m²) | 1.1569 | <0.0001 |
| Coffee intake (cups/day) | 0.9336 | 0.0013 |
| Urine OH/creatinine | 0.9734 | 0.0298 |

| Discriminant function: Sensitivity: 64.4 %, specificity: 64.0 % | Function coefficients |
| Age (years) | -0.3169 | <0.0001 |
| Serum Osteocalcin (BGP - ng/ml) | -0.3010 | <0.0001 |
| BMI (kg/m²) | 0.7294 | <0.0001 |
| Coffee intake (cups/day) | -0.4001 | <0.0001 |
| U-OHP | -0.3123 | <0.0001 |

Entering all variables in the multiple regression yielded an $r^2$ of 0.2195. Including time since menopause in women with intact uterus in the multiple linear regression removed smoking and vitamin D intake and replaced it by tea intake with a total $r^2$ of 0.1948.

Changing the cut point to -0.5 T-score changed the included variables in the logistic function to BMI, daily vitamin D intake, BAP, and U-OHP with a sensitivity of 91.7 % and a specificity of 33.7 % and the discriminant function to: age, BMI, daily vitamin D intake, serum oestradiol, BAP and U-OHP with a sensitivity of 67.4 % and a specificity of 62.8 %.
5.5) Ultradistal forearm

The predictor variables were identified as in the spine and femoral neck.

Table 15: predictor variables in the ultradistal forearm. Only significant variables shown

<table>
<thead>
<tr>
<th>Variable</th>
<th>Expression</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple regression, n=595, (r^2 = 0.2980)</td>
<td>Regression coefficients</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>- (19.0 \times 10^{-4} \pm 6.6 \times 10^{-4})</td>
<td>0.0040</td>
</tr>
<tr>
<td>Daily alcohol consumption or not</td>
<td>-(181.7 \times 10^{-4} \pm 51.0 \times 10^{-4})</td>
<td>0.0004</td>
</tr>
<tr>
<td>Serum Osteocalcin (BGP - ng/ml)</td>
<td>-(17.5 \times 10^{-4} \pm 3.3 \times 10^{-4})</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>43.3 \times 10^{-4} \pm 4.4 \times 10^{-4}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin D intake ((\mu g/day))</td>
<td>18.8 \times 10^{-4} \pm 8.5 \times 10^{-4}</td>
<td>0.0269</td>
</tr>
<tr>
<td>Prior fracture or not</td>
<td>-(170.2 \times 10^{-4} \pm 43.6 \times 10^{-4})</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum BAP (U/l)</td>
<td>-(2.1 \times 10^{-4} \pm 0.7 \times 10^{-4})</td>
<td>0.0041</td>
</tr>
</tbody>
</table>

Unfortunately T-scores were not available for this site, so discriminant analysis and logistic regression were not performed (312).

Inclusion of all independent variables yielded an \(r^2\) of 0.2936. In women with intact uterus, inclusion of time since menopause yielded an \(r^2\) of 0.2940 with serum albumin adjusted calcium, and serum PTH being included in the equation.

Conclusion 10:

Age, BMI, and various biochemical bone turnover markers (especially BAP) were linked to cross-sectional BMD at all three sites with all mathematical methods applied. Vitamin D intake was linked to BMD in the multiple linear regression at all three sites. Small changes in independent variables may alter the outcome of the equation established by the models.

Sensitivity and specificity ranged from approximately 60 % to approximately 70 %. Only 20-25 % of the total variation in BMD could be explained by the independent variables.

6) Results III: Factors of significance to the rate of bone loss

Fig. 25 shows a graphical display of significant bivariate correlations between baseline variables and between these variables and the absolute bone loss over five years. It can be seen, that most of the biochemical variables were positively correlated meaning, that an increase in one parameter would be followed by an increase in one or more of the other (the inner circle). This is an illustration of the coupling of formative and resorptive processes - i.e. if resorption increases it will be followed by an increased formation. However, this increased formation is unable to compensate for the resorption leading to a loss of bone mineral.

Some interesting examples of indirect correlations may be deducted from fig. 25: with increasing age, serum oestradiol (E2) decrease, which was followed by an increase in BAP. This increase in BAP was responsible for the positive correlation between age and BAP.

Likewise, there was a positive correlation between BAP and the absolute loss meaning that with increasing BAP, the loss would become less negative. Theoretically, the loss would diminish or even turn into a gain (positive absolute difference), if the formative processes were able to fully compensate the resorptive processes. Such interrelations have also been observed in other studies with e.g. 1,25-(OH)\(_2\)-D and PTH (313), for bone markers (314), for oestradiol and urinary calcium (315), and for sex steroids, the IGF-system, and age (316,317).

It should be remembered, that the correlations reflects actual correlations, and that the results cannot be extrapolated to e.g. the results of intervention with hormonal substitution.
All of the correlations were weak ($|r| < 0.25$) except for the correlation between BGP and BAP ($r = 0.5$).

From fig. 25 it can also be seen, that the interplay between the different markers and effect parameters was very complicated, and that many parameters were so intercorrelated, that addition of new biochemical markers perhaps only would add to the “carrousel” outlined in the centre.

It thus seemed, that although oestradiol was the central mediator (the only parameter that is negatively related to most of the markers), the interplay was so complicated, that prediction from groups of parameters may be difficult.

**Figure 25**

Bivariate Pearson correlations between variables and the absolute change in spine BMD (absolute change $< 0$ if a loss occurs) in untreated. All correlations except those with BMD loss ($n=226$) covers all 595 participants. A list of abbreviations can be found in section 14, and further explanation of the individual variables can be found in section 3. Light grey lines are positive correlations (e.g. IGF-I increases if IGF-II increases), black lines are negative correlations (e.g. with increasing coffee intake, the reported tea intake decreases).

A) All correlations
B) Groups of variables: This section shows a formal representation of the groups of variables, in 25A, the groups have been somewhat rearranged according to the weight of intercorrelations.

<table>
<thead>
<tr>
<th>Physiology</th>
<th>Loss rate of BMD</th>
<th>Lifestyle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, BMI, waist hip ratio</td>
<td>IGF-I, IGF-II, IGFBP-3, E2</td>
<td>Alcohol intake, smoking, coffee intake, tea intake, pregnancies</td>
</tr>
<tr>
<td>Paternal fracture history, Maternal fracture history</td>
<td>BGP, BAP, 1CTP, P1NP, P1CP, U-Pyr, U-dPYR, U-Ca, U-OHP</td>
<td>Calcium and Vitamin D intake</td>
</tr>
<tr>
<td>Heredity</td>
<td>Bone turnover and balance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum calcium, PTH, sunbathing, Sun bed, calcium intake, vitamin D intake</td>
<td></td>
</tr>
</tbody>
</table>

6.1) In participants not receiving hormonal replacement

6.1.1) Predictor variables:
The included predictor variables were the baseline values of: weekly physical activity, age, current daily alcohol intake or not (1 vs. 0), current smoking or not (1 vs. 0), BMI, waist-hip ratio, calcium intake, vitamin D intake, serum albumin adjusted calcium, serum iPTH, serum oestradiol, serum osteocalcin, serum BAP, urine hydroxyproline/creatinine ratio, urine osteocalcin, serum P1NP, serum P1CP, serum 1CTP, urine pyridinoline/creatinine ratio, urine deoxypyridinoline/creatinine ratio, serum IGF-I, serum IGF-II, serum IGFBP-3, maternal fracture history, paternal fracture history, use of sun bed (never, occasionally, regularly), sunbathing (never, occasionally, regularly), coffee intake, and tea intake.

6.1.2) Lumbar spine (L2-L4)
A ROC curve analysis showed the optimal cut point for loss rate in the lumbar spine to be at a loss greater than -1 SD from the mean loss rate.
Table 16 shows significant parameters for the prediction of the change in BMD from year 1 to year 5 in participants without any sign of spondylosis, who were scanned on the Hologic 2000/w scanner.
In the logistic regression and discriminant function the dependent variable was absolute loss less than 1 SD below mean (≤ -0.09 g/cm²) versus > -0.09 g/cm².
Table 16: predictive parameters, only significant variables shown. In the equations below a negative sign in the multiple regression and discriminant function refers to a decline in predicted BMD. In the logistic regression an OR of less than 1 refers to a decline in BMD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Expression</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple stepwise linear regression ( r^2 = 0.177 ) (n=175)</td>
<td>Regression coefficients</td>
<td>0.0076</td>
</tr>
<tr>
<td>Physical activity (hours/week)</td>
<td>(- 6.7 \times 10^{-4} \pm 2.5 \times 10^{-4} )</td>
<td>0.0066</td>
</tr>
<tr>
<td>Age (years)</td>
<td>(31.4 \times 10^{-4} \pm 11.4 \times 10^{-4} )</td>
<td>0.0348</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>(17.0 \times 10^{-4} \pm 8.0 \times 10^{-4} )</td>
<td>0.0481</td>
</tr>
<tr>
<td>P1CP</td>
<td>(- 1.4 \times 10^{-4} \pm 0.7 \times 10^{-4} )</td>
<td>0.0070</td>
</tr>
<tr>
<td>Serum BAP (U/l)</td>
<td>(3.0 \times 10^{-4} \pm 1.1 \times 10^{-4} )</td>
<td>0.0319</td>
</tr>
<tr>
<td>Urine Calcium/creatinine</td>
<td>(-370.2 \times 10^{-4} \pm 171.1 \times 10^{-4} )</td>
<td>0.0217</td>
</tr>
</tbody>
</table>

Logistic regression:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Expression</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity (hours/week)</td>
<td>0.9597</td>
<td>0.0453</td>
</tr>
<tr>
<td>Maternal fracture history</td>
<td>4.6970</td>
<td>0.0102</td>
</tr>
<tr>
<td>P1CP</td>
<td>0.9879</td>
<td>0.0064</td>
</tr>
<tr>
<td>Serum BAP (U/l)</td>
<td>1.0274</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Discriminant function

<table>
<thead>
<tr>
<th>Variable</th>
<th>Expression</th>
<th>Function coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity (hours/week)</td>
<td>(-0.4511 )</td>
<td>(&lt;0.0001 )</td>
</tr>
<tr>
<td>Maternal fracture history</td>
<td>0.4626</td>
<td>(&lt;0.0001 )</td>
</tr>
<tr>
<td>P1CP</td>
<td>-0.5686</td>
<td>0.0002</td>
</tr>
<tr>
<td>Serum BAP (U/l)</td>
<td>0.5922</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

In the multiple stepwise linear regression inclusion of a term that separated between the groups randomised to no HRT or who chose no HRT by own choice changed the results in table 16 so that \( r^2 = 0.159 \). Physical activity (2p=0.0347), age (2p=0.0103) and urine calcium/creatinine (2p=0.0213) were still included while BMI, P1CP and serum BAP were replaced by vitamin D intake (2p=0.0035), serum oestradiol (2p=0.0046) and IGF-II (2p=0.0166). There was no difference between those on no HRT by own choice and those randomised to HRT (2p=0.8050) in loss rate. Performing the regression by backward elimination with an exclusion probability of 0.10 resulted in a little better \( r^2 \) of 0.168. The included predictor variables were still physical activity (2p=0.0467), age (2p=0.0154), BMI (2p=0.0794) and urine calcium/creatinine (2p=0.0257) while P1CP and serum BAP were replaced by vitamin D intake (2p=0.0057), serum oestradiol (2p=0.0037) and IGF-II (2p=0.0258).

Including all variables in one equation changed \( r^2 \) to 0.168.

Using block inclusion with age, BMI, and waist-hip ratio in block 1 (general variables), physical activity, alcohol intake, coffee intake, tea intake, smoking, solarium, and sunbathing in block 2 (lifestyle variables), paternal and maternal fracture history in block 3 (heredity), oestradiol, BGP, BAP, P1CP, P1NP, ICTP, IGF-I, IGF-II, IGFBP-3, urine calcium/creatinine ratio, urine hydroxyproline/creatinine ratio, urine pyridinoline/creatinine ratio, and urine deoxyripyridinoline/creatinine ratio in block 4 (biochemical variables) did not change the overall picture, but \( r^2 \) decreased to 0.159. In this setting the included predictor variables were age
Due to the many intercorrelations between the different biochemical markers, it was attempted to join together the biochemical variables into one variable, which would express, whether or not the variables were high or low. Thus serum osteocalcin, serum ICTP, serum P1CP, serum P1NP, serum BAP, serum IGF-I, serum IGF-II, serum IGFBP-3, urine calcium/creatinine, urine OH/creatinine, urine pyridinoline/creatinine, and urine desoxypyridinoline/creatinine were all recoded into a binary variable according to whether or not they were higher or lower than their respective median value. A summary variable of all these binomial variables was then computed and used instead of the individual variables.

However, this only meant, that P1CP, serum BAP and urine Calcium/creatinine in table 16 were replaced by paternal fracture history (2p = 0.0119) and vitamin D intake (2p = 0.0247), and that the adjusted squared correlation coefficient dropped from 0.177 to 0.168.

Performing the analysis only with women with intact uterus improved $r^2$ to 0.2468 with age being replace by time since menopause (negative association), and daily vitamin D intake being included in the equation in stead of P1CP and BAP. Weekly physical activity, BMI and urinary calcium/creatinine ratio still being included.

### 6.1.3) Femoral neck

In the femoral neck the optimal cut point was at -0.5 SD below the mean loss rate (separating between $\leq -0.038$ versus $> -0.038$ in the logistic function (sensitivity 61.3 % and specificity 59.6 %) but at -1 SD ($\leq -0.081$ versus $> -0.081$) in the discriminant function in a ROC analysis. As the discriminant function offered the best sensitivity, a cut point of -1 SD is presented in table 16. Predictor variables were identified in the femoral neck as in the lumbar spine.
Table 17: predictive parameters, only significant variables shown. In the equations below a negative sign in the multiple regression and discriminant function refers to a decline in predicted BMD. In the logistic regression an OR of less than 1 refers to a decline in BMD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Expression</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple stepwise linear regression ( r^2 = 0.0612 ) (n=216)</td>
<td>Regression coefficients</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>( 2189 \times 10^{-6} \pm 892 \times 10^{-6} )</td>
<td>0.0150</td>
</tr>
<tr>
<td>Vitamin D intake (µg/day)</td>
<td>( 3164 \times 10^{-6} \pm 1335 \times 10^{-6} )</td>
<td>0.0187</td>
</tr>
<tr>
<td>IGFBP-3 (µg/l)</td>
<td>( 8 \times 10^{-6} \pm 3 \times 10^{-6} )</td>
<td>0.0314</td>
</tr>
</tbody>
</table>

Logistic regression (n=205)
- Sensitivity: 15.4 %, specificity: 98.4 %
- Physical activity (hours/week) 0.9374 0.0012
- Age (years) 1.2234 0.0370
- Serum Calcium (mmol/l) 5230 0.0241
- Serum oestradiol (nmol/l) 41.5 0.0243
- ICTP (µg/l) 0.3974 0.0030
- IGF-II (µg/l) 1.0049 0.0022
- Smoker or not 0.3520 0.0401

Discriminant function (n=243):
- Sensitivity: 70.0 %, specificity: 71.1 %
- Physical activity (hours/week) -0.6478 0.0022
- Age (years) 0.5357 0.0011
- Serum oestradiol (nmol/l) 0.5105 0.0001
- ICTP (µg/l) -0.4664 <0.0001
- IGF-II (µg/l) 0.5131 0.0005

In the multiple stepwise linear regression the addition of a term separating between those on no HRT by own choice and those randomised to no HRT decreased \( r^2 \) to 0.047. Age was replaced by physical activity (2p=0.0411), but otherwise, vitamin D intake (2p=0.0291) and IGFBP-3 (2p=0.0105) were unchanged as predictors. There was no difference between randomised and not randomised (2p=0.4425).

By performing the regression as a backward elimination procedure the \( r^2 \) increased a little to 0.0628. The predictors were still physical activity (2p=0.0511), age (2p=0.0585), vitamin D intake (2p=0.0343) and IGFBP-3 (2p=0.0043) while serum oestradiol was added (2p=0.0486).

Including all predictor variables gave an \( r^2 \) of 0.0308.

Limiting the group analysed to women with intact uterus yielded an equation with vitamin D intake and IGFBP-3, but without age with an \( r^2 \) of 0.0391.

### 6.1.4) Ultradistal forearm

Predictor variables were selected as in the spine and femoral neck. In the logistic regression and discriminant function the optimal cut-point was at -0.5 SD below the mean loss rate in a ROC curve (separating between absolute loss less than ½ SD below the average, i.e. \( \leq -0.0345 \) versus > -0.0345).
Table 18: In the equations below a negative sign in the multiple regression and discriminant function refers to a decline in predicted BMD. In the logistic regression an OR of less than 1 refers to a decline in BMD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Expression</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple stepwise linear regression $r^2 = 0.0818$ (N=216)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>0.0033</td>
</tr>
<tr>
<td>IGFBP-3 (µg/l)</td>
<td></td>
<td>0.0069</td>
</tr>
<tr>
<td>Urine dPYR/creatinine</td>
<td></td>
<td>0.0468</td>
</tr>
<tr>
<td>Logistic regression (N=215)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity: 25.4 %, specificity: 96.8 %</td>
<td>OR</td>
<td>0.0371</td>
</tr>
<tr>
<td>Serum Osteocalcin (BGP - ng/ml)</td>
<td>0.9369</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>BAP (U/l)</td>
<td>1.0303</td>
<td>0.0001</td>
</tr>
<tr>
<td>Discriminant function (N=215):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity: 68.3 %, specificity: 65.5 %</td>
<td>Function coefficients</td>
<td></td>
</tr>
<tr>
<td>Serum Osteocalcin (BGP - ng/ml)</td>
<td></td>
<td>0.0061</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>-0.5585</td>
<td>0.0001</td>
</tr>
<tr>
<td>BAP (U/l)</td>
<td>-0.8155</td>
<td>0.0001</td>
</tr>
<tr>
<td>Urine dPYR/Creatinine</td>
<td>0.4178</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

In the multiple linear regression the addition of a term separating between those on no HRT by own choice and those randomised to no HRT decreased $r^2$ to 0.0733. BMI ($2p=0.0065$) and IGFBP-3 ($2p=0.0037$) were still included as predictors, while urine dPYR/creatinine was replaced by tea intake ($2p=0.0312$). There was no difference between randomised and not randomised ($2p=0.3748$). By performing the regression as a backward elimination procedure the $r^2$ increased a little to 0.1028. The predictors were expanded to: current alcohol intake or not ($2p=0.0682$), BMI ($2p=0.0593$), serum calcium ($2p=0.0617$), vitamin D intake ($2p=0.0900$), IGFBP-3 ($2p=0.0027$), tea intake ($2p=0.0326$) and urine calcium/creatinine ratio ($2p=0.0642$). Including all predictor variables gave an $r^2$ of 0.0831.
If the cut point in the logistic regression and the discriminant function was shifted to -1 SD below the mean, no variables could be included in the equations.
Limiting the multiple regression to the subgroup of women with intact uterus yielded an equation with IGFBP-3 and BMI, but without u-dPYR with an $r^2$ of 0.0516.

**Conclusion 11:**
There is a complex interaction between the independent variables, especially the biochemical markers of bone turnover seems heavily intercorrelated.
The variables included in the equations depended not only on the site of measurement but also on the cut point chosen, i.e. different factors may be active at high and low BMD.
No consensus on selected predictor variables could be achieved between sites or between different mathematical models in the untreated.
6.2) In participants receiving hormonal replacement

Seen in the perspective of the many intercorrelations found in fig. 25 and the poor correlations found above for the untreated the number of variables was reduced in the analysis of the treated women. In this group only the stepwise multiple regression was applied in the light of the conflicting results found in section 6.1 with different models. Furthermore, as a consequence paternal fracture history was not included, as too few observations were available. No ROC analysis could was performed.

6.2.1) Predictor variables:
The included predictor variables were: age, weekly physical activity, current daily alcohol intake or not (1 vs. 0), current smoking or not (1 vs. 0), coffee intake, tea intake, maternal fracture history, use of sun bed (never, occasionally, regularly), sunbathing (never, occasionally, regularly), BMI, waist-hip ratio, calcium intake, vitamin D intake, serum albumin adjusted calcium, serum iPTH, serum oestradiol, serum osteocalcin, serum BAP, urine calcium/creatinine ratio, urine hydroxyproline/creatinine ratio, serum IGF-I, serum IGF-II, and serum IGFBP-3.

6.2.2) Lumbar spine
Table 19 shows significant predictor variables identified in the lumbar spine in participants, who adhered to the same type of HRT, and who had no signs of spondylosis on X-ray.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficients</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple stepwise linear regression $r^2 = 0.280$ (N=83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>$46.8 \times 10^{-4} \pm 11.5 \times 10^{-4}$</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hysterectomised (3) vs. intact uterus (1)</td>
<td>$-149.1 \times 10^{-4} \pm 59.4 \times 10^{-4}$</td>
<td>0.0144</td>
</tr>
<tr>
<td>Urine Calcium/creatinine</td>
<td>$378.5 \times 10^{-4} \pm 154.8 \times 10^{-4}$</td>
<td>0.0170</td>
</tr>
</tbody>
</table>

Hysterectomised subjects seemed to have a lower gain than women with intact uterus. A higher gain in BMD was observed with increasing BMI and urine calcium/creatinine ratio.

6.2.3) Femoral neck
No predictor variables could be identified (i.e. no variables came out significant). Including all variables in the equation yielded a $r^2$ of 0.00.

6.2.4) Ultradistal forearm
Table 20 shows the significant predictor variables identified in the ultradistal forearm.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficients</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple stepwise linear regression $r^2 = 0.129$ (N=83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity (hours/week)</td>
<td>$-2.9 \times 10^{-4} \pm 1.3 \times 10^{-4}$</td>
<td>0.0279</td>
</tr>
<tr>
<td>Smoker (1) or not (0)</td>
<td>$-94.2 \times 10^{-4} \pm 32.5 \times 10^{-4}$</td>
<td>0.0050</td>
</tr>
</tbody>
</table>

Increasing physical activity and smoking were both associated with a higher loss (lower gain).
Conclusion 12:
Predictive power was generally low, no consensus on predictor variables could be achieved between skeletal sites.

6.3) Influence of time on correlations
As time may influence the correlations found above, fig 26 presents some examples of changes in correlation coefficients between baseline variables and changes in BMD at different time points.

Fig 26
Changes in Pearson correlation coefficients (r) in the spine at different time intervals (DIF0-1: change in BMD from inclusion to year 1, DIF1-2: change in BMD from year 1 to year 2, DIF2-3: change in BMD from year 2 to year 3, DIF3-5: change in BMD from year 3 to year 5). The numbers (e.g. 0.006) denotes the significant correlations (2p values). Insignificant values of 2p (>0.05) are not shown.

Several of the correlations changed with time. The correlation coefficient for weekly physical activity changed from being positive to negative, while maternal fracture history changed from slightly negative to positive, and the effect of age became more positive. The same changes were observed for many other predictor variables (data not shown).

Many parameters changed with time (e.g. did BMI increase from a mean of 24.8±4.0 to 25.9±4.4 kg/cm² (2p<0.001).

Conclusion 13:
Correlations between independent variables and changes in BMD varies significantly with time, initially negative correlations may turn to positive correlations. Parameters associated with BMD (e.g. BMI) may also change significantly with time.
7) Results IV: Combined studies of treated and untreated

As can be deducted from fig. 14-16 and section 6 (fig. 26) the interaction between time, treatment and included predictor variables was very complex. The results for the untreated showed, that accurate prediction was not possible in this group. However, there was a significant difference between both the randomised and the non randomised groups and those, who actually adhered to the original treatment schedule or changed between treatment or not in baseline characteristics. (table 4-6).

This section deals with a combined model for treatment and predictor variables using the fact, that not only the BMD but also e.g. the weight and thus the BMI changed with time (see conclusion 13). Furthermore, changes in e.g. weight may - at least in Hologic scanners - lead to artefacts in the determination of BMC, area of interest and thus BMD (264,318).

To address these problems, a repeated measures ANOVA was used. The grouping variables (between subjects factors) were: treatment or not, maternal fracture history, smoking, and shift of scanner. Seen in the perspective of the many intercorrelations found in fig. 25 and the poor correlations found above for the untreated and treated separately, the number of variables was reduced to those, who came out significant in either the cross sectional or longitudinal study. Besides, IGF-I, IGF-II and IGFBP-3 values were only present in 110 subjects on follow-up (293). As the absolute difference proved as efficient as the linear regression, only the initial BMD and the BMD after 5 years was included in the analysis. The continuous covariates were thus: age, weekly physical activity, BMI, total alkaline phosphatase (replaced BAP, as BAP was not available at the five year visit), OHP, calcium intake, vitamin D intake, coffee intake, IGF-I, IGF-II, IGFBP-3. These were included with their values at baseline and after 5 years.

A multiple regression analysis was also performed with the baseline values as potential predictor variables. As only the baseline variables were included in this analysis, more independent variables could be included (see table 22).

7.1) Lumbar spine

In this setting treatment with HRT was associated with higher gain in BMD than no treatment within the individual subject (p < 0.001). However, there was no effect of maternal fracture history (p = 0.599) or smoking status (p = 0.956) between the subjects. There was a significant difference in the level of BMD with or without treatment between those with or without a maternal fracture history (p = 0.003). Table 21 shows the effect of the continuous covariates.

Table 21: Covariates in the repeated measurements ANOVA (n=110)

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td>-0.00017</td>
<td>0.893</td>
</tr>
<tr>
<td>Age</td>
<td>0.00001</td>
<td>0.999</td>
</tr>
<tr>
<td>Total alkaline phosphatase</td>
<td>-0.00129</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI</td>
<td>0.01072</td>
<td>0.003</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>-0.00008</td>
<td>0.129</td>
</tr>
<tr>
<td>Vitamin D intake</td>
<td>-0.00235</td>
<td>0.748</td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.00001</td>
<td>0.983</td>
</tr>
<tr>
<td>IGF-II</td>
<td>0.00007</td>
<td>0.627</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>-0.00005</td>
<td>0.258</td>
</tr>
<tr>
<td>Coffee intake</td>
<td>0.00507</td>
<td>0.284</td>
</tr>
<tr>
<td>Urine OH/creatinine</td>
<td>-0.00120</td>
<td>0.635</td>
</tr>
</tbody>
</table>
In this analysis increasing total alkaline phosphatase was associated with a lower BMD (negative coefficient), while increasing BMI was associated with higher BMD.

The HRT treatment group and reports maternal fracture history were subject to a statistical interaction, because those who received HRT, and who also reported a maternal fracture history, also had a lower initial BMD (0.9790±0.1363) than those without a maternal fracture history on HRT (1.0385±0.1599, 2p=0.01). However, the increase in BMD was almost the same among the treated with or without a maternal fracture history (0.0421 vs. 0.0459, 2p=0.99).

Reducing the equation to e.g. studying IGF-I alone did not change the results, IGF-I was still not a significant covariate.

Table 22 shows the significant predictor variables identified by multiple stepwise linear regression in those, who either adhered to the same type of HRT, or who remained without HRT in the entire treatment period. The dependent variable was the change in BMD from year 1 to year 5, i.e. bypassing the shift of scanners. The included predictor variables were: weekly physical activity, age, current daily alcohol intake or not (1 vs. 0), current smoking or not (1 vs. 0), BMI, waist-hip ratio, calcium intake, vitamin D intake, serum albumin adjusted calcium, serum iPTH, serum oestriodiol, serum osteocalcin, serum BAP, urine calcium/creatinine ratio, urine hydroxyproline/creatinine ratio, serum IGF-I, serum IGF-II, serum IGFBP-3, maternal fracture history, paternal fracture history, use of sun bed (never, occasionally, regularly), sunbathing (never, occasionally, regularly), coffee intake, tea intake, and treatment or not (1 vs. 0).

Table 22: Multiple linear regression (n= 320) in those on unchanged HRT or unchanged no HRT with BMD change as dependent variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficients</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables in the equation r² = 0.376</td>
<td>3.9<em>10^-4 ± 2.0</em>10^-4</td>
<td>0.0476</td>
</tr>
<tr>
<td>Physical activity (hours/week)</td>
<td>24.9<em>10^-4 ± 8.6</em>10^-4</td>
<td>0.0043</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0<em>10^-4 ± 6.0</em>10^-4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum oestriodiol (nmol/l)</td>
<td>-257.8<em>10^-4 ± 102.5</em>10^-4</td>
<td>0.0124</td>
</tr>
<tr>
<td>Paternal fracture history</td>
<td>-324.9<em>10^-4 ± 156.7</em>10^-4</td>
<td>0.0390</td>
</tr>
<tr>
<td>IGF-II (µg/l)</td>
<td>0.4<em>10^-4 ± 0.1</em>10^-4</td>
<td>0.0040</td>
</tr>
<tr>
<td>Treatment or not</td>
<td>721.3<em>10^-4 ± 58.9</em>10^-4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

With increasing physical activity and baseline oestriodiol the change in BMD tended to become more negative (less positive), while increasing age, BMI, and IGF-II were associated with a higher gain in BMD. Treatment was associated with a higher gain and paternal fracture history with a smaller gain in BMD.

7.2) Femoral neck

The results of the repeated measures ANOVA are much similar to those for the lumbar spine found in section 7.1. The treatment was highly significant (p < 0.001) within the individual subject, while maternal fracture history was less than borderline significant as a between subjects factor (p = 0.118), and there was no effect of smoking (p = 0.692). As seen in table 23, BMI was a less significant predictor, and was only significant within the individual subject.
Table 23

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity (hours/week)</td>
<td>0.00046</td>
<td>0.686</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.00200</td>
<td>0.693</td>
</tr>
<tr>
<td>Total alkaline phosphatase (U/l)</td>
<td>-0.00054</td>
<td>0.143</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.00888</td>
<td>0.006</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>0.00003</td>
<td>0.525</td>
</tr>
<tr>
<td>Vitamin D intake (µg/day)</td>
<td>-0.00176</td>
<td>0.785</td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>0.00036</td>
<td>0.364</td>
</tr>
<tr>
<td>IGF-II (µg/l)</td>
<td>-0.00014</td>
<td>0.292</td>
</tr>
<tr>
<td>IGFBP-3 (µg/l)</td>
<td>0.00001</td>
<td>0.816</td>
</tr>
<tr>
<td>Coffee intake (cups/day)</td>
<td>-0.00034</td>
<td>0.936</td>
</tr>
<tr>
<td>Urine OH/creatinine</td>
<td>-0.00270</td>
<td>0.226</td>
</tr>
</tbody>
</table>

Table 24 shows the significant predictor variables identified by a multiple linear stepwise regression with the change in BMD from year 1 to year 5 as the dependent variable.

Table 24: Multiple linear regression in subjects on unchanged HRT or unchanged no HRT (n= 327)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficients</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables in the equation: ( r^2 = 0.207 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>18.1<em>10⁻⁴ ± 7.7</em>10⁻⁴</td>
<td>0.0197</td>
</tr>
<tr>
<td>IGFBP-3 (µg/l)</td>
<td>9.4<em>10⁻⁶ ± 3.2</em>10⁻⁶</td>
<td>0.0035</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>-448.7<em>10⁻⁴ ± 207.9</em>10⁻⁴</td>
<td>0.0317</td>
</tr>
<tr>
<td>Treatment or not</td>
<td>479.3<em>10⁻⁴ ± 53.9</em>10⁻⁴</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Increasing age and IGFBP-3 were both associated with a higher gain in BMD (smaller loss). Treatment was associated with a higher gain than no treatment while those with a high waist-hip ratio tended to have a higher loss (lower gain) of BMD than those with a small waist-hip ratio - i.e. those with an “apple” shape lost more bone than those with a pear shape.

### 7.3) Ultradistal forearm

In this case the treatment had an effect within the individual subject (p < 0.001), but not between the subjects (p = 0.503), while there was little difference between the subjects when considering maternal fracture history (p = 0.181) or smoking (p = 0.714). However, there was an interaction between smoking and maternal fracture history (p = 0.024). Table 25 shows the results of a repeated measures ANOVA in the ultradistal forearm.
Table 25: Repeated measures ANOVA for the ultradistal forearm

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity (hours/week)</td>
<td>0.00014</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.00040</td>
</tr>
<tr>
<td>Total alkaline phosphatase (U/l)</td>
<td>-0.00027</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.00369</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>0.00001</td>
</tr>
<tr>
<td>Vitamin D intake (µg/day)</td>
<td>0.00015</td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>0.00010</td>
</tr>
<tr>
<td>IGF-II (µg/l)</td>
<td>0.00004</td>
</tr>
<tr>
<td>IGFBP-3 (µg/l)</td>
<td>-0.00001</td>
</tr>
<tr>
<td>Coffee intake (cups/day)</td>
<td>0.00079</td>
</tr>
<tr>
<td>Urine OH/creatinine</td>
<td>-0.00075</td>
</tr>
</tbody>
</table>

The interaction between maternal fracture history, smoking and BMD in ultradistal forearm was the result of the fact that those who had a maternal fracture history and smoked tended to have a lower initial BMD in the ultradistal forearm (0.3684±0.0581) than those without a maternal fracture history (0.3893±0.0493) and the non-smokers with (0.3868±0.0471) or without a maternal fracture history (0.3850±0.0461).

Table 26 shows the significant predictor variables identified by a stepwise multiple linear regression with the change in BMD from year 1 to year 5 as the dependent variable.

Table 26: Multiple linear regression in subjects on unchanged HRT or unchanged no treatment (n=325)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficients</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables in the equation: ( r^2 = 0.243 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>( 8.7\times10^{-4} \pm 2.9\times10^{-4} )</td>
<td>0.0030</td>
</tr>
<tr>
<td>IGFBP-3 (µg/l)</td>
<td>( 5.3\times10^{-6} \pm 1.6\times10^{-6} )</td>
<td>0.0013</td>
</tr>
<tr>
<td>Treatment or not</td>
<td>( 263.6\times10^{-4} \pm 27.3\times10^{-4} )</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Treatment, increasing BMI and IGFBP-3 were all associated with a higher gain in BMD.

**Conclusion 14:**
BMI seemed to be the only independent variable associated with BMD (except in the femoral neck in the multiple regression). Several interactions between independent variables were present (as previously shown in fig. 25).

**8) Results V: Testing of the models versus another study group**

Although the correlations found in section 6 were weak, the power of these predictions were tested against the Odense Centre with 555 participants.

The testings were performed for the cross-sectional prediction and the longitudinal prediction of bone loss. In the longitudinal group the untreated group (no HRT) was chosen and tested in the lumbar spine, as this was the site with the highest \( r^2 \) (0.177 vs. 0.0612 in the femoral neck and 0.0818 in the ultradistal forearm).

Table 27 gives baseline parameters.
Peter Vestergaard  
PhD Thesis: Prediction of changes in bone mineral in postmenopausal women

Table 27: Baseline comparison of the Aarhus and the Odense centre, values of Gaussian distributed variables are presented as mean and SD, others as median and range

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aarhus (n=595)</th>
<th>Odense (n=555)</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.0±2.9</td>
<td>49.9±2.8</td>
<td>0.43 (a)</td>
</tr>
<tr>
<td>BMD of lumbar spine (g/cm²)</td>
<td>1.0275±0.136</td>
<td>1.0339±0.137</td>
<td>0.43 (a)</td>
</tr>
<tr>
<td>BMD of ultradistal forearm (g/cm²)</td>
<td>0.3899±0.049</td>
<td>0.4004±0.049</td>
<td>&lt;0.01 (a)</td>
</tr>
<tr>
<td>BMD of femoral neck (g/cm²)</td>
<td>0.7993±0.112</td>
<td>0.7956±0.117</td>
<td>0.58 (a)</td>
</tr>
<tr>
<td>Weekly physical activity (hours)</td>
<td>19.8±12.8</td>
<td>21.1±13.6</td>
<td>0.11 (a)</td>
</tr>
<tr>
<td>Serum osteocalcin (ng/ml)</td>
<td>18.1±6.3</td>
<td>17.6±13.0</td>
<td>0.39 (a)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 (16.8-43.4)</td>
<td>24.6 (17.4-48.3)</td>
<td>0.03 (b)</td>
</tr>
<tr>
<td>BAP (U/l)</td>
<td>73.5 (16-246)</td>
<td>60 (12-145)</td>
<td>&lt;0.01 (b)</td>
</tr>
<tr>
<td>Prior fracture after 15 years of age (yes/no)</td>
<td>113/482</td>
<td>13/542</td>
<td>&lt;0.01 (c)</td>
</tr>
<tr>
<td>Paternal fracture history (yes/no) (d)</td>
<td>14/581</td>
<td>12/543</td>
<td>0.83</td>
</tr>
<tr>
<td>Maternal fracture history (yes/no) (d)</td>
<td>118/477</td>
<td>105/450</td>
<td>0.70</td>
</tr>
</tbody>
</table>

(a) t-test for two independent samples, (b) Mann-Whitney test, \( \chi^2 \) test for contingency tables, (d) prior fracture of hip or forearm

The differences were, that the Odense subjects tended to be a little heavier, to have more dense ultradistal forearms, and to have somewhat lower bone specific alkaline phosphatase values. Furthermore, there was a marked difference with a much lower occurrence of prior fractures in the Odense centre.

### 8.1) Testing of cross-sectional prediction

#### 8.1.1) Multiple regression

At the Odense centre, IGF-I, IGF-II, IGFBP-3, serum oestradiol, PTH, and U-Ca were not available. Rerunning the equation without these parameters and replacing albumin adjusted calcium by total calcium yielded the following equation:

\[
BMDTOT = -44.4 \times 10^{-4} \times \text{AGE} - 27.1 \times 10^{-4} \times \text{SERUM OSTEOCALCIN} + 55.9 \times 10^{-4} \times \text{BMI} - 13.3 \times 10^{-4} \times \text{SERUM BAP} + 1.2480
\]

With an adjusted \( r^2 \) of 0.193. The Equation was almost identical to the one in table 13 (section 5.3) except for the presence of age as a predictor, and the adjusted \( r^2 \) was almost identical (0.193 vs. 0.191).

Using this new equation revealed systematic deviations between predicted and observed values of BMD (fig. 27). With high BMD values there was a trend to underestimate actual measured BMD. Equal results were found studying the Copenhagen centre.
Similar observations as those presented in fig. 27 for the lumbar spine were done for the femoral neck (data not shown).

Although the computed BMD increased with increasing measured BMD, the increase in predicted (computed) BMD did not match the measured BMD in a 1:1 relation. The predictive formula used a constant (1.2480), from which deviations were calculated, however, the deviations were small compared to this constant, meaning that the increase in predicted BMD did not follow the actual increase in measured BMD. The mean of the squared deviations between observed and expected values \(\sqrt{\sum (\text{predicted BMD} - \text{observed BMD})^2/n}\) was 0.122.

Rerunning the prediction for the stepwise multiple linear regression at the Odense centre with albumin adjusted calcium replaced by total calcium gave the following equation:

\[
\text{BMDTOT} = -39.2*10^{-4} * \text{SERUM OSTEOCALCIN} + 34.0*10^{-4} * \text{BMI} - 881.5 * \text{PRIOR FRACTURE HISTORY} - 331.5 * 10^{-4} * \text{MATERNAL FRACTURE HISTORY} - 10.7 * 10^{-4} * \text{SERUM BAP} + 1.0705
\]

The standard errors of the mean for the coefficients were:

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERUM OSTEOCALCIN</td>
<td>0.0011</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0015</td>
</tr>
<tr>
<td>PRIOR FRACTURE HISTORY</td>
<td>0.0383</td>
</tr>
<tr>
<td>MATERNAL FRACTURE HISTORY</td>
<td>0.0155</td>
</tr>
<tr>
<td>SERUM BAP</td>
<td>0.0004</td>
</tr>
<tr>
<td>CONSTANT</td>
<td>0.0432</td>
</tr>
</tbody>
</table>
The adjusted $r^2$ was 0.135 with regression df = 5 and residual df = 372. The magnitude of the predictive power was slightly different ($r^2 = 0.135$ and 0.193 at the two different centres respectively).

Running the equation on both the Aarhus and the Odense Centre together with centre, age, BMI, serum osteocalcin, BAP, maternal fracture history, and prior fracture history as independent variables, and all variables being entered into the equation yielded the following result:

\[
\begin{align*}
\text{AGE:} & \quad -23.1 \times 10^{-4} \pm 14.6 \times 10^{-4}, \quad 2p = 0.11 \\
\text{SERUM OSTEOCALCIN:} & \quad -9.2 \times 10^{-3} \pm 4.0 \times 10^{-4}, \quad 2p = 0.02 \\
\text{BMI:} & \quad 66.8 \times 10^{-4} \pm 9.4 \times 10^{-4}, \quad 2p < 0.001 \\
\text{SERUM BAP:} & \quad -12.8 \times 10^{-4} \pm 1.7 \times 10^{-4}, \quad 2p < 0.001 \\
\text{PRIOR FRACTURE:} & \quad -237 \times 10^{-4} \pm 139 \times 10^{-4}, \quad 2p = 0.088 \\
\text{MATERNAL FRACTURE:} & \quad -179 \times 10^{-4} \pm 101 \times 10^{-4}, \quad 2p = 0.077 \\
\text{CENTRE:} & \quad -64.8 \times 10^{-4} \pm 43.3 \times 10^{-4}, \quad 2p = 0.13 \\
\text{CONSTANT:} & \quad 1.0953 \pm 0.0731, \quad 2p < 0.001 \\
\end{align*}
\]

Adjusted $r^2 = 0.121$

Again the constants were within the same range, but the constant (1.0953) was smaller than the mean BMD in both Aarhus and Odense.

Testing this prediction from the Odense Centre against the other centres yielded the same results shown in fig. 27, i.e. an underestimation of the high BMD values (the same was seen when testing the Aarhus equation on the Aarhus Centre and the Odense equation on the Odense Centre).

A comparison of the regression coefficients can be found in table 28.

Table 28: Re-running the multiple regression on the Aarhus Centre, the Odense Centre, and the two centres combined yielded the following results forcing the same predictors (age, serum BAP, serum osteocalcin, and BMI) into the equations yielded the following results:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aarhus (mean±SEM)</th>
<th>Odense (mean±SEM)</th>
<th>Aarhus+Odense</th>
<th>2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-41.2<em>10^{-4}±19.6</em>10^{-4}</td>
<td>-23.0<em>10^{-4}±23.3</em>10^{-4}</td>
<td>-33.8<em>10^{-4}±15.7</em>10^{-4}</td>
<td>0.55</td>
</tr>
<tr>
<td>Serum osteocalcin</td>
<td>-37.0<em>10^{-4}±9.9</em>10^{-4}</td>
<td>-35.2<em>10^{-4}±11.0</em>10^{-4}</td>
<td>-37.8<em>10^{-4}±7.3</em>10^{-4}</td>
<td>0.90</td>
</tr>
<tr>
<td>Serum BAP</td>
<td>-9.9<em>10^{-4}±2.1</em>10^{-4}</td>
<td>-10.2<em>10^{-4}±3.5</em>10^{-4}</td>
<td>-8.4<em>10^{-4}±1.7</em>10^{-4}</td>
<td>0.95</td>
</tr>
<tr>
<td>BMI</td>
<td>57.9<em>10^{-4}±13.7</em>10^{-4}</td>
<td>39.7<em>10^{-4}±14.4</em>10^{-4}</td>
<td>46.7<em>10^{-4}±9.9</em>10^{-4}</td>
<td>0.36</td>
</tr>
<tr>
<td>Constant</td>
<td>1.2237</td>
<td>1.1505</td>
<td>1.1928</td>
<td>-</td>
</tr>
<tr>
<td>Residual sum of squares</td>
<td>6.9885</td>
<td>5.6254</td>
<td>12.7667</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>505</td>
<td>405</td>
<td>910</td>
<td>-</td>
</tr>
</tbody>
</table>

None of the coefficients (age, Serum BAP, Serum Osteocalcin or BMI) deviated significantly at the Aarhus and the Odense Centre (2p >> 0.05 in all cases). The models at the two centres did behave differently than the combined model ($F = 2.76$, df1 = 4, df2 = 906, $p = 0.026$) as a result of the difference in BMI, BAP (cf. table 27), and the fact that age was a significant predictor at the Aarhus Centre, but not at the Odense centre.

However, as above the constant (1.2237 in the model for the Aarhus Centre, 1.1505 in the model developed on the Odense Centre, and 1.1928 in the combined model) was below the mean BMD for the lumbar spine (1.0339 g/cm²) at the Odense centre, which may explain the lower predicted values as the deviations from the constant were small.
8.1.2) Logistic regression

Upon comparing the results of the logistic regression on the actual measured BMD (expressed as T-scores) in the lumbar spine the following results were obtained:

Table 29:

<table>
<thead>
<tr>
<th>T-score</th>
<th>Predicted &lt; -0.5</th>
<th>Predicted ≥ -0.5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed &lt; -0.5</td>
<td>137</td>
<td>85</td>
<td>222</td>
</tr>
<tr>
<td>Observed ≥ -0.5</td>
<td>155</td>
<td>17</td>
<td>172</td>
</tr>
<tr>
<td>Total</td>
<td>292</td>
<td>102</td>
<td>394</td>
</tr>
</tbody>
</table>

Sensitivity: 137/222 = 62%, specificity: 17/172 = 10%, positive predictive value: 137/292 = 47%.
Kappa: -0.298, i.e. only modest agreement in the opposite direction of the expected.
The kappa value was negative because the logistic function - as the multiple regression shown above - tended to underestimate the high BMD values.

Analysis of both the Aarhus and the Odense Centre in one model with age, BMI, serum osteocalcin, BAP, and centre as independent variables showed that centre was not associated with BMD (2p = 0.52), and that no interactions were present between centre or age, BMI, serum osteocalcin or BAP.

8.2) Testing of longitudinal prediction

In the subgroup from Odense, no data on IGF-I, IGF-II, IGFBP-3, P1CP, P1NP, ICTP, serum oestradiol, or U-Ca were available, so the regression was rerun on the Aarhus centre without these parameters on the lumbar spine.
This yielded the following equation:

\[ \text{BMD loss from year 1 to year 5} = 0.002783 \times \text{AGE} + 0.001856 \times \text{BMI} - 7.63115 \times 10^{-4} \times \text{WEEKLY PHYSICAL ACTIVITY} + 2.35517 \times 10^{-4} \times \text{SERUM BAP} - 0.235129 \]

with an \( r^2 = 0.136 \), which was slightly less than in the first equation (\( r^2 = 0.177 \)).
Again there was a systematic deviation between predicted and observed change in bone mineral density (fig. 28). With high losses (mean < 0.0), there was a trend towards predicting low bone losses or even gains while low losses (mean close to 0.0) yielded a prediction towards high bone losses. The mean of the squared deviations between observed and expected change \( \sqrt{\sum (\text{predicted change - observed change})^2/n} \) was 0.0670.
Fig 28

Correlation between computed (expected) change in lumbar spine BMD from year 1 to year 5 compared to actual measured change (untreated subjects).

Rerunning the stepwise multiple linear regression on the Odense Centre with the predictor variables: age, physical activity, actual alcohol use or not, current smoking or not, serum osteocalcin, serum BAP, urine OH/creatinine ratio, BMI, waist hip ratio, calcium intake, serum albumin adjusted calcium, vitamin D intake, paternal fracture history, maternal fracture history, coffee intake, tea intake, use of sun bed, and sunbathing habits gave the following equation:

Expected loss of BMD from year 1 to year 5:

\[-0.003748 \times \text{PHYSICAL ACTIVITY} + 0.004601 \times \text{AGE} - 0.236945\]

The coefficients being:

PHYSICAL ACTIVITY: \(-0.003748\pm0.001715\)

AGE: \(0.004601\pm0.002311\)

CONSTANT: \(-0.236945\pm0.115351\)

Adjusted \(r^2 = 0.055\), residual df = 119, regression df = 2

I.e. the coefficients of this model were within the same magnitude and with the same sign (+ or -) as those found for the Aarhus centre although only age and weekly physical activity were included at a somewhat lower coefficient of determination.
Conclusion 15:
Upon comparison with another study group (external validation) the models perform poorly. Some independent variables seemed more likely to be included (e.g. BMI in the cross-sectional and age in the longitudinal study) than others.

9) Discussion

9.1) Research questions:
Question 1: Cross-sectional bone mineral:
Cross-sectional bone mineral cannot be predicted despite use of many independent variables potentially linked to bone turnover, heredity etc. It was not possible to use the predictive model developed at one centre at another centre despite a high degree of similarity (e.g. ethnicity) between the centres. It is thus unlikely, that models can be postulated and then used in populations, who are ethnically different, cf. the differences found in biochemical bone turnover parameters (72) and bone loss (71) between different ethnic groups.

Question 2: Magnitude of perimenopausal bone loss:
The perimenopausal bone loss amounted to 1 - 2 % per year in this study group which was in accordance with other studies (table 2 compared to table 9). The loss tended to decline with increasing time from menopause. The total loss over the five years amounted to 5.4 - 8.4%, which was a considerable loss. However, although the loss rate declined with time since menopause, a substantial absolute bone loss that exceeds that of the first postmenopausal years must be anticipated in the age-interval until the age of say 70 years (319). It may thus be equally important to prevent the long-term bone loss as to prevent the accelerated although short-lived perimenopausal bone loss.

Question 3: Longitudinal course of bone loss:
This loss is not linear. Although it may not be possible at individual level to separate between say a linear and an exponential function, at group level there was a clear trend towards a declining bone loss in untreated women with a time span of more than three years since menopause indicating a non-linear function. There was a high degree of individual variability. Predictive models that apply linear models may thus only be appropriate for the first few years after menopause. And, as stated above under question 2, although the accelerated perimenopausal bone loss is significant, the long-term bone loss after the perimenopause is equally important as the short-lived accelerated perimenopausal bone loss.

Question 4: Fast losers:
No specific group of fast losers was identifiable.

Question 5: Prediction of short-term bone loss:
This prediction was not possible, as systematic deviations from the predictions were observed. It did not seem that addition of new biochemical markers of bone turnover would contribute significantly, as all biochemical markers were significantly intercorrelated.

Question 6: Time dependence:
Inclusion of time-dependent variables (e.g. BMI in section 7) only produced borderline significant associations, and it may be questionable whether the association with weight/BMI was real. As the cross-sectional prediction was poor, it is unlikely, that longitudinal inclusion of markers would improve predictive power. The predictive power of longitudinal measurements of BMD would rest on the high probability, that the next measured BMD would be close to the previous. Addition of extra markers would thus not likely contribute to the prediction.
9.2) General discussion:
This study group seemed comparable to those reported in table 2, and the loss rates (approximately 1 - 2 %/year) seemed close to those reported by most studies in table 2. In this study there was no apparent group of “fast losers” as postulated by Christiansen et al. (151), as the bone loss seemed to be normally distributed at all sites in accordance with the findings by Keen et al. (154). No “bimodality” was apparent in the normal distributions, i.e. no separate group with a high or low loss rate could be discriminated by the eye alone. However, this does not rule out the existence of a combined normal distribution, i.e. the normal distributions presented could consist of several underlying independent normal distributions, who added up to a normal distribution. This possibility is however less likely, as no strong predictor variables could be identified.

The study group in this thesis was rather large compared to many previous studies (table 2 - e.g. the study by Keen et al. (154), who used a somewhat comparable group of 141 women was among the largest study groups in table 2) which means that it was unlikely, that even small differences between a hypothetical group of fast losers and normal women should have been overlooked.

The observed sensitivities and specificities in the cross-sectional study were close to those found by Ribot et al. (191) in a large similar group of peri- and postmenopausal women.

The fact that age was strongly related to cross-sectional bone mineral, but not to loss rates in most previous studies (table 1 and 2), could be due to the fact, that most of the longitudinal studies (table 2) only followed the patients for two years or even less after the menopause, meaning that the loss rate was the tangent to the curve outlined in fig. 1 and fig. 4, and that the age span was so narrow, that age dependence could not be expected except in very large samples. Furthermore, within the first two years the seemingly linear decline would mean no dependency on age - a dependency first disclosed when the loss-rate declined after the third year. The linear models could be applied for the first two to three years after the menopause, because the loss seemed to be linear in this period, while the loss when studied for five years or more tended to be exponential. It thus follows, that models, who only studied the first two years, may be inappropriate to predict the loss for the years to follow, as the curve was no longer linear after the first three years. In contrast to our observation of a linear loss over the first 3 years in the spine, femoral neck and ultradistal forearm, Falch et al. (66) reported a linear loss in the forearm for the first 6 years after the menopause. However, in the forearm we only observed a minor deviation from the linear loss, although the exponential model eventually proved a little better than the linear model.

Also van Beresteijn et al. (259) noted, that the deviation between a linear loss model and an exponential loss was small in the forearm over a period of 8 years.

In table 2 it can be seen, that most of the previous studies have used only the change in the forearm as the predictor (91,93,98,99,102,143-147,156,157,256,259,262). A few studies have focused on total body bone mineral (209,214) or total body bone mineral and distal radius (219). Various combinations of spinal QCT and radius BMD (76), forearm and hip (11), spine and proximal femur (94,96,97,104,260), spine & femur & distal radius & whole body (100), spine & hip & forearm (257) or lumbar spine alone (92,95,101,148,154) have also been utilised.

Many of the models have used multiple linear regression (91,93-95,97-99,102,143,145-147,209,257,259). However, in several of the studies all predictor variables have been included in the models not stating, whether or not the individual predictors were significant (93,99,102,143,147). Many studies have eliminated significant predictor variables by using different
types of forward (91,93-95,97,98,145,154,257) or backward (209) elimination procedures in the multiple linear regression to isolate significant predictors as also implemented in this thesis. Only one study reported the use of discriminant analysis (148) while many used univariate correlations or comparisons (11,76,101,105,156,157,219,256,260,262) or other types of correlations or comparisons (96,100).

Most of the studies have used a time span of two years (11,92,94,95,97,100,143,145,147,157,209,256,257,262) a few have used nine month (99), one (101,104), three (148), four (76,154,260), five (93), six (146), eight (102,259), 10 years (98) or even varying time spans (91,96).

Some of the observed differences between the studies in table 2 may thus be the result of the facts, that different predictor variables, different time intervals and different models have been applied or that different skeletal regions have been studied.

However, even when applying many predictors together as in the model outlined in this thesis, it was only possible to predict a smaller part of the total variation despite the use of several different mathematical and statistical models.

In both cross sectional and longitudinal studies (table 1 and 2) biochemical markers of bone turnover were included. This may seem odd in the cross sectional models, who describe the integrated effects of factors having acted over many years as bone markers tends to be linked to actual bone turnover and are rather variable with time (320). However, the relationship may reflect the actual remodelling space (42), which is large, with a high bone turnover and small, with a low turnover. Indeed, the relationship with the markers was high in the present model indicating, that with high turnover bone mineral was decreased, although this may only represent a transient state.

It has been suggested, that repeated measurements of biochemical bone markers would increase the predictive value of actual bone mineral and of bone mineral loss rate (148,174). However, the study by Riis et al. (174) was performed under HRT treatment, thus perhaps merely reflecting the effect of HRT on bone turnover and thus bone mineral density. The better predictive effect on BMD of repeated measurements of biochemical markers of bone turnover may also simply be a matter of regression towards the mean, the mean value being better determined with an increasing number of measurements. However, the cost and practical difficulties increasing with the number of measurements needed making this approach less favourable.

Concerning the effect of weight changes on loss rates demonstrated by Brot et al. (209) using Hologic Scanners and Chen et al. (214) using Lunar scanners - we conducted a separate study on overweight subjects undergoing weight loss by very low calorie diet (318) that demonstrated, that the changes in bone mineral content and thus also bone mineral density observed during weight loss may at least in part be related to scanner artefacts in Hologic Scanners due to the change in body composition - a finding also done in vitro by Bolotin (264).

Changing the cut point to separate between those with high and low BMD changed the included variables, and within skeletal sites the different models (multiple regression, logistic regression and discriminant analysis) did not always agree on the included significant variables. Furthermore, exclusion of variables that were not significant in the final equation from the initial setting in the computer also changed the outcome. In the present study of only one of the four DOPS centres, maternal fracture history (fracture of hip or forearm) was not selected as a predictor variable in either the cross-sectional or the longitudinal functions. However, when studying all four centres or the Odense Centre, maternal fracture history was included although as a weak predictor (321). This supports the hypothesis, that the variables included in the mathematical models are very dependent upon the variables included at the start, as the variables are extremely intercorrelated (fig. 25). Inclusion of one extra variable may thus entirely change the model by altering the balance between
the predictor variables. Thus it seems, that the “strong” predictor variables (e.g. age or BMI) will be included with a large degree of probability, while it is a matter of uncertainty, whether the weaker variables will be included. The question of an association between BMD and an independent variable may thus not be assessed with certainty through the use of mathematical models alone. Furthermore, some of the self reported variables (e.g. prior fracture) seemed rather variable from one centre to another making recall bias or reporter bias by the investigator likely (table 27). The differences in BMI and BAP between the Aarhus and Odense Centre (table 27) remains unexplained. The BMI difference was small and may be a result of mass significance. The BAP measurements were performed at the Aarhus Centre for all blood samples. However, a requirement of a model must be, that is able to overcome centre differences such as those observed for BMI and BAP - a criterion clearly not met.

The problem of systematic deviations seemed to be general. i.e., it was not only a problem between the Aarhus and Odense Centre and vice versa, but also between the Aarhus and the Copenhagen Centre and between the Odense and the Copenhagen Centre. In this thesis the problem of prediction has been addressed in a large sample contrary to some of the studies mentioned in table 2. Several mathematical models has been applied - both model types who have been utilised in other studies (the multiple linear regression and the discriminant function - table 2) and types of models who have not been applied in previous studies (the logistic regression and the repeated measures ANOVA) along with a more detailed approach for studying the various intercorrelations (fig. 25).

Comparisons of the predictive models with another centre showed systematic deviations, i.e. it was not possible to generalise the predictive models (both internal and external validity was low). Although the predictions may reveal biologically relevant associations, their power is limited and the transferral to other centres - an otherwise clinically relevant situation was not possible.

It was observed, that many of the variables were significantly intercorrelated, which may account for many of the different correlations outlined in table 2. For example, both Christiansen et al. (145) and Falch et al. (146) included urine Calcium/creatinine ratio in the initial equation, and both used multiple linear regression, but only Christiansen et al. (145) found urine calcium/creatinine to be a significant predictor. Falch et al. (146) in their study included many more physiological variables (menopause age, weight etc.) than did Christiansen et al. (145) and as it can be seen from fig. 25, there was a significant intercorrelation between e.g. age (and thus also menopause age) and urine calcium/creatinine. In this example the simple intercorrelation between a physiological variable (age) and a biochemical variable (urine calcium/creatinine ratio) abolished the predictive value of the biochemical bone marker.

Several of the studies in table 2 must thus be considered insufficient by not including these very important intercorrelations.

A further factor that contributed to the lack of predictive power was time. Both the loss rates and the correlations change over time (table 9,10, and fig. 26). Therefore it is essential to state the time interval studied for the loss period. Several studies in table 2 used a period of two years, but as it can be seen from table 10, the loss continues at the same rate between the 2nd and the 3rd year only to diminish somewhat between the 3rd and the 5th year. Losses predicted for the first few years after the menopause may thus not be valid for the years to follow as also shown by Hui et al. (152).

In this thesis the prediction models have been attempted by the use of all of the models presented in table 2 and several other approached. But despite these attempts no clinically useful models have been achieved. It has been shown, that only moderate improvements in predictive power can be achieved by the use of more predictor variables or other models, and that inclusion of all predictor variables may not in itself improve prediction (table 13 and 16).
Peter Vestergaard
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The fact that cross-sectional forearm BMD was linked to a prior fracture history in the Aarhus Centre, while no such association could be found in other skeletal sites, could be due to the observation, that many of the prior fractures were indeed forearm fractures, while fractures at other locations (spine, femoral neck) were rare. The association between cross-sectional BMD and forearm fracture being due to a low peak-bone mass in the forearm and thus a higher risk of fractures at this site. In the Odense Centre, a prior fracture history was linked to cross-sectional lumbar spine BMD, and a similar observation was reported by Peel et al. (202) for the relation between distal forearm fracture and vertebral BMD. Soroko et al. (239) also showed, that a family history of osteoporosis was associated with a lower BMD in the index person (hip in male and lumbar spine in female index persons) in such a way, that BMD decreased more, if more family members were affected by osteoporosis.

It may thus seem, that inclusion of factors associated with heredity (e.g. maternal and paternal fracture history) would not contribute very much to the prediction of BMD. As stated previously determination of genotype may perhaps not contribute further, as the genes express their effect on BMD through several of the measured biochemical variables in this study (markers of bone turnover, oestradiol, IGF’s etc.). Furthermore, controversy exists in the existing studies to the effect of genotypes - c.f. table 1 and 2 where e.g. the vitamin D receptor genotypes yield conflicting results in the cross-sectional studies and the longitudinal studies, where one study found vitamin D receptor genotype to be of significance to loss rates in Japanese women (261), while another study found no significant effect in Caucasian women (105,245). It should also be mentioned, that the study in Japanese women (261) did not state, if adjustments for covariates had been made.

Concerning the effect of radiological changes in mineral content/distribution in the region of interest, one prior study (101) showed, that X-ray changes in the lumbar spine were associated with changes in BMD. Among other studies concentrating on lumbar spine loss rate in table 2, Iki et al. (95) excluded women with vertebral fractures and women with osteophytes of grade 4 in Nathan’s classification. Peel et al. (92), Reginster et al, and Keen et al. (148,154,322) did not mention, whether vertebral x-rays had been obtained.

With regards to the effects of coffee intake, the cross-sectional study showed some association between a high coffee intake and a low cross-sectional BMD in the femoral neck (section 5.4), but no such association could be found in the longitudinal study, neither in untreated (section 6.1.3), nor in the treated (section 6.2.3), nor in the combined study of treated and untreated (section 7.2). In other studies, the Framingham study (16) found an association between a high coffee intake (above 2.5 cups/day) and an increased risk of hip fracture. In our study group the coffee intake was relatively high (5 cups/day, table 3), which may explain the relation found in the cross-sectional study. However, it is striking, that no association was present in the longitudinal study, as coffee is believed to exert its negative effects on bone mineral through an increased calcium excretion in urine (12,301). Harris et al. (12) found higher loss rates of bone with increasing intake of coffee, a finding not reproduced in our study group. Cooper at al. (8) found - in accordance with this thesis - that a mean caffeine consumption of 250 -350 mg/day was not linked to BMD of lumbar spine or forearm after adjustment for age, but that there was a negative relation with cross-sectional femur BMD, but it was concluded, that the effects of coffee were moderate, but that a high coffee intake in elderly subjects with a low calcium intake might be potentially harmful.

Among the HRT treated we found - in contrast to the PEPI trial (112) - that initial BMD was of no significance to the gain in BMD. However, the finding of the PEPI trial (112) may have been the result of the natural intercorrelation between BMD0 and the difference BMD(36 month) - BMD0. The result, that extension of the observation interval for BMD gives as good a prediction as several measurements, is in accordance with Davis et al. (87).
The finding of independence between the absolute bone loss and the mean of absolute BMD is in accordance with Davis et al. (162).

The decreasing rate of bone loss observed with increasing age was in accordance with Davis et al. (161) and Smith et al. (83).

The predictive power in this thesis in the cross-sectional study was within the same range as found by other studies (50). A low discriminatory power was also found by Feingold et al. (323), however, they only used age, BMI and ethnicity as predictors.

Although this thesis does not represent a final proof, that no single or a few independent variables can be used to predict BMD, from a practical point of view it seems unlikely, that addition of new predictor variables to the many studied here will add to accurate daily clinical prediction of low bone mineral density (induction proof: Neither addition of e.g. IGF-parameters nor urinary pyridinolines did improve the prediction substantially, and maternal fracture history etc. did not contribute as well).

In conclusion it seems, that despite extensive knowledge of bone turnover, growth factors, heritable factors and many other factors, it was not possible to give an accurate prediction of cross-sectional or longitudinal change in BMD. It also seems unlikely, that addition of further factors as potential predictor variables would contribute further to prediction.

10) Conclusions and perspectives

10.1) Conclusions

1) The use of linear loss models for bone loss after the menopause is only appropriate within the first three years after the menopause, after that period the bone loss rate will diminish.

2) Measurement of actual bone loss by two scannings with an interval of 5 years is at least as effective as repeated measurements of bone loss and constructions of loss functions.

3) It is not possible to predict regional cross sectional bone mineral or the loss rate of bone mineral after the menopause through the use of proxy-variables (biochemical and other variables) to such a degree, that it will be useful in general screening without actually measuring bone mineral content in the subjects.

4) Predictive values of the models were so poor on an individual level, that the models at best could only give a hint to the change in bone mineral due to the variability of especially the biochemical bone markers. Scanning with measurement of bone mineral may thus be more efficient than the models for practical purposes.

5) Generalisation of the models was not possible as systematic deviations from predictions were found.

6) There is a complex interaction between BMD and predictor variables and between treated and untreated meaning, that even with a reasonably secure prediction of low BMD the treatment response to HRT (mediated in part through compliance) may also be influenced by the predictors.

10.2) Perspectives

Predictive models does not seem to be an effective way of selecting women at high risk of having low BMD. In an actual clinical situation, QDR scanning may be a more efficient way of predicting fracture risk. However, in younger age groups a general screening approach may not be economically favourable (57) as fracture risk is low.
Although there is no doubt about the involvement of biochemical markers in bone turnover in some diseases, e.g. Paget’s disease, these markers do not seem reliable to screen for idiopathic osteoporosis in the general population and may thus not prove efficient in monitoring changes over time. The value of hormones (oestradiol) and growth factors (IGF’s) also seemed limited, and the measurement of these did not seem to contribute to daily clinical decision making. The perspective is thus, that expenditures to biochemical analyses can be reduced in primary osteoporosis screening but not in the differential diagnosis between skeletal disorders.

11) English summary

**Aim:** To study the possibilities of establishing a simple, economically affordable, easy-to-use algorithm to select women at high risk of having low bone mineral at menopause or at high risk of losing a large amount of bone after the menopause. The latter with special reference to the possibilities of identifying a subgroup with a high bone loss (fast losers).

**Material and methods:** A total of 595 women aged 45-58 years, from 3 to 24 month past last menstrual bleeding among women with intact uterus or experiencing climacteric symptoms among hysterectomised women participating in the Danish Osteoporosis Prevention Study at the Aarhus Centre. This cohort of 595 was used to develop the algorithms on. The algorithms were then validated using 555 comparable subjects from the Odense Centre of the Danish Osteoporosis Prevention Study. The Danish Osteoporosis Prevention Study is a comprehensive cohort study using a partly randomised study design in a pragmatic approach. Multiple linear regression, logistic regression, and discriminant analysis was used to study the associations between a number of independent variables measured at inclusion and BMD of the forearm, lumbar spine, and femoral neck. The associations were studied both in a cross-sectional design and in a longitudinal design following the participants for five years. The bone loss over the five years was described using both linear and exponential models. In the cross-sectional design the independent variables studied were: weekly physical activity (hours), age (years), current daily alcohol intake or not (1 vs. 0), current smoking or not (1 vs. 0), scanner type (1000/w vs. 2000/w), serum osteocalcin (ng/ml), serum BAP (U/l), urine hydroxyproline/creatinine ratio, urine calcium/creatinine ratio, BMI, waist hip ratio, calcium intake, vitamin D intake, serum albumin adjusted calcium, serum iPTH, serum oestradiol, serum IGF-I, serum IGF-II, serum IGFBP-3, paternal fracture history (1 vs. 0), maternal fracture history (1 vs. 0), prior fracture history (1 vs. 0), coffee intake, tea intake, use of sun bed (never, occasionally, regularly), sunbathing (never, occasionally, regularly), and total number of live births. A separate analysis also including years from menopause was performed in subjects with intact uterus. In the longitudinal study the independent variables were: weekly physical activity, age, current daily alcohol intake or not (1 vs. 0), current smoking or not (1 vs. 0), BMI, waist-hip ratio, calcium intake, vitamin D intake, serum albumin adjusted calcium, serum iPTH, serum oestradiol, serum osteocalcin, serum BAP, urine calcium/creatinine ratio, urine hydroxyproline/creatinine ratio, serum P1NP, serum P1CP, serum 1CTP, urine pyridinoline/creatinine ratio, urine desoxypyridinoline/creatinine ratio, serum IGF-I, serum IGF-II, serum IGFBP-3, maternal fracture history, paternal fracture history, use of sun bed (never, occasionally, regularly), sunbathing (never, occasionally, regularly), coffee intake, and tea intake.

**Results:**

**Cross-sectional study:**
There was an intricate interaction between the independent variables, especially prominent for the biochemical markers of bone turnover that were significantly intercorrelated. A radiological
diagnosis of spondylosis (osteophytes or other changes in calcification) within the region of interest (L2-L4) of the lumbar spine off-set the measurements of BMD in this region so these patients had to be excluded.

Untreated subjects:
Lumbar spine: There was a general agreement between the multiple regression, the logistic regression, and the discriminant function that serum osteocalcin, and serum BAP were significantly negatively associated with cross-sectional BMD, while BMI was positively associated. The optimal cut point for separating between those with high and those with low BMD was at a T-score of -0.5 yielding a sensitivity of 73.4 % in the logistic function and 67.5 % in the discriminant function while the specificities were 66.5 and 69.4 % respectively.
Femoral neck: The optimal cut point in a ROC analysis was a T-score of -1. At this site the functions did not agree on the included predictor variables. Age and serum Osteocalcin were generally associated with lower BMD, while BMI was associated with a higher BMD. The models were not concordant as the multiple regression pointed to vitamin D intake as a positive association, while smoking and high serum BAP were negatively associated with BMD. However, the logistic and discriminant functions pointed towards high coffee intake and U-OHP as negative predictors. The sensitivity was 63.7 and 64.4 % in the logistic and discriminant functions with specificities of 66.3 and 64.0 % respectively.
Ultradistal forearm: At this site only multiple regression was performed. This showed age, daily alcohol consumption, serum osteocalcin, prior fracture, and serum BAP to be negatively associated with BMD, while vitamin D intake and BMI were positively associated with BMD. The equation explained 30% of the total variation.

Longitudinal study:
This study showed, that loss rates were normally distributed without signs of a separate group of “fast-losers” in the shape of a bimodal distribution. Correlations between independent variables and BMD changed with time, e.g. did a maternal fracture history change from a weak negative association at baseline to a positive association at five years. The loss of bone diminished with time making the exponential model give a little better fit than the linear loss model. However, when observed over the entire five year period, a BMD measurement at baseline and after five years yielded the same results as a regression based on five measuring points.
Lumbar spine: The optimal cut point in a ROC analysis lay at a loss of more than -1 standard deviations below the mean for the entire group. There was a general agreement on physical activity as a predictor of a higher loss of bone in both the multiple regression, the logistic regression, and the discriminant function. However, the models did not agree on any other predictors. In the logistic function a sensitivity of 21.4 and a specificity 98.6 % of was achieved. In the discriminant function a sensitivity of 72.4 % and a specificity 69.0 % of was reached.
Femoral neck: The optimal cut point in the ROC analysis was at more than 0.5 standard deviations below the mean of the entire population. The functions only agreed on increasing age at inclusion being associated with a smaller bone loss, but did otherwise disagree on the included variables. The sensitivity of the logistic function was 15.4 % and the specificity was 98.4 %. The discriminant function had a sensitivity of 70.0 % and a specificity of 71.1 %.
Ultradistal forearm: At this site no agreement on any predictor variables among the functions. The logistic regression had a sensitivity of 25.4 % and a specificity of 96.8 %. The discriminant function had a sensitivity of 68.3 % and a specificity of 65.5 %.
Treated subjects:
In the femoral neck no predictors could be identified at all, and there was no agreement between spine and ultradistal forearm on predictor variables.
When trying to implement the developed predictive model on another centre, the model performed poorly with systematic deviations between predicted and observed values.

Conclusions:

1) Measurement of actual bone loss by two scannings is at least as effective as repeated measurements of bone loss and constructions of loss functions.

2) It is not possible to predict regional cross sectional bone mineral or the loss rate of bone mineral after the menopause through the use of proxy-variables (biochemical and other variables) to such a degree, that it will not be useful in general screening without actually measuring bone mineral content in the subjects.

12) Danish Summary

**Formål:** At studere mulighederne for at opstille en simpel, billig, lettilgængelig metode til at identificere kvinder, der er i høj risiko for at have eller at udvikle lavt knoglemineralindhold ved eller efter menopausen. Dette med specielt henblik på at identificere kvinder i risiko for at undergå et stort/hurtigt knogletab efter overgangsalderen.

**Materiale og metoder:** I alt 595 kvinder i alderen 45-58 år fra 3 til 24 måneder efter sidste menstruation eller med klimakterielle symptomer blev brugt til at undersøge mulighederne for at opstille ovennævnte metode. Kvindele deltog i Danish Osteoporosis Prevention Study ved Århus Amtssygehus. De opstillede metoder blev siden validere på en gruppe på 555 kvinder fra Odense, der også deltog i Danish Osteoporosis Prevention Study. Danish Osteoporosis Prevention Study er et kohortestudie, der der delvist randomiseret.

Multiple linear regression, logistisk regression, og diskriminant analyse blev brugt til at studere mulige associationer mellem en række uafhængige variable målt ved inklusionen og BMD i underarm, lændergym samt lårbevulds. Undersøgelserne blev foretaget dels i et tværsnits-design, dels i et longitudinalt design med en opfølgningsperiode på 5 år.

Knogletabet over 5 år blev beskrevet ved hjælp af lineære og eksponentielle modeller.

I tværsnitsstudiet blev følgende uafhængige variable undersøgt: ugentlig fysisk aktivitet (timer/uge), alder (år), dagligt forbrug af alkohol eller ej (1 vs. 0), ryger eller ej (1 vs. 0), type af scanner (1000/w vs. 2000/w), serum osteocalcin (ng/ml), serum BAP (U/l), serum hydroxyprolin/creatinin ratio, serum calcium/creatinin ratio, BMI, hofte-talje ratio, calcium indtag, vitamin D indtag, serum albumin korrigeret calcium, serum iPTH, serum ostadiol, serum IGF-I, serum IGF-II, serum IGFBP-3, optræden af frakturer hos faderen (1 vs. 0), optræden af frakturer hos moderen (1 vs. 0), tidligere frakturer hos deltageren selv (1 vs. 0), kaffe indtag, indtagelse af te, brug af solarium (aldrig, af og til, regelmæssigt), solbadning (aldrig, af og til, regelmæssigt), og totale antal levende-fødsler. Der blev foretaget separat analyse for gruppen af kvinder med intakt livmoder.

I det longitudinale studie var de uafhængige variable: fysisk aktivitet (timer/uge), alder (år), daglig indtagelse af alkohol eller ej (1 vs. 0), rygning eller ej (1 vs. 0), BMI, hofte-talje ratio, calcium indtag, vitamin D indtag, serum albumin korrigeret calcium, serum iPTH, serum ostadiol, serum osteocalcin, serum BAP, serum calcium/creatinin ratio, serum hydroxyprolin/creatinin ratio, serum P1NP, serum P1CP, serum ICTP, serum pyridinolin/creatinin ratio, serum desoxy-pyridinolin/creatinin ratio, serum IGF-I, serum IGF-II, serum IGFBP-3, knoglebrud hos moderen, knoglebrud hos faderen, brug af solarium (aldrig, af og til, regelmæssigt), solbad (aldrig, af og til, regelmæssigt), kaffe indtag og indtag af te.
Resultater
Tværsnitsstudie:
Der var omfattende interaktioner mellem de forskellige uafhængige variable, især de biokemiske knoglemarkører. Optræden af spondylose på rtg. columna (osteofytter eller andre ændringer i calciumindhold) i den skannede region (L2-L4) forstyrrede målingerne af BMD, så patienter med spondylose blev ekskluderet af analysen af lænderyg.

Ubehandlede:
Lænderyg:
Der var generel enighed mellem multipel regression, logistisk regression og diskriminant funktionen om, at serum osteocalcin og serum BAP var negativt associerede til tværsnits BMD, medens BMI var positivt associeret til BMD. Det optimale punkt for at separere mellem de med højt og de med lavt BMD lå ved en T-score på -0,5. Dette gav en sensitivitet på 73,4 % i den logistiske funktion og 67,5 % i diskriminant-funktionen, medens specificiteterne var hhv. 66,5 og 69,4 %.

Lårbenshals:
Det optimale punkt for adskillelse mellem de med høj, og de med lav BMD lå ved en T-score på -1,0 ved ROC analyse. Alder og serum osteocalcin var generelt associeret med lavere BMD, medens BMI var associeret med højere BMD i alle tre modeller.

Modellerne adskilte sig med hensyn til andre variable. Den multiple regression udpegede vitamin D indtag som værende positivt associeret med BMD, medens rygning og høj serum BAP var negativt associeret med BMD. Den logistiske funktion og diskriminant funktionen pegede på højt kaffe-indtag og høj OHP som værende forbundet med lav BMD. Sensitiviteten var 63,7 og 64 % i den logistiske funktion og diskriminant funktionen, medens specificiteteren var hhv. 66,3 og 64,0 %.

Ultradistale underarm:
Denne lokalisation blev kun undersøgt med multipel regression, der viste, at alder, dagligt alkoholindtag, serum osteocalcin, tidligere fraktur og serum BAP var forbundet med lavere BMD, medens vitamin D indtag og BMI var positivt associerede med BMD. Ligningen kunne forklare 30 % af den totale variation.

Longitudinale studie:
Denne del viste, at tabshastighederne for BMD var normalfordelte uden tegn på eksistensen af en specifik gruppe med hurtigt knogletab - dvs. der var ingen bimodalitet. Korrelationerne mellem de uafhængige og den afhængige variable skiftede over tid, således at f.eks. optræden af tidligere maternelt brud var initialt svagt negativt relateret til BMD før sidenhen at blive mere positivt relateret til tværsnits BMD ved 5 års-studiet.

Knogletabet aftog med tid, hvilket gav den eksponentielle model et lidt bedre fit end den lineære model. Det viste sig dog, at to BMD målinger - én ved baseline og én efter 5 år - gav de samme resultater som en tabshastighed beregnet ud fra fem målepunkter indenfor den samme periode.

Lænderyg:
Den optimale adskillelse mellem de med høj og de med lav BMD opnåedes ved ROC analyse ved at skelne mellem de med en tabshastighed på mere end 1 standarddeviation under gennemsnittet for gruppen. Der var generel enighed om, at fysisk aktivitet var prædiktor for en højere tabsrate ved både multipel regression, logistisk regression og diskriminant funktion. Modellerne kunne dog ikke opnå konsensus om andre prædiktorer. Ved logistisk regression nåedes en sensitivitet på 21,4 % og en specificitet på 98,6 %. I diskriminant funktionen var sensitiviteten 72,4 % og specificiteten 69,0 %.

Lårbenshals:
Det optimale afskæringspunkt ved ROC analyse lå ved mere end ½ standarddeviation under middelværdien for tabshastigheder i hele populationen. Funktionerne var kun enige om, at stigende alder ved inklusionen var forbundet med et mindre knogletab. Sensitiviteten i den logistiske
funktion var 15,4 % og specificiteten var 98,4 %. Diskriminant funktionen havde en sensitivitet på 70,0 % og en specificitet på 71,1 %.

Ultradistale underarm: For denne lokalisation var der overhovedet ingen overensstemmelse mellem de tre matematiske funktioner om prædiktor variable. Den logistiske regression havde en sensitivitet på 25,4 % og en specificitet på 96,8 %. Diskriminant funktionen havde en sensitivitet på 68,3 % og en specificitet på 65,5 %.

Deltagere, som modtog hormonbehandling:
I lårbenhalsen kunne der slet ikke identificeres prædiktorer, og der var ikke overensstemmelse mellem ultradistale underarm og lænderyg om eventuelle prædiktor variable.

Andre forhold:
De udviklede matematiske modeller afveg systematisk fra det observerede, når de blev overført på Odense centret.

**Konklusione:**
1) Det er muligt at begrænse antallet af BMD målinger ved at bruge et passende langt tidsinterval mellem målingerne.
2) Det er ikke muligt at forudsige BMD eller BMD tabshastigheder efter overgangsalderen med sikkerhed ved brug af proxy-variabel (biokemiske eller andre). Det er således mere effektivt direkte at måle BMD.
## 13) List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Explanation</th>
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<tbody>
<tr>
<td>ICTP</td>
<td>Carboxyterminal peptide of procollagen type 1</td>
</tr>
<tr>
<td>BAP</td>
<td>Serum Bone Specific Isoenzyme of Alkaline Phosphatase (U/l)</td>
</tr>
<tr>
<td>BGP</td>
<td>Serum Osteocalcin (ng/ml)</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone Mineral Content (g)</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density (g/cm²)</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual Energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>DHEAS</td>
<td>Dehydroepiandrostenedione</td>
</tr>
<tr>
<td>DOPS</td>
<td>Danish Osteoporosis Prevention Study</td>
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<tr>
<td>DPA</td>
<td>Dual Photon Absorptiometry</td>
</tr>
<tr>
<td>E2</td>
<td>Serum oestradiol</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicular Stimulating Hormone</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormonal Replacement Therapy</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Insulin-like growth factor I</td>
</tr>
<tr>
<td>IGF-II</td>
<td>Insulin-like growth factor II</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Insulin-like growth factor binding protein 3</td>
</tr>
<tr>
<td>P1CP</td>
<td>Carboxyterminal propeptide of type 1 collagen</td>
</tr>
<tr>
<td>P1NP</td>
<td>Aminoterminal propeptide of type 1 collagen</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>QCT</td>
<td>Quantitative Computed Tomography</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
</tr>
<tr>
<td>SPA</td>
<td>Single Photon Absorptiometry</td>
</tr>
<tr>
<td>TGF-beta</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>U-Ca</td>
<td>Urinary Calcium/creatinine ratio (mmol/mmol)</td>
</tr>
<tr>
<td>U-dPYR</td>
<td>Urinary Desoxypyridinoline/creatinine ratio (nmol/mmol)</td>
</tr>
<tr>
<td>U-OHP</td>
<td>Urinary Hydroxyproline/creatinine ratio (micromoles/mmol)</td>
</tr>
<tr>
<td>U-PYR</td>
<td>Urinary Pyridinoline/creatinine ratio (nmol/mmol)</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor genotype</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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</table>

See section 3 for further explanation
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