BONE MINERAL MASS AND BONE TURNOVER PARAMETERS IN OSTEOPOROSIS

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BONE MINERAL MASS AND BONE TURNOVER PARAMETERS IN OSTEOPOROSIS

BOT MINERAAL GEHALTE EN BOTOMBOUW PARAMETERS IN OSTEOPOROSE

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Aan mijn ouders

CONTENTS

LIST OF ABBREVIATIONS

ADFR	activate-depress-free-repeat
AOM	age of menopause
AP	alkaline phosphatase
BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMD	bone mineral density
BMU	bone multicellular unit
BSP	bone sialoprotein
CV	coefficient of variation
DPA	Dual photon absorptiometry
dPYR	desoxypyridinoline
DXA	Dual energy X-ray absorptiometry
EP	estrogen/progestagen
FFM	fat free mass
Fneck/FN	femoral neck
FR	fracture rate
^{153}Gd	gadolineum^{153}
GF	glomerular filtrate
GH	growth hormone
GHD	growth hormone deficient
gHa	gram hydroxy-apatite
HAL	hip-axis length
HPLC	high performance liquid chromatography
HRT	hormone replacement therapy
IC	intercept
ICTP	carboxyterminal telopeptide of type I collagen
IGF-I	insulin-like growth factor I
IRMA	immunoradiometric assay
N	number
ND	nandrolone decanoate
OC	osteocalcin
OH-P	hydroxyproline

P	propability
PICP	carboxyterminal propeptide of type I collagen
PINP	aminoterminal propeptide of type I collagen
PTH	parathyroid hormone
PYR	pyridinoline
QCT	quantitative computer tomography
rhGH	recombinant human growth hormone
RIA	radio immuno assay
rmANOVA	repeated measures analysis of variance
sAP	skeletal alkaline phosphatase
SD	standard deviation
SEM	standard error of the mean
SDI	spine deformity index
SOS	speed of sound
SPA	single photon absorptiometry
TBFM	total body fat mass
TBLTM	total body lean tissue mass
TBMC	total body mineral content
TmP/GFR	tubular reabsorption of phosphate, corrected for glomerular filtration rate
TRAP	tartrate resistant alkaline phosphatase
U	units
US	ultrasound
WHO	world health organization
YSM	years since menopause

CHAPTER 1

GENERAL INTRODUCTION

1.1 HISTORY

In the past decades osteoporosis has been recognized as an important public health problem. Several causes for this problem can be pointed out. The most probable cause for the development of osteoporosis is the loss of ovarian function in women and the increasing age of people[1-3], thereby increasing the incidence of osteoporosis. Other causes or risk factors for the development of osteoporosis are immobilization and dietary deficiencies[4]. Finally, the outcome of osteoporosis is an increased risk for the development of fractures (chapter 1.3).
The terminology associated with osteoporosis was developed in the nineteenth century by German pathologists to distinguish diseases of bone. Pommer stated in 1926 that in osteoporosis the formation of bone by osteoblasts was not able to replace the bone resorbed by osteoclasts. Pommer performed extensive histomorphometric analysis of bone, thereby distinguishing various forms of osteoporosis (senile, immobility), osteomalacia and osteitis fibrosa cystica[5].
The first reports concerning calcium homeostasis and bone metabolism were published in the first decades of this century, especially studies performed in animals[6,7]. In 1923 Robison postulated that alkaline phosphatase (AP) plays a role in bone formation[8] and a few years later an increased concentration of serum AP was described in Paget's disease[9].
Early this century, the first radiological studies of bone were published. In the 1930s and 1940s routine x-ray methods were used to detect osteoporosis[10]. Osteomalacia and osteoporosis could not be separated by means of radiographic methods and both were often considered together.
In 1941 Albright et al. brought some clarification and defined osteoporosis pathologically as 'a condition in which there is lack of bone tissue, but that tissue which remains is fully calcified'[11]. This clearly differentiated it from osteomalacia, a condition in which bone matrix mineralization is delayed or has failed. In the same article Albright reviewed the clinical history of 42 patients with osteoporosis[11]. In this paper he founded several etiologic factors, i.e. diet-related, disuse and postmenopausal state. He stated that the major argument for the existence of postmenopausal osteoporosis was the frequency in which osteoporosis occurred in women after the menopause. A few years later Albright et al. showed the reducing effect of estrogen substitution on calcium loss in the urine in postmenopausal women[12]. It was concluded that osteoporosis of the postmenopausal state had to be attributed to the decrease in estrogen production following the menopause.
The following years the diagnosis of osteoporosis was made by using bone biopsies of the iliac crest in which a decrease of cancellous (trabecular) bone was observed, while with the use of tetracycline labelling also an impression of bone remodelling was obtained[13]. At present a standardized nomenclature for bone histomorphometry is used[14].

In the sixties further progress in the diagnosis of the disease was made, as new diagnostic possibilities were made available. The first bone densitometric methods were able to measure bone mineral mass in the forearm[15,16]. These instruments were improved rapidly and at present several different devices with high precision are available to measure at various sites in the skeleton (see chapter 2).
Similar progress has been made in the development of several biochemical markers of bone resorption and formation. Until 1970 only urinary hydroxyproline as a resorption marker[17] and serum alkaline phosphatase as a formation parameter could be measured, whereas nowadays a variety of different turnover parameters are available. The interpretations of the different markers are still controversial and the relationships to one another are not completely understood, but the use of a combination of several turnover parameters seems to be promising for identification of subjects at risk for fractures and for follow-up of treatment[18,19] (see chapter 3).
Associated with the progress in diagnostic possibilities, is the increase in therapeutical options. Until 10 years ago, hormone replacement therapy was the most important treatment. The last decade different treatment modalities have been developed (see Chapter 4).
Currently osteoporosis is recognized as an important health problem in the Western world and Asia. Osteoporosis is also seen as one of the most common age-related metabolic diseases. With a growing elderly population the problem will probably augment in the years to come in the Western world, but to an even greater extent in other parts of the world, i.e. the Asian countries[20]. Even after correction for these demographic changes the incidence of hip and other osteoporotic fractures is steadily increasing. It has been calculated that the number of hip fractures in the whole world will increase from 1.7 million in 1990 to 6.3 million in 2050, indicating the magnitude of the problem[21].

1.2 DEFINITION OF OSTEOPOROSIS

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to an increase in bone fragility[22]. In contrast with earlier consensus definitions this definition does not longer state the presence of a non-traumatic fracture necessary for the diagnosis of osteoporosis.
Two categories of osteoporosis can be distinguished: primary and secondary. Primary osteoporosis occurs in the absence of known causes (except for the estrogen deficiency of the postmenopausal state) that may affect bone structure and quality. The two main representatives of this group are: postmenopausal osteoporosis, in which the loss of ovarian function (and thereby loss of estrogen) leads to an exponential bone loss[23] and age-related osteoporosis[24]. Other representatives of primary osteoporosis are: idiopathic osteoporosis,

mainly occurring in young adult and middle aged persons and juvenile osteoporosis, occurring before puberty[24]. Secondary osteoporosis represents a state, in which another disease or condition, known to affect bone turnover and leading to bone loss, is present (Table 1.1).

Table 1.1 *Causes of secondary osteoporosis*

- Hyperparathyroidism	- Malabsorption, liver cirrhosis
- Hypercortisolism	- Calcium and Vitamin D deficiency
- Hyperthyroidism	- Drugs, i.e. corticosteroids, ethanol
- Hypogonadism	- Immobilization
- Connective tissue disorders	- Myeloma

1.3 RISK FACTORS AND EPIDEMIOLOGY

1.3.1 Risk factors

As our population ages, the number of people at risk for developing fractures increases. Furthermore, fracture risk is correlated with bone mass, which in turn is inversely related with age and menopausal state[25-27]. Low bone mass in the elderly is determined by the peak bone mass and the age-related bone loss. In women the accelerated postmenopausal bone loss, superimposed on the age-related bone loss, plays an important additonal role. In figure 1.1 the factors associated with the development of a low bone mass are presented. They will be discussed in this paragraph.

The peak bone mass is already achieved in early adulthood[28,29]. After the age of about 20 - 25 years no further increase in bone mass is observed. The bone mass at skeletal maturity differs between the sexes and is about 30 % higher in men than in women[28,30]. The peak bone mass is largely determined by genetic factors, i.e. in dizygotic twins much greater differences in bone mass are observed than in monozygotic twins[31,32]. It has also been observed that daughters from women with osteoporosis have reduced bone mass in the lumbar spine and femoral neck[33]. Compared to daughters of non-osteoporotic women the loss was about 5 - 7 %[33]. Recently, it has been shown that common allelic variants in the gene encoding the vitamin D receptor are correlated with the bone density[34]. However, between an Australian population[34] and a European population[35] opposite results were obtained. This has to be worked out further, but vitamin D receptor polymorphism seems to be correlated with bone density[34,35].

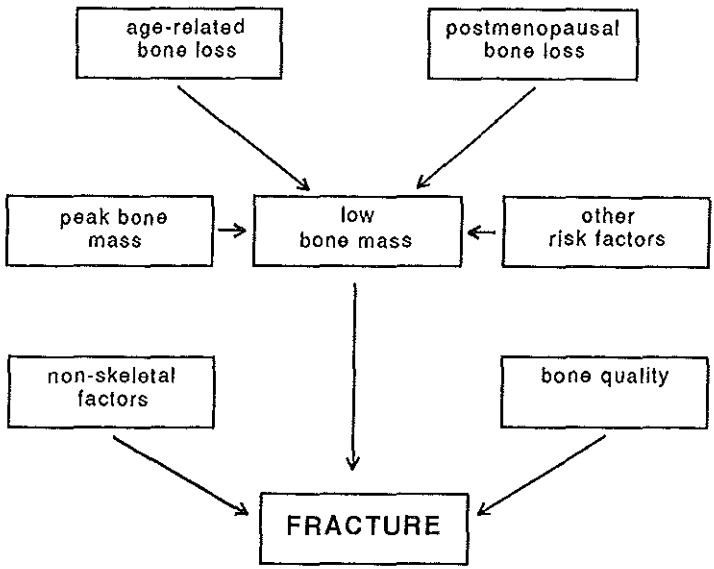

Figure 1.1 *Factors associated with the development of low bone mass and subsequent increased fracture risk*[23-29,36,37,49-54].

Also environmental factors are known to influence peak bone mass, such as diet and exercise[36]. So, if one wants to increase the peak bone mass, in order to prevent osteoporosis from developing later in life, prevention programs have to be started early in life. Physical activity by itself has been shown to increase bone mass, especially those activities which require resistance to an external force[37]. Increased dietary intake of calcium during puberty has shown to increase peak bone mass in young people already using approximately the recommended dietary allowance of calcium[38]. The overall effects of these potential interventions are of about 20 - 30 %, in terms of increased peak bone mass, compared to controls.

Shortly after peak bone mass has been reached age-related bone loss starts[39]. Three patterns of age-related bone loss in women have been described. Riggs et al. demonstrated loss commencing in 'young adulthood' and continuing in a linear matter throughout life[40]. A second model suggested that cancellous bone behaves in a similar way as cortical bone, i.e. it is maintained until the menopause, when bone loss commences[41]. The third model describes that cancellous bone reaches a peak in the mid-30s, and is followed by a progressive loss[40,41]. This last model is considered to be the most accurate and shows a loss of bone which starts

between the age of 30 and 40 years, in men as well as women. Approximately 0.3 - 0.5 % of bone per year is lost[42-45]. In a longitudinal study a progressive bone loss in the femoral neck, in men as well as women over 60 years old, was found of about 1 % per year[46]. In this study rates of loss in the femoral neck showed an increase in both sexes with age. However, no significant loss in the lumbar spine was found[46]. A different pattern of bone loss between the appendicular and axial skeleton has been suggested, i.e. a more pronounced loss at the axial skeleton before the menopause[44].

Next to the age-related bone loss, in women a super-imposed bone loss occurs as a result of the menopause. This is an exponential loss, which starts around or just before the menopause and continues until about 10 years after the menopause. During this period an amount of bone of approximately 15 % of cancellous and cortical is lost[27,47,48]. After this accelerated loss, the rate of bone loss will return to its premenopausal level[49,50]. Although numerous exogenous risk factors related to low bone mass have been identified, these factors only explain 30 % of the variation in bone mass[51]. The risk factors other than genetic, that are recognized nowadays are presented in Table 1.2.

Table 1.2 *Non-genetic factors for developing low bone mass*

- Nulliparity	- High ethanol intake
- Underweight	- Cigarette smoking
- Inactivity	- Low calcium intake
- Previous diseases that reduce bone mass (e.g. hypogonadism, hyper(para)thyroidism, hypercortisolism)	

Next to a low bone mass, other parameters are of importance for the development of a fracture, i.e. the quality of bone and some non-skeletal factors (Fig. 1.1).

The loss of trabeculae has been shown to occur more often in women than in men. In a transversal study of normal iliac bone, it has been shown, that the mean number of trabeculae declines with age in men as well as women[52]. It was concluded that the disappearance of entire elements is the main event in age-related bone loss. Furthermore, in the same population, a lower trabecular width in women, than in men was observed. This may partly explain the higher incidence of osteoporosis in women[52].

A sedentary life style and the age-related increase in falls are the two main non-skeletal factors which are related to the development of fractures[53]. It has been shown that even a moderate exercise program already has a positive effect on the incidence of fractures. This is probably only to a small extent related to increased bone mass[54,55], but more importantly

to improved dexterity and to increased muscle mass[56].
Falls are a common feature as age increases and although only a minority of the falls result in a fracture, falls certainly are a risk factor for the development of fracture. There are several circumstances, which can increase the risk of falling, i.e. the use of sedatives (including alcohol), inappropriate footwear, impaired visual perception, travel under bad weather conditions[53,57].

1.3.2 Epidemiology of osteoporotic fractures

The vast majority of osteoporotic fractures occur in elderly women. These comprise vertebral compression fractures, Colles fractures at the distal radius, hip fractures, and to a lesser extent fractures at other sites[58].In the United States about 1.3 million fractures each year are attributable to osteoporosis, including about 250.000 hip, 250.000 wrist and 500.000 vertebral fractures[58]. In the Netherlands the age-adjusted incidence of hip fractures in women 50 years of age and over is about 350/100.000. For men an incidence of 150/100.000 has been reported[59].
There are large differences in the incidence of osteoporotic fractures between various geographic areas. Fracture rates seem to be higher in the temperate zones than in the tropics, i.e. in the northern parts of Europe and of the United States higher rates of fractures are observed than in the southern parts. Within Europe the difference in incidence of hip fracture between the northern and southern countries is about eleven-fold for women and about seven-fold for men (highest in the scandinavian countries). The ratio between men and women is similar throughout Europe[60]. The explanation for these differences is not clear but may indicate genetic differences and/or environmental factors. Incidence rates of osteoporosis and fractures also are higher in whites than in blacks, indicating that the geographical variation may be related to race and thereby to genetic differences[61].
At this moment the best available possibility to assess the fracture risk of an individual is the performance of a bone mass measurement. In several prospective studies an association between baseline bone mineral mass and fracture risk is observed[53,62,63].
Unfortunately a single bone mineral mass measurement is not always able to differentiate completely between normal and abnormal[64]. A measurement at one site even does not predict the bone mass at another site within the same patient, and it is suggested that a measurement at the site of interest is the best predictor for the fracture risk at that specific site[65-67].
The risk factor for the development of osteoporosis, given by the bone mineral mass or density (BMD), is expressed as T-score or Z-score, according to WHO consensus criteria. The T-score is defined as the difference in standard deviations compared to the mean peak bone mass. The Z-score is defined as the difference in standard deviations compared to the mean bone mineral mass of age-matched controls. A T-score of -1 to -2.5 is considered as osteopenia. A T-score of $<$ -2.5 is considered as osteoporosis, whereas a combination with

a non-traumatic fracture is considered as severe osteoporosis. The expression in T-scores is the preferential way of expressing the risk of osteoporosis at this moment. In the past this risk was expressed in Z-scores, in which a Z-score of < -1 was considered to represent a person at risk for the development of osteoporosis[68].

It is also possible to determine bone turnover parameters in serum and/or urine. At this moment, however, no single measurement is available which can be used in the fracture risk assessment for an individual. In the future a combination of a single bone mass measurement with a combination of bone turnover parameters may be used for the fracture risk assessment, but several problems have yet to be solved (see chapter 3.1).

CHAPTER 1

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CHAPTER 2

BONE MASS MEASUREMENTS

2.1 INTRODUCTION

The measurement of bone mineral mass or bone mineral density (BMD) has been the subject of intensive research over the past decades. Bone mineral content (BMC) determines about 70 % of bone strength[1-3]. Also breaking strength of bone appears to be linearly related to mineral content[4]. However, bone mass is not the only determinant of bone strength. Also other bone-related parameters have a certain impact on the bone strength: (1) geometric changes affect the modulus of elasticity of bone, such as the increased circumference of long bones with age; (2) compared to compact cortical bone smaller changes in the cancellous bone mass may be required to reduce bone strength (loss of connectivity); (3) the bone marrow fat content increases with age and changes the mechanical behaviour of bone[5].
As there are no routine measurements to assess the strength of bone in an individual, and bone mass seems to be a major determinant of bone strength, bone mineral mass is measured and used to predict fracture risk[6-8].
The different measurement sites of bone are moderately related to one another. It has been shown that they show different changes with age and in their response to different factors which affect bone mass in general. Because of the variability in response throughout the skeleton it is difficult to extrapolate directly from one site, at which measurement is feasible, to another as to fracture risk[9-11].
A large variety of non-invasive techniques are available for the assessment of bone mass. There are wide differences in precision and complexity. For example radiogrammetry has important methodological problems, which is reflected in poor precision[12]. It has however been demonstrated to be a rather good method in a large population-based study with respect to prediction of fracture[13]. Neutron activation analysis has, because of its high costs and technical complexity, not found widespread implementation[14]. More attractive are measurements which are easy and fast to perform, with a good accuracy and a high reproducibility. It is also important to measure specific regions in the skeleton. The measurements of bone mineral mass by means of photon or x-ray absorptiometry or CT-scanning have made this approach possible. In table 2.1 several aspects of the different non-invasive bone mass measurement methodes are presented. Furthermore, a brief overview of the various techniques and instruments that are commonly used for bone mass measurement are presented in this chapter.

Table 2.1 *Several parameters of the different bone mass measurement methods.*

Technique	Precision (%)	Accuracy (%)	Radiation (mrem)	Measurement site	Scan time (min)	Costs	References
SPA	1-2	2-5	5	Proximal forearm Distal forearm Calcaneus	10	in between	14-16,21,23
DPA	2-4	3-5	10-15	Lumbar spine Hip Total body	15-25	in between	14,21,28,29
DXA	1-2	1-2	1	Lumbar spine Lateral spine Hip Forearm Total body	3-10	in between	30,31,36-40
Radiographs	-	-	1-10	All bones	-	low	42,43
Radiogrammetry	1-2	-	10-20	Metacarpalia	-	low	13,52
Radiographic densitometry	1	?	10-50	Phalanx	-	low	53,54
QCT	2-5	5-10	50-70	Vertebrae	10	high	55,56
Ultrasound	1-2	1-3	-	Calcaneus Patella	5-10	low	62,64,68,69

2.2 ABSORPTIOMETRY

2.2.1 Single photon absorptiometry

Photons are absorbed by bone and, to a much lesser degree, by soft tissues. When a beam of photons passes through bone, the amount of photon energy that is absorbed is proportional to the amount of mineral in the bone.

In single photon absorptiometry (SPA), which was first described in 1963[15], a iodine-125 source, emitting 28 keV X-rays is used to provide transmission measurements through the tissues of the selected site. In the case of SPA, usually the distal radius is measured. To make the soft tissue thickness constant throughout the scan, the limb is immersed in water or surrounded by water bags; hereby the only remaining variable at each measuring point is the bone mass present in the beam path. The beam is passed in a line across a part of the skeleton. The attenuated beam is detected by a NaI crystal-photomultiplier as light pulses and transformed into a digital read[16]. The result is expressed as arbitrary units (U) per unit of region of interest or after calibration as grams (g) hydroxy-apatite (Ha) per cm. By division of this result by the bone width (measured at individual scans) the data are expressed as U or g/cm^2 and are thereby corrected for skeletal size[17].

SPA can produce accurate measurements only at sites that have very little soft tissue. Therefore it can be performed at the calcaneus and the forearm. The latter is generally used[18].

In the forearm, the cross-sectional areas of the radius and ulna vary along their length and also the proportion of cortical to trabecular bone varies, particularly distally in the bones. 40-50 % of bone in the ultradistal site consists of cancellous bone, but this falls to 10 % within the next 2 cm proximally. To overcome this problem there is an automatic detection (of 8 mm) used of the distance between radius and ulna; from there on measurements commence, distally as well as proximally.

An important problem when measuring with SPA is the percentage of fat surrounding the bone. The penetration of the beam through fatty regions is greater, and the transmitted intensities are therefore higher than expected[19]. This may become an even greater problem when therapy is started for osteoporosis which may induce body composition changes. It is therefore important to correct for the fat mass, which is performed by using an algorithm, which corrects the raw BMC value, based on various amounts of fat in the surrounding tissue[20].

In our laboratory the coefficient of variation (CV) for this technique was determined by measuring 50 healthy subjects. It proved to be 1.9 % and 1.0 % for the distal and proximal site, respectively[21], similar to what others have found in healthy people[22]. Long-term reproducibility is also good, a CV of 1.8 % over a 7 year period has been found[23]. As the

half-life of ^{125}I is rather short, the source has to be replaced every 3 to 4 months to maintain an adequate intensity for good precision.

2.2.2 Dual photon absorptiometry and dual X-ray absorptiometry

Dual photon absorptiometry (DPA) and dual X-ray absorptiometry (DXA) permit the determination of bone mineral content at sites, where there is much surrounding soft tissue, notably the femoral neck, the lumbar vertebrae or even the whole skeleton. Sources emitting two different energies are used.

In 1975, the Gadolineum^{153} (^{153}Gd) source, that emits photons both at 42 and 100 keV was introduced. These photon energies appeared ideal for measuring bone through thick soft tissue layers. The half-life of ^{153}Gd is 242 days and the working life of the source is 12-18 months.

In the 1980's, DXA was introduced and has replaced DPA. DXA is based on the same principle as DPA but uses an x-ray tube instead of an isotope as the photon source[24]. X-rays at two photon energies are generated, either by rapid switching of the generator voltage between high and low kV in synchrony with the main supply[25], or by using a constant potential generator with a rare earth filter (samarium or cerium). There appears to be no particular advantage of one of both approaches[26,27].

The absorption of photons is dependent on three variables; the photon energy, the kind of absorbing material and the thickness of the absorber. With bone mineral mass measurement, the absorber consists of soft tissue and bone mineral in bone tissue. To eliminate the effect of the soft-tissue mass on the results of bone mineral measurement, two different measurements (one with low and one with high energy) are performed. The underlying principle is that the mass attenuation coefficients of two different tissues (i.e. soft tissue and bone) differ as a function of photon energy. By using two photon energies it is possible to calculate the attenuation in bone independent from the attenuation by the soft tissue. After calibration the BMC is calculated as gHa. BMD is calculated by dividing BMC by the surface of the projected regions of interest (gHa/cm^2).

DPA of the lumbar vertebrae has a high precision, with a CV of 1-4 %[28] for the lumbar spine, but is limited by the need for exact relocation for reproducibility. In our laboratory the CV in osteoporotic women proved to be 3.7 and 2.3 % for BMC and BMD respectively[29]. The precision may be influenced by source change.

The precision of DXA is higher than with DPA, with an error of about 1 %. In our laboratory the coefficient of variation for normal women is 1.1 $\pm$ 0.2 % for the lumbar BMD and 1.4 $\pm$ 0.2 to 2.3 $\pm$ 0.3 % for the various regions in the proximal femur[30]. Because of the higher photon fluxes in the X-ray tube, the radiation beam can be highly collimated, resulting in high resolution images, improved study precision, shorter scanning

times and reduced radiation dose to the patient[31].
Besides the problem of precision, DPA and DXA measurements in the lumbar spine may be affected by aortic calcification and osteophytes[11,29,32-34].
With the increased photon flux with DXA, lateral projection of the spine became more accessible. The advantage of a lateral projection measurement is that it is possible to perform isolated measurements of the vertebral body without inclusion of the posterior spinal elements and of possible aortic calcifications[32]. However, differences in overlying fat amount may introduce another bias in the lateral projection[35].
Next to measurements of the lumbar spine (anteroposterior and lateral) it is also possible to measure the proximal femur at three regions of interest; the femoral neck, Ward's triangle and the trochanteric region[36]. The femoral neck represents an area with predominantly cortical bone, whereas the Ward's triangle represents an area with mainly cancellous bone. It has also been shown that DXA-measurements can be used to assess bone mineral mass in the forearm with comparable results as SPA measurements (correlation $r=0.99$)[37].
Besides the measurements of bone mineral mass in the various regions of the skeleton it is also possible to measure total body bone mineral and determine soft tissue composition, showing a higher precision with DXA than with DPA for bone density as well as fat mass and lean body mass. With DXA a three-compartment analysis of the total body is made, consisting of total body mineral content (TBMC), total body lean tissue mass (TBLTM) and total body fat mass (TBFM). TBLTM is largely a combination of total body water and total body protein. Fat free mass is the sum of TBMC and TBLTM. It is concluded that DXA provides precise composition analysis with a low radiation exposure (1-3 mrem) and a scanning time of about 20 minutes[38]. The coefficient of variation for the various total body measurements has been reported to be around 0.8 ± 0.5 %[39]. Correlations between these measurements and other body composition measuring methods have been found with an r-value of about 0.80[40].

2.3 RADIOLOGY

Radiographic methods for the evaluation of osteopenia are divided into three categories: (1) qualitative, (2) semi-quantitative and (3) quantitative. The qualitative methods include plain radiographs and are usually the initial techniques used in the clinical diagnosis of osteopenia or osteoporosis. Semiquantitative methods are those that are based upon a grading system. Quantitative methods include radiogrammetry and radiographic densitometry.

2.3.1 Radiographs of thoracal and lumbar vertebrae and femoral neck

The normal vertebral body is homogenous in its opacity as seen on a lateral x-ray. As vertebral osteoporosis develops, there is a loss of density in the centre of a vertebral body more than in its cortical shell. Because of the preferential loss of the horizontal trabeculae, the vertical trabecular pattern appears to be accentuated. The advantage of using radiographs of the vertebrae is that it is a rather simple technique, which is widely available. An important disadvantage is that changes are only seen as already a substantional amount of the bone mineral (more than 30 %) has disappeared[41]. On the other hand, vertebral radiographs remain the best method for assessing the presence of vertebral fractures. For screening purposes, however, in a prevention or an early intervention program there is no place for the use of radiographs.

Changes in height of vertebrae have been used as an index of progressive bone loss and various grading schemes have been proposed[42]. It is important to be informed about the lateral view of both thoracic and lumbar spine. The radiographs have to be performed in a standardized position[43].

Until recently there were no agreed-upon criteria for the variability in normal vertebral shape or in the transition from normal to vertebral deformation and vertebral fractures. However, recently a consensus meeting has given guidelines for the procedure for taking the radiographs as well as for special considerations with respect to longitudinal studies[44]. In the same consensus report criteria for the diagnosis of prevalent fractures as well as incident fractures are described[44].

For quantitative measurement of vertebral bodies, typically six points are marked, defining the anterior, middle and posterior heights of each vertebral body[45].

Several definitions have been suggested to define normal vertebral bodies and fractures[46,47], leading to a (semi-)quantitative measurement. With this method normal values are obtained from a representative sample of groups that have a very low prevalence of vertebral deformity (e.g. premenopausal women). The relative vertebral heights of patients with osteoporosis are compared to the relative vertebral heights of the normal subjects. This comparison allows the identification of deformed vertebrae, as well as a quantification of the extent of deformation due to these fractures, which is expressed as spine deformity index (SDI)[47]. An important disadvantage of this technique is that it is very time-consuming and therefore expensive. Recently McCloskey et al. developed another semi-quantitative technique for assessing vertebral deformities based on lateral spine radiographs, which showed a high specificity. This technique is based on differences in the ratio within a vertebra of anterior, central and posterior heights, which are compared to reference values, obtained in controls. In patients a cut-off of -3 standard deviations was used in assessing the number of vertebral fractures[48]. On the other hand, it has also been shown that a rather simple approach of a

semiquantitative grading of vertebral fractures, i.e. visual inspection without direct vertebral measurement showed a good correlation with quantitative morphometry[43]. As the different quantitative techniques for the assessment of vertebral deformities are very time-consuming, the only application of these techniques at present are the use in population studies and clinical trials. Also in these studies quantitative vertebral morphometry should be compared with simple visual grading[44].

Recently it has been shown that the hip axis length (HAL) (i.e. the distance from the greater trochanter to the inner pelvic brim) is correlated with the risk for developing a hip fracture. With every standard deviation increase in HAL, nearly a doubled risk for hip fracture was observed, independent of other known risk factors[49]. In this group there was no correlation with height. The increased risk of hip fractures in white women, compared to asian and black women may also be related to the HAL, as it has been shown that white women have significantly longer HAL's. Also body composition may be related to this difference[50]. One can speculate about the possibilty to use the HAL in combination with BMD. For instance one may decide not to start prevention therapy, because of a short HAL in combination with a modest decline in BMD, whereas on the contrary a longer HAL may be of decisive value to start preventive therapy with a similar BMD. Whether this rather simple technique can be used in the risk assessment for future development of a hip fracture has yet to be established.

2.3.2 Radiogrammetry and radiographic densitometry

In radiogrammetry the midshaft of the second metacarpal of the nondominant arm or of several metacarpals of one or both hands are measured[51]. The method requires a high quality radiograph taken under standardized conditions. The outer and inner diameters of the metacarpal cortex are measured[51]. The correlation with other measurements of bone mass is reasonable. The main advantage of this techniques are the low cost, the reproducibility and the easy performance of the technique in large groups of patients or normal persons[13,52].

In radiographic densitometry or quantitative radio-microdensitometry (QMD) standardized radiographs of a phalanx provide a measure of BMD. The density of the bone on the radiograph is analyzed with an optical microdensitometer together with a simultaneously radiographed aluminium reference wedge[53,54].

2.3.3 Quantitative CT-scanning

Quantitative CT-scanning (QCT) is the only technique which can measure a real bone mineral density expressed per unit volume of bone. It is also the only technique that is able to distinguish the density of cortical and cancellous bone. Standard CT-scanners are all potentially capable to measure at the level of the lumbar spine. With QCT, a standard phantom is measured along with the lumbar vertebrae. The value of the attenuation of the radiation by

the bone, which is compared to that of the phantom, is expressed as mg K_2HPO_4/cm^3. These values are used as a true measure for bone mineral density.
The precision with this measurement is in phantoms about 1-2 %, but less favourable in vivo. In our institute a precision of about 2.5 - 2.7 % in osteoporotic women was found at the level of the lumbar spine[29].
The accuracy of the QCT measurements is between 5 an 10 %[55]. The amount of marrow fat is for a great deal responsible for this low accuracy. With age, the amount of intravertebral fat increases and bone red marrow mass falls, leading to an underestimation of the bone mineral mass. This problem can be overcome by using dual energy QCT, in stead of single energy QCT[56,57].
In the past, an important disadvantage of QCT, was the high radiation dose of about 200 mrem, which has been reduced to about 50 mrem. However, this is still a much higher radiation dosage, when compared with DXA.
Nowadays high resolution CT scanning with a resolution of about 0.25 mm (as opposed to 1 mm) is under investigation. With this high resolution it may be possible to quantify cancellous bone structure in a more precise manner[58]. With micro CT-scanning it is possible to measure also structure of bone and not alone bone mass, but this technique is only available for in vitro studies[59].
In the future it may be possible to perform bone mass measurements and even get an impression of bone structure by using magnetic resonance imaging (MRI). The main advantages of this technique are the high resolution and the fact that no radiation is used. However, at present this technique can only be used in in vitro situations[59,60].

2.4 ULTRASOUND MEASUREMENTS

In recent years a new technique for the non-invasive measurement of bone has been developed. This technique is based on the use of ultrasound waves, which means that it is a non-ionizing technique. Media for which the velocity of ultrasound are not equivalent throughout the medium, are known as dispersive. Bone is an example of a dispersive medium, and cancellous bone is more dispersive than cortical bone. Because ultrasound is a mechanical wave, its attenuation is suggested to depend on both the structural and material properties of the propagating medium[61]. There are two sites, which are attractive to measure, i.e. the os calcis and the patella. Most often the os calcis is measured[62].
Two types of measurements are performed, speed of sound (SOS) and broadband ultrasound attenuation (BUA). The system consists of a tank with water or a coupling gel is administered around the os calcis. Two broadband ultrasonic transducers (emitting and

receiving) are present. The heel or knee is positioned between the transducers, in which the calcaneus or patella acts as a frequency selective absorber for the ultrasonic waves. After scanning, the SOS and BUA are calculated by a computer program, correcting for the influence of water[62]. SOS is expressed in m/sec and BUA in dB/Hz. The in vivo reproducibility is about 3-4 %. One of the main problems with this new technique is related to the inhomogeneity of the os calcis and to the sensitiviy of BUA to small changes in density and structure[63]. A high variability of the measurements is related to the position of the transducers[64]. One of the most critical steps therefore is correct foot positioning. Because this influences reproducibility due to interindividual anatomical variations and variable amounts of soft tissue below and behind the heel, the site of measurement may vary from one subject to another. A new technique, BUA imaging, may diminish these variances[65].

Ultrasound measurements are suggested to be related to the compressive strength and load bearing capacity. If these measurements are able to characterize the complex, mineral and nonmineral properties of bone, it might be useful in predicting fracture risk[62]. Ultrasound measurements might therefore be complementary to BMC and/or BMD measurements [62].

Calcaneal BMC has been found to predict spine mineral content and spine fracture incidence, although the calcaneus is a mainly cancellous bone and not a common fracture site[66].

A correlation of about 0.6-0.7 between calcaneal ultrasound measurements and absorptiometry of the femoral neck and/or lumbar spine has been reported[66,67]. In another cross-sectional study, however, only weak correlations of 0.3-0.5 between ultrasound measurements and femoral neck and lumbar spine BMD were observed[68]. In this particular study it was also not possible to identify subjects with low BMD by means of ultrasound measurement. A correlation of 0.99 between DXA- and ultrasound measurements has been observed in calcaneal bones, obtained from deceased people (personal communication J.W. Kuiper). Recently also high correlations of ultrasound of the calcaneus and densitometry of the proximal femur with the failure loads of cadaveric femurs have been found[69]. No extra information was obtained with the ultrasound measurement as compared to densitometric methods[69].

The main question at present is, whether this ultrasound technique offers information on bone quality, additional to the results of bone mineral mass measurements. Present data indicate that ultrasound is another density measurement.

The main advantages of this technique at this moment, are the short scanning time, the low cost and no use of radiation.

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CHAPTER 3

BONE TURNOVER PARAMETERS

3.1 INTRODUCTION

Research has been focused on the identification of markers of bone mineral metabolism, which can provide more insight in the process of bone turnover, and may therefore be used in the identification of disease.

The bone turnover markers, which can be determined in serum or urine samples, are the easiest to collect, since other parameters of bone turnover are more difficult to assess, i.e. bone histomorphometry or radiokinetic methods[1].

Bone remodelling or turnover refers to the sequential processes of resorption and subsequent formation, which is essential for the maintenance of bone mass. This bone remodelling process was first described by Frost[2]. Bone is remodelled by osteoclasts and osteoblasts, working in combination in a cycle of activity that lasts between 3 and 6 months[3]. In 1899, it was already recognized that bones remodel in response to the forces applied to them[4]. The resorption of bone by osteoclasts and its replacement by osteoblasts are normally 'coupled', which ensures that the processes of bone destruction and formation are more or less matched[5]. Both are subject to local and humoral regulation, by which bone resorption and formation can be stimulated or inhibited[6]. This coupled process is altered in the period between midlife and older age: resorption exceeds formation, leading to a reduction of skeletal mass. This proces has been extensively studied by means of bone histomorphometry [7-9] and by recording changes in biochemical markers[10]. In the cancellous bone much more remodelling sites are present than in the cortical bone, because of the higher surface to volume ratio in cancellous bone. Consequently, disorders of remodelling become earlier manifest in cancellous bone than in the cortical regions.

It is possible to assess bone turnover by measuring in serum and/or urine markers of bone formation and resorption. In figure 3.1 and table 3.1 the various markers of bone turnover, which are available, are listed.

These markers will be discussed in the following paragraphs, in which they will be divided into formation and resorption markers. Despite this separation into two groups, it should be borne in mind, that in disease states in which both events remain coupled, either of these groups of markers will reflect the overall rate of bone turnover.

The biochemical markers can be used for two purposes; follow-up of treatment and the prediction of the rate of bone loss. In postmenopausal osteoporosis changes up to 50-100 % in biochemical markers of bone turnover have been reported. The reproducability of the different assays are in the order of 85 to 95 %. As the changes in biochemical markers occur earlier during treatment, compared to the changes of bone mass measurements, they might be used as early indicators of a therapeutic response.

The other purpose for which biochemical markers can be used is the prediction of the rate

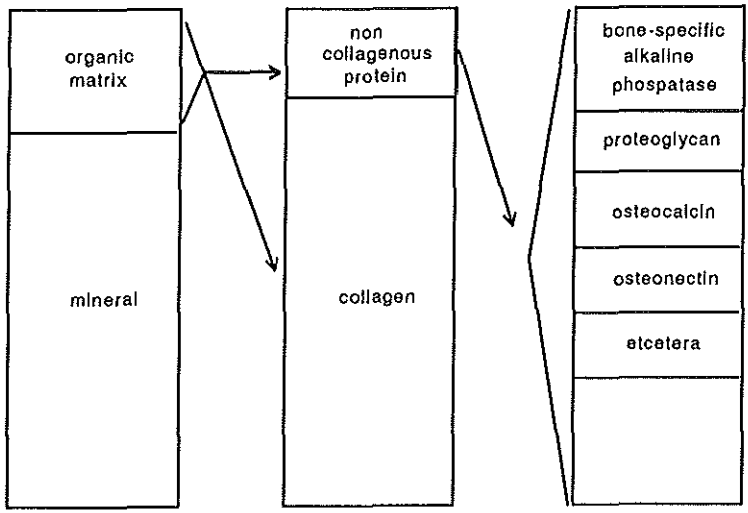

Figure 3.1 *Biochemical composition of bone.*

Table 3.1 *Biochemical markers of bone turnover.*

Formation	Resorption
- (Bone specific) alkaline phosphatase	- Urinary hydroxyproline
- Osteocalcin	- Tartrate resistant acid phosphatase
- Pro-collagen type-I	- Urinary pyridinoline and deoxypyridinoline crosslinks
- Osteonectin	- Telopeptides of collagen type-I
- Bone sialoprotein II	- Fasting urinary calcium

of bone loss, and therefore indirectly the fracture risk. It has been shown that biochemical markers reflect the rate of bone loss in postmenopausal women[11-13]. In the study of Christiansen et al., the biochemical parameters were only related to the bone loss at the proximal forearm[12].

The rate of bone loss may also be a determinant of bone quality, i.e. a fast bone loss may

result in a deterioration of the bone structure of the cancellous bone, which will not be reflected in bone density measurement. On the other hand, biochemical markers reflect turnover states throughout the skeleton, and might therefore be less predictive for bone loss at specific skeletal sites[14].

There are a number of other potential problems in using biochemical parameters. Different assay methods have different performance characteristics. Accuracy and precision may differ within and between laboratories. Another problem is that these entities vary from day to day, and also show distinct seasonal and circadian fluctuations[15].

Theoretically, a combination of multiple biochemical measurements may offer greater efficiency but is also more complicated to apply. However, because of the correlation between the various bone turnover parameters, it is not clear whether different markers provide different information. The relative levels of specific markers of bone formation and bone resorption may also be of importance[16].

3.2 BIOCHEMICAL MARKERS OF BONE FORMATION

3.2.1 Total and bone specific alkaline phosphatase.

Serum total alkaline phosphatase activity (sAP) is the oldest known biochemical marker of bone turnover[17]. Already in 1923, it was postulated that sAP reflects bone formation[18] and a few years later an increased level of sAP in Paget's disease of bone was described[19].

Because sAP is not only produced in osteoblasts but also in other tissues, for example liver, kidney, intestine and placenta, its measurement lacks specificity[20,21]. In older people changes in sAP levels frequently represent liver disturbances because of medications. Changes in sAP levels therefore do not always represent metabolic bone disease[20].

In osteoporosis the lack of sensitivity and specificity is of great importance because the majority of the patients have normal values of sAP[22]. In other bone diseases a better correlation between severity of the disease and value of sAP is found. In primary and secondary hyperparathyroidism sAP correlates positively with radiological and histological involvement of the skeleton[23]. Also in Paget's disease of bone a positive correlation between radiological changes and sAP has been found[24,25].

Because of the rather low sensitivity and specificity of sAP measurement, several attempts have been made to differentiate the several isoenzymes of sAP, especially bone and liver sAP. The isoenzymes are encoded by a single gene and differ therefore only in their posttranslational modifications[26].

Initially, the activity of the various iso-enzymes was assessed with the use of differentially effective activators and inhibitors (heat, phenylalanine and urea), electrophoresis or

separation by polyclonal antibodies[26-28]. These methods did only slightly improve specificity compared to total sAP.
Recently monoclonal antibodies, recognizing only the bone sAP have become available. This new technique is based on a two-site immunoradiometric assay (IRMA), which involves specific monoclonal antibodies to measure bone specific AP in serum[29]. The first clinical reports suggest that this approach results in a higher sensitivity and specificity[30].

3.2.2 Serum osteocalcin (Bone Gla-protein)

Osteocalcin (OC), also called bone Gla protein (BGP) is a small non-collagenous protein that is specific for bone tissue and dentin. OC was discovered in 1975[31]. At first gamma-carboxyglutamic acid (Gla) residues were found in coagulation factors derived from liver tissue, in which under the influence of vitamin K, glutamic acid (Glu) was carboxylated to Gla[32]. Later on these Gla residues were also discovered in non-hepatic tissues. In mineralized tissues, a protein rich in Gla residues was found, named osteocalcin[31,33]. OC contains three residues of gamma-carboxyglutamic acid[32,34], which have a high affinity for Ca^{2+}.
OC is synthesized de novo only by odontoblasts[35] and osteoblasts[36,37]. Its synthesis is directly stimulated by 1,25-$(OH)_2$ vitamin D_3[37,38]. In mature bone, OC is the most abundant non-collagenous protein, comprising 1-2 % of the total protein content of bone and about 25 % of the non-collagenous matrix proteins[33,39].
A small fraction of the newly synthesized OC is released into the circulation ("leakage"), where it can be measured[40,41].
Until now the exact function of OC is unknown although it has been postulated that OC may play a regulatory role in both bone resorption and mineralization[39]. The involvement of OC in mineralization is illustrated by its expression in bone at the time of mineralization[42-45].
OC levels are valid markers of bone turnover when coupling between formation and resorption is present and are a specific marker of formation when turnover is uncoupled. This is best illustrated in patients with multiple myeloma (M. Kahler), in which an inverse relation between OC and severity of the disease has been found, i.e. the lowest values of OC in the patients with the most extensive lytic lesions[46]. After treatment, OC showed a rise, correlated with diminishment of the disease activity and an increase in osteoblastic activity[46].
In hyperparathyroidism a positive relationship between disease activity and OC has been shown. The level of OC correlates with levels of PTH, serum calcium and adenoma mass[47]. With ^{47}Ca kinetics[38] or bone histomorphometry the OC level has been shown to correlate with bone formation and mineralization rates[47].
In postmenopausal osteoporosis OC is a valuable marker of bone turnover with high correlations between OC values and histomorphometric measurements of bone formation[48,49]. OC is in the lower range in patients with low turnover and is significantly increased in the

patients with higher turnover[50]. In untreated postmenopausal women, a single measurement of OC in combination with urinary excretion of deoxypyridinoline has been reported to reflect spontaneous rate of bone loss as assessed over a follow-up period of 2 years ($r=0.77$)[50].

Because vitamin K is essential for the carboxylation of OC and, thereby for its ability to bind Ca^{2+}, vitamin K antagonists (anticoagulants) are able to increase the proportion of noncarboxylated OC relative to carboxylated OC[51,52]. There are some experimental indications that the use of vitamin K antagonists may induce slower bone formation and even slower fracture healing[52,53]. Nevertheless bone mass appears not to be affected[54]. It has also been shown that administration of vitamin K increases the total OC concentration as well as the OC-hydroxyapatite binding capacity[55].

In elderly women, an increased level of serum undercarboxylated OC (ucOC) has been found, despite normal levels of vitamin K[55]. Recently it has been shown that ucOC is a marker for the risk of hip fracture in healthy elderly women, suggesting that serum ucOC reflects some changes in bone matrix associated with increased fragility[56]. The exact mechanism of this undercarboxylation is still unclear, but a relationship with vitamin D seems likely, as ucOC was negatively correlated with 25-OH vitamin D_3 and administration of vitamin D induced a reduction of ucOC[56].

Taken together, OC is a useful biochemical marker, in characterizing patients with changes in bone turnover, as well as in monitoring treatment.

3.2.3 Carboxyterminal propeptide of type I collagen

Type I collagen represents 90 % of the organic matrix of bone and is produced by the osteoblasts[57]. Therefore, markers of collagen synthesis, may be of relevance as biochemical markers of bone formation. During formation the carboxyterminal propeptide of type I collagen (PICP) is split off from its precursor, procollagen, in a 1 : 1 molar ratio of newly formed collagen. PICP is not incorporated in bone but is released into the extracellular fluid[58]. In contrast, part of the aminoterminal extension peptide of collagen type I (PINP) is incorporated into bone matrix, where it has been identified as the 24 Kd phosphoprotein of bone[59].

Recently radioimmunoassays for the measurement of PICP and PINP have been developed [60-62]. Although assays for PICP and PINP both measure native procollagen similarly, the serum concentration for PINP is almost a 100-fold higher than that of PICP[60]. This strongly suggests that there is a preferential release of PINP from bone and/or that the clearance of PINP is retarded compared to PICP, or both[60].

Measurement of PICP seems to give more representative results than do PINP assays in primary hyperparathyroidism and Paget's disease[60,63].

In a recent study an increase of PICP shortly after the menopause was observed, indicating an increased bone turnover. This increase could rapidly be reversed by administration of hormone replacement therapy[64]. The same study, however, did not show any advantage for PICP compared to measurement of osteocalcin. Charles et al. studied several groups of patients, i.e. thyreotoxicosis, osteoporosis and normals and found a significant correlation between the measurement of PICP and ^{47}Ca kinetic studies[65]. This correlation, however was rather weak with a r-value of about 0.50.
PICP and PINP are resistent to defrosting, whereas osteocalcin is not. Other advantages for the measurement of PICP or PINP are not present.

3.2.4 Other bone proteins

Next to the above mentioned markers of bone formation, two other bone-related proteins: osteonectin and bone sialoprotein II (BSP), are secreted by the osteoblasts and are potential markers of bone formation. Both can be measured by RIA[66,67]. These measurements however do not reflect bone turnover in a precise manner because significant amounts of both proteins are produced in other cells, i.e. platelets and fibroblasts, which contribute to the concentrations measured in serum[68]. Currently, no monoclonal antibodies, which detect only osteonectin and BSP from bone, are available.

3.3 BIOCHEMICAL MARKERS OF BONE RESORPTION

3.3.1 Urinary hydroxyproline

A commonly used measurement of bone resorption and, indirectly, of bone turnover is the urinary excretion of hydroxyproline (OH-P). OH-P is found only in collagen and represents about 13 % of the total amino acid content of mature collagen[69].
OH-P once released from collagen is not reused for collagen biosynthesis and is released into the urine[17]. Since the bone matrix contains half of human collagen, and has a rapid turnover compared to other collagen pools, like the skin, urinary OH-P is utilized as a marker of bone resorption.
Despite the information provided by urinary OH-P, it has several disadvantages. OH-P is not specific for collagen-type I, which is the bone specific collagen. In practice, urinary OH-P also originates from other sources such as diet (gelatin), the C1q fraction of complement, turnover of soft connective tissues and degradation of the aminoterminal propeptides from collagen biosynthesis[70]. Only about 10 % of the OH-P released by the breakdown of collagen circulates in the peptide-bound form, and these peptides that contain OH-P are filtered and excreted in the urine without further metabolism. The other 90 % of the OH-P, released

during breakdown is degraded to free amino acids (serine and glycine) that circulate in plasma and are almost completely reabsorbed in the kidney after filtering. Finally it is completely oxidized in the liver[71].

OH-P is usually measured in a 24 h urine collection, and the patient is ordered to avoid food with a high gelatin content for at least two days before the collection. Because this has led to problems with patient compliance and interpretation of the results nowadays the OH-P/creatinine ratio in an early 2-hour morning specimen after an overnight fast is measured[72]. With this measurement OH-P is a good parameter of bone resorption. In the same sample also calcium excretion as a marker of resorption can be measured. Despite the easier collection of urine, the other above mentioned problems with respect to OH-P still exist.

When the OH-P excretion in urine is used in the evaluation of postmenopausal osteoporosis it seems important to look also at the other parameters of bone turnover. It has been claimed that urinary OH-P values together with body mass index, serum alkaline phosphatase, and urinary calcium measurements can designate nearly 80 % of postmenopausal women with accelerated bone loss[73]. In this study, however only the forearm was taken into account. Data with respect to the same variables and the lumbar spine are not available.

3.3.2 Tartrate-resistant acid phosphatase (TRAP)

Acid phosphatase is a lysozomal enzyme that is present primarily in bone, prostate, platelets, erythrocytes and the spleen. The different isoenzymes can be separated by electrophoretic methods. In serum two main isoenzymes are present, isoenzyme 5 is produced by osteoclasts, and is resistant to tartrate (TRAP), whereas isoenzyme 3 is inhibited by tartrate[1]. The function of TRAP is still unknown. Plasma TRAP levels are elevated in a variety of metabolic bone diseases, for example in Paget's disease of bone[75], in vertebral osteoporosis[76] and certain categories of osteopetrosis[77]. These elevations correlate well with other markers of bone turnover[75]. At this moment it has not been shown that TRAP is a more sensitive marker for bone resorption than urinary OH-P[78]. As there is currently no monoclonal antibody against the bone isoenzyme of TRAP available, there is no place for the determination of TRAP as a marker of bone resorption.

3.3.3 Urinary excretion of the collagen pyridinium crosslinks

Pyridinium crosslinks are products of a unique series of reactions during the maturation of collagen fibrils that lead to the formation of pyridinoline (Pyr) and deoxypyridinoline (dPyr) also called hydroxylysylpyridinoline and lysylpyridinoline, respectively. Bone collagen contains both Pyr and dPyr. Release of these components from bone undergoing resorption constitutes the main source of both crosslinks in urine[79,80]. Pyr as well as dPyr have been identified in many connective tissues, such as bone, cartilage, and, to a lesser extent, other

connective tissues, except skin[81]. Pyr is present in greater amounts than dPyr most of these tissues (molar ratio Pyr:dPyr 10:1), whereas dPyr is present in a higher proportion especially in bone tissue (molar ratio Pyr:dPyr 3.5:1)[82].
Because, compared to other connective tissues, bone tissue has a high turnover, it is believed that the pool of pyridinoline crosslinks in the urine is mainly derived from bone. The crosslinks can be measured by fluorometry after reversed phase high pressure liquid chromatography (HPLC) of cellulose bound extracts of hydrolyzed urine[83].
Compared to the measurement of urinary OH-P, the measurement of the crosslinks seems to have some advantages: they are relatively specific for bone, they are not metabolized further before their urinary excretion and they are not influenced by dietary denatured collagen[84].
In early postmenopausal women an increment of the crosslinks in urine has been shown, whereas a decline after hormone replacement therapy was observed[11,22,85]. Also in other (non-) metabolic bone diseases, such as primary hyperparathyroidism and neoplastic bone disease, an elevated excretion of both crosslinks has been found[86,87].
Since the current method of measuring both crosslinks in the urine (HPLC) is rather complicated, several methods have been developed to measure the pyridinoline crosslinks more directly[88].

3.3.4 Telopeptide of collagen type I

The cross-linked carboxyterminal telopeptide of collagen type I (ICTP) is a 9 kD fragment of type I collagen which is released into the extracellular fluid during the resorption of mature bone collagen. Its concentration in serum has been reported to correlate with histomorphometric measurements of bone resorption[89]. However, in another study this was not confirmed[90]. Charles et al. found weak correlations in high and low bone turnover states between ICTP and ^{47}Ca kinetics[65]. The use of ICTP as a marker of bone resorption in serum has to be evaluated further, before it can be used in clinical practice.
Recently, urinary levels of the cross-linked N-terminal telopeptide of type I collagen have been reported to be a more specific and sensitive marker of bone resorption[91]. The peptide which is measured in this assay appears to be unique for bone collagen degradation and seems to be promising[92]. Also a urinary marker with a peptide sequence specific for the C-telopeptide α_1 collagen chain (Crosslaps) has been introduced recently and is under investigation for the use as marker of bone resorption.

3.3.5 Fasting urinary calcium

The total daily excretion of calcium is dependent on calcium intake during day-time. The fasting urinary calcium, however, does not come from the diet but is derived from the

tissues, presumably bone or bone cells[93]. For this reason it can be used as a marker of bone resorption. An elevated value generally means an increase in net bone resorption and is seen in postmenopausal osteoporosis and hyperparathyroidism[93]. As also OH-proline in urine is measured in a 2 hours fasting urine sample, both measurements can be performed in the same sample.

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CHAPTER 4

TREATMENT MODALITIES

4.1 INTRODUCTION

In the discussion of the treatment of osteoporosis, two situations have to be distinguished. First, the early detection of those at risk for developing osteoporosis (secondary prevention) and secondly the treatment of established osteoporosis (tertiary prevention). The goal of primary prevention is to prevent a disease from developing at all. Prevention of bone loss is most effective in the earliest stages of osteoporosis (secondary prevention), i.e. before perforation and removal of cancellous bone structures has led to irreversible destruction of bone microstructure[1]. However, it has been shown that arrest of further bone loss also in patients with established osteoporosis reduces the number of further fractures. The ultimate goal of the several different treatment strategies is to decrease the incidence of new osteoporosis-related fractures.

As osteoporosis is considered to be a consequence of a disbalance between resorption and formation, drugs used to treat osteoporosis can be grouped into those that decrease bone resorption and those that increase bone formation. In table 4.1 the drugs, currently used or under investigation, are listed.

Table 4.1. *Medications used in the prevention and treatment of osteoporosis.*

Antiresorptive drugs	Formation inducing drugs
- Estrogen or hormone replacement therapy	- Fluoride
- Anabolic steroids	- Parathyroid hormone
- Bisphosphonates	- Growth hormone ?
- Calcitonine	

Unknown or complex action
- Calcium
- Vitamin D derivatives
- Growth hormone ?

In table 4.2 the proposed working mechanisms for the various medications are presented. It is striking to observe, that the treatment that is used most often, i.e. estrogen substitution does not have a fully understood mechanism of action.

Recently, the impact of bone resorption inhibitors on the so-called "bone-remodelling transient" has been reviewed by Heaney[2]. The gain of bone induced by an inhibitor of bone resorption will only be transient because the associated rate of change in bone mass persists

Table 4.2 *Proposed mechanism of action of the various treatment modalities.*

Medication	Proposed working mechanism	References
Estrogen	Reduction of activation frequency and remodelling mechanism via an unknown mechanism, maybe via local mediators, i.e. TGF-β, IGF's, IL-6, IL-11, etc.	3-6
Calcitonin	Inhibition of osteoclast recruitment and osteoclastic activity directly	7
Bisphosphonates	Induction of changes in the membrane permeability of the osteoclast, which may account for structural changes at the ruffled border or cytoskeleton Decrease in acid production by the osteoclasts	8,9
Anabolic steroids	Decrease bone resorption and increase muscle mass, and may increase collagen synthesis	10,11
Fluoride	Stimulation of osteoblastic activity with incorporation of fluorapatite crystals in the bone hydroxyapatite	12
Parathyroid hormone	Induction of bone formation in low dosages, probably via IGF-I	13,14
Growth hormone	Via IGF-I effects, locally or systemically.	15
Calcium	Diminishment of PTH release Correction of subclinical calcium deficiency	16 17
Vitamin D	Correction of subclinical deficiency Increased intestinal Ca-absorption	18
1,25- $(OH)_2D_3$	Increased calcium absorption	19,20

for only one remodelling cycle. After resorption has been diminished, also formation will be reduced, leading to a lower level of bone turnover[2]. It was calculated, that dependent on the rate of bone turnover an increase in bone mineral mass of about 5 - 10 % could be obtained in the first 6 to 12 months after treatment with a bone resorption inhibitor was started. So, with an anti-resorptive agent, in patients with high bone turnover a higher increase in bone mineral mass will be obtained compared to those with a low bone turnover (see also Fig 4.1). However, after the remodelling space has been filled in, age-related bone loss will eventually continue.

The restricted effect of an anti-resorptive agent forms also the basis of the so called "activate-depress-free-repeat (ADFR)" schedule. In this schedule initially an activator of bone turnover

is given, followed by a depressor (or inhibitor) of bone resorption. After a period in which no medication is used, this schedule is repeated. The theory behind this approach is that a sequential or intermittent therapeutic schedule results in an uncoupling of bone turnover with ultimately a more pronounced increase in bone mass.

The ideal agent or combination of agents should not only induce a substantial increase in bone mass but also have to induce the development of new trabeculae. Only an increase of the thickness of the cortex and available trabeculae, together with a restoration of connectivity would lead to a considerable increase in bone strength. However, such a restoration of connectivity has not been observed up to now.

Until now, no good formation inducing agent is available. Only fluoride has been shown to cause impressive increases in lumbar bone mass. However, this has not resulted in a decrease of fracture rate. On the contrary a small increase in the number of hip fractures was observed[22]. However, this might have been related to the dosage of fluoride used. In an additional analysis of the same group of patients, 6 years after start of treatment it has been shown that a decrease in vertebral fractures was related to the serum fluoride level[22]. It is

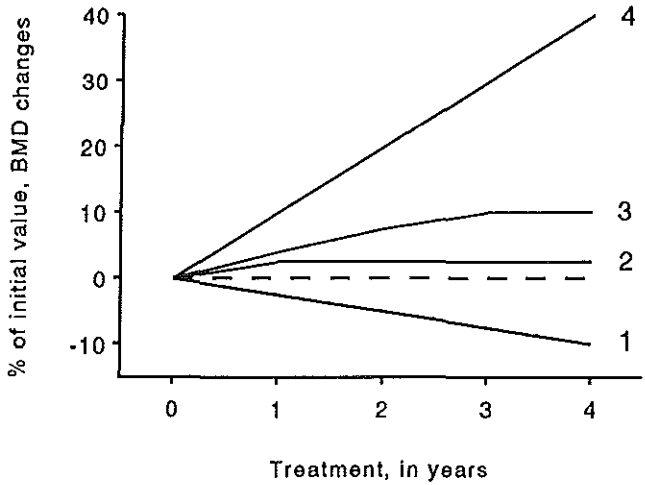

Figure 4.1 *Patterns of change of BMD in the lumbar spine in women with osteoporosis during treatment with various regimens (Modified from Riggs et al.[26]). The dashed line represents no change. When no treatment is given (1), bone loss continues. With an anti-resorptive drug (2 and 3) an increase of about 3 - 10 % can be reached with a waning effect after 2 - 3 years. With low turnover (2) a lower increase is reached, compared to high bone turnover (3). With a formation stimulator (possibly in combination with an anti-resorptive agent) higher positive changes might be obtained (4).*

concluded that lower dosages of NaF, than presently used, may be indicated in the treatment of postmenopausal osteoporosis[22,23]. This implicates that fluoride has a rather narrow therapeutic window.

The other available formation inducing agent is parathyroid hormone (PTH), which has been shown to increase bone formation when administered intermittently[13]. However, this may be an indirect effect, via insulin-like growth factor I (IGF-I), which has been shown to increase during intermittent, but not during continuous administration of PTH[24]. In an animal model (sheep) PTH has been shown to increase bone turnover in elderly sheep, but this effect was completely blunted in the presence of an anti-resorptive agent, tiludronate[25]. In this study, the hypothesis has been made that an increased resorption is a prerequisite for the anabolic effect of PTH. As IGF-I may be important in the local environment of the osteoblast, the administration of growth hormone (GH) may be another attractive agent for formation induction.

In figure 4.1 the potential gain in bone mineral density for different treatment modalities is shown [26].

Osteoporosis is most frequently seen in postmenopausal women. However, also in men and in patients using corticosteroids or suffering from diseases interfering with the bone turnover (see also Table 1.1) it is frequently observed. In table 4.3 the medications most often used for the different patient-groups are mentioned.

Table 4.3 *Medications used in different groups of patients.*

Postmenopausal	Corticosteroid-induced	Male
- HRT	- Bisphosphonates	- Bisphosphonates
- Calcitonin	- HRT	- $1,25\text{-}(OH)_2D_3$
- Bisphosphonates	- Calcitonin	- Calcitonin
- Fluoride	- Anabolic steroids	- Calcium
- Anabolic steroids	- $1,25\text{-}(OH)_2D_3$	
- $25\text{-}(OH)D_3$, $1,25\text{-}(OH)_2D_3$	- Calcium	
- PTH*	- Thiazide diuretics	
- Growth hormone*		
- Calcium		
- Thiazide diuretics		

(* Under investigation)

In the next chapters the various therapeutic options for prevention of developing and treatment of established postmenopausal osteoporosis will be discussed. However, first a few

general remarks about some of the medications and their administration will be made.
At present a wide variety of estrogen preparations are available. Estrogen replacement therapy can be given as unopposed or opposed therapy. In opposed therapy the estrogens can be given cyclically or continuously, with cyclic progestagen, inducing regular monthly bleedings, or estrogen/progestagen can be given as a continuous treatment, thereby avoiding bleedings. This also prevents the increased risk of the development of endometrial carcinoma found in patients treated with estrogen alone[27]. So far, no sufficient data are available with respect to continuous treatment and its impact on endometrial carcinoma[27].
The measured effects of the various treatment schemes, based on estrogen therapy, on bone mass and bone turnover are more or less alike.
With respect to the bisphosphonates it may be important to make a differentiation between the amino bisphosphonates and the non-amino bisphosphonates. Pamidronate and alendronate are representatives of the former group, whereas etidronate, clodronate and tiludronate represent the latter. The non-amino bisphosphonate, etidronate, has been suggested to prolong the total remodelling time, which may induce osteomalacia[28]. The spatial distribution of etidronate in bone has been suggested to differ from that of an amino bisphosphonate.
Autoradiographic studies were done with the amino bisphosphonate alendronate in animals, in which bone remodeling was accelerated by injections of PTH-related peptide. After 24 hours the alendronate was concentrated on the bone surface in the vicinity of osteoclasts. It seemed as though the drug only bound to regions where bone remodeling existed[29].
No comparable studies with etidronate were performed, but because of the more polar structure of etidronate it is possible that it penetrates the bone cell lining better, and thereby is deposited throughout the bone surface. No indication for impairment of bone mineralization and the subsequent development of osteomalacia have been observed in a long term clinical study of osteoporotic patients with etidronate[30]. However, in patients with Paget's disease, treated with low dosages of etidronate, focal osteomalacia and increased incidence of pathological fractures have been reported[31]. This complication was dose-dependent. For the other non-amino bisphosphonates clodronate and tiludronate no such data are known. In general they produce little or no inhibition in mineralisation at doses which show antiresorptive action[32].

4.2 PREVENTION OF DEVELOPING OSTEOPOROSIS

Prophylactic regimens aim at preserving bone mass at a given level. This is not comparable to the intention of reaching an optimal peak bone mass, which can be considered as primary prevention. It has been shown, that for reaching a maximal peak bone mass exercise as well

as a sufficient calcium intake at a young age are important determinants. Moderate weight bearing exercise induces a higher peak bone mass[33], whereas extreme exercise may induce amenorrhoea with negative effects on the bone mass[34,35]. Also an adequate calcium intake seems to be necessary to obtain a maximal peak bone mass. In normal women, aged 20 - 30 years, the gain of bone mass in the third decade depended for a large part on the daily calcium intake, i.e. with a daily intake of 200 mg Ca no change in bone mas was found, whereas with an intake of 1500 mg an increase of about 15 % during the third decade was observed[36]. This amount of calcium intake seems mandatory, also for older women, at risk for, or with osteoporosis[17,37]. Most of the treatment regimens, used in the prevention or treatment of postmenopausal osteoporosis, do make sure this amount of calcium intake is reached. However, during the first years after the menopause no real effects of extra calcium supplementation have been observed[38,39]. A possible explanation for this observation is, that the increased calcium released by the bone itself, meets the requirements for bone formation. The aim in a prophylactic regimen (secondary prevention) is generally different from regimens used in the treatment of established osteoporosis, which focus on increasing bone mass to a level above the fracture threshold. Prophylaxis aims at preserving bone mass and structure after the menopause, thus preventing desintegration of the trabecular network and cortical thinning. Pharmacological intervention to decrease postmenopausal and age-related bone loss should be undertaken in persons who, because of low or relatively low bone density, are deemed to be at increased risk for osteoporosis.

After the menopause, it seems obvious to use hormone replacement therapy to counteract estrogen deficiency. Estrogen treatment indeed was the first prophylactic agent and has been used for over 20 years. Actually it is the only compound used on a large scale. As there is an increased risk of the development of endometrium carcinoma[27], when using estrogen alone, many schemes use a combination of estrogen with a progestagen. Also various ways of administration are available and show comparable results with respect to bone mass (Table 4.5). When estrogen therapy is started at the menopause it is unclear how long it has to be continued to maintain the positive effects on bone density. However it has been calculated that for long-term preservation of bone mineral density, at least a period of seven years is warranted in which estrogen therapy is used[40]. Discontinuation leads to accelerated bone loss like that which follows the natural menopause[41,42].

Next to the beneficial effect of estrogens on preserving bone mass, it is important to take into account other effects of long-term administration of estrogens in postmenopausal women. The adverse effect with respect to increased endometrium carcinoma, when administering estrogen alone has already been mentioned. Uncertainty exists with respect to breast carcinoma[43-46]. An increased incidence appears to be observed[46], which is not reduced with the addition of progestagens. Results on mortality are contradictory, some studies show no increased

mortality, probably because of more frequent surveillance[45], whereas another study showed a relative risk of 1.45 among women who had used estrogen for a period of 5 year or more[46]. Another important and maybe even more important effect than the one on bone mass, is the beneficial effect of estrogen on cardiovascular disease[47]. This effect can partly be explained by the decrease of LDL-cholesterol and the increase of HDL-cholesterol[47]. Another pathogenetic mechanism may be the direct relaxing effect of estrogen on the arterial wall[48] or by a direct inhibition of cholesterol deposition within the arterial wall[49].

Although these positive effects are more impressive in women using estrogen alone, they also can be found in women treated with a progestagen in combination with estrogen. In a meta-analysis of the recent literature, relative risks have been calculated for the above mentioned factors[50]. These are depicted in Table 4.4. With addition of a progestagen the beneficial effect of estrogen on cardiovascular disease is moderately diminished, according to the somewhat higher relative risk values.

When looking at these figures, one might speculate whether the most important reason for the administration of estrogen would not be to decrease cardiovascular events,

Table 4.4 *Calculated relative risk of several diseases for a 50-year-old white woman treated with long-term hormone replacement (modified from Grady et al.[50]).*

Disease	Relative risk	
	Estrogen therapy	Estrogen plus progestagen
Coronary heart disease	0.65	0.65 - 0.80
Stroke	0.96	0.96
Hip fracture	0.75	0.75
Breast cancer	1.25	1.25 - 2.00
Endometrial cancer	8.22	1.00

thereby also reducing the incidence of hip fracture. For the Netherlands an increase in the age-adjusted incidence of hip fractures in a cohort analysis of 15 years (1972-1987) has been found in women as well as men[51]. Some data indicate a decrease of up to 50 % in the incidence of osteoporosis related fractures with the postmenopausal use of HRT[52].

Also bisphosphonates and calcitonin are used in the prevention of osteoporosis, but until now, only on an experimental basis. The effects of these medications on fracture risk have yet to be evaluated.

In Table 4.5 the various treatment modalities are presented, especially with respect to their

Table 4.5 *Effects of preventive treatment in osteoporosis.*

Treatment	Duration of treatment or follow-up	Subjects	Design	Number (treated/placebo)	Years since menopause	Age (range)
Estrogen:						
Mestranol 23.3 μg	upto 5 years	oophorect. women	db-bl	63/57	0-6	-
Mestranol 23.3 μg	6-12 years	oophorect. women	db-bl	58/42	3-9	-
* Conj. estrogen 0.625 mg + 5 mg methyltestosteron	2 years	postmenop. women	rand	20/18	1-32	55-65
Ethinyl-estradiol 25 μg or 50 μg	2 years	postmenop. + oophorect. women	rand	19/18	mean 5.4	33-62
* Conj. estrogen 2.5 mg + 10 mg medroxyprogesterone	10 years	postmenop. women	rand	< and > 3 yr postm. < 3 yr 30/21 > 3 yr 37/41	< or > 3 yr	-
17β-estradiol gel 3 mg percutaneously	2 years	postmenop. women	db-bl	11/11	0.5-3	47-54
17β-estradiol 50 ug/day transdermal + 10 mg medroxyprogesterone	1 year	postmenop. women	rand	17/17	2-4	43-58
Calcitonin:						
Calcitonin 50 IU/day 5 days/wk, intranasally	1 year	postmenop. women	rand	30/30	< 3	-
Calcitonin 100 IU/day, intranasally	2 years	postmenop. women	db-bl	19/20	2.5-5	48-55
Bisphosphonate:						
Tiludronate 100 mg 6 months, daily	1 year	postmenop. women	db-bl	28/31	3-10	50-60
Etidronate 400 mg 3 months, daily	6 months	oophorect. women	db-bl	10/10	0	44-51
Thiazide diuretics:						
Chlorthalidone 12.5 - 25 mg day	2.6 years	elderly women	rand	43/70	not mentioned	mean 72

oophorec., oophorectomised; postmenop., postmenopausal; db-bl, double blind; rand., randomised.
* The combination of an estrogen with a progestagen was given cyclically, i.e. 21 days out of 28 the estrogen and from day 14-21 the progestagen was added. Bone mass measurement in the various studies compared to initial values.
When ↓ is mentioned in bone turnover parameters, these represent significant changes.

Site of measurement	Bone mass		Bone turnover parameters		References
	Therapy	Placebo	Therapy	Placebo	
bmc metacarpalia	+ 1 %/yr	- 2 %/yr	OH-Proline ↓ Ca in urine ↓	no change	53
bmc metacarpalia	no change	- 1 %/yr	not mentioned		54
bmc radius bmc metacarpalia	no change - 1 %/yr	- 2.7 %/yr - 1.3 %/yr	not mentioned		55
bmc proximal radius	no change	- 2.5 %/yr	not mentioned		56
bmc 3rd metacarpal	< 3 yr postmenopausal: + 10 % > 3 yr postmenopausal: no change	- 10 % - 10 %	not mentioned		57
bmc proximal radius bmc distal radius bmc L2-4	no change no change + 1 %/yr	- 4 %/yr - 4 %/yr no change	OH-Proline ↓ Alk. Phosp ↓ Osteocalc. ↓	no change no change no change	58
bmc forearm	+ 4.3 %	- 3.5 %	OH-Proline ↓ Alk. Phosp ↓ Ca in urine ↓	no change no change no change	59
bmc L2-4	+ 1.4 %/yr	-3.2 %/yr	no changes	no changes	60
bmc proximal radius bmc distal radius bmc L2-4	- 1.5 %/yr -1 %/yr + 1.5 %/yr	-1.5 %/yr -1.5 %/yr -3 %/yr	Alk. Phosp. no change Osteocalc. no change OH-Proline ↓	no change ↓ ↓	61
bmc L2-4	+ 1.5 %/yr	-2.5 %/yr	OH-Proline ↓ Alk.Phosp no change	no change no change	62
bmc proximal radius	no change	no change	OH-Proline no change Ca in urine ↓ Alk. Phosp. no change Osteocalc. ↓	no change no change no change no change	63
bmc proximal radius bmc distal radius	- 0.3 %/yr + 1.5 %/y	- 0.9 %/yr + 0.4 %/yr	not mentioned		64

Table 4.6 *Effects of treatment in postmenopausal osteoporosis. Anti-resorptive agents.*

Treatment	Concurrent treatment	Duration of treatment	Design	Number (treated/placebo)	Years since menopause
Estrogen:					
Conjugated estrogen 1.25 mg/day	none	1 year	db-bl-pc	11/10	mean 6
17β-estradiol, 0.1 mg patch, day1-21, + 10 mg medroxy-progesteron, day 11-21 cycle of 28 days	none	1 year	db-bl-pc	36/39	5-28
17β-estradiol 2 mg + 1 mg norethisterone continuously	Ca, 500 mg daily	1 year	db-bl-pc	16/15	mean 16
Anabolic steroids:					
Nandrolone decanoate 50 mg/3 weeks, i.m.	Ca, 500 mg daily	1 year	db-bl-pc	19/17	not mentioned
Bisphosphonate:					
Etidronate, 400 mg, 2 weeks on, 13 weeks off treatment	Ca, 500 mg daily Vit D 400 IU, daily	150 weeks	db-bl-pc	33/33	7-34
Etidronate, 400 mg, 2 weeks on, 13 weeks off treatment	Ca, 500 mg, day 18-91	2 years	db-bl-pc	105/104	mean 19
Etidronate 400 mg, 2 weeks on, 13 weeks off treatment	Ca, 500 mg, day 18-91	3 years	db-bl-pc	77/78	mean 19
Pamidronate 150 mg/day	Ca, 1 g daily	2 years	db-bl-pc	26/22	mean 18
Pamidronate 150 mg/day	Ca, 500 mg daily	1 year	randomised (GH + Pam vs Pam)	10/11	5-27
Calcitonin:					
Salcatonin 50 IU/day	Ca, 500 mg daily	2 years	db-bl-pc	40	mean 21
Salcatonin 100 IU/day			randomized	43	
Salcatonin 200 IU/day			group	41	
Placebo			comparison	40	

All patients suffered from postmenopausal osteoporosis with osteoporotic fractures. The concurrent treatment was also given to the placebo-treated patients. Bone mass measurement in the various studies compared to initial values. Bone mass changes during whole study period. YSM, years since menopause; FR, new vertebral fracture rate/1000 patient years; SDI, spine deformity index; db-bl-pc, double blind placebo-controlled; FN, femoral neck; TB, total body.

Age (mean or range)	Site of measurement	Bone mass Therapy	Bone mass Placebo	FR or SDI Therapy	FR or SDI Placebo	References
mean 64	bmc L2-4	+ 8.3 %	- 5.0 %	not done		68
	bmc FN	+ 2.6 %	- 2.0 %			
54.6 - 72.1	bmc L2-4	+ 5.3 %	+ 0.2 %	FR: 230	580	69
	bmc FN	+ 1.0 %	+ 1.0 %			
	bmc midradius	+ 1.0 %	- 2.6 %			
mean 64	bmc L2-4	+ 8.0 %	no change	not done		70
	bmc distal radius	+ 8.0 %	no change			
	bmc proximal radius	no change	no change			
mean 66	bmc distal radius	+ 3.0 %	no change	not done		71
	bmc proximal radius	+ 3.0 %	+ 1.0 %			
57-75	bmc L2-4	+ 5.0 %	- 5.0 %	SDI: - 0.1	- 0.25	72
	bmc distal radius	no change	- 4.0 %			
mean 65	bmc L2-4	+ 4.0 %	+ 1.0 %	FR: 44	68	73
	bmc FN	+ 2.0 %	no change			
mean 65	bmc L2-4	+ 5.0 %	+ 1.5 %	FR: 59	132	74
	bmc FN	+ 2.0 %	no change			
mean 66	bmc L2-4	+ 7.0 %	- 1.5 %	FR: 130	240	75
	bmc FN	+ 1.0 %	- 1.0 %			
	bmc TB	+ 2.0 %	-1.0 %			
		GH+Pam:	Pam:			
55-72	bmc L2-4	no change	+ 5.0 %	not done		76
	bmc FN	no change	no change			
	bmc distal radius	no change	+ 4.0 %			
	bmc proximal radius	no change	no change			
mean 70	bmc L2-4	+ 2.2 %		FR: 43	146	77
		+ 2.2 %				
		+ 3.6 %				
			+ 1.1 %			

Table 4.7 *Effects of treatment in postmenopausal osteoporosis. Formation inducing drugs and drugs with unknown action.*

Treatment	Concurrent treatment	Duration of treatment	Design	Number (treated/placebo)	Years since menopause
Fluoride:					
50 - 60 mg/day	Ca, 1500 mg, daily Vit D 400, IU, daily	4 years	randomized	33/27	not mentioned
75 mg	Ca, 1500 mg, daily	4 years	db-bl-pc	66/69	9-33
PTH:					
no placebo-controlled studies					
1,25 (OH)$_2$D$_3$:					
2 dd 0.25 μg	Vit D, 400 IU, daily	2 years	db-bl-pc	12/15	mean 17
2 dd 0.25 μg	none placebo-group 1 g Ca, daily	3 years	prospective single blind	314/308	mean 15
Growth hormone:					
7 IU, daily for 2 months after 5 months (2 + 3), 3 months rest period. Schedule repeated 3 times	Calcitonin 100 IU months 3 -5	2 years	randomized	7/7	not mentioned

All patients suffered from postmenopausal osteoporosis with osteoporotic fractures. The concurrent treatment was also given to the placebo-treated patients. Bone mass measurement in the various studies compared to initial values. Bone mass changes during whole study period. YSM, years since menopause; FR, new vertebral fracture rate/1000 patient years; SDI, spine deformity index; db-bl-pc, double blind placebo-controlled; FN, femoral neck.

Age (mean or range)	Site of measurement	Bone mass Therapy	Placebo	FR or SDI Therapy	Placebo	References
50-81	not done			FR: 304	419	78
58-74	bmc L2-4 bmc FN bmc radius	+ 8.2 %/yr + 1.8 %/yr - 1.8 %/yr	+ 0.4 %/yr - 0.9 %/yr - 0.7 %/yr	FR: 446 Total new non-vertebral fractures: 61	525 24	21
mean 64	bmc L2-4 bmc distal radius	no change + 2.6 %	- 8.0 % - 3.2 %	FR: 250	333	79
mean 64	not done			FR: 88-99	103-315	80
mean 63	bmc radius TB neutron activation analysis	no change + 4.6 %	no change -1.0 %	not done		81

effects on bone mass and on biochemical markers. In all studies mentioned in table 4.5 the subjects used a calcium intake of about 1000 mg per day.
As can be seen in table 4.5 estrogen, calcitonin and bisphosphonates all are able to prevent bone loss in the early postmenopausal years. It has also been shown, however, that when treatment is started several years after the menopause a reduction of further loss can be obtained but not a gain of bone mass towards premenopausal values. The most illustrative study has been performed by Lindsay et al. in oophorectomised but otherwise normal women, where treatment with estrogen was started immediately, 3 and 6 years after oophorectomy respectively[53]. In the women in whom treatment was started within 3 years of oophorectomy an increase of bone mass towards the values obtained in women who started treatment immediately after oophorectomy was observed. In women started 6 years after oophorectomy no further loss, nor an increase was observed[53,54]. This indicates that the best period of initiating treatment with the intention of preventing bone loss is within 3 years after the menopause.
Most of the earlier studies have used rather high dosages of estrogen. In later on studies 0.625 mg of conjugated equine estrogens, 25 μg of ethinyl estradiol or 1 mg of estradiol valerate turned out to be the minimum dosages needed to reduce bone turnover effectively[65,66]. It has also been shown that circulating levels in serum between 60 and 90 pg/ml are capable of reducing bone turnover efficiently[67].

4.3 TREATMENT OF ESTABLISHED OSTEOPOROSIS

Treatment of established osteoporosis remains a problem. In the introduction several remarks have been made with respect to the theoretical basis of various treatment schemes. The ultimate goal of treatment is to achieve a reduction in (further) fractures. It is important to take into consideration that an increase of bone mass, does not perse indicate a reduction in fractures. The skeleton in postmenopausal osteoporosis is reduced in mass, and the tissue may be qualitatively abnormal. It is clear that the loss of tissue in cancellous bone produces important architectural changes, with elimination of complete trabeculae[1].
Most available agents inhibit resorption on the surface of the existing trabeculae, the hope being that the outcome will be an increase in the size of the individual trabeculae, and to decrease further loss of connectivity. Thereby the bone tissue would be capable of withstanding stress more effectively.
In the following tables some of the results, which are available, of anti-resorptive regimes as well as of some of the formation inducing agents are shown. In several studies also fracture rates have been calculated. It is, however, important to take into account the small

size of several studies. This may introduce an important bias, i.e. small differences in number of new fractures observed in small groups are represented as more impressive absolute differences, when they are calculated as new fractures/ 1000 patient years.

4.4 CORTICOSTEROID INDUCED OSTEOPOROSIS

As this subject as well as osteoporosis in men (chapter 4.6) are beyond the scope of this thesis, only some brief remarks with respect to the various treatment options will be made. Glucocorticoid-induced osteoporosis has been recognized since 1932 when Cushing first described skeletal decalcification as a characteristic feature of adrenal hyperplasia secondary to adrenocorticotrophic hormone production by pituitary tumours[82]. Glucocorticoid-induced bone loss became a significant problem as therapeutical corticosteroids became available[83]. The metabolic effects of corticosteroids on bone and mineral metabolism are extensive. several pathogenetic factors, which can be divided in systemic and skeletal effects are mentioned in table 4.8. The secondary hyperparathyroidism which occurs after long-term administration with glucocorticosteroids is considered to be one of the most important pathogenetic factors[84]. The risk of hip fracture has been calculated to increase 50 % in patients using long-term corticosteroids compared to non-users. 30 to 35 % of the patients using long-term corticosteroids have vertebral fractures.
Because corticosteroids are used for a variety of diseases, the corticosteroid-induced bone loss is an important problem. For this reason various schemes for prevention and treatment have been studied. No sufficient data do exist to allow recommendation of any definite treatment protocol.

Table 4.8 *Pathogenetic factors in the pathogenesis of glucocorticoid osteoporosis*[84,85].

Systemic effects	Skeletal effects
- Decrease gastrointestinal calcium absorption and increase renal excretion leading to secondary hyperparathyroidism.	- Direct inhibition of osteoblast function: decrease replication, differentiation and life span.
- Decrease gonadal hormone secretion	- Decrease production of prostaglandin E_2 and IGF-I
- Inhibit IGF-I production and action	- Increase sensitivity to parathyroid hormone and $1,25(OH)_2D_3$.
- Cause muscle wasting	

As bone loss in patients receiving glucocorticosteroids is dose related, it is prudent to describe the lowest effective dose and to use topical preparations when possible. Many attempts to prevent bone loss associated with glucocorticosteroids have been carried out to improve calcium retention by increasing calcium absorption and decreasing urinary excretion, thus reversing secondary hyperparathyroidism. With $1,25OH_2D_3$ administration in combination with calcium bone loss at the lumbar spine was prevented but not at the femoral neck[85], where it was increased by the addition of intranasally calcitonin[86].
During glucocorticoid treatment thiazide diuretics have been shown to improve gastrointestinal absorption of calcium and to decrease urinary calcium excretion without any clear effects on bone mass[87,88].
The use of gonadal hormones to prevent and treat glucocorticoid osteoporosis has not been adequately studied. In postmenopausal women, however, it seems a logical treatment to apply. Also premenopausal women, who have become amenorrheic during treatment with glucocorticosteroids could benefit from this treatment, as estrogen is intensely related to bone mass. As serum levels of testosterone can be reduced in men during glucocorticoid therapy[89] and testosterone deficiency can cause osteoporosis[90], testosterone replacement may be indicated. Nandrolone decanoate has been shown to increase forearm bone density in glucocorticoid-treated male and female patients[91]. Also calcitonin has been used in the treatment of glucocorticoid-induced osteoporosis with only small positive effects on bone mass[92].
The most promising agents with respect to the use in glucocorticoid-induced osteoporosis are the bisphosphonates. Significant increases in spinal bone mass with oral pamidronate after one year of treatment have been found, which were maintained after two years[93]. Also with intermittent etidronate positive results with respect to prevention of bone loss at the lumbar spine have been observed[94]. However, no effects on the femoral neck have been observed, mostly in uncontrolled, short term studies in fairly small patient populations. Therefore longer controlled studies are needed to clarify the place of bisphosphonates in the treatment of corticosteroid-induced osteoporosis.

4.5 OSTEOPOROSIS IN MEN

Although fractures related to osteoporosis are more frequent in women, also in men an increased incidence of osteoporotic fractures occur with advancing age[95]. In men, of the age of 65 and more, the incidence of hip fracture is 4-5/1000, compared with 8-10/1000 in age-matched women[95,96].
The reasons for the difference in fracture incidence between men and women are

multifactorial. First, men obtain a greater skeletal mass during puberty, leading to a higher peak bone mass, a greater diameter of the bone and greater cortical thickness[97]. Secondly, women loose more bone with age than do men, partly because of the loss of estrogens at menopause. Thirdly, a sexual difference in the character of age-related changes in cancellous bone structure contributes to a greater fracture risk in women[97].
Compared to women, where estrogen loss is an important pathogenetic factor for the development of osteoporosis, in men causes of secondary osteoporosis can be identified in a majority of the patients[98]. In Table 4.11 the various forms are presented.
Age-related bone loss is an important feature of osteoporosis in men as well as women. In some men, this age-related bone loss may suffice to cause non-traumatic fractures, but

Table 4.11 *Osteoporosis in men*

1.	Primary		
2.	Secondary:	Hypogonadism	Immobilization
		Glucocorticoid excess	Rheumatoid arthritis
		Alcoholism	Osteogenesis imperfecta
		Gastrointestinal disorders	Systemic mastocytosis
		Hyper(para-)thyroidism	Homocystinuria

frequently other conditions may adversely affect fracture risk. A low peak bone mass, deficient calcium intake, and a lack of physical activity are some of the factors mentioned[99,100].
The pathophysiology in male osteoporosis is only minimally explored. However, causes of secondary osteoporosis have been shown to be present in 30 - 60 % of men with vertebral fractures[101,102]. Glucocorticosteroid use is one of the most prominent causes of osteoporosis in men, accounting for up to 20 % of the men evaluated[101,103]. Also alcohol abuse (about 10 %) and hypogonadism (about 8 %) are frequently found in male patients with osteoporosis[101-103].
For the treatment of male osteoporosis it is important to treat any underlying cause. The treatment of glucocorticosteroid-induced osteoporosis has been discussed in the previous chapter. In hypogonadism, androgen replacement has been shown to increase bone mass[104,105].
Primary or idiopathic osteoporosis is a diagnosis by exclusion, which results after causes of secondary osteoporosis have been excluded. With detailed biochemical and histomorphometric analyses no consistent features of primary osteoporosis have been found[98,103]. So far, no appropriate treatment advices are available. Some beneficial effects of

pamidronate in a heterogenous patients group have been found[106]. Also with etidronate positive results have been obtained, with an increase in spinal BMD of 9 % after one year of treatment[107]. Although specific data are scarce, there is no reason, why bisphosphonates (and other antiresorptive agents) would not be effective in men as they are in women[108]. In men with idiopathic osteoporosis low levels of IGF-I have been reported[109], and it has been shown that administration of growth hormone in elderly men resulted in an increase of lumbar bone mineral density[110]. Whether this is a relevant treatment potential has to be sorted out further.

In general, the various treatment options, which are available for women, could be transferred to men as well, with the exception of HRT.

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CHAPTER 5

SCOPE OF THE THESIS

SCOPE OF THE THESIS

The aim of this thesis was to obtain insight in longitudinal bone mass measurements and bone turnover parameters in postmenopausal osteoporosis and growth hormone deficient patients, treated with a combination of medications. These combinations of medication consisted of a known inhibitor of bone resorption, which was combined in randomized studies with a potential activator of bone formation. The reason for this was to observe, whether it was possible not only to diminish bone resorption but on the other hand also increase bone formation, i.e. increase of activation frequency and thereby reach a netto positive result.
Another part of the thesis consists of normative data of DXA measurement in the lumbar spine as well as in the proximal femur in normal caucasian Dutch women.
In the ***chapters 1-4*** a general overview of the current methods with respect to bone mass measurements, bone turnover parameters and treatment options, which are avialable at present, has been given.
In ***chapter 6*** and ***7*** the data of bone mass measurements and turnover parameters are presented, obtained in females with established postmenopausal osteoporosis. These women were treated for 3 years with a combination of hormone-replacement therapy with or without the anabolic steroid nandrolone-decanoate. ***Chapter 6*** describes the effects after two years of treatment. ***Chapter 7*** describes the effects in the same groups in terms of bone mass and turnover after the treatment has been stopped for a period of one year.
Chapter 8 describes the effect on the same parameters in females with postmenopausal osteoporosis, all treated with the bisphosphonate pamidronate, whereas half of these women were (double-blind) treated with recombinant human growth hormone (rhGH).
Chapter 9 describes the same combination of medications, but now applied to growth hormone deficient patients. In this group of patients the pamidronate was initially given two weeks before adding rhGH to all patients.
Finally in ***chapter 10*** cross-sectional normative data of bone mass measurements for the Dutch female population are presented, which can be used in further studies as a representative normative data set.

CHAPTER 6

CAN NANDROLONE ADD TO THE EFFECT OF HORMONAL REPLACEMENT THERAPY IN POSTMENOPAUSAL OSTEOPOROSIS?

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SUMMARY

Thirty six women with postmenopausal osteoporosis (31 of them with at least one non-traumatic vertebral compression fracture) were matched pair-wise as to age, years since menopause and body mass index and randomized to receive either cyclical estrogen-progestagen replacement treatment (group 1) or the same treatment plus nandrolone decanoate (group 2). During the first year of treatment in both groups the forearm BMC (SPA) rose proximally and distally 2-3%. Over 2 years the increments of forearm BMC in both groups were up to 4.5%. Lumbar BMD (DPA) rose in both groups nearly 10% over the first year and 12% over 2 years. The cancellous bone density of L3 (QCT) showed in 6 months an increase of 21% in group 1 and 29% in group 2 to subsequently stay at that level. All these changes from the basal levels were highly significant but there were no significant differences between the 2 groups. These 2 conclusions were also drawn with regard to the induced fall of serum alkaline phosphatase (-23%), osteocalcin (-35% to 44%) and procollagen I (-15% to 22%) and of the fasting urinary hydroxyproline (-33% to 36%). No significant increase in the number of new deformed vertebrae occurred in 2 years.

INTRODUCTION

With many prospective studies it has amply been demonstrated that estrogen deprivation causes an increase of the bone resorption rate and that conversely substitution with estrogens may largely or completely inhibit bone resorption[1-6]. Also, fracture incidence in postmenopausal women appears to be lowered by estrogen use[7-11], but prospective controlled studies of the efficacy of estrogens in patients with established postmenopausal osteoporosis are scarce[12]. Most of the above mentioned prospective trials have been carried out in normal postmenopausal women, albeit in some studies after bilateral oophorectomy. Characteristically, with this treatment, as with other antiresorptive therapeutic modalities, a modest initial gain in bone mineral density may be observed, limited to the first 1-2 years of treatment. In the forearm this potential initial gain in bone density will -depending on the time elapsed since the menopause- amount to 3% at most[4,5]. This phenomenon is generally ascribed to the coupling of bone formation to bone resorption (or: dependence of osteoblastic on osteoclastic activity) in the basic multicellular units (BMU)[13]. A transient increase of bone (mineral) mass after sudden inhibition of bone resorption is then explained by the far longer life-time of the osteoblasts as compared to that of osteoclasts.

The possible antiresorptive activity of androgens (anabolic steroids) is less well documented than that of estrogens with or without progestagens, although histomorphometric evidence

of it has been obtained[14]. Treatment of women suffering from postmenopausal osteoporosis with stanozolol for 2.5 years induced a rise of total body Ca (neutron-activation), but no change in forearm bone mineral content (BMC; by single photon absorptiometry, SPA) and no consistent effect on the urinary hydroxyproline excretion[15]. More recent studies of the effect of the anabolic steroid nandrolone decanoate in postmenopausal osteoporosis include two open non-randomized comparative trials of around 6 and 12 months, respectively, in which significant increases of forearm BMC and lumbar vertebral bone mineral density (BMD; by quantitative CT-densitometry, QCT) were noted[16,17]. In two double blind-studies, one comparative and placebo-controlled lasting 2 years and the other placebo-controlled during 1 year, rises of 4 and 3%, respectively, of the forearm BMC have been observed over one year[18-20]. No increase of the lumbar vertebral BMD (by dual photon absorptiometry, DPA) was found[20], while the absolute urinary hydroxyproline excretion appeared to be reduced and serum alkaline phosphatase did not change[19].There are also other indications that anabolics such as nandrolone may enhance the activity of osteoblasts, such as raising the serum osteocalcin and (temporarily) procollagen type I levels[21,22]. Although in osteoblasts estrogen as well as androgen receptors have been found[23,24], the mechanism of action of both types of steroids on bone metabolism, such as the established inhibition of bone resorption by estrogens, has not yet been clarified.

To investigate the posibility that anabolic steroids, such as nandrolone decanoate, stimulate bone formation rather than inhibit bone resorption (or stimulate bone formation in addition to inhibiting bone resorption) and thus break through the above mentioned coupling of bone formation to bone resorption we compared in women with postmenopausal osteoporosis the effects of treatment with a combination of hormonal replacement therapy (HRT) and nandrolone decanoate with those of treatment by HRT alone. In other words the possible effects of nandrolone decanoate were studied under conditions, where bone resorption was already depressed by cyclical treatment with a classical combination of estrogen and progestagen. In this comparative randomized single blind trial of two years duration we used various types of bone mass measurement as well as biochemical parameters of bone metabolism.

METHODS AND MATERIALS

A. Patients and treatment.

Women, aged 50-70 years, with postmenopausal osteoporosis (at least one non-traumatic vertebral compression fracture, wedge or crush) or osteopenia were matched pair-wise with regard to age (difference < 5 years), years since menopause (YSM, difference < 5 years) and body mass index (BMI): weight (kg)/height(m^2) (difference < 3.0). Osteopenia (5 patients) was defined as a lumbar BMD value (by DPA) of > 2 SD below the normal mean of age-matched controls (25). After informed consent had been obtained the patients were randomized pair-wise to receive either hormonal replacement therapy (HRT) or HRT plus nandrolone decanoate (ND, Deca-Durabolin (R), Organon International BV, Oss, The Netherlands).

HRT was given as estradiol valerate (Progynova (R), Schering-Nederland BV, Weesp, The Netherlands) 2 mg orally daily on day 1 through 25 each month and medroxyprogesterone acetate (Provera (R), Upjohn-Nederland, Ede, The Netherlands) 10 mg orally daily on day 16 through 25 each month. ND was administered intramuscularly in a dosage of 50 mg once every 4 weeks. When needed according to the dietary history calcium suppletion up to a total intake of at least 1000 mg was given in the form of Ca Sandoz effervescent tablets (n = 28). Signed informed consent was obtained as well as permission by the Universital Hospital Medical Ethical Committee.

Treatment was single blinded, i.e. the biochemical data, the radiographs (vertebral deformity) and the bone densitometric data were all coded.

Initially 42 patients were randomized to enter the study. Seven of these patients wanted to terminate their participation in the trial after 6 to 21 months (only one of them being replaced), so that for evaluation after 2 years treatment the data of 36 patients are available. The characteristics of both treatment groups of patients have been listed in Table I. Five of the 7 patients who stopped treatment did so because of voice problems, 2 because of the extent of uterine bleeding. In all patients the diagnosis osteoporosis (osteopenia) was confirmed by biochemistry and iliac crest biopsy.

B. Bone mineral mass measurements.

1. SPA of the right forearm was performed at the (ultra)distal and proximal site with intervals of 3 months according to Nilas et al.[26] with a Nuclear Data 1100a scanner. The (fat-corrected) results are expressed as bone mineral content (BMC, arbitrary units (U)/cm) and bone mineral density (BMD, U/cm^2). In our hands the coefficient of variation (C.V.) for BMD in normal individuals is 1.9 at the distal site and 1.0% at the proximal site. During the first 18 months of the study a calibration-phantom was scanned daily. A slight drift of -1.8%

was observed. Subsequently a new software version including a master calibration was installed. This calibration was run weekly. No drift was seen any more: at yearly intervals the BMC of the phantom amounted to 37.6, 37.5 and 37.6 U/cm, respectively.

2. DPA of the 2nd through 4th lumbar vertebrae was done at 3 months intervals according to Krolner and Nielsen[27] with a NOVO BMC-Lab 22a scanner. Results are expressed as BMC (g hydroxyapatite = HA) and BMD (g HA/cm^2). For BMD in osteoporotic women a C.V. of 2.3% was found. During the study three ^{153}Gd-sources have been used. There were no software changes. Immediately before and after a source change a calibration-phantom (supplied by the manufacturer) consisting of 3 cylindrical chambers with diameters of 1, 3 and 5 cm and containing a mineral equivalent solution (402 mg K_2HPO_4/ml, equivalent to 398 mg HA/ml) was measured. Daily quality control was performed by scanning an aluminum tube supplied by the manufacturer. No drift of the BMC value of the aluminum tube was observed. With the three sources the BMC value of the tube was 32.6 $\pm$ 0.2 (SD), 32.5 $\pm$ 0.2 and 32.7 $\pm$ 0.2 g HA equivalent, respectively.

3. QCT of the trabecular (cancellous) compartment of the 2nd and 3rd lumbar vertebrae was carried out at intervals of 6 months with the method we previously described as QCT_{trab}[28]. Scans were made at 120 kVP. A simultaneous calibration device was used containing different concentrations of K_2HPO_4 in water as bone mimicking material. The CT-system was calibrated before each session according to the recommendations of the manufacturer. The results are expressed as mg K_2HPO_4 equivalent/ml. In osteoporotics the C.V. for the cancellous bone compartment amounted to 2.7%[28]. Fractured vertebrae were not measured.

C. Radiological examination.

Standardized lateral view radiology of the thoracic and lumbar vertebral column (5th thoracic through 4th lumbar vertebrae) was performed yearly. The radiology of the lumbar vertebral column was carried out at long distance (2 m). Pre-existing and new vertebral fractures (deformities) were evaluated using as definitions: a) loss of anterior and/or mid-height of $\geq$ 20% of the posterior height of the vertebral body and b) loss of posterior height of $\geq$ 20% of the posterior height of both or one of the adjacent vertebral bodies. Heights were measured using a digital ruler. The coefficient of variation of the indices was $<$ 3%.

D. Biochemistry.

Every 3 months were determined:

1. Serum Ca, P, alkaline phosphatase, creatinine and albumin with standard methods.
2. Serum osteocalcin and procollagen type I with RIA (INC, Stillwater, USA and Formos Diagnostica, Oulunsalo, Finland, respectively) and
3. Fasting (2 hr) urinary hydroxyproline by Hypronosticon (Organon Technika, Oss, the

Netherlands). The data obtained were corrected for the creatinine clearance (glomerular filtrate, GF) and expressed as nmol/100 ml GF.

At zero time and after 3 months:

4. 17ß-Estradiol by RIA (Diagnostic Products Corporation, Los Angeles, USA).

Every 6 months:

5. Serum total and HDL-cholesterol with standard methods.

All blood samples were taken in the non-fasting state.

E. Phoniatric investigations.

The patients were examined by the phoniatrician and the speech-pathologist of the Division of Phoniatrics of the Department of Otorhinolaryngology. These examinations were performed before and after one year of medication and consisted of a standard voice anamnesis, a logopaedic voice evaluation, observation of the vocal cord aspect and determination of the voice field, the maximum phonation time, voice breaks and pitch perturbation.

F. Statistics.

Within each of both treatment groups changes (either absolute or relative) over time have been tested by means of the Wilcoxon signed ranks test (two-sided). Between the two treatment groups absolute values and percent changes from baseline have been compared and tested by means of the Mann-Whitney U-test (two-sided).

RESULTS

1. Forearm densitometry (SPA).

At both sites in the forearm the initial (fat-corrected) BMC values by SPA of the HRT- and the HRT + ND-group (group 1 and 2, respectively) were practically identical (Table 6.1). The course of the proximal and distal forearm BMC during treatment is presented in Fig. 6.1 A and B. In group 1 the BMC rose proximally 2.2% over the first year and 2.8% over 2 years ($P < .02$ and $= .005$, respectively). In group 2 the corresponding numbers were +3.2 and +4.5% ($P = .001$ and $< .001$, respectively). At the distal site the rise of the BMC consisted in group 1 of 3.1% over the first year and 4.4% over 2 years (n.s. and $P = .02$, respectively) and in group 2 of 2.3 and 4.5% (n.s. and $P = .002$, respectively). At both sites the BMC (and BMD; data not shown) increments observed after 1 and 2 years of treatment were not significantly different in both treatment groups.

Table 6.1 *Pretreatment patients characteristics*

	HRT (1)	HRT + ND (2)	*P-value*
n	18	18	
n with vertebral fractures	15	16	
Age (yrs, ± S.D.)	60.7 ± 3.9	62.6 ± 4.2	n.s.
YSM (yrs, ± S.D.)	13.7 ± 6.2	14.2 ± 7.7	n.s.
BMI (kg/m^2, ± S.D.)	24.8 ± 2.9	25.5 ± 4.9	n.s.
Bone densitometry:			
BMC forearm (SPA)			
prox. (U/cm)	32.4 ± 1.2	32.1 ± 1.5	n.s.
distal (U/cm)	31.6 ± 1.2	31.0 ± 1.5	n.s.
L2-L4 (DPA):			
BMC (gHA)	26.5 ± 1.4	28.8 ± 1.6	n.s.
BMD (gHA/cm^2)	0.62 ± 0.02	0.64 ± 0.03	n.s.
BMD L3 (QCT)			
(mg K_2HPO_4/ml)	44.3 ± 6.1	42.1 ± 4.2	n.s.
Biochemistry:			
Serum:			
Alkaline phosphatase (U/l)	68.2 ± 6.8	64.8 ± 4.9	n.s.
Osteocalcin	4.0 ± 0.4	4.1 ± 0.5	n.s.
Procollagen	115.6 ± 10.6	111.9 ± 10.3	n.s.
Urinary (fasting 2h):			
Hydroxyproline (nmol/100 ml GF)	15.2 ± 4.4	16.8 ± 7.0	n.s.

YSM: years since menopause
HRT: hormonal replacement therapy (estrogen + progestagen)
ND: nandrolone-decanoate

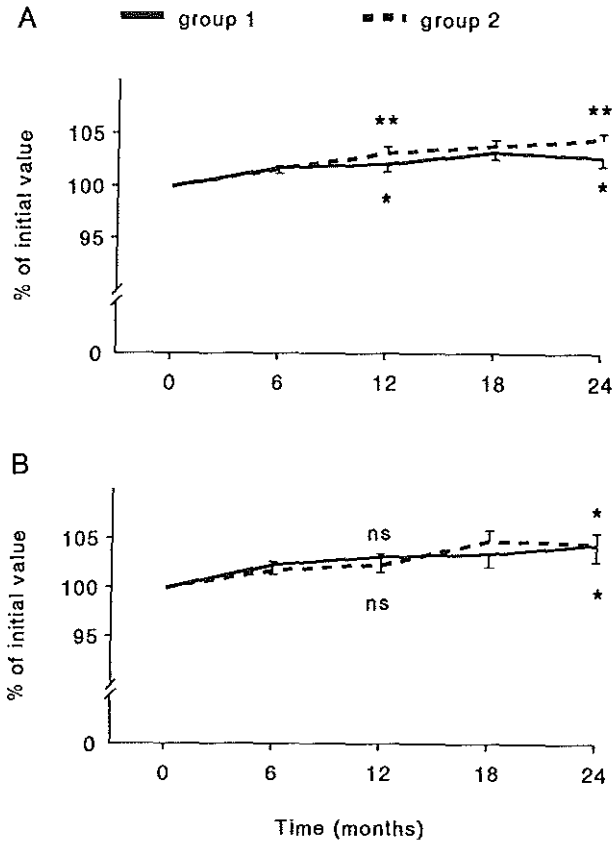

Figure 6.1 *Course of the BMC (by SPA) of the forearm (U/cm). Group 1: cyclical treatment with estrogen and progestagen; group 2: cyclical treatment with estrogen and progestagen, combined with nandrolone (both groups n = 18). Means ± S.E.M. A: Proximal site, B: Distal site. Statistical analysis was performed at 12 and 24 months.*

2. Lumbar spine densitometry.

DPA.

The initial BMC and BMD values of the 2nd through 4th lumbar vertebrae were not different in the 2 groups (Table 6.1).

The effect of treatment on lumbar BMC is depicted in Fig. 6.2. In group 1 the BMC increased over the first year by 9.9 and over 2 years by 12.5% ($P < .001$ for both), while in treatment group 2 the respective increments were 9.2 and 12.2% ($P = .001$ and $< .001$,

respectively). The course of the BMC (and of the BMD; data not shown) was in both treatment groups not significantly different.

QCT.

The basal BMD values of L_3 (n = 16 in both treatment groups, Fig. 6.3) were not significantly different between in group 1 and 2. It rose in 6 months by 21.0 and 29.0% (*P = .002* and *.001*), respectively, to stay at about that level for the duration of treatment. Again, there was no significant difference between the extent of the increase of the QCT of L_3 (and of L_2; data not shown) in both treatment groups.

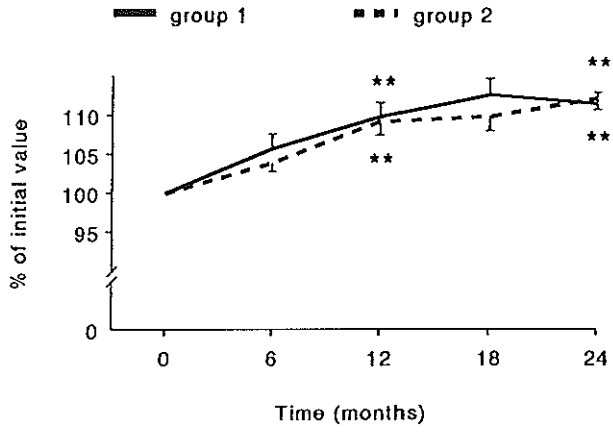

Figure 6.2 *Course of the BMC (by DPA) of the 2nd through 4th lumbar vertebral body (g hydroxyapatite/cm²). See also the legend to Fig. 6.1.*

3. Vertebral fractures (deformities).

Using the standardized radiographs of the 15 patients of group 1 and 16 patients of group 2, who initially had vertebral compression fractures, the average number of deformed vertebral bodies (for definition see Methods, section C) per patient was before treatment 3.53 in group 1 and 2.81 in group 2. After 2 years treatment the corresponding numbers were 3.73 and 3.13 (n.s. within both groups). None of the 5 patients initially without vertebral fractures showed evidence of subsequent compression.

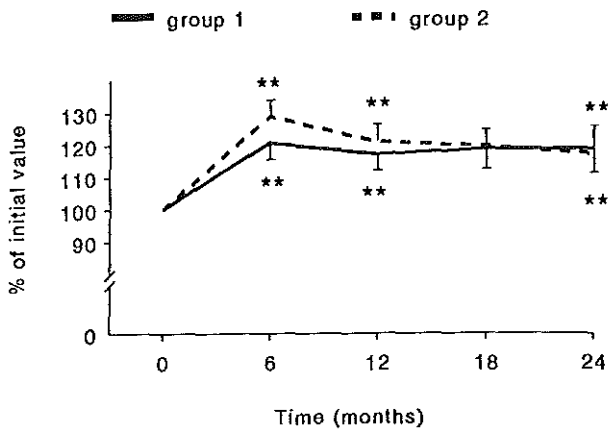

Figure 6.3 *Course of the cancellous BMD (by QCT) of the 3rd lumbar vertebral body (mg K_2HPO_4/ml). See also the legend to Fig. 6.1. Group 1: n = 14, group 2: n = 15. Statistical analysis was performed at 6, 12 and 24 months.*

4. Biochemical investigations.

In both treatment groups the serum Ca level (group 1 2.39 ± 0.02 (S.E.M.) and in group 2 2.36 ± 0.02 mM) did not change significantly. Serum P, however, fell in both groups: in group 1 from 1.20 ± 0.03 to 1.06 ± 0.03 mM as measured after 6 months and in group 2from 1.17 ± 0.04 to 0.95 ± 0.03 mM after 3 months ($P < .001$ in both instances). In group 1 the P level was after 3, 12, 18 and 24 months significantly higher than in group 2. In group 1 serum creatinine (68.0 ± 2.7 μM) did not change over the study period, whereas in group 2 it rose from 72.2 ± 2.1 to 80.3 ± 1.9 after 3 and to 85.5 ± 2.5 μM after 12 months ($P \leq .001$) to subsequently stay at that level. In the two groups of patients the initial average values of serum alkaline phosphatase, osteocalcin and procollagen I and the fasting urinary hydroxyproline were not significantly different (Table 6.1). In both groups an identical decrease (of 23 %) of the serum alkaline phosphatase was seen, that was complete after 6 months of treatment ($P \leq .002$, Fig. 6.4A). Over the same period serum osteocalcin fell by 28% in group 1 and 27% in group 2, while the maximal decreases were 35 and 44% after 12-24 months (Fig. 6.4B; $P < .01$). Serum procollagen type I decreased by 22 and

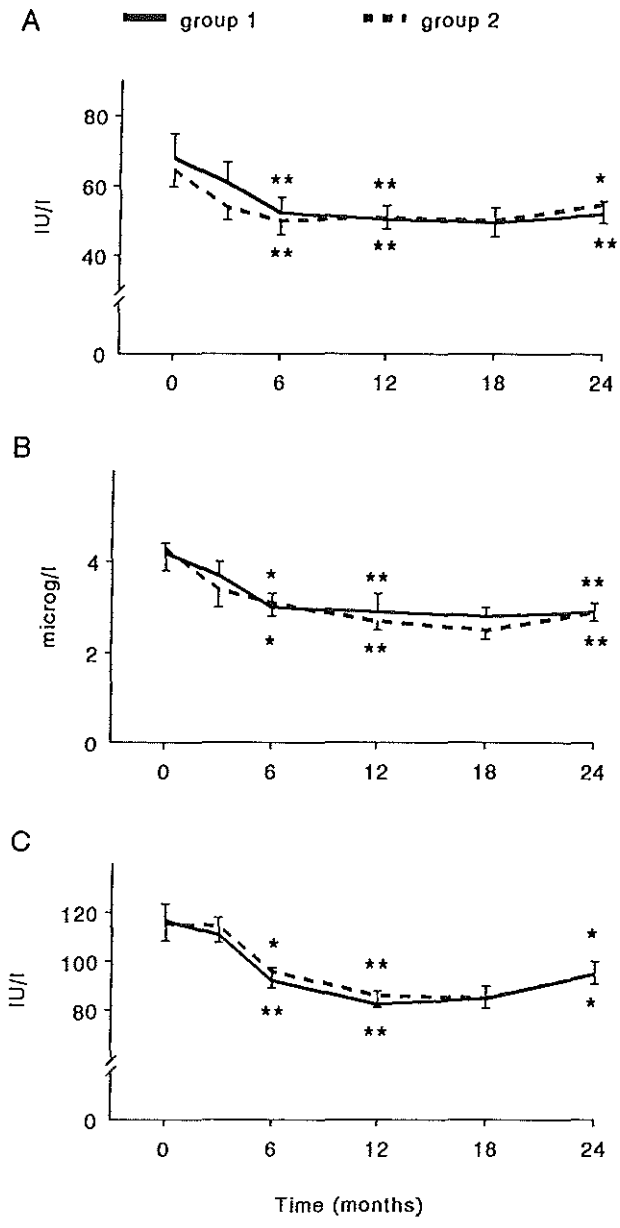

Figure 6.4 *Course of serum alkaline phosphatase (A), osteocalcin (B) and procollagen I (C) during cyclical treatment with estrogen and progestagen (group 1) and cyclical treatment with estrogen and progestagen, combined with nandrolone (group 2). All groups n = 18, except for the groups of C: n = 17. Means ± S.E.M. Statistical analysis was performed at 6, 12 and 24 months.*

15% in group 1 and 2, respectively, after 6 months treatment (Fig. 6.4C; $P < .001$ and $.05$, respectively). Of the 2 hour fasting urinary hydroxyproline a similar fall was observed in both groups, that was maximal at 12 months: minus 36% in group 1 and minus 33% in group 2 (Fig. 6.5; $P < .01$).

Before treatment the average serum estradiol level was in group 1 and 2 33.6 ± 4.6 (S.E.M.) pM and 34.1 ± 5.9 pM, respectively. After 3 months of treatment with 2 mg estradiol valerate the estradiol concentration had risen to 264 ± 37.5 pM and 250 ± 60.6 pM, respectively.

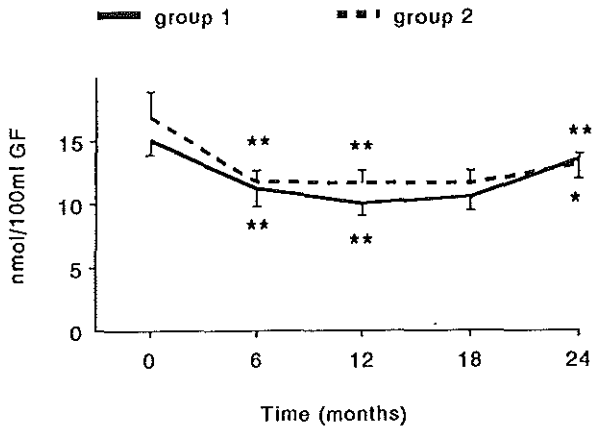

Figure 6.5 *Course of 2h fasting urinary hydroxyproline, corrected for glomerular filtration during cyclical treatment with estrogen and progestagen (group 1) and cyclical treatment with estrogen and progestagen, combined with nandrolone (group 2). Both groups n = 18. Means ± S.E.M. Statistical analysis was performed at 6, 12 and 24 months.*

5. Adverse effects.

A. Phoniatric evaluation.

After one year of medication a considerably higher percentage of patients of group 2 as compared to group 1 complained of change of voice timbre, voice unsteadiness, voice

lowering and loss of high frequencies (Table 6.2). Logopedic evaluation revealed a significantly higher percentage of patients in group 2 than in group 1 with an increase of voice creakiness and voice instability (Table 6.2). Likewise, with voice field measurements a significantly lower mean frequency during speech and lower highest frequency was observed in group 2. With regard to the vocal cord aspect, maximum phonation time, voice breaks and pitch perturbation no significant differences between the 2 groups were found. The results of the phoniatric studies will be reported more extensively elsewhere.

B. Serum total and HDL-cholesterol.

Because it was not possible to obtain fasting blood samples on follow-up, plasma triglyceride levels could not be taken into account. In group 1 serum total cholesterol showed a decrease from 6.49 ± 1.39 (S.D.) to 5.91 ± 1.06 mM ($P < .01$) after 6 months. In group 2 no change was seen: 6.42 ± 1.31 and 6.35 ± 1.69 mM.

In contrast, HDL-cholesterol was unchanged after 6 months treatment with oestrogen-progestagen-cycles (1.45 ± 0.49 and 1.42 ± 0.43 mM), but fell in the patients who were additionally treated with nandrolone decanoate: from 1.38 ± 0.29 to 1.22 ± 0.18 mM ($P < .01$).

C. Other.

Liver enzymes did not change in the 2 groups. There were also no complaints or signs of increased hair growth, facial or elsewhere.

Table 6.2 *Phoniatric evaluation after 1 year treatment*

	HRT (1)	HRT + ND (2)	*P-value*
n	17	22	
voice timbre change %	6	64	< .01
voice unsteadiness %	0	52	< .01
voice lowering %	12	86	< .01
loss of high frequencies %	18	62	< .01
increase voice creakiness %	0	55	< .01
voice instability %	0	55	< .01
mean frequency Hz	222	195	< .01
highest frequency Hz	731	505	< .01

DISCUSSION

In this study HRT induced an impressive increase in both trabecular and cortical bone mineral mass. This increase was not enhanced by the addition of nandrolone decanoate to the treatment. Most of the gain in bone mineral mass during HRT was seen during the first year. The increase in bone mineral mass and density we found during two years of estrogen-progestagen-treatment at the forearm and at the lumbar vertebrae (by DPA; up to 12%) is comparable to the recently reported effect on bone mass of continuous estrogen-progestagen-treatment of proven postmenopausal osteoporosis[12]. At these sites and with these methods this rise appears to be larger than that regularly seen in normal early postmenopausal women treated with estrogen or a combination of estrogen and progestagen[3-5]. We did not include an untreated control group. The rise in lumbar BMC we observed could be compared to a loss of lumbar BMC of about 4% over 2 years in untreated established postmenopausal osteoporosis[29]. The increment of the lumbar bone mineral mass seen in both our treatment groups is also larger than that reported by Storm et al. to occur over 1-2 years in osteoporotic women treated with the antiresorptively active bisphosphonate etidronate[29]. In contrast to HRT etidronate did not induce an increase in forearm BMC. In this respect it may be relevant that - in contrast to for instance etidronate[29] and PTH (1-34) or (1-38)[30,31] - HRT induces an increase of appendicular as well as axial bone mass.

Preliminary histomorphometric results obtained in these patients after 2 years treatment have been reported[32]. In both groups cortical thickness rose significantly, while trabecular bone volume did not change. There was no significant difference between the course in both groups. These results do not contradict those reported here, as it has been shown that the correlation between the histomorphometrically estimated trabecular bone volume in the iliac crest and the results of bone mineral mass measurements elsewhere is not high[33].

The observation of a faster and more extensive rise of the lumbar BMD measured by QCT as compared to that measured by DPA might be explained by the fact that with QCT the trabecular (cancellous) compartment of the vertebral body is selectively measured. Secondly, the results of single energy QCT may be greatly influenced by the bone marrow fat content[34,35] in that sense that a reduction in the proportion of fat marrow could show up as an increase in BMD. Conceivably the hormonal intervention may have led to such a reduction. However, one would then have expected a significantly larger (apparent) increase of the QCT values in the patients additionally treated with nandrolone. Furthermore, the increases of the trabecular BMD of 21% and 29% in the HRT and the HRT + ND group, respectively, are too high to be largely ascribed to marrow fat loss.

The fall in biochemical parameters of bone resorption (fasting urinary hydroxyproline) and of osteoblastic activity (serum alkaline phosphatase, osteocalcin and procollagen type I) was

also comparable in both treatment groups. As expected, the decrease of urinary hydroxyproline was faster and tended to be larger than that of the other parameters. Geusens and Dequeker[19], treating osteoporotic patients with nandrolone only, saw a considerable and persisting lowering of the urinary excretion of hydroxyproline.

The fact that an anabolic agent like nandrolone over 2 years does not add to the effect on bone mass of an estrogen-progestagen cyclic substitution scheme that as to dose level is considered to be sufficiently high to prevent bone loss[36] and that led to serum estradiol values within the normal preovulatory range, indicates that the anabolic agent acts mainly by inhibiting bone resorption and that bone resorption is already maximally inhibited by the estrogen-progestagen combination at the dose level used. An alternative explanation would be an interaction between nandrolone and estradiol, for example at the receptor level. We have also to consider the possible anabolic properties of medroxyprogesterone. One cannot, however, expect a major effect of the dosage used on bone mineral mass, especially in the spine[37]. Based on the 90% confidence limits of our data an additional effect of nandrolone of up to 3.5% for lumbar BMC or up to 2.5% for lumbar BMD cannot be excluded (Type II error). For the forearm BMC this would amount to zero proximally and 3% distally. With nandrolone monotherapy other investigators found an increase of forearm BMC of 4-5% over 1-2 years[18,19].

In established osteoporosis a vertebral fracture incidence of 35-54 per 100 patient-years has been reported[29]. In 31 patients about 25 new vertebral deformities would have been expected in 2 years. In both groups the average number of deformed vertebrae per patient observed before and after 2 years treatment did not show a significant increase. Further compression of already deformed vertebrae has not been taken into account, but given the limited number of patients studied and the relatively short follow-up we do not want to attach too much importance to these fracture data in contrast to the results of the bone mass measurements.

Adverse effects mainly concerned voice changes that occurred in the great majority of the nandrolone treated women and that apparently were not prevented by the simultaneous treatment with estrogen. The possible reversibility of voice changes is still under investigation. With regard to the lipoprotein profile nandrolone neutralized the favorable effect of the estrogen-progestagen cyclic treatment on total cholesterol, while a slight reduction in HDL-cholesterol was induced by this steroid. It is clear that these adverse effects need consideration, whether nandrolone is given as mono- or as adjuvant therapy.

Acknowledgments.

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CHAPTER 7

THE COURSE OF BONE MASS DURING AND AFTER HORMONAL REPLACEMENT THERAPY WITH AND WITHOUT ADDITION OF NANDROLONE DECANOATE.

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SUMMARY

Thirty three women with postmenopausal osteoporosis were matched pair-wise as to age, years since menopause and body mass index and randomized to receive either cyclical estrogen-progestagen replacement treatment (group 1) or the same treatment plus nandrolone decanoate (ND) (group 2). Both groups were treated during 3 years and subsequently followed for another year off treatment.

One year after cessation of the treatment the distal forearm bone mineral content was in group 2 significantly higher than in group 1. Bone mass measurements in the axial skeleton already showed a significant difference in favour of group 2 after 3 years treatment, which persisted during the year off treatment. The decline in lumbar bone mineral mass and density in the one year off treatment was similar in both groups. Correction for body mass did not change these results. Bone turnover parameters did not show significant differences between the two groups after cessation of treatment. A higher muscle mass, induced by ND, could partly explain the differences between the groups since even one year after treatment was stopped an increased serum creatinine level was still observed in group 2.

INTRODUCTION

There are many prospective studies which clearly demonstrate that estrogen deprivation causes an increased bone loss, which can be prevented by estrogens[1-5]. In addition epidemiological studies indicate that hormone replacement therapy (HRT) reduces the incidence of osteoporotic fractures[6-8].

Also in patients with established osteoporosis beneficial effects of HRT have been reported. Previously we and others[9-11] showed that in osteoporotic women treatment with HRT for 1-2 years resulted in a considerable increase in both appendicular and axial bone mineral mass. Furthermore, we observed that combined treatment of HRT plus nandrolone decanoate (ND) did not result in a further rise of bone mass, as measured after 2 years. Other studies have demonstrated that treatment with anabolic steroids only can increase bone mass in women with established osteoporosis[12-15].

To obtain more insight in the longterm effects of HRT versus HRT plus ND we extended our previous observations (9) up to 3 years. This study intended to investigate the possible potency of anabolic steroids to stimulate bone formation rather than inhibit bone resorption (or stimulate bone formation in addition to inhibiting bone resorption) and thus break through the coupling of bone formation to bone resorption.

Because data on the course of bone mass measurements and of bone turnover parameters

after cessation of these treatment modalities are currently not available we also followed these parameters during the first year off treatment.

METHODS AND MATERIALS

A. Patients and treatment.

Women, aged 50-70 years, with postmenopausal osteoporosis (at least one non-traumatic vertebral compression fracture, wedge or crush) or osteopenia were matched pair-wise with regard to age (difference $\leq$ 5 years), years since menopause (YSM, difference $\leq$ 5 years) and body mass index (BMI): weight (kg)/height (m^2) (difference $\leq$ 3.0). Osteopenia (5 patients) was defined as a lumbar BMD value (by DPA) of $\geq$ 2 SD below the normal mean of age-matched controls. The patients were randomized pair-wise after informed consent had been obtained to receive either hormonal replacement therapy (HRT) or HRT plus nandrolone-decanoate (ND, Deca-Durabolin^R, Organon International BV, Oss, The Netherlands).

HRT was given as estradiol valerate (Progynova^R, Schering-Nederland BV, Weesp, The Netherlands) 2 mg orally, day 1 - 25 of each month and medroxyprogesterone acetate (Provera^R, Upjohn-Nederland, Ede, The Netherlands) 10 mg orally, day 16 - 25 of each month. ND was administered intramuscularly in a dosage of 50 mg once every 4 weeks. When needed according to the dietary history calcium suppletion up to a total of at least 1000 mg in the form of a Ca-Sandoz^R effervescent tablet containing 0.5 g of elementary calcium was given (n=28). Compliance was measured by means of tablet counting. Treatment was single blinded, i.e. the biochemical data and the bone densitometric data were all coded.

Initially 42 patients were randomized to enter the study.

Nine of these patients wanted to terminate their participation in the trial after 6 to 33 months (only one of them replaced), so that for evaluation after 3 years treatment the data of 34 patients are available. Five of the 9 patients who stopped treatment did so because of voice problems (all five received HRT + ND), 2 because of extent of uterine bleeding (HRT), one moved elsewhere (HRT) and one died of myocardial infarction (HRT). In the fourth year one patient started again with therapy because of depression after stopping HRT. The remaining 33 patients (group 1; n=16, group 2; n=17) could be followed for one year after the treatment was stopped. The initial average age and YSM were 60.6 $\pm$ 4.1 (S.D.) and 14.0 $\pm$ 6.4 years, respectively, in the HRT- and 61.9 $\pm$ 4.4 and 14.1 $\pm$ 6.3 years, respectively in the HRT + ND group.

In all patients the diagnosis of primary osteoporosis was confirmed by biochemistry and iliac crest biopsy.

B. Bone mineral mass measurements.

1. SPA of the right forearm was performed at the (ultra)distal and proximal sites with intervals of 3 months according to Nilas et al.[16] with a Nuclear Data 1100a scanner. The (fat-corrected) results are expressed as bone mineral content (BMC, arbitrary units (U)/cm). The coefficient of variation in normal subjects in our hands is 1.9 % for the distal site and 1.0 % for the proximal site[17].

2. DPA of the 2nd through 4th lumbar vertebrae was done every 3 months according to Krolner and Nielsen[18] with a NOVO BMC-Lab 22a scanner. Results are expressed as BMC in g hydroxyapatite (HA). The coefficient of variation in osteoporotic women in our laboratory is 2.3 %[17].

3. Single energy QCT of the trabecular (cancellous) compartment of the 2nd and 3rd lumbar vertebrae was carried out at intervals of 6 months with the method we previously described as QCT_{trab}[17]. Scans were made at 120 kVP. The results are expressed as bone mineral density (BMD, mg K_2HPO_4 equivalent/ml). The coefficient of variation in twenty osteoporotic women in our laboratory is 2.7 %[17]. Fractured vertebrae were not measured.

C. Radiogrammetry of the thoracic and lumbar vertebrae.

New fractures were defined as a reduction of 15 % or more of the anterior or mid vertebral height as compared to the posterior vertebral height.

D. Biochemistry.

Every 3 months the following parameters were determined:

1. Serum Ca, P, alkaline phosphatase, creatinine, albumin and haemoglobin with standard methods.
2. Serum osteocalcin and procollagen type I with RIA (INC, Stillwater, USA and Formos Diagnostica, Oulunsalo, Finland, respectively) and
3. Fasting (2 hr) urinary hydroxyproline by Hypronosticon (Organon Technika, Oss, The Netherlands) and Ca by a standard method. The hydroxyproline data obtained were expressed as nmol/100ml glomerular filtrate (GF).
4. Every six months serum total and HDL-cholesterol were determined by standard methods in the non-fasting state.

E. Statistics.

Within each of both treatment groups changes (either absolute or relative) over time have been tested by means of Wilcoxon signed ranks test (two-sided). Between the two treatment groups absolute values and percent changes from baseline have been compared and tested by

means of the Mann-Whitney U-test (two sided).

RESULTS

Bone mass measurements.

The changes with time within the treatment groups on and off treatment are shown in Fig. 7.1 A and B for the forearm (SPA) and 7.2 and 7.3 for lumbar spine (DPA and QCT, respectively). The differences within the two groups after 36 months *vs* 24 months were only significant ($P < .05$) for the QCT-measurement in group 1. The statistical analysis of the differences between the two groups at the time points 1,2,3 and 4 years (QCT, because of technical reasons at 3 1/2 instead of 4 years) are given in Table 7.1.
Correction of the bone mass measurements for body mass index (BMI) did not change these results.

Table 7.1 *Statistical differences between treatment groups*

	1	2	3	3 1/2	4 years
SPA prox	N.S.	N.S	N.S.		N.S.
SPA dist	N.S.	N.S.	N.S.		.05
DPA	N.S.	N.S.	.05		<.05
QCT	N.S.	N.S.	<.05	.05	
Creatinine	<.001	<.001	<.001		<.01
Hemoglobin	<.001	<.001	<.001		N.S.

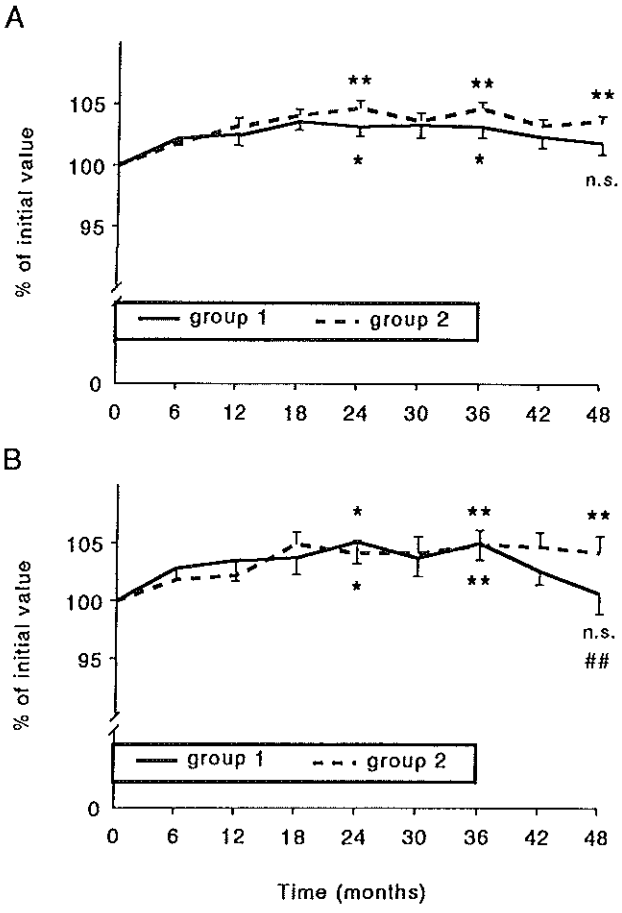

Figure 7.1 *Course of the BMC (by SPA) of the forearm (U/cm). Group 1 (n=16): Cyclical treatment with estrogen and progestagen; Group 2 (n=17): Cyclical treatment with estrogen and progestagen, combined with nandrolone decanoate. Treatment for both groups discontinued after 36 months. Means ± S.E.M. A:Proximal site, B: Distal site.*
*Statistical analysis was performed after 24,36 and 48 months vs t=0: * P < .05, ** P < .01, *** P < .001. T=48 months vs t=36 months: # P < .05, ## P < .01 and ### P < .001.*

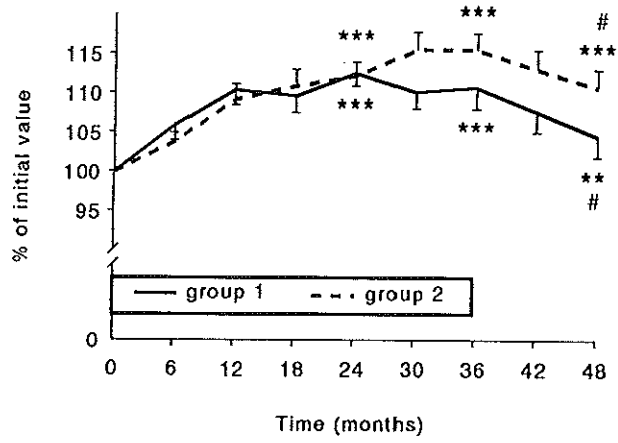

Figure 7.2 *Course of the BMC (by DPA), of the 2nd through 4th lumbar vertebral body. See also the legend to Fig. 7.1.*

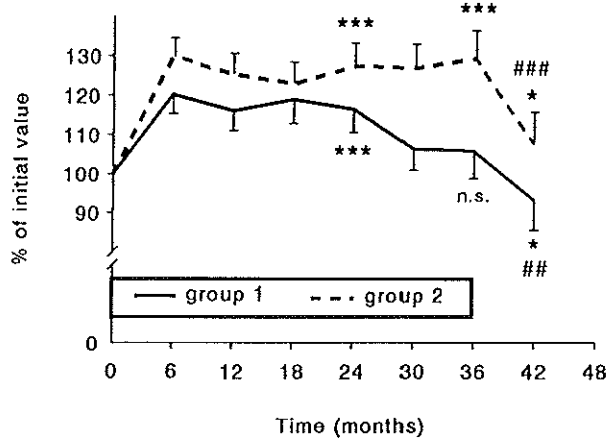

Figure 7.3 *Course of the BMD of the cancellous bone (by QCT) of the 3rd lumbar vertebral body. Group 1: n=13, group 2: n=14. Statistical analysis was performed after 24,36 and 42 months vs t=0: * P < .05, ** P < .01, *** P < .001.*
T=42 months vs t=36 months: ## P < .01 and ### P < .001.

Biochemical investigations.

Serum Ca was initially not different between both groups and in neither group a significance change in serum Ca was observed. The changes over time within the treatment groups of serum alkaline phosphatase, osteocalcin, procollagen type I and urinary hydroxyproline are depicted in Fig. 7.4 and 7.5. There were no statistical differences at the various time points between both groups. Serum creatinine was unchanged in group 1 during the total study, while in group 2 it showed an increase from 71.7 $\pm$ 2.2 to 80.0 $\pm$ 2.1 umol/l after 3 months and 88.1 $\pm$ 2.6 umol/l after 3 years of treatment and a decline to 80.2 $\pm$ 2.3 umol/l one year after termination of the treatment (*P* t=3yrs *vs* t=0 $<$ *.01*, *P* t=4yrs *vs* t=3yrs $<$ *.05*; Fig. 7.6).

In group 1 the hemoglobin level (8.3 $\pm$(S.E.M.) 0.2 mmol/l) also did not change during treatment, whereas in group 2 a rise was seen that amounted to 16 % after 3 years ($p < .001$) with a rapid decline until one year after the end of the treatment (*P vs* t=0 NS, *P vs* t=3yrs $<$ *.001*; Fig. 7.7).

Both for hemoglobin and serum creatinine the values in group 2 were significantly higher than in group 1 from 3 months onwards. At 4 years this was the case with regard to creatinine only (Table 7.1).

Vertebral fractures.

Over the third year of observation 1 new vertebral fracture has been observed only in group 1, while after the end of treatment in both groups 2 new fractures have been found (NS). In the terms we previously reported before and after 2 years treatment[9], this would for group 1 amount to 3.73 fractures per patient after 2 years and 3.91 after 4 years and for group 2 3.13 and 3.25 respectively.

Compliance.

No differences between the two groups were observed by means of tablet counting. The nandrolone decanoate administration was performed by the general practitioner. The patients attended to all scheduled hospital visits within one week. The patients also reported monthly withdrawal bleedings, except for five women who had undergone a hysterectomy.

The administration of nandrolone decanoate did not influence the serum estradiol levels: after 3 months of treatment estradiol had risen from 33.6 $\pm$ 4.6 (S.E.M.) to 264 $\pm$ 37.5 pM in group 1 and from 34.1 $\pm$ 5.9 to 250 $\pm$ 60.6 pM in group 2.

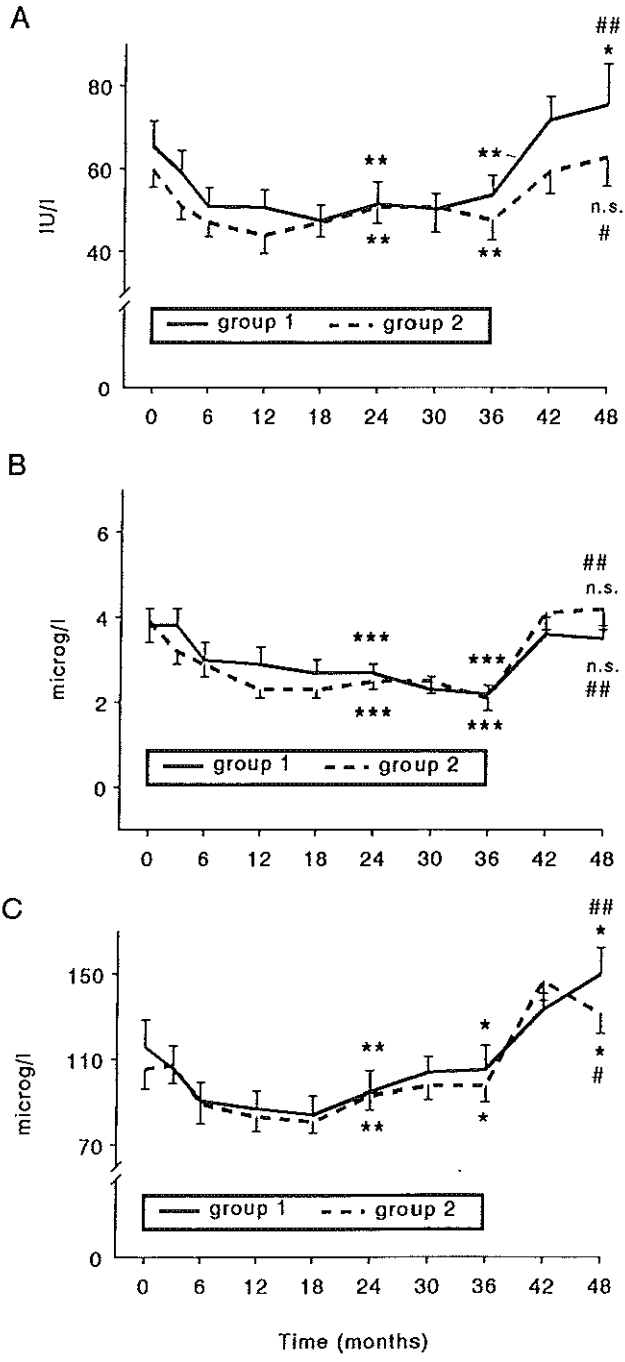

Figure 7.4 *Course of serum alkaline phosphatase (A), osteocalcin (B) and procollagen I (C) during the treatment period and one year after termination of the treatment. Group 1: n=16, group 2: n=17. See also the legend to Fig. 7.1.*

Adverse effects.

Phoniatric evaluation. As reported previously[9] a considerably higher percentage of the patients in group 2 as compared to group 1 had subjective and objective voice changes: For example, voice timbre changes, loss of high frequency and voice instability were found in around 60 % of the patients in group 2 and in 0-18 % of group 1. Elsewhere these changes are reported extensively[19]. The reversibility of the voice changes is still under investigation. No complaints or signs of increased hair growth, facial or elsewhere were mentioned. We saw no effects on the plasma level of liver enzymes.

Serum total and HDL-cholesterol. In group 1 serum total cholesterol fell from 6.39 ± 1.31 (S.E.M.) to 5.86 ± 1.14 mM ($P < .01$), 6.07 ± 1.06 and 6.24 ± 1.29 mM after 6 months, 1 and 3 years respectively. No change of total cholesterol was seen in group 2: initial value 6.86 ± 1.34 and 6.88 ± 1.72, 6.68 ± 1.06 and 6.36 ± 0.70 mM after 6 months, 1 and 3 years respectively.

HDL-cholesterol on the other hand did not show a consistent fall either in group 1: 1.61 ± 0.55 initially and 1.47 ± 0.48, 1.63 ± 0.65 and 1.25 ± 0.33 mM ($P < .01$) after 6 months, 1 and 3 years or in group 2: 1.37 ± 0.26 initially and 1.21 ± 0.18, 1.21 ± 0.16 and 1.06 ± 0.24 mM ($P < .01$) after 6 months, 1 and 3 years.

One year after terminating treatment total cholesterol amounted to 6.37 ± 1.10 mM in group 1 and 6.60 ± 1.01 mM in group 2. HDL-cholesterol again did not show a significant change.

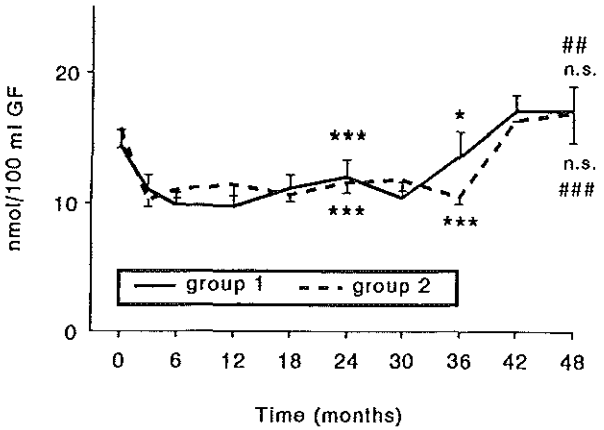

Figure 7.5 *Course of fasting 2h urinary hydroxyproline in both treatment groups. See also the legend to Fig. 7.1.*

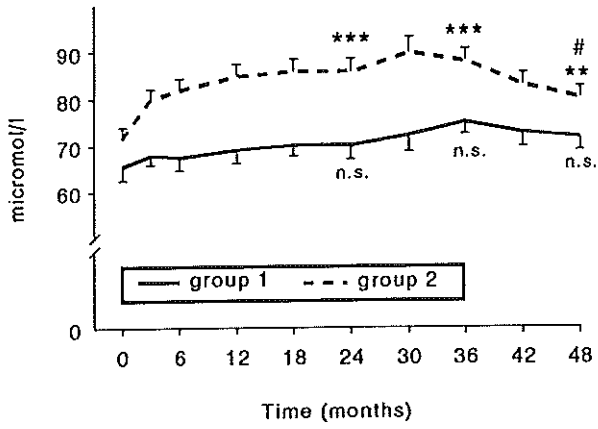

Figure 7.6 *Course of serum creatinine. Means ± S.E.M. See also the legend to Fig. 7.1.*

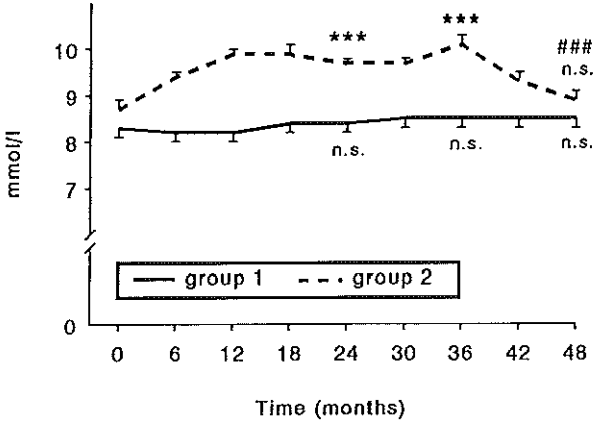

Figure 7.7 *Course of the hemoglobin level. See also the legend to Fig. 7.1.*

DISCUSSION

We previously described the effect of 2 years treatment of patients with postmenopausal osteoporosis with estrogen and progestagen (HRT) on bone mineral mass and biochemical parameters. No additional effect of nandrolone decanoate on bone mineral mass in the forearm and the lumbar vertebrae was observed[9]. The same groups were treated for another year in the same way. As compared to the initial values after 2 years treatment a gain of bone mineral mass at the forearm of up to 4.5 % and in the lumbar vertebrae (by DPA) of up to 12.5 % was observed, while QCT measurements showed a gain of the BMD up to 29 %. Our SPA and DPA values are comparable (not identical) with the effect of cyclical and continuous estrogen-progestagen treatment of proven postmenopausal osteoporosis[10,11].
As could be expected there was no further increment in the HRT-group during the third year of treatment. However, in the HRT + ND group a further increase for lumbar BMC (DPA) of 3.5 % was found resulting in a significant difference between both groups at the end of the third year of treatment (Table 7.1). Lumbar BMD (QCT) appeared to fall during the third year in group 1 only, also resulting in a significant difference between the groups after 3 years.
The favourable effect of additional treatment with ND on bone mass, as found after 3 years, is still present 1 year after cessation of treatment. Geusens et al. reported a maintenance of bone mineral mass in cortical bone after withdrawal of nandrolone decanoate in osteoporotic women[20]. In our study the rate of bone loss in the axial skeleton appears to be comparable in both groups. In other words, the beneficial effect of HRT + ND in the third year of treatment is not followed by a higher loss during the period off treatment. Although we did not observe significant differences between the biochemical parameters in both groups, the time course of especially serum alkaline phosphatase and urinary hydroxyproline showed in the HRT group a tendency towards a higher turnover during the third year. This might implicate that during this period the anti-resorptive action of HRT alone is less effective than in the HRT + ND group. This possibility is also compatible with the observation that the differences in bone mass measurements between both groups are primarily found at sites with a relatively high amount of cancellous bone. At the forearm bone loss was only observed at the distal site in the HRT group.
We also looked at the possibility whether the favourable effect of additional treatment with ND could be induced by indirect effects. A possible role for changes in body mass index seems unlikely, since correction of the bone mass measurements for BMI did not change our results. However, this does not exclude a possible influence of changes in muscle mass. It is well-known that anabolic steroids induce an increase in muscle mass [21,22], while it is also known that there exists a positive correlation between muscle strength and bone mass[23]. The

highly significant increase in serum creatinine in the HRT + ND group, compared to the HRT group, clearly indicates an increase in muscle mass. Therefore, the observed differences in the course of bone mass in the two groups could at least partly be explained by this effect of ND on muscle mass, although muscle strength was not measured in this study. Also we cannot rule out the possible effect of increased muscle mass on the activity level and thereby on the feeling of well being in the group treated with ND.

Finally we have to take into account that especially the lumbar QCT measurements could be influenced by changes in the bone marrow. In this respect the observed rise of hemoglobin in the HRt + ND group might indicate an increase in the amount of red marrow. Since single energy QCT, as used in our study, will show an apparent increase of BMD when the amount of red marrow increases[24], the QCT measurements have to be interpreted with some caution. However, if one compares the course of lumbar bone mass as obtained with DPA this phenomenon can not explain the differences in axial bone mass between the groups at the end of the third year of treatment.

There were no differences in compliance between the two treatment groups. We observed no influence of the ND on the estradiol levels. Furthermore, the estradiol dosage used is generally considered adequate, which was confirmed by the estradiol levels measured.

Taken together our study shows that treatment with HRT in women with postmenopausal osteoporosis will initially result in a considerable increase of both axial and appendicular bone mass. That the beneficial effects of additional treatment with ND is not apparent before the third year of treatment might indirectly indicate that during longterm treatment with HRT of patients with postmenopausal osteoporosis some escape will occur from the antiresorptive activity of HRT. Up to now such a partial escape has not been reported in long-term HRT[2,3], but this may be due to the fact that most long-term data have been obtained in normal postmenopausal women and not in patients with postmenopausal osteoporosis.

Acknowledgements.

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CHAPTER 8

TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS WITH A COMBINATION OF GROWTH HORMONE AND PAMIDRONATE. A PLACEBO-CONTROLLED TRIAL.

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SUMMARY

Objective.
It is known that growth hormone (GH) can induce accelerated bone turnover in GH deficient people as well as healthy elderly people. In this study we examined the effect of recombinant human GH (rhGH) on bone mineral mass and bone turnover in the presence of the bone resorption inhibiting agent, pamidronate. Also effects on body composition were studied.
Methods.
21 postmenopausal osteoporotic women were treated with the bisphosphonate pamidronate during twelve months. During the initial 6 months rhGH was administered (0.0675 IU/kg, 3 times/week) in a placebo-controlled fashion (10 vs 11 patients).
Measurements.
Bone mineral content (BMC) of the lumbar spine and femoral neck was measured with dual energy X-ray absorptiometry and BMC of the distal and proximal forearm with single photon absorptiometry. Body composition was measured with bioelectrical impedance and total body dual energy X-ray absorptiometry. Furthermore serum IGF-I and biochemical indices of bone turnover were measured.
Results.
The group treated with rhGH showed a two- to three-fold increase in serum IGF-I levels. No effects on bone mineral mass were observed in the group treated with rhGH, after the initial 6 months of treatment with rhGH nor after the total period of 12 months. In women treated with pamidronate, however, a consistent increase of about 5 % at the lumbar spine and somewhat lower in the distal forearm was reached from 6 months onwards. In both groups no change in BMC at the femoral neck and forearm was observed.
Compared to baseline the biochemical measurements of bone turnover showed a decrease of about 50 % in the pamidronate-treated group, whereas in the group additionally treated with rhGH this effect was blunted.
The body composition measurements showed clear effects of rhGH administration, i.e. a decrease of fat mass of about 5 % and an increase of lean body mass of about 3 %. However, these effects disappeared after the treatment with rhGH was stopped; fat mass as well as lean body mass returned to initial values.
Conclusions.
The present study suggests that treatment with rhGH blunted both the pamidronate-induced accumulation of bone mineral mass and the reduction of biochemical markers of bone turnover. Furthermore the positive effect of rhGH on body composition disappears completely after cessation of treatment with rhGH.

CHAPTER 8

INTRODUCTION

Several approaches for the medical treatment of postmenopausal osteoporosis are available at present. Among these are estrogens, bisphosphonates, anabolic steroids, calcitonin, fluoride and vitamin D preparations[1-7]. Most of these treatment schemes are effective by diminishing bone resorption, whereas only fluoride is able to increase osteoblastic activity[8,9]. Recently Heaney argued that much of the apparent gain in bone mass produced by several agents currently employed to treat osteoporosis can be explained by the so called "bone-remodelling transient"[10]. In other words the gain of bone induced by an inhibitor of bone resorption will only be transient because the associated rate of change in bone mass persists for only one remodelling period. A more fundamental change in bone mineral mass can only be obtained if a given drug can induce a change in the balance between formation and resorption at each individual remodelling locus.

Recent evidence suggests a role for growth hormone (GH) as an osteotrophic factor. It is known that GH is responsible for longitudinal bone growth through a direct stimulation of chondrocytes and osteoblasts[11,12]. In adults GH also activates bone remodelling[13,14], and probably diminishes bone loss as compared to controls[14]. Furthermore, several studies indicate that in GH deficient (GHD) adults treatment with recombinant human GH (rhGH) results in an increase of bone turnover parameters and after long-term treatment even a rise of bone mineral mass has been observed[15-18]. In addition studies of patients with endogenous high levels of GH (acromegaly) have shown a stimulated bone turnover and an increased bone mineral mass[19,20].

Normal aging and GHD show some striking common features. Both GHD as well as normal aging are associated with decreases in protein synthesis, decreases in percentages of lean body and bone mass as well as with increases in percentage body fat[21-26]. It is possible that reduced GH and IGF-I secretion may account, at least in part, for one or more of the above effects of aging[27,28] and that elderly individuals might benefit from treatment with rhGH. In several studies administration of rhGH for periods varying from a few weeks to twelve months, resulted in improvement of nitrogen balance, an increase in lean body mass, and a decrease in percentage body fat[14,29-31].

Very little is known about the effects of rhGH in the treatment of postmenopausal osteoporosis. In a small study in which GH was administered in sequence with calcitonin in women with postmenopausal osteoporosis a small favourable effect was observed[32]. In the present placebo-controlled study we tested whether rhGH might have an additional anabolic effect on bone formation in postmenopausal osteoporotic women treated with the bone resorption inhibitor pamidronate. Furthermore, the effects of rhGH on body composition were evaluated.

MATERIALS AND METHODS

Patients and treatment.

After review and approval of the protocol by the Ethical Committee of our institution, written informed consent was obtained from the patients. All 23 patients were caucasian postmenopausal osteoporotic women with at least one non-traumatic vertebral fracture (not the lumbar vertebrae 2 - 4). The presence of vertebral fractures was assessed by clinical reading. The basal number of vertebral fractures did not differ between both groups. Patients with any disorder known to affect bone mass were excluded from this study, as were women with a history of malignancy, as well as patients with a known history of diabetes mellitus or hypertension. All patients were treated with pamidronate. The patients were randomly assigned to receive either rhGH or placebo.

All patients received during 12 months pamidronate as an enteric-coated tablet, 150 mg per day, orally on an empty stomach. During the initial six months rhGH or placebo was self administered by means of subcutaneous injection of 0.0625 IU/kg with a maximum of 4 IU, 3 times per week (monday, wednesday, friday). In a pilot-study this dose resulted in high normal IGF-I levels. Treatment was double blinded with regard to rhGH or placebo. rhGH (Humatrope) and placebo was supplied by Eli Lilly Co. Indianapolis, IN., USA. Placebo vials were indistinguishable from rhGH vials.

In patients with a calcium intake below 1000 mg (according to dietary history), a Ca-Sandoz effervescent tablet containing 0.5 g elemental calcium was given. This applied to 5 patients in the GH-group and 6 patients in the placebo group. Compliance was measured by means of tablet and vial counting.

Bone mineral mass measurements.

Single photon absorptiometry (SPA) of the right forearm was performed at the distal and proximal sites with an interval of 3 months using a Nuclear Data 1100a scanner[33]. The (fat-corrected) results are expressed as bone mineral content (BMC; arbitrary units, U/cm). The coefficient of variation in our hands is 1.9 % for the distal site and 1.0 % for the proximal site.

Dual energy X-ray absorptiometry (DXA; Lunar DPX-L, Lunar Radiation, Madison, WI, USA) was performed at the lumbar vertebrae 2-4 (L2-4) and in the left proximal femur: the femoral neck, Ward's triangle and trochanteric region according to the instructions of the manufacturer[34]. BMC is expressed as grams of bone mineral (g/cm). For osteoporotic women the coefficient of variation is 1.5% for the lumbar spine and 1.8, 2.4 and 2.8% in the femoral neck, Ward's triangle and trochanteric region, respectively.

Quality assurance, including calibration with the standard of the machines (SPA and DXA),

was performed routinely every working day. No drift was observed during the whole study period.

Body composition.

Body composition was measured after an overnight fast by the bioelectrical impedance method (BIA) with a body composition analyzer (RJL-systems BIA-101 Detroit, MI, USA) as described previously[35,36]. Through regression equation, fat free mass (FFM) was calculated. Fat mass was calculated by subtracting FFM from body weight (Lukaski *et al.*, 1986).

Body composition was also measured by using the DPX-L densitometer. The instrument measures fat mass, lean mass and bone mineral content. The method is well validated[37].

Biochemistry.

At baseline and after 1, 3, 6, 9, and 12 months the following fasting serum parameters were determined:

* Calcium, phosphate, alkaline phosphatase, creatinine, albumin, glucose and glycosylated hemoglobin (HbA_1C) with standard methods.
* Insulin-like growth factor-I (IGF-I), osteocalcin and procollagen type 1 (PICP) with radioimmuno-assay (RIA) (Medgenix Diagnostics, Brussels, Belgium; Incstar Corporation, Stillwater, USA and Orion Diagnostica, Espoo, Finland respectively).

At baseline and after 3, 6, 9 and 12 months the following urinary parameters were determined:

* Fasting (2h) hydroxyproline by Hypronosticon (Organon Technika, Oss, The Netherlands) and excretion of free deoxypyridinoline by enzyme linked immuno sorbent assay (Pyrilinks D, Metra biosystems, Palo Alto, USA).

All samples were collected after an overnight fast on the day after administration of rhGH or placebo.

Statistics.

Values are expressed as means $\pm$ SEM. At baseline differences between groups were tested by Student's t-test for unpaired data, with a P value of $< .05$ for significance.

Statistics within and between treatment groups were performed by means of repeated

Table 8.1 *Clinical and biochemichal characteristics of the patients*

characteristic	rhGH (n=10)		placebo (n=11)		
	mean	range	mean	range	*P*
Age (yr)	63.5	57-74	62.8	55-72	NS
YSM (yr)	20.2	7-30	14.4	5-27	NS
BMI (kg/m^2)	25.0	19.6-27.4	25.6	19.0-32.5	NS
Height (cm)	162.5	149-179	160.5	153-178	NS
Weight (kg)	65.3	56.0-77.5	66.7	45.0-89.1	NS
BMC_{spine} (gHa)	32.7	23.4-39.7	31.8	21.2-44.6	NS
$BMC_{fem\ neck}$ (gHa)	3.5	3.1-4.4	3.6	1.9-6.0	NS
BMC_{ward} (gHa)	1.6	1.2-2.4	1.7	1.0-3.5	NS
BMC_{troch} (gHa)	8.2	4.6-14.3	7.3	3.2-11.6	NS
BMC SPA_{dist}(U/cm)	29.4	22.2-38.2	27.8	14.0-40.3	=.05
BMC SPA_{prox}(U/cm)	30.8	19.5-38.4	30.3	16.3-42.9	NS
BIA fat (%)	39.6	33.3-47.3	37.7	28.1-55.2	NS
DXA fat (%)	32.1	21.5-38.4	35.1	26.1-46.9	NS
Calcium (mmol/l)	2.28	2.10-2.40	2.29	2.15-2.44	NS
Phosphorus (mmol/l)	1.13	0.99-1.27	1.19	0.81-1.41	NS
Creatinine (μmol/l)	67	48-91	78	49-113	NS
APase (U/l)	57.2	44-82	79.5	37-124	<.05
IGF-I (nmol/l)	20.0	9.6-33.0	24.2	13.2-48.0	NS
Osteocalcin (μg/l)	3.0	1.0- 6.5	4.7	0.6- 8.5	NS
Procollagen I (μg/l)	104.2	44-286	108.1	74-178	NS
24 hr urinary:					
Calcium (mmol/mmol creat)	0.61	0.15-1.09	0.46	0.20-0.89	NS
2 hr fasting urinary:					
OH-proline (mmol/mol creat)	20.8	9.6-39.4	23.5	10.2-60.0	NS
free deoxypyridinoline (nmol/mol creat)	80.3	36.7-171.0	51.3	14.6-88.1	NS

NS: no significance between groups

measures analysis of variance (rmANOVA). The dependent variable is assumed to have a linear trend relationship with time; in the model the slope is different between the two treatment groups. In the GH group the model includes additional effects after stopping rhGH in month 9 and 12.
If the rmANOVA resulted in a significant main effect between treatment groups, we performed a Student's t-test posthoc analysis for paired data within groups and for unpaired data between groups, with a P value of $< .05$ for significance.

RESULTS

Side effects, baseline values.

Initially 23 patients entered the study. 12 patients were treated with pamidronate only (placebo-group). One of these patients decided to stop at 4 months, because of gastric complaints, related to the use of the bisphosphonate. The 11 patients of the other group were treated with APD and rhGH (rhGH-group). In this group one patient was taken out at 3 months because of cardiac complaints related to fluid retention (minor cardiac failure, which was treated with diuretics). The remaining 21 patients (placebo-group; n=11, rhGH-group; n=10) completed the 12 months study period and were eligible for evaluation. Except for 1 patient complaining temporarily of gastric complaints no side effects were observed in these patients. rhGH injections were tolerated well, no dose adjustments were necessary.
The baseline values are presented in Table 8.1. No significant differences as to age, or years since menopause (YSM) were found between both groups. Also anthropometric data were comparable. The initial bone mass measurements showed no significant differences except for the distal forearm BMC, measured with SPA (Table 8.1). Body composition did not show any differences at baseline when calculated with BIA or measured with DXA. The baseline biochemical measurements showed a higher serum alkaline phosphatase ($P < .05$) in the placebo-treated group, otherwise there were no differences (Table 8.1).

Longitudinal bone mass measurements.

The time course of the different bone mineral mass measurements are shown in Fig. 8.1 A-D. For the lumbar spine a rapid increase in the placebo-group was observed after three months for the lumbar spine as well as the distal forearm, with a much slower increase during the subsequent nine months. For the rhGH-group no significant changes were observed during (0-6 months) and after cessation (6-12 months) of treatment. In the femoral neck (as well as the Ward's triangle and trochanteric region) no significant changes during the whole treatment period (12 months) for either group were observed.

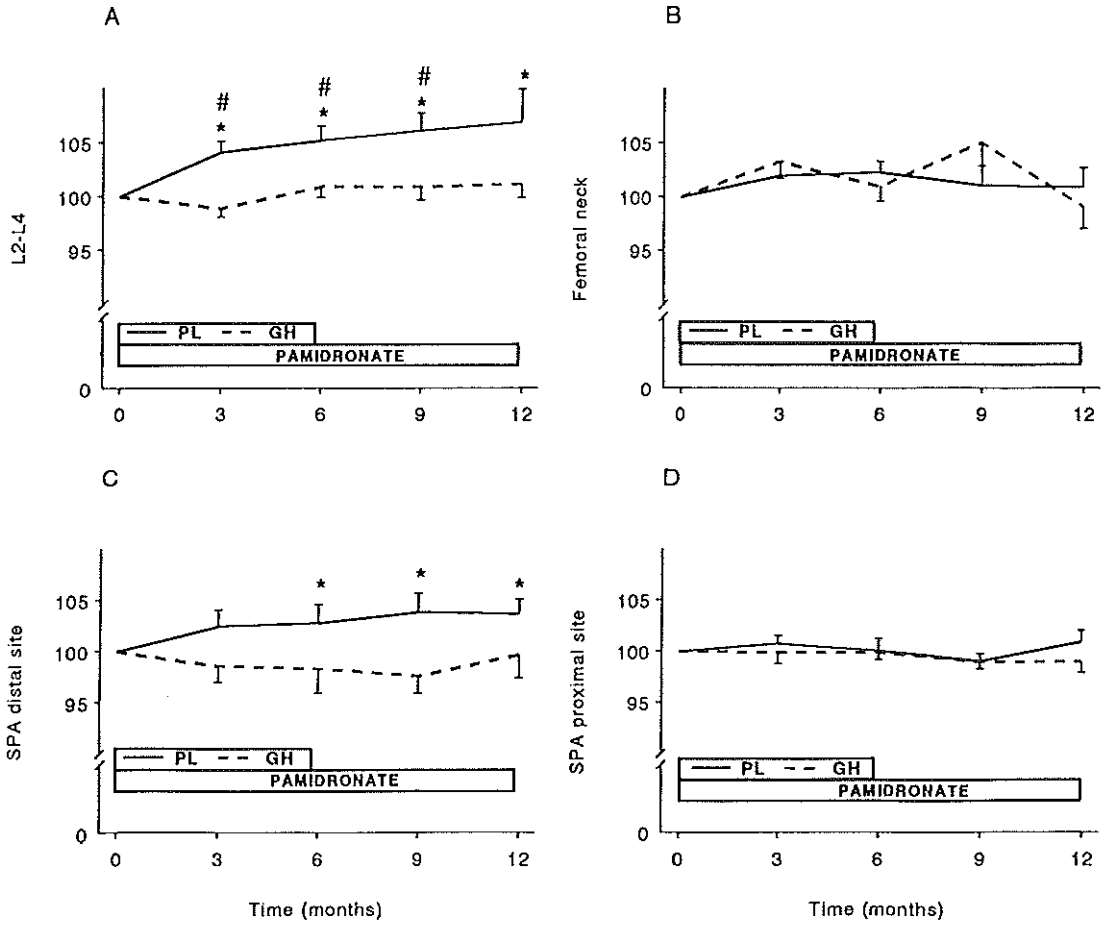

Figure 8.1 *Course of the BMC. Treatment with Pamidronate during twelve months. Treatment with rhGH (GH) or placebo (PL) during first 6 months. Treatment periods are denoted by the bars. Measurement at 0, 3, 6, 9 and 12 months. Mean ± SEM. The measurements are presented as percentage of initial value. Measurements by means of DXA: lumbar vertebral bodies 2-4 (A); Femoral neck (B) and SPA: distal site (C); proximal site (D) radius. For statistical analyses see material and methods section. Significance within groups versus T=0: * $P < .05$, ** $P < .01$.*
Significance between groups: # $P < .05$, ## $P < .01$.

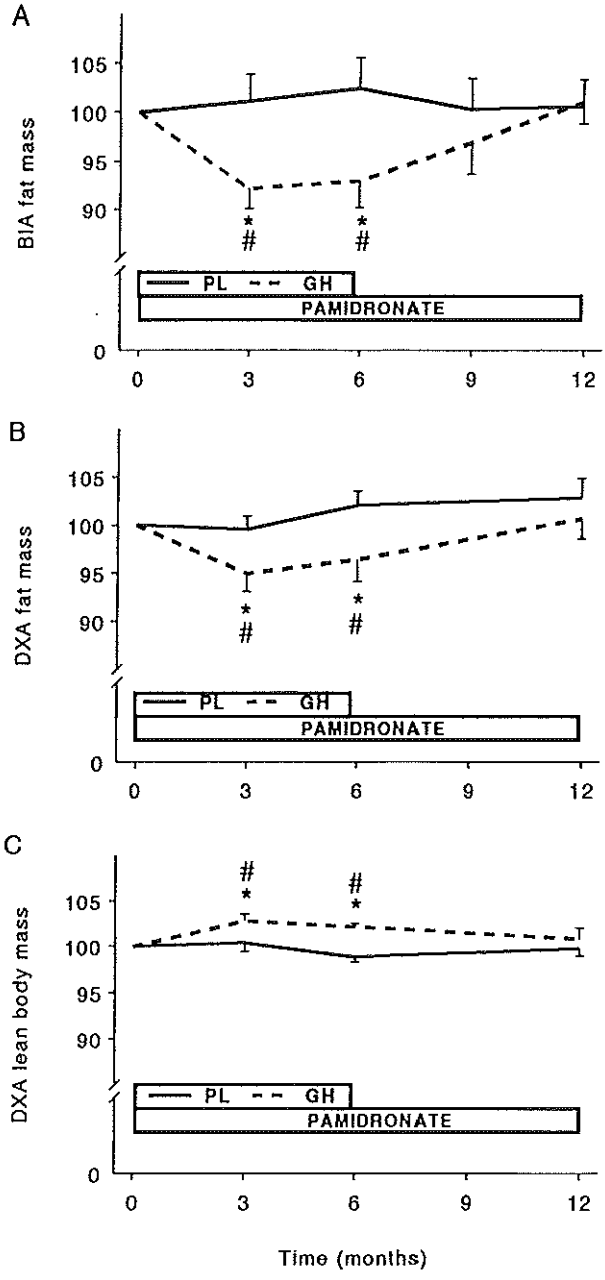

Figure 8.2 *Course of the fat mass: calculated from impedance (BIA) (A) and DXA (B). Course of lean body mass measured with DXA (C). See also the legend to Fig. 8.1.*

For the forearm a similar pattern as in the lumbar spine was found, however no significance wasreached between both groups. The proximal forearm showed no clear changes in either group.

Body composition.

Baseline measurements for body composition showed a correlation between fat mass assessment by BIA and DXA ($r = 0.74$, $P < .001$). Also the changes in time measured by the two methods were well correlated ($r = 0.85$, $P < .001$).

In the placebo-group no changes in body composition were observed during the year of treatment. In the rhGH-group, irrespective of the method used, a significant decline of the percentage fat of about 5-7 % compared to initial values occurred. After the treatment with rhGH was withdrawn an increment of fat mass occurred, reaching pretreatment levels within 3 months, when measured by BIA (Fig. 8.2A). Total body DXA measurements were not performed at 9 months (Fig. 8.2B). When lean body mass was analysed by DXA, again no changes in the placebo-group were found whereas a significant increase in lean mass of about 2.5 % after 3 months was found in the rhGH-group (Fig. 8.2C). Again, these changes disappeared after treatment with rhGH was stopped at six months and returned to pretreatment values.

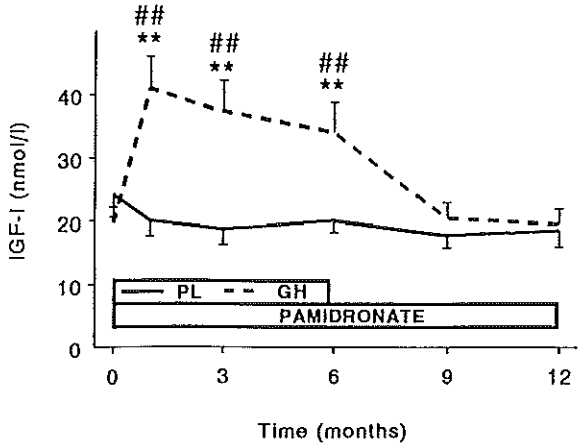

Figure 8.3 *Course of serum IGF-I. Treatment with Pamidronate during twelve months. Placebo-controlled treatment with rhGH during first 6 months. Measurement at 0, 1, 3, 6, 9 and 12 months. Mean ± SEM. The measurements are expressed in nmol/l. See also the legend to Fig. 8.1.*

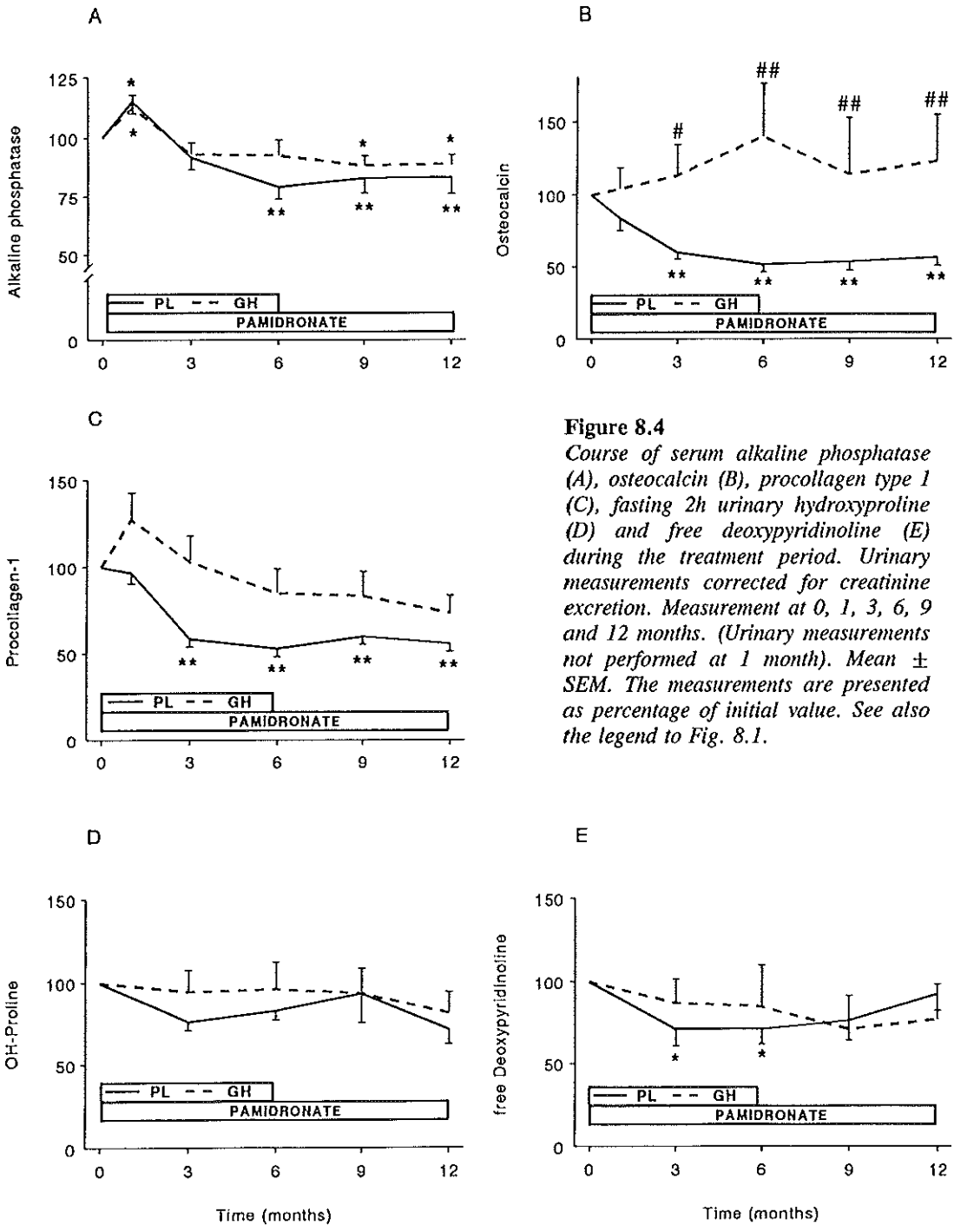

Figure 8.4

Course of serum alkaline phosphatase (A), osteocalcin (B), procollagen type 1 (C), fasting 2h urinary hydroxyproline (D) and free deoxypyridinoline (E) during the treatment period. Urinary measurements corrected for creatinine excretion. Measurement at 0, 1, 3, 6, 9 and 12 months. (Urinary measurements not performed at 1 month). Mean ± SEM. The measurements are presented as percentage of initial value. See also the legend to Fig. 8.1.

Biochemical parameters.
Baseline serum IGF-I levels did not differ between the groups. They also did not differ from the values found in a control population of women (n = 115), aged 55 years and over (18.1 ± 3.2 nmol/l vs 22.1 ± 6.4 nmol/l (in our patients)). Serum IGF-I increased rapidly after 1 month in the rhGH-group and stabilized during rhGH-treatment. After cessation of rhGH-treatment a rapid return to pretreatment values was observed (Fig. 8.3).
Serum Ca, P and creatinine did not change during the treatment period between or within groups. During the treatment period no changes in glucose metabolism (glucose, glycosylated Hb) were observed in both groups.
The relative changes of serum alkaline phosphatase, osteocalcin, procollagen type 1 over time within and between both treatment groups are depicted in Fig. 8.4 A-C. In the placebo-group as well as in the rhGH-group a significant increase in alkaline phosphatase was found after 1 month. From 6 months onwards in the placebo-group and from 9 months onwards in the GH-group a significant decline of serum alkaline phosphatase was found. For osteocalcin a rapid decrease in the placebo-group was observed, whereas in the rhGH-group a non-significant increase was observed. From 3 months onwards a significant difference between both treatment groups existed. Procollagen type 1 showed a small increase after 1 month in the rhGH-group, followed by a non-significant decline (compared to initial values). In the placebo-group an instant decline was observed from initiation of treatment onwards.
In the placebo-group, urinary OH-proline (corrected for urinary creatinine) showed a rapid although not significant decline, whereas in the rhGH-group no changes at all were observed (Fig 8.4D). Also urinary free deoxypyridinoline, which is a more specific marker of bone resorption, showed only in the placebo-group a temporarily significant decline.

DISCUSSION

In this study postmenopausal osteoporotic women were treated for a period of one year with pamidronate. In a placebo-controlled manner, half of the women received also rhGH during the initial six months. No additional effect of rhGH treatment was found on bone mineral mass. On the contrary, the beneficial effect of pamidronate on bone mass was blunted by the additon of rhGH.
In the placebo-group, treated with pamidronate only an increase of bone mineral mass at sites representing cancellous bone (lumbar spine and distal forearm) was observed, whereas no significant changes were observed at sites merely containing cortical bone (femoral neck and proximal forearm). A similar gain of bone mineral mass, explained by the so-called "transient"[10], has been reported by other investigators using bisphosphonates[4,38]. In the rhGH-

group a rapid and consistent increase of circulating IGF-I levels was observed, indicating that the dose of rhGH used (0.0625 IU/kg, 3 times/wk) was sufficient to reach a level which is supraphysiological for this age group. After withdrawal of rhGH administration, IGF-I values returned to baseline values. Other investigators recently showed that in postmenopausal women a comparable dosage of rhGH as used in our study induces a prompt and sustained increase in bone remodelling[14]. However, in our osteoporotic women the simultaneous addition of pamidronate appears to attenuate this rhGH-induced increase in bone turnover. Therefore, the absence of a clear change in the activation of bone remodelling units, as reflected by the markers of bone turnover, might explain that in the rhGH-group no change of bone mineral mass was observed. It became only apparent after the study that the baseline bone turnover rate appeared somewhat lower in the rhGH treated patients. However, this does not detract from our main conclusion that combined treatment of rhGH and pamidronate, for a relatively short period of six months, does not result in a positive effect on bone mineral mass.

The present combination of therapeutic modalities in the treatment of postmenopausal osteoporosis was based on the assumption that it might be possible to activate bone formation by rhGH in the presence of an anti-resorptive therapy (pamidronate). However, this turned out not to occur. Several possible explanations can be given. First, we have to take into account that the postmenopausal women we have treated are not growth hormone deficient and may therefore be less sensitive to supraphysiological dosages of growth hormone. However, several studies have indicated that both short-[30] and long-term[14] treatment with comparable dosages rhGH resulted in an increased bone turnover. Compared to controls, in which a bone loss of 2-3 % was observed, the rhGH treated group did not show a change in bone mass measurement[14]. In the present study we also observed in the rhGH treated patients at least initially a trend towards an increase of all bone formation markers, which was not accompanied by an increase of bone resorption. However, this apparent shift of the balance towards formation did not result in a clear rise of bone mass.

Secondly, the treatment period might have been too short to observe an osteotropic effect of rhGH. In GHD patients only long-term treatment with rhGH beyond six months resulted in a sustained increase of bone mineral mass[18].

Thirdly, the present scheme of continuous rhGH and pamidronate treatment might have inhibited potential beneficial effects of rhGH on osteoblastic function. Whether it may be necessary to administer rhGH cyclically in tandem with pamidronate remains to be seen. No extensive data with respect to cyclical treatment are currently available. Only one study [32] using GH in sequence with the bone resorption inhibitor calcitonin did not support the idea that such an approach indeed results in a more favourable response than could be expected from treatment with an inhibitor of bone resorption alone.

Fourthly, we can not exclude the possibility that the rhGH-induced increase of bone turnover may have made the bone tissue less sensitive to the bisphosphonate.
Finally, one can argue that the number of patients in this study is too small. However, based on the present results it is highly unlikely that inclusion of more patients will change the results towards a more favourable effect of combined treatment versus treatment with pamidronate alone.
rhGH treatment affects body composition with a decrease of fat mass and increase of lean body mass[24,39,40]. Body composition in this group of elderly patients also changed accordingly during rhGH treatment with a decrease of fat mass and a rise of lean body mass. The changes observed were comparable to those obtained in GHD adult individuals during rhGH replacement therapy[40,41]. One might speculate, that increased lean body mass might (and thereby muscle mass) benefit strength and possibly coordination , which may eventually lead to a reduction in fall and fracture rate[42].
Still, the methodology used in this study to measure body composition merits some further comments. The validation of the BIA method has not been carried out for different patient groups. The hydration status significantly influences the outcome, in the sense that a slight dehydration, as might be the case in the elderly, overestimates the percentage body fat[43,44]
The DXA method is better validated and measures fat directly[45]. The DXA technique does not distinguish between intra- and extracellular water. The increase in fat free mass as observed in our patients during GH treatment indicates an accumulation of body water. The decrease in fat mass, as measured by DXA, however, might be a true phenomenon. As the changes in body composition, measured by BIA and DXA are closely in parallel, these changes might therefore have clinical significance.
Taken together our results clearly show that simultaneously started combined treatment with rhGH and pamidronate during six months offers no new approach in the treatment of postmenopausal osteoporosis. Even if long-term treatment with rhGH results in a favourable shift of the balance between bone resorption and formation, then it is still questionable whether such an effect justifies long-term treatment given the side-effects of GH-treatment and its high costs. Nevertheless, further studies are needed to elucidate whether in selected cases with a low bone turnover administration of rhGH in sequence with an inhibitor of bone resorption is a useful option.

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CHAPTER 9

COMBINED TREATMENT OF GROWTH HORMONE (GH) AND THE BISPHOSPHONATE PAMIDRONATE VS TREATMENT WITH GH ALONE IN GH-DEFICIENT ADULTS: THE EFFECTS ON RENAL PHOSPHATE HANDLING, BONE TURNOVER AND BONE MINERAL MASS.

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SUMMARY

Objective.

A potential drawback of GH replacement therapy in GH deficient (GHD) patients is the initial decrease in bone mass. The present study investigates the effects of the addition of pamidronate to GH replacement therapy in adult GHD subjects, on serum levels of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), renal phosphate handling, bone turnover and bone mineral content (BMC).

Design.

Six GHD adult patients were studied for two periods of six months with a wash out period of three years. In the first period they were treated with conventional replacement therapy and GH. In the second study period GH treatment was identical, while after two weeks 150 mg pamidronate per day was added.

Results.

In the first study period (GH only) there was a significant increase of phosphate reabsorption, without a change in serum PTH and 1,25-$(OH)_2D_3$ levels. This suggests a specific effect of GH or IGF-I on renal phosphate handling. This, indeed was supported by the close correlation between serum IGF-I levels and TmP/GFR ($r=0.75$, $P < .0001$). When GH was administered together with pamidronate, this correlation was less, but remained significant ($r=0.44$, $P < .001$).

The increase in bone turnover and decrease in BMC, as initially observed during GH replacement therapy alone, were attenuated by simultaneous pamidronate administration. The percentual decline of lumbar spine BMC (measured with dual-photon absorptiometry) at six months was -3.1 ± 1.5% during GH replacement therapy alone, versus +3.8 ± 2.0% during the administration of the combination of GH and pamidronate (measured with dual-energy x-ray absorptiometry). At the distal and proximal forearm the changes amounted -0.5 ± 3.4% versus +4.5 ± 1.8% and -1 ± 1.2% versus +1.2 ± 1.1% respectively.

Conclusions.

This study shows that the addition of a bisphosphonate to GH replacement therapy in GHD adults counteracts the GH (or IGF-I) induced increase in renal phosphate reabsorption. Furthermore, it reduces GH-induced bone turnover and prevents the initial decrease in bone mineral content as seen during GH treatment alone, resulting in a beneficial effect on bone mineral mass. Pamidronate might therefore be an important adjunct to GH replacement therapy in adults with GHD and severe osteopenia during the early phase of GH-induced stimulation of bone turnover.

CHAPTER 9

INTRODUCTION

Several studies indicate that GH increases renal phosphate reabsorption and stimulates the renal production of the active vitamin D metabolite 1,25-dihydroxyvitamin D (1,25-$(OH)_2D_3$[1,2]. In other words at the level of renal phosphate handling GH appears to act as a parathyroid hormone (PTH) antagonist, while at the level of the renal 1α-hydroxylase an opposite more PTH-like action appears to take place[3]. This apparent dichotomy in the interaction between GH and PTH prompted us to compare two periods of GH replacement therapy in the same GH deficient (GHD) individuals; one period with GH treatment alone and a second period whereby GH treatment was combined with the bisphosphonate pamidronate. It is known from other studies that pamidronate treatment results in an increase of PTH levels[4-6]. Recent reports also indicate a high prevalence of osteopenia in subjects with growth hormone deficiency (GHD) of both childhood[7,8] and adult onset[9,10]. Other investigators showed an increased susceptibility for fractures in this group, which is compatible with the inverse relationship between bone mineral density (BMD) and fracture risk[11-13]. Also a positive correlation between circulating insulin-like growth factor (IGF-I) and BMD in individuals with acquired GHD has been reported[14,15]. Based on these observations clinical studies were initiated to establish the potential effects of GH replacement therapy on bone turnover and bone mass[16-18]. Several reports indicate that GH replacement results in a considerable increase of bone turnover as indicated by an increase of markers of bone resorption and bone formation[18-23]. This increase in bone remodeling might result in an increase of remodeling space with a subsequent loss of bone mineral mass. This indeed was observed in a group of GHD adults we have followed on GH replacement therapy for six months[24]. However, long-term treatment with GH up to 30 months leads, after an initial downwards trend, to a sustained increase of bone mineral content (BMC) as measured in the axial and appendicular skeleton[25-28]. In other words long-term GH replacement therapy appears to result in a favourable shift in the balance between bone resorption and bone formation. In the present study we investigated the effects of GH replacement therapy in a group of GHD patients in combination with an inhibitor of bone resorption, the bisphosphonate pamidronate. Given the fact that bone turnover is initiated with bone resorption, we added in the present study pamidronate two weeks after the initiation of GH replacement therapy.

Taken together, the present study performed in GHD subjects compares the effects of GH alone and GH with pamidronate on parameters of renal phosphate handling, bone turnover and bone mineral mass.

MATERIALS AND METHODS

Patients.

Eight patients with long-standing GHD were treated with GH alone for six months[24]. The baseline characteristics of the six patients who agreed to enter the second part of the study three years later are shown in Tables 9.1 and 9.2. Age of onset of GHD was between 13 and 19 years of age. Two patients received GH treatment during childhood (no.4 and 6) more than 10 years earlier. In the first study period patients 1, 3 ,4 and 6 received 25 μg/kg.day with a maximum of 1.48 mg (4 U) per day. Patient 2 received only half this dose (12.5 μg/kg.day). This lower dose was given because this patient already spontaneously complained of signs and symptoms of fluid retention. Patient 5 received 18 μg/kg.day. She complained of fluid retention in the initial part of the study at which time the dose was reduced. Patients received GH replacement therapy for six months at the same dose in the two study periods. rhGH (Humatrope) was generously supplied by Eli Lilly Co., Indianapolis, IN.. In the second study period, from day 16 onward, the patients were cotreated with pamidronate 150 mg per day orally. Measurements were performed at baseline, 1 month, 3 months and six months in the first study period and again at baseline, two weeks, 3 months and six months in the second study period. During and in between both treatment periods conventional pituitary replacement therapy remained unaltered.

The aims and methods of the study were explained to the patients, and written informed consent was obtained before the study, which was approved by the ethics committee of the University Hospital Dijkzigt (Rotterdam, The Netherlands).

Bone mass measurements.

Lumbar bone mass (lumbar vertebrae, L2-L4) was measured, during the first study period using Dual-Photon Absorptiometry (DPA, Novo BMC-Lab 22a scanner). In the second study period a Lunar DPX Bone Densitometer (Lunar Corp., Madison, WI) was used. The short-term coefficients of variation (CV) in normal subjects for DPA and DXA were 2.3% and 1.1% respectively[29,30] correlation between both methods, as measured on the same day, was high (based on 252 lumbar spine measurements, r=0.984, β=1.29 $\pm$ 0.015, $P < .001$). The results are expressed as BMC (g hydroxyapatite (Ha)). Quality assurance including calibration, was performed routinely every morning. No drift was observed during both study periods.

Single-Photon Absorptiometry (SPA) of the right forearm was performed at the distal and proximal sites using a Nuclear Data 1100a scanner[31]. The (fat-corrected) results are expressed as BMC (arbitrary units (U) per cm). The CV in our institution is 1.9% for the distal site and 1.0% for the proximal site[29].

Measurements and laboratory tests.

Routine methods were used for the measurements of total alkaline phosphatase (APase) and inorganic phosphate (Pi). The renal tubular reabsorption maximum of phosphate corrected for glomerular filtration rate (TmP/GFR) was calculated according to Bijvoet[32]. Serum osteocalcin and the carboxyterminal propeptide of type I collagen (PICP) were determined by commercially available kits (INCSTAR Corp., Stillwater, MN and Orion Diagnostica, Espoo, Finland respectively).

24-hour Urinary excretion of hydroxyproline (Organon Technika, Oss, The Netherlands) expressed as mmol OH-proline per mol creatinine was assessed at each visit. Prior to urine collections patients were on a gelatine free diet for three days.

25-hydroxyvitamin D_3 (25-(OH)D_3), 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and intact PTH (1-84) were determined using commercially available kits (INCSTAR Corp., Stillwater, MN).

Statistical analysis.

Paired T-tests were performed to analyse the effects on renal phosphate handling, bone turnover parameters and BMC between the two periods as well as within the two study periods. Results are expressed as mean ± SEM. $P < .05$ was considered significant.

Table 9.1 *Baseline clinical and biochemical characteristics of the six patients included in the study*

Patient no.	GH daily dose (μg)	Sex	Cause of pituitary deficiency	Diagnosis[a]	Peak GH (μg/L)[b]
1	1480	m	Craniopharygeoma	TAGDI	2.3
2	740	f	Idiopathic	TAG	1.7
3	1480	f	Craniopharygeoma	TAG	1.1
4	1288	m	Congenital	TAG	4.2
5	1110	f	Craniopharygeoma	TAG	2.9
6	1480	m	Craniopharygeoma		3.2

[a] T, thyroid deficiency; A, adrenal deficiency; G, gonadal deficiency; DI, diabetes insipidus.
[b] Peak GH, maximal GH level after iv injection of GHRH (1 μg/kg BW).

RESULTS

All patients gained weight during the three years interval. Since height did not change, this also resulted in a significant increase of BMI (Table 9.1). PTH levels were significantly different at the beginning of the two study periods. Although none of the other parameters differed significantly, there was a tendency to a somewhat lower bone turnover and higher BMC at the beginning of the second study period (Table 9.2).
GH replacement therapy was well tolerated. In the second study period, one patient was treated with furosemide 20 mg for two weeks in the third and fourth week of GH therapy because of tense feeling in the fingers due to mild edema. Also in the second study period two of the six patients complained of gastrointestinal discomfort after the initiation of pamidronate. This disappeared spontaneously within two weeks.
The rise of IGF-I levels in the second study period was similar to that reported for the first study period[24].

Biochemical measurements.
Despite the higher baseline serum PTH levels during combined treatment, the rise of PTH levels was more prominent than during treatment with GH alone (Fig. 9.1A). The increase of PTH levels was significantly higher on combined treatment ($P < .05$). A similar trend was observed for $1,25\text{-}(OH)_2D_3$ levels, although no significant difference between treatment periods could be observed (Fig. 9.1B). $25\text{-}(OH)D_3$ levels did not change in either treatment period (results not shown). Serum phosphate increased from 1.19 $\pm$ 0.03 to 1.48 $\pm$ 0.03 mmol/L in the first study period and from 1.10 $\pm$ 0.03 to 1.35 $\pm$ 0.03 mmol/L in the second study period ($P < .05$ baseline *vs* six months). TmP/GFR increased in both treatment periods, with a less prominent rise during combined treatment, reaching significance at three months (Fig. 9.1C). Although the correlation between serum IGF-I and TmP/GFR was highly significant ($P < .001$) during both treatment periods, addition of pamidronate weakened this relationship (r=0.75 *vs* r=0.44) (Fig. 9.2).
In both treatment periods an increase in markers of bone formation (ic. osteocalcin, APase and PICP) was observed. However, versus baseline the increase of the biochemical markers of bone turnover was markedly blunted in the second study period from the moment that GH replacement therapy was combined with pamidronate (Fig. 9.3 A-C).
Urinary hydroxyproline as a measure of bone resorption rose significantly in both treatment periods. It reached a maximum after 1 month during treatment with GH alone, whereas an attenuated response was observed after addition of pamidronate (Fig. 9.3D). 24-hour Urinary calcium excretion increased significantly, but was not significantly different between the two periods (data not shown).

Table 9.2 *Clinical and biochemical characteristics of the patients*

characteristic	GH (period I)		GH + APD (period II)		
	mean	range	mean	range	*P*
Age (yr)	33.7	25-46	36.7	28-49	
Height (cm)	162.3	137-182	163.2	138-181	NS
Weight (kg)	73.3	47-95	80.8	52-102	0.004
BMI (kg/m^2)	27.6	18.1-33.8	30.3	19.8-36.2	0.007
Serum calcium (mmol/L)[a]	2.21	2.16-2.23	2.17	2.08-2.42	NS
Serum phosphate (mmol/L)	1.19	1.11-1.28	1.10	0.98-1.22	NS
$1,25\text{-}(OH)_2D_3$ (pmol/L)	80	57-103	77	54-114	NS
PTH (pg/mL)	20	13-32	26	15-32	0.017
TmP/GFR (mmol/L)	1.0	0.8-1.2	1.1	0.9-1.2	NS
APase (U/L)	71	39-150	58	37-97	NS
Osteocalcin ($\mu g/L$)	4.1	2.6-7.5	3.2	1.2-8.0	NS
Procollagen I ($\mu g/L$)	85.7	56-116	72	21-139	NS
Urinary OH-proline (mmol/mol creat)	20.8	9.6-39.4	23.5	10.2-60.0	NS
BMC SPA_{DIST}(U/cm)	30.9	18.4-42.6	32.9	25.2-42.2	NS
BMC SPA_{PROX}(U/cm)	33.8	22.3-44.5	34.7	25.5-44.9	NS

NS: no significance between groups

[a] serum calcium corrected for serum albumin.

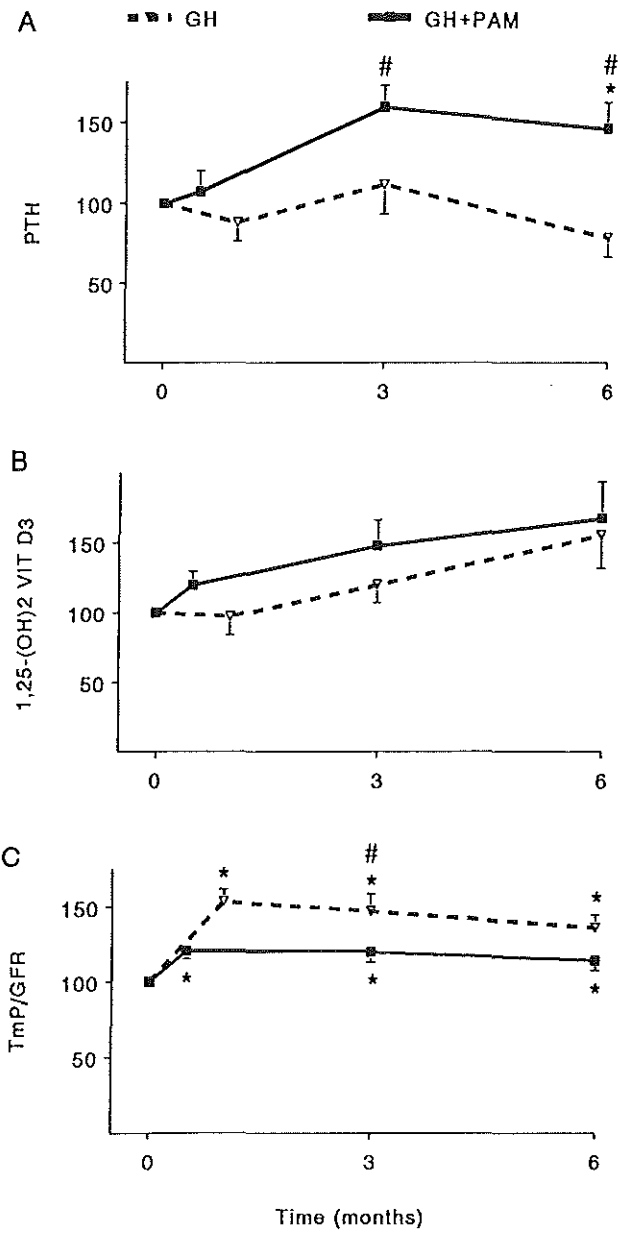

Figure 9.1

Time course of:
A. Serum PTH-levels as percentage of initial value.
B. 1,25-dihydroxyvitaminD_3 levels.
C. TmP/GFR.
*Results are presented as mean ± SEM. * $P < .05$ vs. baseline. # $P < .05$ difference between periods.*

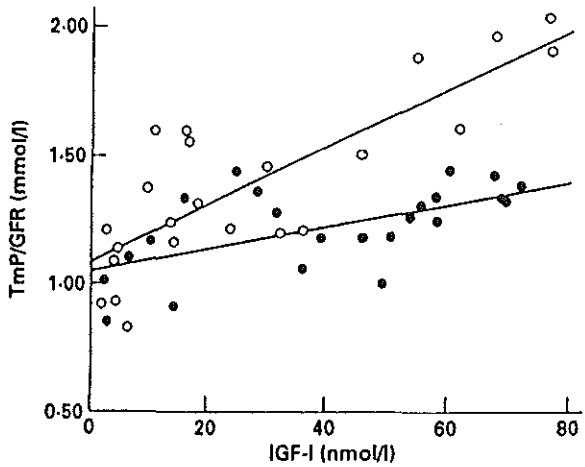

Figure 9.2 *Correlation between TmP/GFR and IGF-I. ○; GH (r = 0.75). ●; GH + pamidronate (r =0.44). P < .001 both periods.*

Bone mineral content.

The baseline date for bone mineral mass are shown in Table 9.1. The changes of the bone mass measurements during the two treatment periods are shown in Fig. 9.4. Only BMC data are shown, because bone mineral density data showed similar changes.

Lumbar spine. In the first treatment period there was a significant decrease in BMC after three and six months of GH replacement therapy (-1.5 ± 1.3% and -3.1 ± 1.5%, respectively). In the second treatment period there was an increase in BMC, which already reached significance versus baseline at six months (3.8 ± 2.0%). The difference between the periods was already nearly significant at three months and reached significance at six months ($P = .052$ and $P = .04$ respectively)

Distal forearm. In the first treatment period there was a significant decrease in BMC as measured in the distal forearm at three months. At six months BMC was still lower vs baseline, however not significantly (-4.8 ± 2.0% and -0.5 ± 3.4%). In the second treatment period there was an increase at three and six months (2.5 ± 0.7% and 4.5 ± 1.8%). The difference between the periods was significantly different at three months, but not at six months of therapy ($P = .036$ and $P = .239$ respectively).

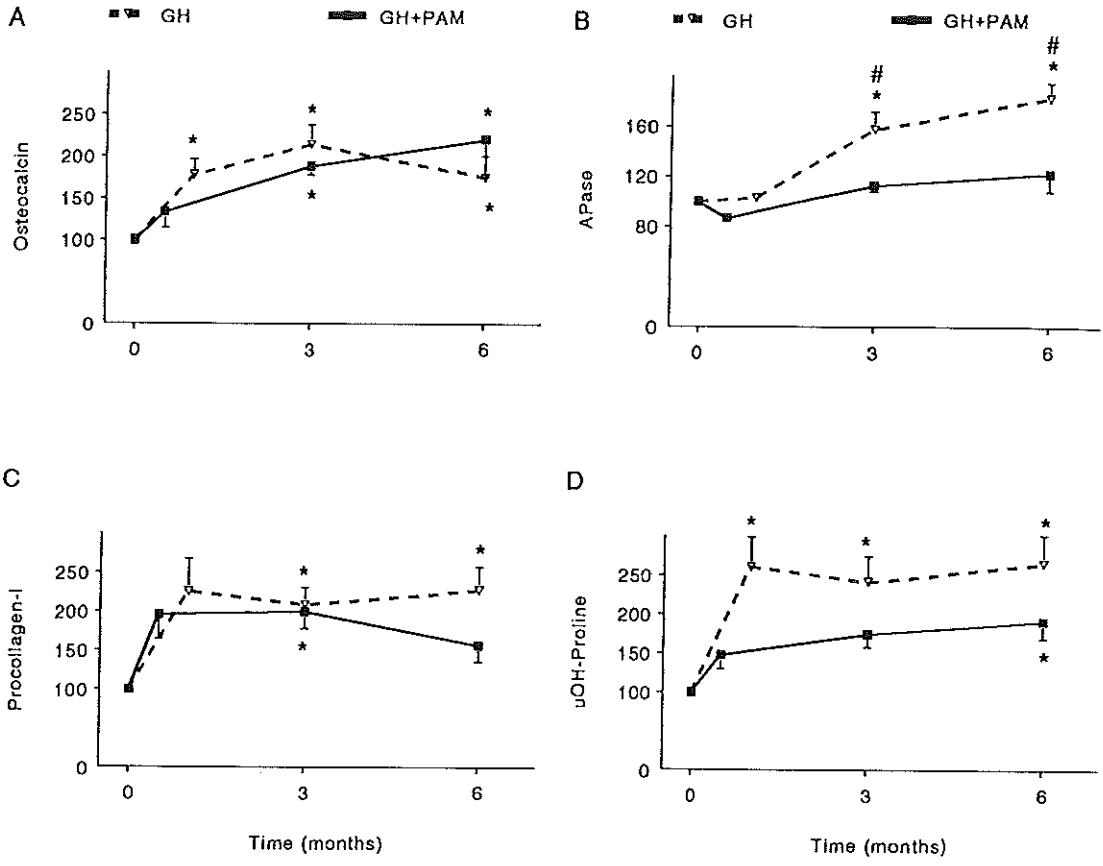

Figure 9.3 *Time course of bone turnover parameters, presented as percentage of initial value.*
A. Osteocalcin, B. APase, C. PICP, D. Urinary OH-proline.
See also legend to Fig. 9.1.

Proximal forearm. The initial decline in BMC in the first treatment period was significantly greater than in the second treatment period. After six months in the second treatment period BMC levels were above baseline (-1.4 ± 0.6% and -1 ± 1.2% in the first period versus -0.3 ± 0.4% and 1.2 ± 1.1% at three and six months, respectively). Changes were not significantly different between periods.

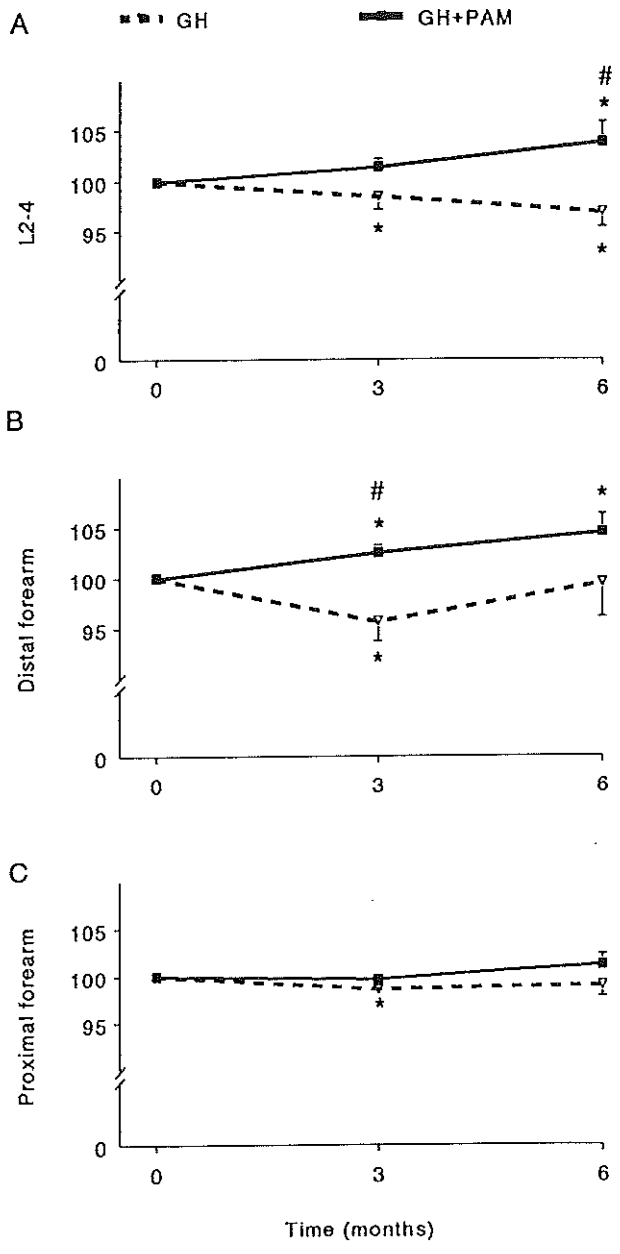

Figure 9.4 *Time course of bone mass measurements (BMC), presented as percentage of initial value.*
A. Lumbar spine, B. Distal forearm, C. Proximal forearm.
See also legend to Fig. 9.1.

DISCUSSION

Our study indicates that addition of pamidronate to GH replacement therapy in GHD adults attenuates the GH-induced increase of the renal phosphate threshold and prevents the initial bone loss as observed during treatment with rhGH alone.

As observed during the first study period GH induced a pronounced increase in renal phosphate reabsorption. This increase was not accompanied by changes of the phosphate regulating hormones PTH and 1,25-$(OH)_2D_3$. Therefore, a direct effect of GH or IGF-I on renal phosphate handling is more likely, which is also supported by the high correlation observed between IGF-I and TmP/GFR. Also recent in vivo and in vitro studies[34,35] suggest a similar mode of action. That GH does not affect the PTH-induced phosphaturic response is provided by the observation that the rise of PTH levels, as observed after the addition of pamidronate, is accompanied by a lowering of the renal phosphate threshold. In other words it appears that the GH-induced increase in phosphate reabsorption is counteracted by a PTH-induced decrease. Based on these observations it is unlikely that GH at the level of renal phosphate handling acts as a PTH-antagonist[3].

Recent studies also indicate that GH or IGF-1 stimulates renal 1α-hydroxylase activity [3]. In the first study period we did not observe a significant rise of 1,25-$(OH)_2D_3$ levels, which might be explained by the prominent increase of serum phosphate levels during GH treatment. It is known that hyperphosphatemia is a potent inhibitor of 1α-hydroxylase activity[36]. The lower phosphatemic response after addition of pamidronate might, therefore, explain the trend towards higher 1,25-$(OH)_2D_3$ levels during the second study period. Of course, it is also possible that this represents a direct effect of the raised PTH levels on the renal 1α-hydroxylase.

When we compare the pretreatment values of biochemical markers of bone turnover and bone mass between both study periods, there appears to be a tendency to a somewhat lower bone turnover and a somewhat higher BMC at the start of the second study period. Nevertheless, the differences in these markers did not reach significance, which exclude an important "carry-over" effect of GH treatment from the first to the second study period. The somewhat higher bone mass at the start of the second study period could be related to the increase in body weight between both study periods. Several studies have shown a positive relationship between body weight and bone mineral measurements.

As shown by the results obtained during the first study period GH leads to an increase in bone turnover. During an initial phase the GH-induced increase in the number of activated bone remodelling units will result in a larger bone surface undergoing bone resorption and subsequently will result in bone loss. An initial decrease in BMC was indeed found during treatment with GH alone in our patients, as well as by others[26]. During a second phase the

coupling between bone resorption and formation will result in a new balance, which will limit bone loss[33]. However, Vandeweghe et al observed that longterm treatment with rhGH, beyond six months, even resulted in an increase of bone mass. Therefore, this suggests that besides an increase in activation frequency of bone remodelling units, longterm rhGH treatment may result in a loss of synchronization between resorption and formation with an apparant shift towards bone formation.

Several studies in postmenopausal women have shown that a reduction of bone turnover, as can be induced by pamidronate, is accompanied by a transient increase of bone mass (for review see Heaney[33]). However, in the present study treatment with pamidronate only attenuated the GH-induced increase in bone turnover, but did not result in a reduction below pretreatment level. Therefore, it is unlikely that the observed gain in BMC during additional pamidronate treatment can simply be explained by the so-called "transient". A shift of the bone balance towards formation is more likely. As stated above this shift towards formation may be related to GH treatment. It is known that IGF-I can stimulate osteoblast-like cells[37]. Furthermore the effects of GH treatment can be indirect via an increase in muscle mass and physical activity. However, an anabolic effect on bone of the raised PTH levels during pamidronate treatment can not be excluded. Although the mechanism for the anabolic action of PTH is poorly understood, GH is required for its effect in animals[38]. As the volume to surface ratio of trabecular bone is higher than that of cortical bone, changes in bone turnover are more reflected in trabecular bone. This also explains the more pronounced change of BMC at the distal radius and lumbar spine, compared to the proximal forearm.

This study shows that the effects of GH-replacement therapy and bisphosphonates on renal phosphate handling are partly antagonistic and that these effects are probably not mediated through PTH. GH therapy results in an increased bone turnover and consequently in bone loss. Inhibition of bone turnover with the bisphosphonate pamidronate, prevents this initial loss and even results in an increase of bone mass. Additional studies are needed to see whether pamidronate interacts with long-term effects of GH on bone mass in GHD patients.

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CHAPTER 10

BONE MINERAL DENSITY IN HEALTHY DUTCH WOMEN; SPINE AND HIP MEASUREMENTS USING DUAL-ENERGY X-RAY ABSORPTIOMETRY.

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SUMMARY

Objective.
To investigate in healthy normal Dutch women the age-associated changes in bone mineral density (BMD) and the effect on bone mass of menopause and potential risk factors.

Methods.
In 260 healthy Dutch women BMD was measured in the lumbar spine and three regions of the proximal femur (Ward's triangle, femoral neck and trochanter), using Dual Energy X-ray Absorptiometry (DXA). The subjects were interviewed using a structured questionnaire on age, reproductive history and gynaecological status, height, weight and consumption of tobacco and alcohol.

Results.
In 125 premenopausal women a small age-related bone loss was observed at both the lumbar spine and proximal femur, while in postmenopausal women (n=135) a 2-3 times higher age-related loss was observed. Expressed in years since menopause this postmenopausal loss was found to be exponential ($P < .001$). After adjustment for age there appears to be a relationship between actual age of menopause and BMD at the lumbar spine and femoral neck. After adjustment for age and actual age of menopause we observed a small negative effect of breastfeeding, whereas parity, current alcohol use and smoking showed no additional effect on BMD in this cohort. For all women (n=260) a highly significant correlation between BMD and BMI was found.

Conclusions.
In healthy Dutch women we observed a small premenopausal and an accelerated postmenopausal bone loss in both the lumbar spine and proximal femur. Except for breastfeeding no other risk factors could be identified.

INTRODUCTION

A low bone mineral density (BMD) has been identified as a major determinant of the occurrence of non-traumatic fractures[1-4]. As the incidence of both hip and vertebral fractures increases with age, much attention have been paid to age-related bone loss[5-18]. During the last decade Dual Photon Absorptiometry (DPA) was the most widely used technique for the measurement of bone mineral mass. The new generation densitometers use instead of an isotope source (DPA) an X-ray tube (Dual Energy X-ray Absorptiometry (DXA)). This has resulted in a significantly improved scan precision and has decreased radiation dose and scanning time[19-21].

For the interpretation of bone mass data in women it is important to emphasize that, besides age and menopausal state, also other determinants like body mass index (BMI), nutritional factors (e.g. calcium, vitamin D, alcohol), medication (e.g. glucocorticoids, thiazides, thyroid hormone) and smoking may influence bone mass[22-28]. In addition genetic, geographical, cultural and socio-economic factors may also explain the differences observed in BMD's within and between populations[29,30]. Therefore, it is important that for a proper interpretation of bone mass measurements, values assessed in normal healthy women of the same population are available.
In the present study we have measured spine and hip BMD in 260 healthy Dutch women, aged 20-80 years, to determine normal ranges for the different measurement sites. In addition, the influence of potential risk factors was analysed.

SUBJECTS AND METHODS

Subjects.

All subjects were recruited from a call for volunteers in a national television programme, as part of an international multi-centre study to cross-calibrate bone mineral mass measurement data obtained with different DXA-machines (COMAC-BME)[31]. Only healthy ambulatory caucasian white women were included in the study. Subjects with known structural and/or metabolic bone diseases, arthritis or neurologic conditions were excluded. Subjects who were recruited were given a questionnaire, comparable to the one used in the MEDOS study[32]. Those who reported in the questionnaire previous hyper(para)thyroidism, hysterectomy or oophorectomy before the age of 50, menopause before the age of 45 and subjects who had been treated with oestrogens, androgens, fluoride, vitamin D, corticosteroids or thiazide diuretics were also excluded. Women, who ceased menstruating for more than 6 months were classified as postmenopausal. The other women were classified as premenopausal.
Besides exclusion criteria, the questionnaire contained questions about whether the women had given birth (parity) and if so, how many children were born and whether they had fed their children by breast. Also questions about smoking (present or past) and current alcohol usage (number of days/week) were included.
Of the five hundred and eighty women who completed the questionnaire, finally two hundred and sixty women were considered eligible to enter the study (total exclusion 55 %). The age ranged from 20 to 80 years. One hundred and thirty five women were considered premenopausal. Informed consent was obtained from all volunteers.

Bone mass measurements.

BMD was determined using DXA (Lunar DPX-L, Lunar Radiation, Madison, Wisconsin) according to the instructions of the manufacturer[19]. The measurement sites were the lumbar vertebrae 2-4 (L2-4) and in the left proximal femur the femoral neck (Fneck), Ward's triangle and trochanteric region. BMD is expressed in gram hydroxyapatite/cm^2 (gHa/cm^2). Quality assurance including calibration with the standard of the machine was performed routinely every morning. No drift was observed during the whole study period (= 3 months). All measurements were performed by one of three experienced persons using the same instrument.

Short-term precision was assessed by measuring BMD in 10 persons two times.

The coefficient of variation ($\pm$ S.D.) for lumbar BMD was 1.1 $\pm$ 0.2 %. The coefficients of variation in the femoral neck, Ward's triangle and trochanteric region were 1.4 $\pm$ 0.2, 2.1 $\pm$ 0.3 and 2.3 $\pm$ 0.3 %, respectively.

Data analysis.

Means and standard deviations (SD) or standard errors of the mean (SEM) of anthropometric and BMD data were calculated in five year-age strata for pre- and postmenopausal women. Adjustment for mean body mass index (BMI, kg/m^2) was made by using multiple regression analysis. Age-associated changes of BMD, after adjustment for BMI, were analysed by means of linear regression analysis, in which a division in pre- and postmenopausal women was made. In addition regression with a polynomial model (quadratic) in the postmenopausal women was performed to detect a non-linear association between years since menopause (YSM) and BMD.

The measure of YSM represents a mixture of age and age of menopause and equals, after adjustment for age, age of menopause (AOM). Therefore, we decided to analyse the effect of AOM on BMD by adding this variable to the model, thereby separating the effect of age and menopause completely.

By using stepwise multiple regression analysis the effects on BMD of parity, breastfeeding, smoking (package-years; calculated amount of years in which 1 package of cigarettes/day has been smoked) and current alcohol use (number of days/week on which alcohol was used) were analysed after adjustment for age, BMI and AOM.

Statistical computations were made using Statgraphics (Statistical Graphics Corporation, Inc., Maryland, USA).

CHAPTER 10

RESULTS

Characteristics of the subjects.

General characteristics of the study group as a whole and after division between pre- and postmenopause are presented in Table 10.1.

Of all the women, 195 (75 %) had given birth (number of children 2.2 ± 1.0 (range 0-6)). Of these 195 women, 147 (75 %) had breastfed their children for a period of 1 month or more (number of children breastfed 1.1 ± 1.1, (range 0-6)). Of all the women 69 (27 %) used at present no alcohol, 147 (56 %) used 1-3 times a week alcohol and the rest (44 women, 17 %) used four or more times per week alcoholic drinks. With respect to smoking the following numbers were obtained: 108 (42 %) women had never smoked, 102 (39 %) had smoked in the past (package-years 7.9 ± 7.2 (range 1-38)) and 50 (19 %) were current smokers (package-years 14.7 ± 10.2 (range 1-41)).

Table 10.1 *Data of whole group*

	All	Premenopausal	Postmenopausal
Number (n)	260	125	135
Age (years) range	49.7 ± 13.3 20 - 80	39.1 ± 10.0 20 - 55	59.5 ± 7.0 45 - 80
Age of menopause range			50.4 ± 2.5 45 - 56
Years since menopause range			9.4 ± 6.6 0 - 30
BMI (kg/m^2) range	24.5 ± 4.0 17.5 - 42.5	23.4 ± 4.1 17.6 - 42.5	25.5 ± 3.6 17.5 - 42.5

Age-associated bone mineral mass.

In Table 10.2, the distributions of BMI and the bone mass measurements at the various sites are listed according to age and menopausal state. For height a significant decline was noted

Table 10.2 *Characteristics of the women, stratified by age and menopausal state.*

Group (age)	PREMENOPAUSAL								POSTMENOPAUSAL						
	20-25	26-30	31-35	36-40	41-45	46-50	51-55	regression coefficient[#]	46-50	51-55	56-60	61-65	66-70	71+	regression coefficient[#]
N	17	14	16	10	26	22	20		10	28	47	22	17	10	
age,years															
mean	22.6	28.0	33.5	37.8	42.8	47.4	52.4		49.1	53.2	58.1	62.9	67.6	74.6	
sd	1.6	1.7	1.1	1.6	1.6	1.3	1.5		1.0	1.6	1.5	1.5	1.3	3.4	
menopausal age															
mean									47.8	50.3	50.6	51.0	50.7	51.0	
sd									1.7	2.1	2.6	2.2	2.5	3.0	
years since menopause															
mean									1.9	3.0	7.5	11.9	16.9	23.4	
sd									1.1	2.6	2.7	3.2	2.4	3.5	
BMI, Kg/m^2															
mean	22.2	25.4	22.1	25.0	23.3	23.8	23.1	.0184	24.9	25.9	25.9	24.7	25.1	25.9	.0004
sd	5.6	5.1	1.7	6.9	4.0	2.6	2.5		3.7	4.5	3.4	3.5	3.4	2.5	
BMD (gHa/cm^2)															
L2-4															
mean	1.279	1.318	1.203	1.350	1.217	1.199	1.218	-.0031**	1.195	1.168	1.102	1.047	1.027	1.009	-.0084**
sd	.118	.132	.127	.127	.152	.148	.143		.174	.186	.171	.131	.136	.130	
Femoral neck															
mean	1.010	1.076	0.927	1.021	0.916	0.941	0.965	-.0031**	0.986	0.916	0.863	0.844	0.794	0.816	-.0056**
sd	.102	.132	.100	.170	.112	.097	.115		.115	.139	.141	.113	.071	.089	
Ward's triangle															
mean	0.983	1.064	0.849	0.956	0.832	0.832	0.867	-.0061**	0.892	0.822	0.737	0.711	0.670	0.714	-.0069**
sd	.142	.189	.137	.180	.122	.114	.129		.142	.160	.154	.123	.083	.083	
Trochanteric region															
mean	0.797	0.882	0.752	0.850	0.777	0.815	0.815	-.0005	0.875	0.804	0.782	0.773	0.749	0.732	-.0037*
sd	.094	.118	.095	.163	.105	.115	.109		.159	.139	.129	.117	.116	.120	

* $P < .05$, ** $P < .001$ (difference from zero). [#] regression coefficient: coefficient from univariable regression analysis.

in the premenopausal as well as in the postmenopausal group.
For all sites of measurement, except for the Ward's triangle, a significant difference between the slopes, representing the relation between age and BMD, of the pre- and postmenopausal women was found ($P < .01$). Compared to the premenopausal group a 2-3 times higher

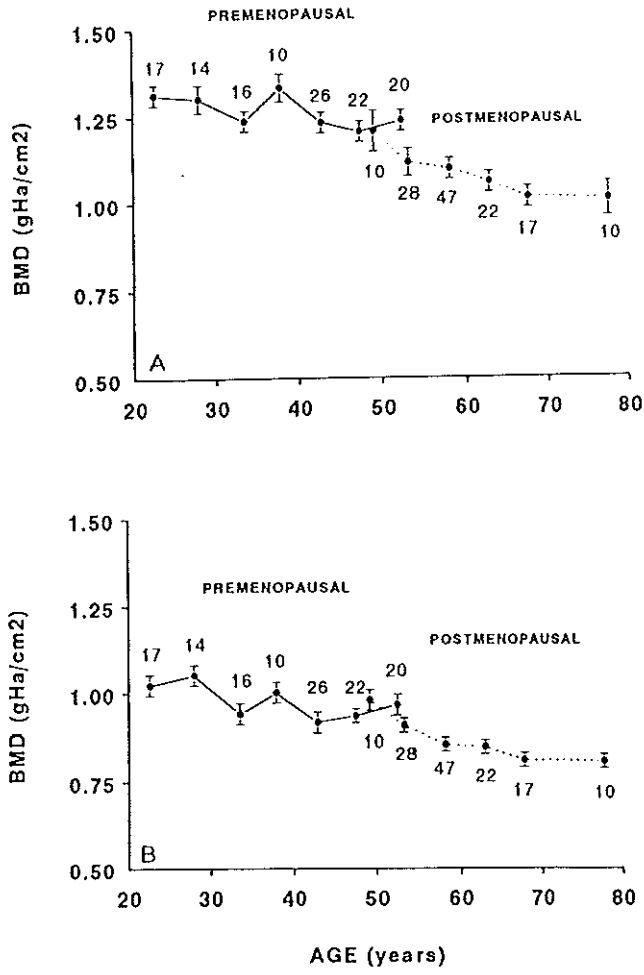

Figure 10.1 *Age associated BMD in pre- and postmenopausal women, stratified in five-year strata of age, adjusted for mean BMI. BMD expressed as gHa/cm^2 ± SEM. The numbers of patients for each five-year strata are depicted above (premenopausal women) or below (postmenopausal women) the curves. A. Lumbar vertebrae 2-4. B. Femoral neck.*

apparent loss was found in the postmenopausal women, at the various sites measured, except for the Ward's triangle. In Fig. 10.1 the BMI-adjusted BMD values for the lumbar spine and femoral neck are depicted.
For the premenopausal women the calculated apparent loss per year was 0.2 % for the lumbar spine, 0.2 % for the femoral neck, 0.4 % for the Ward's triangle whereas no loss in the trochanteric region was observed. For the postmenopausal women a calculated loss per year of 0.8 % for the lumbar spine, 0.8 % for the femoral neck, 1 % for the Ward's triangle and 0.6 % for the trochanteric region was found, respectively.
Comparing the youngest premenopausal group with the oldest postmenopausal group a loss of 20 % for the trochanteric region and of up to 27 - 30 % for the other sites was observed.
In Table 9.3 the various regression coefficients of the multivariable analyses are presented for the pre- and postmenopausal group respectively, showing a significant contribution to the BMD of age and BMI in the premenopausal and of age and BMI and AOM in the postmenopausal group.

Bone density and menopause.
During the first 10 years after menopause we found an exponential decline of the BMD at all sites measured (Fig. 10.2). For our study population, it was calculated that in the first 10 years after the menopause approximately 60 - 70 % of total postmenopausal loss occurred. By using multiple regression analysis the BMD values of the postmenopausal women were corrected for age. By using this approach the BMD at a given age of menopause can be predicted. The data shown in Fig. 10.3 depict the expected BMD of the lumbar spine and femoral neck of a woman at the age of 59.6 years (that is, the mean age of postmenopausal women in our group) as a function of the age of menopause. It is clear from these data that an earlier time of menopause will result in a lower BMD at a given age, compared to women with a later time of menopause.

Bone mineral density and other variables.
In all four skeletal regions studied the BMD showed a highly significant correlation with the BMI. This applied to the premenopausal ($r = 0.30 - 0.42$, $P < .001$) as well as to the postmenopausal group ($r = 0.28 - 0.47$, $P < .001$). BMI was correlated neither with age nor with AOM.
Using multiple stepwise regression analysis we looked at the effects of the following variables after adjustment for age and years since menopause: parity, breastfeeding, smoking (package- years) and current use of alcohol (days/wk). Only for breastfeeding a small but significant effect was found in the lumbar spine ($P < .05$) and the femoral neck ($P < .05$).

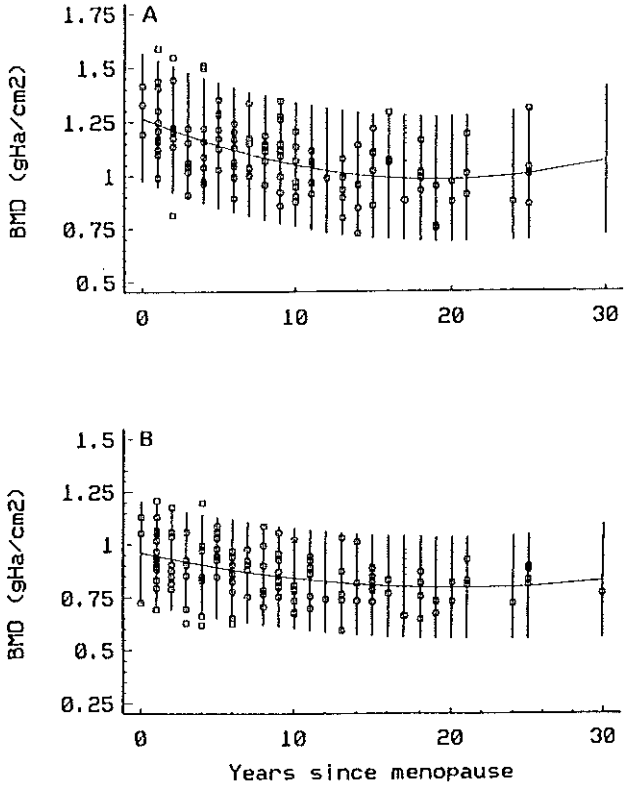

Figure 10.2 *Curves of BMD against years since menopause (YSM). BMD expressed as gHa/cm². Predicted and observed values with 95% interval for predictions.*
A. Lumbar vertebrae 2-4: $Y = 1.261 + (-0.0282 * YSM) + (0.00076 * YSM^2)$, $P < .01$.
B. Femoral neck: $Y = 0.961 + (-0.0157 * YSM) + (0.00038 * YSM^2)$, $P < .01$.

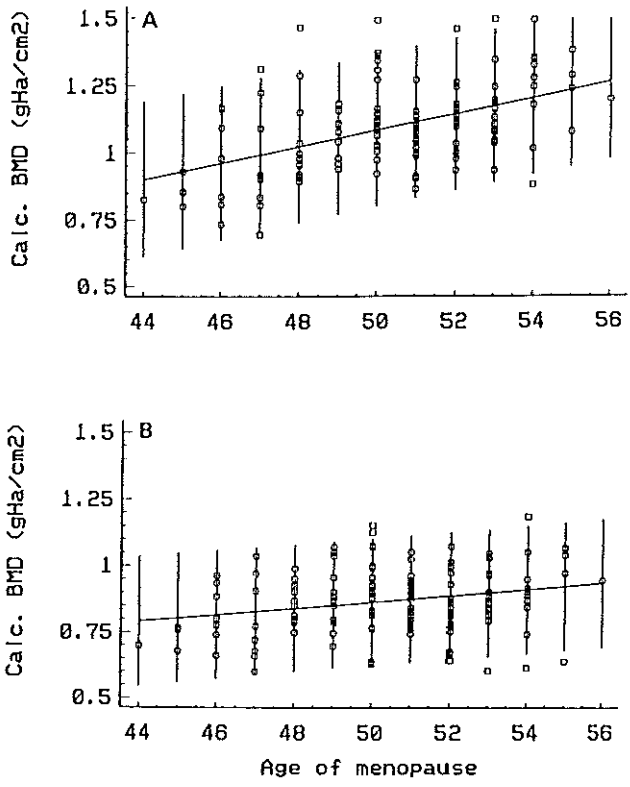

Figure 10.3 *The relation between actual age of menopause and calculated BMD at the mean age of the postmenopausal group examined. Predicted and observed values with 95% interval for predictions.*
*A. Lumbar vertebrae 2-4: L2-4$_{measured}$ - (-0.011347 * (AGE - 59.6)), P < .001.*
*B. Femoral neck: Fneck$_{measured}$ - (-0.006629 * (AGE - 59.6)), P < .001.*
(means multiply, 59.6 represents the mean age of the postmenopausal women).*

Table 10.3 *Results of regression analysis**

Univariable analysis:

Premenopausal:

	ic	age	*P*
L2-4	1.365	-0.0031	<.05
Fneck	1.089	-0.0031	<.01

Postmenopausal:

	ic	age	*P*
L2-4	1.597	-0.0084	<.001
Fneck	1.198	-0.0056	<.001

Multivariable analysis

Premenopausal:

	ic	age	*P*	BMI	*P*
L2-4	1.011	-.0034	<.01	0.0156	<.001
Fneck	0.823	-.0032	<.01	0.0116	<.001

Postmenopausal:

	ic	age	*P*	BMI	*P*
L2-4	1.234	-.0085	<.001	0.0144	<.001
Fneck	0.823	-.0057	<.001	0.0146	<.001

	ic	age	*P*	BMI	*P*	AOM	*P*
L2-4	0.003	-.0115	<.001	0.0118	<.001	0.0293	<.001
Fneck	0.415	-.0066	<.001	0.0138	<.001	0.0098	<.001

* Intercept (ic (g/cm^2)), age (g/cm^2/year), BMI ($g/cm^2/(kg/m^2)$) and AOM (g/cm^2/year) coefficients and *P*-values from univariable and multivariable regression analysis, stratified by site of measurement and menopausal state.

DISCUSSION

The aim of the present study was to determine for the Dutch female population, age-related changes in BMD as measured with DXA . In addition, the influences of various potential risk factors on bone mass have been studied.

In the premenopausal women we observed a small (0.2 - 0.3 %/yr) although significant bone mineral loss in the lumbar spine and proximal femur. These results are comparable to those obtained by others, using similar [13] and other bone mass measurement techniques [15-17].

For the postmenopausal women we observed a three times higher age-related bone loss in the lumbar spine and a two times higher loss in the proximal femur. The apparent total bone loss in the lumbar spine of about 30 % appears comparable to that found in a previous study of our group using DPA[6].

In the first ten years after the menopause, bone loss in both the lumbar spine and proximal femur appears to be exponential. For the population studied about 60-70 % of total postmenopausal bone loss seems to occur during this period. This implies a postmenopausal loss of about 15%. Our results, therefore, indicate an important menopause-related bone loss superimposed on the age-related bone loss[8,9]. Also other investigators obtained similar results for a comparable cohort[7].

Our data also indicate that an early menopause is a risk factor for osteoporosis. From the regression between actual age of menopause and BMD we calculated that a one year later onset of the menopause compensates for two years of age-related bone mineral loss.

In this cross-sectional study, the strict exclusion criteria used will have resulted in a certain degree of selection bias. However,the use of these criteria is the only way to minimize the interference of potential confounders like degenerative changes of the spine[34], immobilization and metabolic bone diseases. Therefore, our data represent healthy Dutch women. Of course this is different from a population based study like the Rotterdam Study.

This Rotterdam study clearly shows the confounding effect of osteoarthritis on lumbar spine BMD measurements, and indicates that in elderly subjects, hip measurements give a better impression of BMD[18]. In cross-sectional studies, like our investigation, cohort effects may also affect the slope of the regression, between age and BMD, upwards if one assumes that younger persons will have lower age-adjusted BMD's compared to older persons. In this respect life style factors as physical activity and diet could play an important role[35,36]. Furthermore, this may explain the apparent increment of BMD when the results are plotted against YSM (Fig 10.2 A and B, right hand party of the curves).

The highly significant correlation between BMD and BMI, as also found by several other investigators[22,24,25], can be explained by the endogenous production of estrogen in fat tissue[37]. Because the correlation with BMI was also found in premenopausal women other factors such

as increased weight bearing may also be of importance.
Of the additional potential risk factors (parity, breastfeeding, cumulative smoking and current alcohol use) we examined, only for breastfeeding a small but significant negative effect on bone mineral mass was observed. In another cross-sectional study in healthy Dutch women also a negative effect of breastfeeding was found and it was concluded that duration of breastfeeding was of more importance than parity[25]. On the other hand a recent prospective study of one year recently showed a fast decrease of bone density in breastfeeding women, but this loss was regained within a year[33].
We noticed no effects of smoking or use of alcohol. In our group 19 % of the women were current smokers. This is somewhat lower than the percentage of smokers (40 %) in large health interview surveys in the Netherlands[38]. In our group 73 % of the women used alcohol once a week or more. This is comparable to the normal Dutch population, in which 70 % uses alcoholic drinks once a week or more[39]. Another study showed a more important effect of tobacco or alcohol (ab)use[26]. In this study however the subjects under investigation used on the average much higher amounts of alcohol and nicotine than the group we studied.

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CHAPTER 11

DISCUSSION OF THE MAJOR CONCLUSIONS

DISCUSSION OF THE MAJOR CONCLUSIONS

The central theme of this thesis is the question whether combination of an antiresorptive drug (estrogen or pamidronate) with a potential stimulator of bone formation (nandrolone decanoate) or bone turnover (growth hormone) might result in a larger increase in bone mass than the use of the antiresorptive agent only.

Normally bone formation and bone resorption are coupled, but in the treatment of osteoporosis the ideal situation would be a shift in the balance betweeen the two processes in favour of the bone formation. In terms of bone mineral mass, a transient (positive) effect of bone mineral mass can be expected from an anti-resorptive agent, which will only be temporary until a new equilibrium has been established. When uncoupling can be established a more continuous increase of the bone mineral mass would be expected.

It is important to distinguish a situation of uncoupling from a state of low bone turnover. With a low bone turnover state the number of bone multicellular units (bmu's) is diminished, but at the level of the bmu's the process of turnover can still be coupled. This implies, that with a drug that decreases bone turnover, remodelling space will be filled in, but ultimately a new steady state will be reached. Even when there is a negative balance at the bmu level, this mechanism will lead to some increase in bone mass although after the new steady state is reached again bone loss can occur[1]. With uncoupling at the level of the bmu an additional effect on bone formation is warranted with of course no effect on bone resorption. In other words a specific stimulation of the activation of osteoblasts will induce a certain degree of uncoupling. The response to treatment with fluoride is a typical example of stimulation of bone formation.

Looking at our HRT + Nandrolone - trial no evidence has been obtained to support that uncoupling really took place. What is actually seen is an apparent correction by nandrolone of a fall in bone mass during the third year of HRT. This might be due to a waning effect of the estrogen-antiresorptive effect and a perseverance of a nandrolone-antiresorptive effect. In a theoretical model this effect has been described by Adami and Kanis[2]. This waning effect was also present in the biochemical parameters, in which an increase of some of the biochemical bone turnover parameters was observed during the third year of treatment in the HRT-group, whereas in the HRT + ND group bone turnover was still suppressed.

Another explanation for the differences, occurring during the third year of treatment, would be an indirect effect of nandrolone on bone mass by inducing an increase of skeletal muscle mass. During the fourth year of follow-up, in which no treatment was given, both groups

showed comparable changes in bone mineral mass as well as in bone turnover parameters. Bone resorption as well as formation parameters showed a rapid (coupled) increase. This implies that the extra effect of nandrolone-decanoate (during the third year) is only temporary and tend to disappear shortly after treatment has stopped.

Taken together; ND appears not to be a stimulator of bone formation, but has to be looked upon as an anti-resorptive agent. The effects of ND in the presence of HRT are rather late, indicating that HRT itself induces a maximal antiresorptive effect, during the first two years of treatment.

GH is a clear example of a stimulator of bone turnover, as is also supported by the studies described in this thesis (chapter 8 and 9).

In postmenopausal osteoporotic women, treatment with the combination of pamidronate and GH for half a year showed no effects on bone mineral mass. Only minor effects on bone turnover parameters were found. However, these rather small effects did indicate a small increase in bone formation, i.e. osteocalcin as well as procollagen-1 were increased with no effects on bone resorption parameters (Fig. 8.4). It is possible that a shift of the coupling at the bmu level in the direction of bone formation could explain this observation. However, the rather small changes in bone formation indices with no change in bone resorption parameters might explain that no changes in the bone mineral mass were observed in the patients treated with GH + pamidronate.

In the patients treated with pamidronate-only the decrease in bone turnover, as shown by a decrease in bone turnover parameters, resulted in the expected gain of bone mineral mass. It remains possible that the treatment period and the period of follow-up were too short to observe an increase in bone mineral mass in the patients treated with the combination of GH + pamidronate.

Also in GH-deficient adults treated with rhGH, vandeWeghe et al. showed an increase in bone mineral mass after a follow-up period of up to three years, whereas in the first year a decrease in bone mineral mass was observed, although bone formation indices were increased[3].

In our GH-deficient patients, who were treated with a combination of GH and pamidronate as well (no control group with pamidronate-only was studied), several differences were found.

A possibly important difference between both patient groups is the increase mainly in cancellous bone in the GH-deficient group, whereas initially treatment with GH and pamidronate resulted in a small decrease of bone mineral mass in the osteoporotic women

studied (Fig. 9.4, cf Fig 8.1).

It is important to realise that both patient groups can not be analysed together, as the osteoporotic patients were not GH-deficient according to their serum IGF-I levels.

A possible explanation for the more important changes in the GH-deficient patients is a more significant shift of bone formation at the level of the bmu. Furthermore, pamidronate inhibited the GH-induced increase in remodelling space.

In a recent study in which elderly sheep were treated with human PTH (1-34), also no increase of bone formation was found in the presence of the anti-resorptive agent tiludronate[4]. This seems comparable to the results found in our osteoporotic women, treated with a combination of GH and pamidronate. One might speculate that the anabolic effect of GH or PTH on bone is not maintained when a bisphosphonate is coadministered. Because bisphosphonates are selective inhibitors of osteoclastic bone resorption that do not directly affect osteoblastic bone formation, it is suggested that activation of bone resorption may be a prerequisite for an anabolic agent to be active.

Another possible explanation for the differences found between both groups is the way in which medication was initiated. The osteoporotic women started with the combination of GH and pamidronate at the same moment, whereas the GH-deficient patients were treated initially with GH-only for two weeks. From then onwards pamidronate was added. In this scheme at first activation is stimulated and thereafter resorption is counteracted. It is possible that in the osteoporotic women the anti-resorptive agent already had induced a diminished turnover, after which GH could not act anymore as a stimulator of activation. This might implicate a very rapid effect of pamidronate on bone turnover.

The additional treatment with GH resulted in a positive effect of GH on lean body mass and a decrease in fat mass. This represents the anabolic effect of GH, as has been observed in many other studies in which GH-deficient patients were treated with GH. Only few results are available in healthy people, but these results also indicate an increase in lean body mass and a decrease in fat mass[5].

The studies presented in this thesis support the view that bone turnover parameters are better indicators of a response to treatment than early follow-up BMD measurements. In all the studies presented differences in bone turnover parameters were visible as early as 3 months, whereas the earliest changes in bone mineral mass measurements were found after 6 months of follow-up at the earliest.

When various study results are translated to a single patient it is important to take into account some variables. The two main variables with respect to bone mass meaurements or

bone turnover parameters are the coefficiency of variation (CV) of the method and the rate of change in the measurement results induced by treatment.
Although the bone mass measurements have a high precision it is not feasible to examine the short-term effects of treatment because of the rather small changes induced.
For example a period of follow-up for 1 year is necessary for bone mass measurements with a CV of 2 % and a change in bone mass of 5 %.
Although the CV for the various bone turnover parameters is much higher than for the bone mass measurements the required duration of follow-up to obtain significant changes is shorter because of the higher rate of change.
For example a period of follow-up of about 0.5 year is needed when changes in the order of 20 - 40 % are present with a CV for the method of 5 - 15 %
At present no really good discriminative single biochemical measurement with respect to people at risk for fractures is available. In the future new medications or combinations of medications will be developed for the treatment of established osteoporosis. Another subject of future research will have to be the assessment of the value of the various parameters measured longitudinally with respect to the effects of treatment. Until those results are available, we will have to use a variety of measurements, bone mineral mass as well as biochemical measurements, to predict and follow the effect of treatment in an individual.

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SUMMARY

SAMENVATTING

SUMMARY

Osteoporosis is a disease, in which a lot of interest has been shown during recent years. This is for a large part the result of the increasing age of the population, because the occurrence of osteoporosis increases with age.
Next to the diminishment of the amount of bone also a diminished stuctural quality of the bone occurs in osteoporosis. The ultimate result of osteoporosis, is an increased incidence of fractures, especially fractures of the hip, spine and wrist.
Osteoporosis can be divided in a primary and secondary form, the secondary being the result of an underlying disease. Primary osteoporosis is related to a lot of risk factors, but the most important cause is occurrance of the menopause. With the loss of estrogen production, an increased resorption of bone occurs, which leads to a decline in bone mineral mass. Because this loss of estrogen production is important for the genesis of osteoporosis, the incidence of osteoporosis in women is higher than in men. However, because also other factors are important, osteoporosis is not strictly a disease of women.
The fractures, especially the hip fractures, induce a high morbidity and also a rather high mortality.
For the above mentioned reasons, it is certainly worthwhile to try to prevent the occurrence of osteoporosis. Next to the prevention of osteoporosis it is also necessary to prevent further bone loss in patients with established osteoporosis.
In ***chapter 1*** several risk factors and epidemiological aspects of osteoporosis are described. Because a low or decreased bone mass is one of the most important factors for the development of fractures, it is important to be able to measure the bone mass as accurately as possible. Whereas in the past only few possibilities were available to measure bone mass, during the last two decades a number of measuring methods have been developed. These techniques are able to assess the bone mass in a non-invasive manner. Unfortunately, however, these techniques do not give a good impression of the connectivity of the cancellous bone, another important factor for the strength of bone. In ***chapter 2*** the various bone mass measurement techniques are discussed. In conclusion, it can be stated that, at present, various methods are available with a high precision, a good accuracy and a very low radiation dose. These important facts have made it possible to follow the bone mass in a longitudinal manner, that is to describe the natural and pathological course of bone mass, as well as the effect of various treatment schemes to try to increase bone mineral mass. Next to the measurements, which are dependent on a radioactive source or on x-rays also the use of ultrasound techniques for the measurement of bone mass is discussed.
By measuring several biochemical markers of bone mineral metabolism in serum or urine, it is possible to investigate and follow bone turnover. These markers can be divided into two

groups, one related to bone formation, the other related to bone resorption. When the menopause is reached an increase of bone turnover occurs, as is apparent from the increase of the various bone turnover parameters.
The various markers of the bone turnover can be followed during treatment. In general the bone formation and resorption are coupled, which means, that with a decrease in bone resorption also the bone formation is subsequently diminished. As to biochemical parameters until now, no "golden standard" is available. Therefore, it is necessary to use a combination of the various markers of bone turnover to get a reasonable impression of the bone turnover activity. This can be strengthened by having measurements longitudinally.
In recent years several attempts have been made to find a good treatment to prevent osteoporosis or to find a treatment, which can be used for established osteoporosis. The ultimate goal for the treatment of osteoporosis is to prevent or decrease the number of new fractures. In ***chapter 4*** the various treatment schemes, as they are or have been used, are presented. With respect to the prevention of the development of osteoporosis, the most extensively used treatment, with good results, is estrogen substitution. Estrogen substitution has then to be started within a few years after the menopause. About other preventive regimens very few data are available.
A number of studies have been published on the treatment of established osteoporosis, although the results were varying. Generally they were positive with respect to increasing bone mineral mass. In ***chapter 4*** the design of several treatment schedules is presented in tables. The role of estrogens is also paramount here. Finally a brief overview about osteoporosis in men and corticosteroid induced osteoporosis as well as the treatment of both disorders is presented in ***chapter 4***.

The investigations, which are presented in this thesis, were aimed at a combination of several medications and the effects of these combinations on bone mass measurements as well as biochemical parameters of bone turnover. The goal was to investigate, whether it was possible with the various medications to induce uncoupling between bone resorption and bone formation. Normally resorption and formation are coupled to one another. If uncoupling between formation and resorption could be reached it was hypothesized that a more important and more sustained effect on bone mass could be reached.

In ***chapter 6*** the effects of hormone replacement therapy (HRT) for two years with or without the addition of nandrolone decanoate (ND), an anabolic steroid, are described. A prominent increase of bone mineral mass was observed, in the lumbar spine as well as in the forearm Bone turnover was suppressed as measured with biochemical parameters. Between both treatment groups no differences were observed, and no indication as to whether formation

and resorption were uncoupled were found.

Chapter 7 describes the effects in the same groups of patients, after the treatment was continued for another (third) year. Also the effects after one year of discontinuation of treatment were studied. During the third year of treatment a difference in bone mass occurs between both groups, i.e. the group treated with HRT alone showed a little decline in bone mineral mass values, whereas the other group, treated with ND, in addition to HRT showed a continuous rise of bone mineral mass, measured with two independent methods. A possible explanation for this observation, is the waning effect of HRT, thereby showing more clearly the effect of ND. Another explanation might be, the anabolic effect of ND, especially on muscle mass, and thereby inducing indirectly an increase in bone mineral mass. After treatment had been stopped for one year, a comparable decline in bone mineral mass in both groups was observed, although the group also treated with ND had a higher value of bone mass after this year of follow-up. Also biochemical parameters showed a comparable pattern in both groups after treatment was withdrawn, i.e. a rather high increase in bone turnover parameters. The virilizing effects of ND were noticeable at various sites. During the use of ND changes of the voice occurred, which were only partly reversible after the treatment was withdrawn. Muscle mass, extrapolated from serum creatinine values, increased. Also the amount of hemoglobin increased, as a result of increased erytropoiesis. The positive effect of HRT on plasma lipids was blunted by the addition of ND.

In conclusion, the combination of HRT and ND seems not to be a combination to be used routinely in the treatment of postmenopausal osteoporosis. Only minor positive effects of addition of ND with respect to bone mineral mass were found during the third year of treatment, while on the other hand some side effects were observed in the group treated with ND in addition to HRT.

In *chapter 8* the effects of another anti-resorptive agent namely a bisphosphonate, pamidronate, on the bone mineral mass and bone turnover parameters are described in a group of patients with postmenopausal osteoporosis. The patients were treated with growth hormone (GH) in a placebo-controlled manner, as a stimulator of bone turnover. The results showed a decrease of bone resorption when treatment with pamidronate only was given, which resulted in an increased bone mass. In the group, in which the patients were also treated with GH, in addition to pamidronate, this effect on bone mineral mass was not found. The biochemical parameters in this group (GH plus pamidronate) did not show many changes, when compared to the group, treated with pamidronate only. Next to the effect on bone mineral mass and bone turnover also the effects on body composition are described. In the GH-treated group, a clear effect of GH was shown, i.e. a decrease of fat tissue and an increase of lean body mass (indicating an increase of muscle mass). Several hypotheses about the differences between both treatment groups are discussed in this chapter.

In ***chapter 9*** again effects of GH, combined with the administration of pamidronate are presented. In this study, growth hormone deficient patients were studied. The same group of patients was also treated with GH-only 3 years ago. The results, previously obtained, were compared with results obtained in the same group, now treated with a combination of GH and pamidronate. When the patients were treated with GH and pamidronate an increase in bone mass was observed. In the earlier study, a small decrease in bone mass was found. The additional administration of pamidronate seems to antagonize this loss. It is important to emphasize that the bisphosphonate treatment was started two weeks after treatment with GH had begun. This may be an important difference with respect to the treatment in the postmenopausal women, in whom both medications were started at the same time. The effect on bone turnover parameters with the combined treatment of GH and pamidronate was less than with the treatment of GH alone. This is probably due to the anti-resorptive effect of pamidronate.

In ***chapter 9*** also the effect of GH and GH combined with pamidronate on the calciotropic hormones: parathyroid hormone, 25-(OH) Vit D_3 and 1,25-$(OH)_2$ Vit D_3 and their effect on the tubular reabsorption of phosphate are discussed. It is concluded, that GH induces an increase in tubular reabsorption of phosphate, which can be counteracted by simultaneous administration of pamidronate.

In ***chapter 10*** the normal values of bone mineral mass, in the lumbar spine as well as in the proximal femur, are presented, measured in a group of 260 healthy Dutch females, by means of DXA. Also an analysis with respect to risk factors was performed. In the premenopausal women a small age-related loss was observed in the lumbar spine as well as in the proximal femur. In the postmenopausal women a far greater age-related loss (2-3 times) at the various sites of measurement, was found. With respect to risk factors, especially an early menopause was shown to be an important risk factor, whereas also breastfeeding had a small negative effect on bone mineral mass.

SAMENVATTING

Osteoporose is een ziektebeeld, dat de laatste jaren volop in de belangstelling is komen te staan. Dit is vooral een gevolg van de toenemende vergrijzing van de bevolking, aangezien het optreden van osteoporose toeneemt met de leeftijd.
Bij osteoporose treedt naast een vermindering van de hoeveelheid bot, ook een verminderde structurele kwaliteit van het bot op, waardoor het uiteindelijke resultaat van de osteoporose of 'botontkalking', namelijk een toename van het aantal fracturen, en dan met name heup-, wervel- en onderarmfracturen, voor een ieder duidelijk zichtbaar wordt.
Osteoporose kan worden onderverdeeld in een primaire en secundaire vorm, waarbij de secundaire vorm veroorzaakt wordt door een onderliggende ziekte. Primaire osteoporose is gerelateerd aan een groot aantal risicofactoren, maar de voornaamste oorzaak is het optreden van de menopauze. Bij het verliezen van de oestrogeenproduktie, treedt een sterke toename van de botafbraak op, hetgeen leidt tot afname van de botmassa. Aangezien dit verlies van oestrogeen produktie, belangrijk voor het ontstaan van osteoporose, is ook de incidentie van osteoporose bij vrouwen veel hoger dan bij mannen. Aangezien er echter ook andere factoren een rol spelen is osteoporose niet een geslachtsgebonden ziektebeeld.
De fracturen, met name de heupfracturen, leiden tot een hoge morbiditeit en uiteindelijk ook een relatief hoge mortaliteit. Het is dan ook zeker de moeite waard om het optreden van osteoporose te proberen te voorkomen. Naast het voorkomen van osteoporose is het ook noodzakelijk om bij patiënten met osteoporose een verdergaande botafbraak te voorkomen.
In ***hoofdstuk 1*** worden de diverse risicofactoren en epidemiologische aspecten van osteoporose verder toegelicht.
Aangezien een lage of verlaagde botmassa één van de belangrijkste factoren is voor het optreden van fracturen is het van belang deze botmassa zo nauwkeurig mogelijk te kunen vaststellen en te kunnen vervolgen. In het verleden waren slechts beperkte mogelijkheden om de botmassa vast te kunnen stellen beschikbaar. De laatste twee decennia zijn er een aantal meetmethoden ontwikkeld. Deze technieken zijn in staat op een niet-invasieve wijze een indruk te geven van de botmassa. Helaas zijn deze meetmethoden niet in staat een uitspraak te doen over de samenhang van het bot, een factor ook belangrijk voor de sterkte van het bot. In ***hoofdstuk 2*** zijn de diverse botmassametingen besproken. Concluderend kan gesteld worden dat er momenteel een aantal verschillende methoden met een hoge precisie en gevoeligheid beschikbaar zijn. Deze hoge precisie en gevoeligheid alsmede een lage stralingsbelasting, heeft er toe geleid, dat ook in longitudinale studies het natuurlijk en pathologische beloop van de botmassa of een effect van behandeling kan worden vastgesteld.
Naast de meetmethoden welke afhankelijk zijn van radioactieve straling of röntgenstraling wordt ook de toepassing van ultrageluid als mogelijkheid om botmassa te bepalen besproken.

Samenvatting

Door middel van het vervolgen van diverse merkstoffen van botmineraal metabolisme in het bloed of in de urine, is het mogelijk een uitspraak te doen over de ombouwactiviteit of turnover van het bot. Deze merkstoffen zijn onder te verdelen in 2 groepen, de ene groep is gerelateerd aan botaanmaak, de andere aan botafbraak. Bij het optreden van de menopauze treedt een toename van de botombouwactiviteit op, hetgeen zich uit in stijging van de verschillende merkstoffen.
De merkstoffen kunnen worden vervolgd bij het toepassen van verschillende behandelingen. In het algemeen zijn de aanmaak en afbraak van bot gekoppeld, dit betekent dat bij een afname van de afbraak vervolgens ook de aanmaak wordt geremd. In ***hoofstuk 3*** worden de verschillende biochemische merkstoffen van de botombouw besproken. Totnogtoe is er helaas geen "gouden standaard" beschikbaar, zodat in ieder geval voorlopig nog een combinatie van de aanwezige merkstoffen moet worden gebruikt om inzicht te krijgen in de botombouwactiviteit.
De afgelopen jaren is men op zoek geweest naar een goede behandeling om het optreden van osteoporose te voorkomen, dan wel een behandeling toe te passen bij het aanwezig zijn van osteoporose. De behandeling van osteoporose heeft als doel het optreden van fracturen te voorkomen dan wel het aantal te verminderen. In ***hoofdstuk 4*** worden de verschillende behandelingsschema's, zoals deze zijn toegepast, besproken. Wat betreft het voorkomen van osteoporose, is de meest beproefde methode met goede resultaten, het toevoegen van oestrogenen. Dit dient dan binnen enkele jaren na het bereiken van de menopauze gestart worden. Over andere preventieve behandelingen is slechts weinig bekend.
Voor de behandeling, bij het aanwezig zijn van osteoporose, zijn een groot aantal studies verricht met wisselende resultaten. In ***hoofdstuk 4*** wordt de opzet van de verschillende behandelingsschema's in een aantal tabellen toegelicht. Naast oestrogenen zijn een groot aantal andere medicamenten toegepast. Uiteindelijk wordt in ***hoofdstuk 4*** ook kort ingegaan op het voorkomen en de behandeling van osteoporose bij mannen, en osteoporose bij het gebruik van corticosteroïden.

De in dit proefschrift beschreven onderzoeken, richtten zich op de combinatie van de diverse medicamenten en het effect hiervan op zowel de botmassametingen als ook de biochemische botombouwactiviteit. Het doel was om te onderzoeken of het mogelijk was met verschillende medicamenten de koppeling tussen aanmaak en afbraak te doorbreken en op die manier een verdergaand en aanhoudend effect op de botmassa te bewerkstelligen.

In ***hoofdstuk 6*** worden de effecten van hormonale replacement therapie (HRT), gedurende twee jaar behandeling, al dan niet gecombineerd met nandrolone-decanoate (ND), een anabool steroïd, beschreven. Hierbij werd een duidelijke toename van de botmassa

waargenomen, zowel in de lumbale wervelkolom als aan de onderarm. De biochemische parameters toonden een onderdrukking van de botombouw. Tussen de beide groepen konden geen duidelijke verschillen worden waargenomen, en op grond van de gevonden resultaten kon dan ook van ontkoppeling tussen formatie en resorptie niet worden gesproken.

Hoofdstuk 7 beschrijft de effecten in dezelfde groep patiënten, waarbij de behandeling van beide groepen gedurende een derde jaar wordt gecontinueerd. Tevens wordt het effect van het staken van de therapie na 1 jaar beschreven. Gedurende het derde jaar treedt er een verschil op in botmassa tussen beide groepen, waarbij de groep, behandeld met HRT een geringe daling in de botmassa vertoont, maar de andere groep, welke naast HRT ook met ND behandeld een aanhoudende stijging vertoont, gemeten met twee verschillende methoden. Een mogelijke verklaring voor deze observatie is, dat het effect van HRT verdwijnt, waardoor het effect van ND zichtbaar wordt. Een andere verklaring is dat het anabole effect van ND, met name op de spieren, de oorzaak is van een toename van de botmassa. 1 jaar na het staken van de therapie in beide groepen, toonden beide groepen een vergelijkbaar verlies van botmassa, waarbij de groep behandeld met ND op een hoger niveau van botmassa zit, dit tengevolge van een hogere botmassa na het derde jaar. Ook de biochemische parameters gedurende het vierde jaar van follow-up tonen een vergelijkbaar beloop in beide groepen, namelijk een forse stijging van de botombouw parameters.

Het viriliserende effect van ND was waarneembaar op verschillende plaatsen. Bij het gebruik van ND traden forse stemveranderingen op, welke deels reversibel waren na het staken van de behandeling. Spiermassa, gemeten aan een stijging van het serum kreatinine gehalte, toonde een toename. Het hemoglobine gehalte steeg fors bij het gebruik van ND. Dit laatste kan worden toegeschreven aan een positief effect van ND op de erytropoiese. Het positieve effect van HRT op de plasmalipiden werd teniet gedaan door toevoeging van ND.

Concluderend lijkt het niet geïndiceerd de combinatie van HRT en ND in de behandeling van osteoporose routinematig toe te passen.

In ***hoofdstuk 8*** worden de effecten van een bisfosfonaat, pamidronate, op de bot massa en botombouw weergegeven in een groep patienten met postmenopausale osteoporose. Dit bisfosfonaat is een botresorptieremmer. Tevens werden de patienten placebo-gecontroleerd behandeld met groeihormoon (GH) als stimulator van botturnover. De resultaten toonden een goede remming van de botresorptie met pamidronate, gecombineerd met placebo, gepaard gaande met een forse toename van de botmassa. In de groep welke werd behandeld met GH en pamidronate werd dit effect op de botmassa niet waargenomen. De biochemische parameters in deze groep waren minder duidelijk gewijzigd in vergelijking met de placebo-behandelde groep. Naast de effecten op botmassa en botombouw activiteit werd ook naar de lichaamssamenstelling van beide groepen gekeken. In de groep behandeld met GH was een duidelijk effect zichtbaar van dit GH, namelijk afname van de hoeveelheid vetweefsel en

toename van de vet vrije massa (= o.a. spier). Over de verschillen, zoals deze tussen beide groepen werden waargenomen, zijn diverse hypothesen op te stellen welke in de discussie van het hoofdstuk worden toegelicht.

In ***hoofdstuk 9*** worden opnieuw de effecten van GH in combinatie met pamidronate beschreven, echter nu in GH deficiënte patiënten. Dezelfde groep patiënten was in het verleden (3 jaar terug) met alleen GH behandeld geweest. De resultaten welke toendertijd werden verkregen werden nu vergeleken met de huidige resultaten. Hierbij bleek dat met de combinatie van GH en pamidronate een toename van de botmassa optrad. Bij het eerdere onderzoek (alleen behandeling met GH) werd een geringe afname van de botmassa waargenomen. De toevoeging van pamidronate lijkt dit verlies tegen te gaan. Het is van belang te benadrukken, dat de toediening van pamidronate pas twee weken na de start met GH werd gestart. Dit is een duidelijk verschil met de behandeling van de postmenopauzale vrouwen met osteoporose, waar beide medicamenten tegelijkertijd werden gestart. Het effect op de biochemische botombouw parameters was bij de behandeling met zowel GH als pamidronate minder uitgesproken dan eerder. Dit wordt waarschijnlijk veroorzaakt door remming van de botresorptie door pamidronate, waarbij er in de eerdere studie alleen een anabool hormoon, GH, werd gegeven.

In ***hoofdstuk 9*** wordt ook het effect van GH en pamidronate op de diverse calciotrope hormonen; parathyroid hormoon, 25-(OH) Vit D_3 en 1,25-$(OH)_2$ Vit D_3 en hun effect op de terugresorptie van fosfaat in de nier besproken. Geconcludeerd wordt GH een toename van de renale fosfaat terugreabsorptie geeft, hetgeen kan worden tegengegaan door gelijktijdige toediening van pamidronate.

In ***hoofdstuk 10*** worden de normaalwaarden van botmassa, van zowel de lumbale wervelkolom als de heup gepresenteerd, zoals deze in een groep van 260 gezonde Nederlandse vrouwen met behulp van DXA werden verkregen. Tevens werd een analyse verricht naar risicofactoren. In de premenopauzale groep vrouwen werd een gering van de leeftijd afhankelijk verlies van de botmassa waargenomen in zowel de wervelkolom als in de heup. Bij de postmenopauzale vrouwen werd een veel duidelijker (2-3 x zo groot) leeftijdsafhankelijk botmassa verlies waargenomen. Met betrekking tot risicofactoren bleek een menopauze op jonge leeftijd een fors risico met zich mee te dragen, terwijl ook borstvoeding een gering negatief effect op de botmassa had.

NAWOORD

CURRICULUM VITAE

NAWOORD

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CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 7 september 1964 te Genemuiden. Na het behalen van het Atheneum B diploma aan de Rijksscholen Gemeenschap te Meppel, werd in 1982 met de studie geneeskunde aangevangen aan de Erasmus Universiteit te Rotterdam. In 1989 werd het artsexamen afgelegd. Aansluitend was hij tot 1990 werkzaam als wetenschappelijk medewerker bij de afdeling Inwendige Geneeskunde III van het Academisch Ziekenhuis "Dijkzigt" te Rotterdam (hoofd Prof. Dr. J.C. Birkenhäger) op de afdeling Klinische Pathologie (Mw. D.H. Birkenhäger-Frenkel), waarbij de basis voor dit proefschrift werd gelegd. Vanaf januari 1990 was de schrijver werkzaam als arts-assistent op de afdeling Inwendige Geneeskunde III en per 1 december 1990 werd de opleiding tot internist aangevangen op dezelfde afdeling (hoofd Prof. Dr. J.C. Birkenhäger). Op 1 september 1995 werd gestart met de opleiding in het kader van het aandachtsgebied Endocrinologie. Het onderzoek zoals beschreven in dit proefschrift werd deels op de Balans-afdeling (Clinical Research Unit, hoofd Prof. Dr. J.C. Birkenhäger) en deels op de afdeling Nucleaire Geneeskunde (hoofd Prof. Dr. E.P. Krenning) verricht, in samenwerking met de afdeling Radiodiagnostiek (hoofd Prof. Dr. H.E. Schütte).