Tibolone and bone

Tibolone en bot

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1

Introduction: Tibolone as Hormone Replacement Therapy (HRT), a comparison with continuous combined HRT and Raloxifene.

1.1 History and definition of the study objectives

The menopause is defined as the time at which a woman has her final menstruation. The average age at menopause is 51 years. In contrast to the decreased age at which menarche takes place, the age at menopause remained constant over the last century (McKinlay *et al.*, 1992). In the early 18th century, life expectancy of (upper class) women increased from 55 to 65 years (Cope, 1976). It is unsurprising that the first reports concerning the climacteric period date from that period (Titus, 1710; anonymous, 1727). At that time the problems of postmenopausal women were attributed to the cessation of menstrual blood flow leading to accumulation of poisons in the body. In the 19th century Tilt published a work which hypothesised that menopausal problems were caused by ovarian failure (Tilt, 1857). The previous theory of poisons accumulation was abandoned. At the end of the 19th century, Maintzer was the first practitioner to treat young ovariectomised women with bovine ovarian extracts (Maintzer, 1896). At the beginning of the 20th century Starling introduced his concept of "hormones". Subsequently, the knowledge of ovarian function increased. After the isolation of the first estrogen (oestron) from theburine of pregnant women in 1929 (Butenandt, 1929), the concept of estrogen deficiency and "postmenopausal syndrome " was fairly well established, by around 1930. After isolation of estradiol in 1940, commercial hormone replacement therapy (HRT) preparations became available from the 1950s onwards. HRT became more popular after publication of the book "feminine forever" in 1966, a rather uncritically advocate for the use of estrogens. Interest in HRT faced a serious blow in 1975 when unopposed estrogen therapy was associated with a higher risk of endometrial carcinoma

in non-hysterectomised women (Ziel *et al.*, 1975; Smith *et al.*, 1975). From then on the use of progestins to protect the endometrium from estrogen-induced hyperplasia was mandatory. During the last decade, the positive effect of long term HRT on osteoporosis became apparent. In addition, active research into the mechanism of action of estrogens on the cardiovascular and nervous systems is underway. However, in spite of benefits of long-term HRT, compliance with treatment is poor. Scheduled withdrawal bleeding substantially reduces the acceptability of and compliance with HRT (Groeneveld *et al.*, 1998). Since it is the anti estrogenic effect rather than the shedding of the endometrium that is the important factor in preventing atypical hyperplasia or endometrial carcinoma, strategies to avoid withdrawal bleedings are now under development. A first approach is to add a progestin continuously to the estrogen administration in order to induce endometrial atrophy. Although spotting in the first months of therapy is frequent, about 90 % of the postmenopausal women will achieve amenorrhoea. More recently, effects of direct intrauterine administration of progestin, using a levonorgestrel releasing intrauterine device, have been studied. In addition to the protective effects on the endometrium and contraceptive properties, this device also reduces menstrual blood flow, which makes it especially suitable for use in perimenopausal women. Another approach to eliminating progestin-induced withdrawal bleeding is to develop target tissue specificity. The ideal compound would have the positive effects of estrogens on climacteric symptoms, bone and lipid profile, but would lack a stimulatory effect on the endometrium or breast. Tibolone is at present the only target tissue specific compound that alleviates postmenopausal symptoms without the need for withdrawal bleeding.

Another important factor contributing to low compliance is the association of long-term HRT with breast cancer. When HRT is used for longer than 5 years there is a somewhat increased risk (relative risk 1.3) of breast cancer (Beral et al., 1997). In this respect, the development of selective estrogen receptor modulators is of special interest. Raloxifene is first SERM to be developed that has no stimulatory effect on the endometrium and breast but has an estrogen-like effect on bone. The target tissue specificity of tibolone and SERMs will be discussed in the next section. In the present chapter, tibolone will be compared with continuous combined HRT and with raloxifene with respect to the most important aspects of HRT.

The first aim of this thesis is to evaluate the effect of two different doses of tibolone on early postmenopausal bone loss. Furthermore, data on the incidence of vaginal bleeding and effect on the endometrium are presented. Thirdly, bone loss after discontinuation of treatment with tibolone is assessed. Finally, tibolone and its effect on bone will be reviewed.

1.2 Mixed hormonal profile and target tissue specificity of tibolone and raloxifene.

Tibolone is a C19 derived synthetic progestin. An oral dose of tibolone is rapidly absorbed, appearing in the plasma within 30 minutes, and peaking at 4 hours. It is extensively metabolised by the liver into three metabolites: 3α(OH)tibolone, 3β(OH)tibolone and its Δ4 isomer (Sandker *et al.*, 1994). The elimination half-life is about 45 hours, which is comparable to norethisteron (48-72 hours) and norgestrel (36 hours) (Anonymous, 1991).

Endocrinological studies in rats and rabbits showed that tibolone has weak progestagenic estrogenic and androgenic effects (Visser *et al.*, 1984). In classical in vivo endocrinological assays, the estrogenic potency of tibolone is about $1/50^{th}$ that of ethinyl estradiol, the progestagenic potency $1/8^{th}$ that of norethisteron, and the androgenic potency about $1/3^{th}$ that of norethisteron (Vies *et al.*, 1987).

These actions are receptor mediated, as evident from the inhibitory effects of anti-estrogens and anti-progestins. Therefore the effects of tibolone at the receptor level are complex as progestins reduce estrogen-receptor levels, while estrogens increase progestin-receptor concentrations (Markiewicz *et al.*, 1994). The complexity is increased by the fact that the three metabolites of tibolone show different estrogenic and progestagenic capabilities in vitro. Furthermore, the concentrations of metabolites differ depending on the target tissue. It is therefore likely that the estrogenic or progestagenic capabilities of tibolone differ, depending on the target tissue. For example, tibolone and its Δ4 isomer stimulate 17β dehydrogenase activity and glycogen accumulation in proliferative endometrium comparable to stimulation by progesterone. In contrast, the 3α(OH)- and 3β(OH) metabolites increase prostaglandin output in proliferative endometrium (i.e. an estrogenic effect). Furthermore, it was shown that tibolone was extensively metabolised to its Δ4 isomer in the endometrium by 3β hydroxylase/isomerase (Tang *et al.*, 1993). These findings suggest a complex receptor-mediated as well as metabolic regulation of the activity of tibolone at the target tissue level, which probably accounts for tissue specificity in the relative estrogenic/progestagenic activities (Markiewicz *et al.*, 1990). In other words, the tissue specific action of tibolone may be caused by differences in metabolism in combination with the presence of various receptor types and their density.

In contrast to tibolone, SERMs produce tissue specific agonist/antagonist effects after binding to the estrogen receptor (ER) through a mechanism that has not yet been elucidated. An important step in explaining the differential effects of SERMs may be the discovery of a

second ER, ERβ (Kuiper *et al.*, 1996; Mosselman *et al.*, 1996). The classic ER has been renamed ERα. As reviewed recently (Nilsson *et al.*, 1998), these two ERs appear to have unique tissue distributions and their own specific set of functions. Furthermore, in contrast to 17β estradiol (E2), raloxifene (a benzothiophene SERM) showed an ERα selective partial agonist/antagonist function but acted as a pure antagonist through ERβ (Barkhem *et al.*, 1998). The precise mechanism by which SERMs produce their tissue selective agonist/antagonist effect after binding to the ER, which is distinct from that of 17β E2 since it behaves as an estrogen agonist in all tissues, is now the subject of intensive research. It is thought that when a SERM binds to the ER, a specific conformation of the receptor-ligand is induced. Then, depending on structure of the gene-promotor and the sets and concentrations of the co-regulatory proteins present, produces a cell-specific antagonist/agonist effect (Barkhem *et al.*, 1998; McDonnell, 1998). Raloxifene is the first SERM available for prescription that has no stimulatory effect on the endometrium while having an estrogenic effect on bone. A comparison of raloxifene with continuous combined HRT and with tibolone in respect to its effects in postmenopausal women will be made in the present chapter.

1.3 Climacteric symptoms

1.3.1 Hot flushes and nocturnal sweats

The hallmark symptom of the menopause is the hot flush. A hot flush is a sudden, transient sensation ranging from warmth to intense heat that spreads over the upper part of the body, typically accompanied by flushing, perspiration and often followed by a chill. In some instances, there are palpitations and a feeling of anxiety. Whether referred to as hot flashes, hot flushes, night sweats or vasomotor symptoms (terms that are used interchangeably) these episodic events can disrupt a woman's sense of well-being. Although hot flushes are the predominant complaint of western peri- and postmenopausal women, it was not until 1975 that their objective existence was demonstrated (Molnar, 1975). One should bear in mind that several conditions share some clinical features with hot flushes, particularly flushing and sweating. These conditions include hyperthyroidism, panic attacks, carcinoid syndrome and pheochromocytoma.

As many as 75% of all postmenopausal women experience vasomotor symptoms. For 65% these last longer than a year and in 20%, these symptoms continue for more than 5 years (Brenner, 1988). A drop in estrogen levels rather than a steady low estrogen level is associated with hot flushes. The observation that women with gonadal dysgenesis (Turner's syndrome) who never had adult estrogen levels do not experience hot flashes unless they previously used HRT, supports this contention (Casper *et al.*, 1985). Furthermore premenopausal women using gonadotrophin releasing hormone (GnRH)-agonists experience vasomotor symptoms and postmenopausal women on HRT often experience the return of hot flushes when they discontinue medication. Hypotheses about the definitive explanation of the hot flush also include the role of α-adrenergic mechanisms, endogenous opioid peptide and GnRH (Casper *et al.*, 1985). As yet, however, the aetiology of the hot flush remains unknown. Usually within a few weeks of starting replacement therapy vasomotor symptoms are reduced.

The effect of continuous combined HRT on vasomotor symptoms has been reviewed recently (Udolff *et al.*, 1995). Thirteen studies specifically examined vasomotor symptomatology. Of these only four studies used the Kupperman index. All four studies revealed a significant treatment-associated decline in Kupperman scores. The remaining 9 studies made use of diaries or interview to record the incidence and severity of hot flushes. Similarly these studies noted a reduction in the rate of complaints. Furthermore, recently introduced low-dose continuous combined HRT (25μg 17βE2 administrated transdermally or 1mg 17βE2 orally) seem to be as effective as continuous combined HRT with 2mg 17βE2 in alleviating vasomotor symptoms in women 2-3 years after menopause (Stadberg *et al.*, 1996; Mattson *et al.*, 1999).

Tibolone, at a dose of 2.5mg/d has been proven to alleviate vasomotor symptoms when compared with placebo. There are 6 randomised placebo-controlled trials (Aloysio *et al.*, 1987) of which 5 were blinded (Kicovic *et al.*, 1982; Trévoux *et al.*, 1983; Nevinny-Stickel, 1983; Benedek-Jaszmann, 1987, Hammar *et al.*, 1996). All used diaries scoring the incidence and/or severity of flushes. Follow-up varied from 12-52 weeks. All studies found a significant reduction in vasomotor symptoms compared with placebo. In a non-randomised study in which patients with more severe vasomotor symptoms chose treatment with tibolone 2,5 mg/d, the number of hot flushes was reduced from 5 to less than 1 per day. (Rymer *et al.*, 1994). The effect of tibolone on vasomotor symptoms was as effective as sequential HRT regimens (Volpe *et al.*, 1986; Crona *et al.*, 1988; Egarter *et al.*, 1996). Two studies directly compared the effect of tibolone on vasomotor symptoms with that of continuous combined

HRT. One double-blind, placebo-controlled trial (Hammar *et al.*, 1998) involving 423 patients, and an open label, randomised study of 148 women (Al-Azzawi *et al.*, 1999) showed that both treatments effectively reduced vasomotor symptoms. However, in one study there was a small but significant difference in favour of continuous combined HRT (Hammar *et al.*, 1998).

Raloxifene increases hot flushes. In a large prospective randomised trial involving more than 7700 elderly postmenopausal women (Ettinger *et al.*, 1999), 10% of the women with raloxifene 60 mg/d reported hot flushes compared to 6% in the placebo group. Although the incidence of hot flushes was increased, only 1% of the women withdrew from the study for this reason. Another study pooled the treatment groups of 8 randomized parallel clinical trials comparing the adverse effects of raloxifene 60 mg/d with HRT, unopposed estrogen therapy and placebo (Davies *et al.*, 1999). These 8 studies enrolled a total of 2789 women. Twenty-four percent of women on raloxifene reported hot flushes compared with 18% of women in the placebo group. However, discontinuation rates were not increased.

In summary, satisfactory relief of hot flushes was universally reported for continuous combined HRT as well as for tibolone. Unfortunately this effect is solely based on subjective (patient) reporting since no study used objective biophysical monitoring. Although one direct comparative study showed a small but significant difference in favour of continuous combined-HRT, the efficacy of tibolone and continuous combined as well as sequential HRT seem to be comparable for combating vasomotor symptoms. As one might suspect, there is a slight increase in vasomotor symptoms in women using raloxifene. However, data suggest that discontinuation rates are not substantially increased by this adverse event.

1.3.2 Mood and libido

Data on the relationship between changes in the endocrine milieu and psychological symptoms are conflicting. Epidemiological studies in the USA and Europe have failed to document an increase in depressive symptoms around the time of menopause (Hällström *et al.*, 1985; McKinlay *et al.*, 1992). In contrast, other studies of non-clinical populations showed an increased incidence of depressive symptoms in peri- and postmenopausal women (Bungay *et al.*, 1980; Hunter *et al.*, 1986). Interestingly, a longitudinal survey of middle-aged women in the USA showed that depressive symptoms increase after surgical menopause but not after natural menopause (McKinlay *et al.*, 1987). Furthermore, up to 89% of women attending menopause clinics have depressive symptoms (Montgomery *et al.*, 1987). Plasma

levels of estrogens and testosterone are associated with positive moods in healthy non-depressed women who had undergone bilateral oophorectomy (Sherwin, 1988). However, the positive effect of estrogens on mood seems to be dampened by the concomitant use of progestins, which can be attenuated by higher doses of estrogens (Holst *et al.*, 1989; Sherwin, 1991).

There are 3 studies evaluating the effect of tibolone on mood. In a short, randomised cross-over trial involving healthy postmenopausal women, tibolone showed no statistically significant effect on mood compared with placebo (Nevinny-Stickel, 1983). However, in women who had undergone bilateral oophorectomy tibolone as well as unopposed estrogens, had a statistical significant positive effect on mood (Crona *et al.*,1988). Tibolone, proved to be significantly better than continuous combined HRT for improving mood (Egarter *et al.*, 1996). The effects of raloxifene on mood were assessed in women using raloxifene 60 or 120 mg/d. Raloxifene showed no effect on mood compared with placebo (Nickelsen *et al.*, 1999).

Sexual problems seem to be associated with the menopause and not with ageing per se. Epidemiological data serve to point out that between one third and one half of menopausal women recruited from general populations complain of a problem in one or more aspects of sexual functioning (Sherwin, 1994). Since the integrity of the tissues of the female reproductive tract depend on estrogens, degenerative changes due to the hypo-estrogenic state may adversely affect sexual functioning in postmenopausal women. For example, decreased vaginal lubrication or atrophic vaginitis may result in dyspareunia. The positive effect of androgens rather than estrogens on libido or sexual motivation is now well established (Sherwin, 1994). For example, women who complained of loss of libido that had not been relieved by unopposed estrogen therapy reported significant symptomatic relief following treatment with a combined estrogens/testosterone implant (Burger *et al.*, 1987). In this respect it is of interest whether tibolone is superior to HRT, since it possesses weak androgenic proprieties. Five studies evaluated the effect of tibolone on libido and sexual desire. Three of them showed a significant increase in libido compared to placebo or no treatment (Kicovic *et al.*,1982; Rymer *et al.*, *Maturitas* 1994; Palacios *et al.*, 1995). The studies showing no difference to placebo had either a short follow-up (Nevinny-Stickel, 1983) or low baseline scores for loss of libido (Genazzani *et al.*, 1987). These factors seem important since two randomised studies which intensively studied sexuality showed lager effects at longer duration of treatment and women with the lowest sexual desire scores were shown to have the greatest improvement over one year (Palacios *et al.*, 1995; Nathorst-Böös *et al.* 1997). Comparison between tibolone and sequential HRT showed that both treatments increased

libido without a significant difference between treatment groups. However, tibolone was found to be superior in improving all seven items assessing sexual life when compared with continuous combined HRT (Nathorst-Böös *et al.* 1997).

In summary, there some evidence that tibolone has a positive effect on mood, libido and sexual desire in postmenopausal women. In this respect tibolone seems to be superior to continuous combined HRT but not to sequential HRT or unopposed estrogens. Assessments with raloxifene show no influence on mood compared with placebo (Nickelsen *et al.*, 1999).

1.4 Genital atrophy

Estrogen loss due to follicular depletion in the postmenopausal ovary is the major cause of vulvovaginal dysfunction in older women, accounting for most of the anatomic, cytological, bacteriologic and physiologic genital changes that occur. The most common presentations of vulvovaginal complaints are: vaginal dryness/irritation (27-40%) dyspareunia (25%) and urinary frequency and urgency (25%) (Oldenhave *et al.*, 1993; Geelen *et al.*, 1996).

Systemic or topical administration of estrogens has been shown to alleviate symptoms of urogenital atrophy. In general, a minimum treatment period of 3 months is advised for alleviation of the symptoms of vaginal atrophy. In addition, estrogen therapy considerably reduces the incidence of urogenital infections (Raz *et al.*, 1993). Although a significant effect on subjective improvement of all types of incontinence has been demonstrated for estrogen therapy compared with placebo (Fantl *et al.*, 1994), there is no conclusive evidence that estrogens objectively improve incontinence (Formosa *et al.*, 1994). However, a combination of estrogen and α-adrenergic drugs seems to improve genuine stress incontinence subjectively as well as objectively (Hilton *et al.*, 1990).

For continuous combined HRT, specific data on the effect of urogenital atrophy are lacking. Only one study of 59 healthy postmenopausal women states that "all patients noted improvement of their climacteric symptoms and vaginal atrophy" (AinMelk, 1996). A total of 7 studies evaluate the effect of tibolone on the vagina (Punnonen *et al.*, 1984; De Aloyoisio *et al.*, 1987; Rymer *et al.*, 1994; Siseles *et al.*,1995; Egarter *et al.*, 1996; Botsis *et al.*, 1997; Hammar *et al.*, 1998). None of the studies comparing tibolone with placebo or no treatment were double-blinded. Only one double-blind randomised study compared the effect of tibolone on the vagina with continuous combined HRT (Hammar *et al.*, 1998). Outcomes of these studies were either the maturation index of the vaginal epithelium and/or subjective

symptoms scoring (diaries) of vaginal dryness and dyspareunia. When tibolone was compared to no treatment or placebo there was a significantly increased maturation of the vaginal epithelium and significantly less complaints of vaginal dryness and dyspareunia. When tibolone was compared to other HRT regimens, vaginal dryness, dyspareunia and signs of atrophic vaginitis all improved with both treatments. Tibolone seems as effective as conventional HRT in this respect. Like continuous combined HRT, data on the effect of tibolone on urinary incontinence are lacking. For raloxifene, data on the effect on urogenital atrophy are not yet available. When adverse experiences of raloxifene were compared to placebo or HRT, the incidence of events related to vaginal atrophy did not differ (Davies *et al.*, 1999).

1.5 Bone density

1.5.1 Prevention and treatment of osteoporosis

Osteoporosis is defined as a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, that leads to an increase in bone fragility and susceptibility to fracture (anonymous Am J Med; 1993). In contrast with earlier consensus statements this definition no longer requires the presence of non-traumatic fractures. Primary osteoporosis occurs in the absence of known causes for bone loss. The exception to the rule is postmenopausal osteoporosis. Although estrogen deficiency after menopause is a well-known cause for increased bone turnover and accelerated bone loss, postmenopausal osteoporosis is still classified as primary osteoporosis. Other representatives of primary osteoporosis are: age-related osteoporosis, idiopathic osteoporosis and juvenile osteoporosis. When another disease, condition or medication is the cause of accelerated bone loss leading to low bone mass it is referred to as secondary osteoporosis (Table 1).

In the USA, osteoporosis gives rise to approximately 1.5 million fractures annually. Vertebral fractures are most frequent: 700,000 each year. Of all fractures due to osteoporosis, hip fractures are the most serious. Between 10-15% of 250,000 hip fractures victims die within a year of their fracture. Only about half of the survivors are able to return to fully independent living, and approximately a third become permanent nursing home residents (Ray *et al.*, 1997). In the Netherlands the age-adjusted incidence of hip fractures in women over 50 years of age is about 350 per 100,000. The number of hospital admissions for hip

Table 1 Proposed risk factors for osteoporosis.

Factor	Example
Genetic	Race, sex, familial prevalence
Nutritional	Low calcium intake, high intake of: alcohol, caffeine, sodium or animal protein
Life-style	Cigarette use, low physical activity
Endocrine	Menopausal age (oophorectomy), body composition, low premenopausal estrogen levels (anorexia nervosa, hypogonadism), hyper(para)thyreoidy, hypercortisolism.
Disease	Malabsorption, liver cirrhosis, myeloma, connective tissue disorder.
Drugs	Corticosteroids

fractures in Dutch inhabitants of 65 years and older is expected to double between 1990 and 2010 (Boereboom *et al.*, 1992). Postmenopausal white women are most commonly affected because at any age they have lower bone mass than men and white people have a lower bone mass than other ethnic groups.

Postmenopausal bone mass depends on the peak bone mass and the subsequent loss of bone. Bone mass depends on many hereditary and environmental factors. Although various risk factors for osteoporosis have been proposed (Table 1), it has been shown that patient history, anthropomorphic measurements and biomarkers of bone metabolism cannot be used to identify those women at risk for osteoporosis (Slemenda *et al.*, 1990; Cooper *et al.*, 1991; Berning *et al.*, 1993). At this moment the best available method of assessing fracture risk, especially in early postmenopausal women without previous fractures, is the performance of a bone mass measurement (Wasnich *et al.*, 1985; Gärdsell *et al.*, 1993; Cummings *et al.*, 1995). The current "gold standard" method of bone density testing is Dual Energy X-ray Absorptiometry (DEXA) of the spine and hip (Bracker *et al.*, 1998).

Bone mass is expressed as T-scores. This is defined as the difference in standard deviations of the individual bone mass, compared to the mean peak bone mass of a reference population. A T-score of -1 to -2.5 is considered to indicate osteopenia. A T-score of less than -2.5 is

considered as osteoporosis and, with fractures, it is considered to indicate severe osteoporosis. For each standard deviation reduction in bone mass there is an approximately 2-fold increase in the risk of fracture (Marshall *et al.*, 1996).

In theory, the prevention of osteoporosis could be achieved by either increasing peak bone mass, or by decreasing subsequent bone loss. However, except for a healthy active life style, no definite strategy for increasing peak bone mass has yet identified. In elderly prevention of fall may reduce the risk of hip fracture considerably. Although risk factors for falling have been identified (Grisso *et al.*, 1991) there is no specific intervention that has been proven to be widely effective. General measures including an adequate intake of calcium and vitamin D and an active life style can be recommended to everyone, whether or not they present with osteoporosis. A recent study (Kannus *et al.*, 2000) in elderly people (mean age 82 yrs) showed a reduction of 60% of hip fractures with the use of hip protectors. However, an important goal for prevention and therapy of osteoporosis is the restoration of bone resorption and formation to premenopausal levels.

Estrogens are generally considered to be the agent of choice for both treatment and prevention of osteoporosis. The epidemiological and observational studies on reduction of fracture risk are numerous (Weiss *et al.*, 1980; Krieger *et al.*, 1982; Ettinger *et al.*, 1985; Kiel *et al.*, 1987; Naessén *et al.*, 1990; Kanis *et al.*, 1992; Cauley *et al.*, 1995). To date there are three randomised placebo controlled studies showing that HRT reduces the incidence of vertebral fractures (Lufkin *et al.*, 1992), non-vertebral fractures (Koumalainen *et al.*, 1998) and forearm fractures (Mosekilde *et al.*, 2000).

Biphosphanates are a class of compounds that are resistant to enzymatic hydrolysis and therefore have a long skeletal half-life, resulting in reduced bone resorption. In the Netherlands etidronate and alendronate are generally in use for the treatment of osteoporosis. Etidronate has been shown effective for treatment of postmenopausal osteoporosis in two large prospective, randomized controlled trials (Watts *et al.*, 1990; Miller *et al.*, 1997). In addition, etidronate showed to be effective in preventing early postmenopausal bone loss (Herd *et al.*, 1997). In phase III trials, including almost 1.000 osteoporotic patients, alendronate showed an increase of spinal bone density up to 10% (Liberman *et al.*, 1995). In the fracture intervention trial (Black *et al.*, 1996), involving over 2.000 osteoporotic women with pre-existing fractures, alendronate significantly reduced the frequency of vertebral, wrist and hip fractures. In women without pre-existing fractures alendronate significantly reduced vertebral (Cummings *et al.*, 1998) and non-vertebral fractures (Pols *et al.*, 1999). Results from the early intervention cohort indicates that alendronate in a dose of 5 mg/d prevents

postmenopausal bone loss in women under 60 years of age nearly to the same extent than estrogen-progestin (Hosking *et al.*, 1998).

The effect of continuous combined HRT on bone density in healthy postmenopausal women has been studied in 12 studies, which are listed in Table 2. Five of these studies where double-blind and placebo-controlled. Meaningful comparison of these studies is hampered due to different methods of bone mass measurements and sites measured. In addition various combinations and doses of estrogens and progestins were involved. Duration of treatment ranged from 1-10 years. Generally the studies showed a significant increase or maintenance of bone density. This positive effect was seen even after a follow-up of 10 years.

For tibolone, 8 randomised studies (listed in Table 2) showed that with a dose of 2.5mg/d there was an increase in spinal and appendicular bone density in early and late postmenopausal women. Furthermore, a dose of 1.25 mg/d seems to be effective in prevention of postmenopausal bone loss especially in older women. In two large multicentre trials 1.25 mg/d of tibolone seems the preferred dose for preventing early postmenopausal bone loss. These studies will be discussed in detail in chapter 3. Raloxifene in a dose ranging between 30 mg/d and 150 mg/d has been shown to increase bone density in hip and spine ranging from 1-2.2% (see Table 2). Compared to placebo all doses seem to be effective in preventing postmenopausal bone mass. Studies evaluating the effect of continuous combined HRT, tibolone and raloxifene on bone density in osteoporotic postmenopausal women are listed in Table 3. Tibolone and continuous combined HRT seem to increase bone density in hip and spine to the same extent. Data from a large multicentre trial on multiple outcomes of raloxifene (the MORE study) (Ettinger et al., 1999) shows an increase in bone density, which is somewhat less than seen with tibolone or continuous, combined HRT. However, this trial showed a significant reduction of vertebral fracture risk of 50% in women with no pre-existing fracture and 30% in women with pre-existing fractures who used raloxifene 60 mg. Data on the reduction of fracture risk are not available for continuous combined HRT and tibolone. There are no comparative studies with respect to bone density between tibolone and continuous combined HRT. As will be discussed in chapter 3, the effect of tibolone on postmenopausal bone density is comparable with unopposed estrogens or sequential HRT.

In conclusion, tibolone, continuous combined HRT and raloxifene are capable of increasing bone density in healthy and osteoporotic postmenopausal women. For continuous combined HRT this effect has been proven to last for up to 10 years. Although there are no direct comparative studies, the effect on bone density of the three treatment modalities seem to be in the same order of magnitude. Only for raloxifene it has been shown that fracture risk

Table 2 Effects of preventive treatment of osteoporosis for continuous combined (cc) HRT, tibolone and raloxifene.

Reference	medication	subjects	number*	design	yrs since menopause	technique#	site	therapy	placebo	duration
cc-HRT:										
Riis et al., 1988	2mg E2 1mg NET	pmp	21/22	db-bl-pc	0.5-3	SPA/DPA	distal radius proximal radius spine L2-4	no change +1.0% +5.4%	-7.5% -4.5% -3.7%	2 yrs
Christiansen et al,. 1990	2mg E2 1mg NET	pmp	18/19	controlled	0.5-3	SPA	distal radius proximal radius	no change no change	-10% -10%	5 yrs
Williams et al., 1990	5µg EE 0.5mg NET	pmp	12/10	randomized controlled	1-5	seQCT	spine L2-3	+6.8%	no change	1 yr
	5µg EE 1mg NET		14/10			seQCT	spine L2-3	+7.8%	-	
	10µg EE 0.5mg NET		13/10			seQCT	spine L2-3	+11.2%	-	
	10µg EE 1mg NET		14/10			seQCT	spine L2-3	+10.2%	-	
	20µg EE 1mg NET		12/10			seQCT	spine L2-3	+13%	-	
Marslew et al., 1992	2mg E2V 1mg CPA	pmp	19/24	db-bl-pc	0.5-3	SPA DEXA	distal radius spine L2-4	+1.9% +3.9%	-4.8% -2.4%	2 yrs
Fuleihan et al., 1992	0.625mg CEE 2.5mg MPA	pmp	7/0	randomized comparative	1-20	DPA	hip spine L2-4	no change no change	N.A.	1 yr
	0.625mg CEE 5.0mg MPA		8/0			DPA	hip spine L2-4	no change no change	N.A.	
Luciano et al., 1993	0.625mg CEE 2.5mg MPA	pmp	7/0	randomized comparative	2-13	DPA	spine L2-4	no change	N.A.	
	0.625mg CEE 5.0mg MPA		6/0			DPA	spine L2-4	+5.2%	N.A.	
Maclennan et al., 1993	0.3 or 0.625mg CEE with	pmp		db-bl comparative	1-10					1 yr
	2.5 mg MPA or		25/0			SPA	forearm	no change	N.A.	
	0.35mg NET or		25/0			SPA	forearm	no change	N.A.	
	0.03mg LNG or		25/0			SPA	forearm	no change	N.A.	

Table 2 Effects of preventive treatment of osteoporosis for continuous combined (cc) HRT, tibolone and raloxifene **(continued)**.

Reference	medication	subjects	number	design	yrs since menopause	technique#	site	therapy	placebo	duration
Eiken et al., 1996	2mg 17β E2 1mg NET	pmp	19/25	controlled	0.5-2	SPA DPA	proximal radius spine L2-4	no change +15.9%	-17.6% -4.7%	10 yrs
Bush (PEPI) et al., 1996	0.625mg CEE 2.5mg MPA	pmp ovx	168/163	db-bl-pc	1-10	DEXA	hip spine L2-4	+2.0% +5.0%	-2.2% -2.8%	3 yrs
Speroff et al., 1996	1µg EE2 0.2mg NET	pmp	139/137	db-bl-pc	1-5	seOCT	spine L2-3	no change	-7.4%	2 yrs
	2.5µg EE2 0.5mg NET		136/137			seOCT	spine L2-3	no change	-	
	5µg EE2 1mg NET		146/137			seOCT	spine L2-3	+2.2%	-	
	10µgEE2 1mg NET		145/137			seOCT	spine L2-3	+4.2%	-	
Hart et al. 1998	2mg 17β E2 1mg NET	pmp ovx	27/0	prospective observational	0-2	DPA/ DEXA	spine L2-4	+5.5%	N.A	10 yrs
Recker et al., 1999	0.3mg CEE 2.5mg MPA (Ca vit.D)	pmp	54/53	db-bl-pc		DEXA	hip spine forearm	no change +3.2% no change	no change no change no change	3.5 yrs
tibolone										
Lindsay et al., 1980	2.5mg	pmp ovx	33/30	db-bl-pc	0.5-3	SPA	metacarpal	no change	-3.6%	2 yrs
Rymer et al., 1994	2.5mg	pmp	46/45	controlled	0.5-3	DPA	hip spine L2-4	+3.5% +2.5%	-2.5% -3.5%	2 yrs
Lyritis et al., 1995	2.5mg 1g Ca all	ovx	15/10	randomized controlled	-	SPA	distal radius proximal radius	no change no change	-12.4% -15.2%	1 yr

Table 2 Effects of preventive treatment of osteoporosis for continuous combined (cc) HRT, tibolone and raloxifene **(continued).**

Reference	medication	subjects	number*	design	yrs since menopause	technique#	site	therapy	placebo	duration
Berning et al., 1996	2.5mg	pmp	35/23	db-bl-pc	1-3	MDM	phalanx	+5.5%	no change	2 yrs
						seQCT	spine L2-4	+9.1%	-6.4%	
	1.25mg		36/23			MDM	phalanx	+3.1%	-	2 yrs
						seQCT	spine L2-4	+4.0%	-	
Bjarnason et al., 1996	2.5mg	pmp	28/13	db-bl-pc	>10	SPA	distal radius	+1.9%	-2.1%	2 yrs
						DEXA	spine L2-4	+5.1%	no change	
	1.25mg		29/13			SPA	distal radius	+2.2%	-	
						DEXA	spine L2-4	+5.9%	-	
Gallagher et al., 1999	0.3mg	pmp	total of 770	db-bl-pc	?	DEXA	spine study I/II[1]	2.6%[3]/2.6%[3]	-1.6%/-3.0%	2 yrs
	0.5mg Ca				mean age 52		hip study I/II	0.7%[3]/1.3%[3]	-2.0%/-3.2%	
	0.625mg					DEXA	spine study I/II	2.1%[3]/1.8%[3]	-	
	0.5mg Ca						hip study I/II	0.4%[2]/0.6%[3]	-	
	1.25mg					DEXA	spine study I/II	0.7%[2]/1.5%[3]	-	
	0.5mg Ca						hip study I/II	0.8%[3]/-1.4%	-	
	2.5mg					DEXA	spine study I/II	-0.6%/-0.2%[3]	-	
	0.5mg Ca						hip study I/II	0.5%[2]/-1.0%[1]	-	
Breadsworth et al., 1999	2.5mg	pmp	22/20	randomized controlled	1-?	DEXA	hip	no change	-3.9%	2 yrs
							spine L2-4	+3.7%	no change	
raloxifene										
Delmas et al., 1997	30mg	pmp	152/150	db-bl-pc	2-8	DEXA	hip	+1.0%	-0.8%	2 yrs
							spine L2-4	+1.3%	-0.8%	
	60mg		152/150			DEXA	hip	+1.6%	-	
							spine L2-4	+1.6%	-	
	150 mg		147/150			DEXA	hip	+1.5%	-	
							spine L2-4	+2.2%	-	
	0.4-0.6g Ca all									

* treatment group/placebo group; #: for BMD assessment; pmp: postmenopausal; ovx: ovariectomized; db-bl-pc: double-blind placebo-controlled; [1] 2 identical independent studies; [1]P<0.05; [2]P<0.01; [3]P<0.001, treatment versus placebo.
E2: 17β estradiol; NET: norethisterone; EE: ethinyl estradiol; E2V: estradiol valerate; CPA: cyproterone acetate; CEE: conjugated equine estradiol; MPA: medroxyprogesterone acetate; LNG: levonorgestrel.
SPA: single photon absorptiometry; DPA: dual photon absorptiometry; seQCT: single energy quantitative computed Tomography; DEXA: dual energey X-ray absorptiometry.

Table 3 Effects of tibolone, continuous combined (cc) HRT, and raloxifene for treatment of osteoporosis.

Reference	medication	concurrent treatment	subject number*	duration	design	Technique#	site	therapy	placebo	Fracture risk
tibolone										
Geusens et al., 1990	2.5mg	-	14/17	2 yrs	db-bl-pc	SPA/DPA	distal radius proximalradius spine L2-4	no change no change +8.0%	no change no change -4.0%	NS
Pavlov etal., 1996	2.5mg	-	63/43	2 yrs	db-bl-pc	DEXA	hip spine L2-4	+7.2% +2.6%	no change no change	not done
Studd et al., 1996	2.5mg	Ca 1g	31/36	2 yrs	db-bl-pc	DEXA	hip spine L2-4	+2.7% +6.9%	+1.4% +2.7%	not done
cc-HRT:										
Christiansen et al., 1990	2mg E2 1mg NET	Ca 0.5g	16/15	1 yr	db-bl-pc	SPA/DPA	distal radius proximalradius spine l2-4	+8.0% no change +8.0%	no change no change no change	not done
Grey et al., 1994	0.3-0.625mg CEE+5mgMPA		44/19	1/6-3 yrs	retrospective controlled	DEXA	hip spine L2-4	+3.0% +7.0%	no change no change	not done
	50µg E2 5mg MPA		7/19			DEXA	hip spine L2-4	+7.2% no change	no change no change	
raloxifene										
Ettinger et al., 1999	60mg	Ca 0.5g + 400-600 IU vit. D	2557/2576	3 yrs	db-bl-pc	DEXA	hip spine L2-4	+1.0% +3.1%	-1.1% +0.5%	RR 0.5 (95%CI 0.3-0.7)[1] RR 0.7 (95%CI 0.6-0.9)[2]
	120mg		2572/2576					+1.3% +2.9%	-1.1% +0.5%	RR 0.5 (95%CI 0.4-0.6)[1] RR 0.6 (95%CI 0.4-0.9)[2]

*: treatment group/placebo group; # for BMD assessment
pmp: postmenopausal; ovx: ovariectomized; db-bl-pc: dubble blind placebo controlled N.S.: not significant
E2: 17β estradiol; NET: norethisterone; EE: ethinyl estradiol; E2V: estradiol valerate; CPA: cyproterone acetate; CEE: conjugated equine estradiol; MPA: medroxyprogesterone acetate; LNG: levonorgestrel; Ca: elementary calcium; vit. D: vitamin D.
SPA: single photon absorptiometry; DPA: dual photon absorptiometry; seQCT: single energy quantitative computed Tomography; DEXA: dual energy X-ray absorptiometry.
[1]: relative risk and 95% confidence interval in subjects without preexisting fractures; [2]: relative risk and 95% confidence interval in subjects with preexisting fractures

is reduced by 30-40% in (severe) osteoporotic women (Ettinger et al., 1999). Data on fracture risk are lacking for continuous combined HRT and tibolone. However, numerous epidemiological and observational studies as well as three randomised trials show that unopposed estrogens and conventional HRT regimens reduce fracture risk. Since the effect of tibolone on postmenopausal bone density is comparable to estrogens, it is likely that tibolone will reduce fracture risk. However, the magnitude of fracture risk reduction by tibolone remains to be determined.

1.5.2 Add-back therapy in GnRH-agonist treated patients

Gonadotrophin-releasing hormone (GnRH) is a decapeptide, secreted by the hypothalamus into the portal circulation. GnRH controls the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) from the gonadotroph cells of the anterior lobe of the pituitary gland. The pituitary response depends to some extent on the frequency of the GnRH pulse. Continuous infusion will result in the inhibition of pituitary secretion. GnRH-agonists are GnRH-analogues with a longer half-life due to their resistance to cleavage by endopeptidases, causing pituitary down regulation. This "medical castration" results in suppression of gonadal steroids levels. This medication is therefore used for steroid-dependent diseases such as uterine fibroids and endometriosis. Their short-term use in women is of proven benefit in the assisted reproduction and prior to endometrial ablation. Of the long-term indications the management of endometriosis is the most important. Since endometriosis is a chronic disease long-term use of GnRH-agonists would be favourable. However, because of the hypo-estrogenic state and subsequent rapid reduction in bone density, the use of GnRH-agonists is limited to a 6-month course.

Since the oestrogen-dependent growth in endometriosis is believed to respond to cyclical changes in circulating estrogens, this adds weight to the idea that, low steady state oestrogen levels do not stimulate the growth of endometriosis. This in turn leads to the rationale that HRT regimens and tibolone could be used as add back, or replacement, therapy, to reduce the adverse effects of bone loss and vasomotor symptoms, while not altering the therapeutic effect of the GnRH-agonists. GnRH-agonist induced bone loss averages 1% per month during a 6-month course (Pickersgill 1998), which much greater than an average loss of 2-3% annually during the first years after natural menopause (Riggs *et al.*, 1986). Although most studies indicate that bone mass is partially or completely restored after cessation of GnRH-agonist therapy (Pickersgill, 1998) it can be argued that adverse changes in trabecular bone

structure may occur during bone loss, which could lead to permanently reduced bone strength even when the total bone mass has been restored (Compston *et al.*, 1995). Therefore the importance of preventing GnRH-agonist induced bone loss is emphasised.

Studies using HRT regimens with commonly used doses for the prevention of postmenopausal bone loss, do not entirely prevent GnRH-a induced bone loss (Maheux *et al.*, 1992; Moghissi *et al.*, 1996; Maheux *et al.*, 1991; Sugimoto *et al.*, 1993). In contrast, a higher dose of estrogens (Leather *et al.*, 1993; Simber *et al.*, 1996; Makarainen *et al.*, 1996) protected against significant bone loss during GnRH-agonist treatment.

When continuous combined HRT regimens are considered, transdermal 25μg 17β E2 with 5mg medroxyprogestrone acetate did not prevent bone loss at the lumbar spine completely (Howel *et al.*, 1997). In contrast, 1.25mg conjugated estrogens with 5mg medroxyprogestrone acetate or 1 mg 17β E2 with 0.5 mg norethisterone was effective in preserving lumbar spine bone density in a 6-month course with a GnRH-agonist (Gregoriou *et al.*, 1997; Franke *et al.*, 2000). In a study of 12 months'duration in patients treated with a GnRH-agonist, norethisterone in a daily dose of 5mg alone or in combination with conjugated estrogens 0.625 or 1.25mg maintained bone density and alleviated vasomotor symptoms (Hornstein *et al.*, 1998). Another continuous combined HRT regimen with 20μg of ethinyl estradiol with 0.15mg desogestrel reduces vasomotor symptoms but also the efficacy of GnRH-a treatment for endometriosis (Gnoth *et al.*, 1999).

A prospective, randomised, placebo-controlled trial with tibolone 2.5 mg/d in patients treated for 6 months with a GnRH-agonist, showed that tibolone prevented GnRH-agonist induced bone loss and alleviated vasomotor symptoms without altering the efficacy of the GnRH-agonist (Lindsay *et al.*, 1996). When compared to data from other studies, tibolone appears at least as effective as high doses of norethisterone or estrogen-progestagen combinations. In addition histomorphometric studies in trabecular (Compston *et al.*, 1995) and cortical (Bell *et al.*, 1997) bone showed that increased bone turnover as well as adverse structurally changes can be counteracted by 2.5 mg/d tibolone. There are no studies using raloxifene as add-back therapy as it does not alleviate vasomotor symptoms. However, it would be interesting what effect raloxifene may have on endometriosis itself.

In summary, proven regimens preventing GnRH-agonist induced bone loss for up to 6 months include: 2mg estradiol valerate, 100μg transdermal 17β E2 with sequential progestin, conjugated estrogens 1.25mg/d with 5mg/d medroxyprogestrone acetate, 1mg 17β estradiol and 0.5 mg norethisteron, or 2.5 mg/d tibolone, without altering the efficacy of a the GnRH-

agonist in treating endometriosis. Up to 12 months this is proven for 5mg/d norethisterone alone or in combination with conjugated estrogens.

1.6 Safety and compliance aspects

1.6.1 Endometrium and vaginal bleeding

Since unopposed estrogens increase the risk of endometrial cancer, sequential progesterone should be administered to women who have not had a hysterectomy (Grady *et al.*, 1995). One disadvantage of sequential HRT is withdrawal bleeding, which may result in premature cessation of HRT (Groenveld *et al.*, 1998). Long cycle HRT reduces the frequency from 12 to 4 scheduled bleeding episodes a year and may be an attractive alternative towards meeting the needs of postmenopausal women (Ettinger *et al.*, 1994). Although a 2-year study of 1 or 2 mg/d E2 for 84 days with 50μg gestodene for 12 days showed no hyperplasia (Boerrigter *et al.*, 1996), to date no detailed studies determined conclusively long-term safety of long-cycle HRT (Ettinger *et al.*,1994; Williams *et al.*, 1994; Hirvonen *et al.*, 1995). In a discontinued, randomised, multicentre trial of 240 women, there was a significant increase in incidence of simple and complex hyperplasia in women using a long-cycle regimen compared to monthly withdrawal bleeds. This was seen at the 2-3 year point in a planned 5 year study (Studd *et al.*, 1997). Obviously, long-term data as well as data on the optimum dose and duration of administration of estrogens and progestins are needed before such a long-cycle treatment can be safely recommended to all women requesting this kind of HRT.

One approach to the elimination of undesirable uterine bleeding and protection against endometrial hyperplasia is to use continuous combined HRT. Continuous combined HRT involves the use of a lower dose of progestin continuously every day (administrated orally or by a progestin containing intra uterine device) with the aim of rendering the endometrium atrophic and subsequently achieving amenorrhoea (Udoff *et al.*, 1995).

Another approach is that of target tissue specificity. The ideal compound should have the positive effect of estrogens on climacteric symptoms, bone and lipid profile, without a stimulatory effect on endometrium or breast. Tibolone is a synthetic steroid with combined estrogenic progestagenic and androgenic activity. It effectively alleviates climacteric symptoms and prevents postmenopausal bone loss. In the endometrium tibolone is transformed in its Δ4 metabolite, which has no estrogenic activity. Hence, there is no need for

progestin-induced withdrawal bleeding. Another group of compounds, which has either estrogenic or anti-estrogenic effects depending on the target tissue, are so called SERM's. The first SERM available on prescription, without stimulatory effects on the endometrium, is raloxifene. Data on endometrial effects as well as incidence of vaginal bleeding of continuous combined HRT, tibolone and raloxifene will be discussed.

The effects of various continuous combined HRT regimens on the endometrium has been recently reviewed (Udoff *et al.*, 1995) and, since then data from an additional two large clinical trials (CHART study and PEPI-Trial) have been published (Speroff *et al.*, 1996; The writing group for the PEPI-Trial, 1996). Almost all studies, regardless of the hormonal preparations used, reported endometrial atrophy rates between 90-100%, even after only 3 months of treatment (Udoff *et al.*, 1995; AinMelk *et al.*, 1996; Stadberg *et al.*, 1996). The endometrial atrophy rates reported represent the number of patients with atrophic endometrium or insufficient tissue for diagnosis, divided by the total number of patients sampled. It should be stated that, by uncertainty as to whether all patients with insufficient tissue for diagnosis have atrophic endometrium, the endometrial atrophy rates could be falsely elevated. However, with regard to endometrial hyperplasia the three largest studies involving a total of almost 1800 women on a continuous combined HRT regimen reported less than 1% hyperplasia, which was significantly less than with unopposed estrogen therapy, and comparable to that in placebo groups (Woodruff *et al.*, 1994; Speroff *et al.*, 1996; The writing group for the PEPI-Trial, 1996). The estrogen doses used were 0.625 mg/d conjugated estrogens or 1-10µg ethinyl estradiol, while the daily progestin dose was as low as 2.5mg medroxyprogesteron acetate or 0.5mg norethisteron.

Thirty-two studies, which evaluated the bleeding patterns of various continuous combined HRT regimens, showed that vaginal bleeding was relatively common during the first months of treatment (Udoff *et al.*, 1995). The incidence of vaginal bleeding varied widely ranging from 0 to 93% in the first 6 months. After 6 months the incidence of vaginal bleeding was less than 25% in 22 out of 23 studies with a follow-up longer than 6 months (range 0-38%). Data also suggest that the incidence of bleeding is inversely related to the number of years since menopause (Archer *et al.*, 1994). The different dosages and types of estrogens and progestins used hamper comparison of these results. Moreover, whereas some studies used doses fixed, other varied the dose in an attempt to decrease vaginal bleeding. Data on the effects of different estrogen and progestin doses on the incidence of breakthrough bleedings are conflicting. Some studies show that lower estrogen dose was associated with higher rates of amenorrhoea whilst others reported the opposite. For progestins, some studies found no

relation between progestin dose and amenorrhoea while others reported higher rates of amenorrhoea with increasing progestin dose (Udoff *et al.*, 1995). Interestingly, the occurrence of breakthrough bleeding during continuous combined HRT with estradiol and dydrogesterone in postmenopausal women was related to serum estradiol levels rather than to dydrogesterone levels. However, further studies are needed to test the hypothesis that oestrogen is a major factor in incidence of vaginal bleeding during continuous combined HRT (Weijer vd *et al.*, 1999).

More recently adverse progestagenic effects were reduced to a minimum when postmenopausal patients using unopposed estrogen therapy were switched to a levonorgestrel-containing intrauterine device (LNG-IUD). Atrophic endometrium was seen in all postmenopausal women using LNG-IUD in combination with 1.5 mg/d percutaneous 17β E2 for 1 year (Suvanto Luukkonen *et al.*, 1998). In another study of postmenopausal women using subdermal implants of 17β E2 and a LNG-IUD, 72% were amenorrhoeic for at least 3 months after 1 year of treatment (Suhonen *et al.*, 1995). Since it was found that the LNG-IUD reduces blood loss considerably (Anderson *et al.*, 1990), the LNG-IUD would be especially advantageous in perimenopausal women who have a high incidence of irregular and heavy menstrual blood loss due to anovulatory and hypoprogestagenic cycles (Metcalf *et al.*, 1981). In addition, the use of these intrauterine devices bridge the gap between contraception and estrogen replacement therapy. In perimenopausal patients, frequent and irregular spotting will trouble the first 6 months of use. However, after 12 months, amenorrhoea has been reported in 61-83% of perimenopausal women using LNG-IUD (Wollter Svenson *et al.*, 1997; Andersson *et al.*, 1992).

Clinical studies of tibolone, which include data on the endometrium and vaginal bleeding compared to placebo or no treatment, are listed in Table 4. In all these studies there was no evidence of endometrial stimulation by tibolone. In total there were 2 women with endometrial cancer, of whom one had hyperplasia at the baseline endometrial biopsy (Ginsburg *et al.*, 1996). There was no evidence that these malignancies were directly caused by tibolone. Furthermore, an incidence of 2 out of 434 postmenopausal women (Ginsburg *et al.*, 1996) using tibolone is no more than might normally be expected in the light of the calculated "presumed" occurrence of 2-3 cases of endometrial cancer per 300 women (Dadelszen *et al.*, 1994). World wide at that time, six cases of endometrial hyperplasia and adenocarcinoma in patients on tibolone have been reported (Dadelszen *et al.*, 1994). All of these women presented with vaginal bleeding. Two out of four women who had endometrial cancer in that series also had other significant risk factors for cancer (one being obese and the

Table 4 Clinical studies of tibolone with data on endometrium and/or vaginal bleeding.

Reference	study design	duration	population	treatment	vaginal bleeding (incidence)	endometrial thickness or histology	pathology or other measurements
Punnonen et al.,1984	nonrandomized, open, non-comparative	14 wks	69 postmenopausal women	2,5mg/d tibolone	one subject (1,4%)	atrophic 54 subjects; slightly proliferative 13 subjects, of whom 11 had an atrophic endometrium at baseline	
De Aloyosio et al., 1987	randomized, open parallel group	16 wks	168 postmenopausal women	2,5mg/d tibolone, placebo or no treatment	3 drop outs due to bleeding. No incidence given. Total of 54% drop outs in Org OD 14 group.	No alterations in endometrium morphology in all subjects	
Rymer et al., 1994	nonrandomized, open parallel group.	104 wks	100 women mean age 50 yrs	2,5mg/d tibolone or no treatment.	tibolone:12 (20%) no treatment: 5 (9%)	No evidence of endometrial stimulation at 52 and 104 wks.	
Habiba et al., 1996	observational	N.A.	37 women bleeding on tibolone	2,5mg/d tibolone	63% began to bleed within 6 mths	no hyperplasia	10 women polyps, 4 had fibroids and 2 subseptate uterus
Ginsburg et al., 1996	observational	mean 35 mths	47 women bleeding on tibolone out of 434 women.	2,5mg/d tibolone	(12.6%)	simple hyperplasia in 2 women early adenocarcinoma in 2 women	11 women polyps, 7 had fibroids
Bjarnason et al., 1996	db-bl-pc	104 wks	91 women >10 yrs after menopause	2,5mg/d tibolone (n=35) 1,25mg/d tibolone (n=36) placebo (n=20)	(20%) (11%) (5%)		
Gallagher et al., 1999	db-bl-pc	104 wks	770 early postmenopausal women (mean age 52 yrs)	1st yr 0,3-2,5mg/d tibolone placebo 2nd yr 0,3-2,5mg/d tibolone placebo	 (27-42%) (13%) (18-35%) (12%)	Endometrial hyperplasia in 3 women (1 in placebo and 2 in the 2,5 mg/d tibolone)	
Berning et al., 2000	db-bl-pc	104 wks	94 women 1-3 yrs after menopause	2,5mg/d tibolone (n=35) 1,25mg/d tibolone (n=36) placebo (n=23)	(51%) (44%) (22%)	No evidence of endometrial stimulation in women who bled during treatment or placebo.	1 woman with uterine fibroids

Wks: weeks; mths: months; N.A.: not applicable; db-bl-pc: double-blind placebo-controlled.

other having been on unopposed estrogens for 15 yrs); none of these women had endometrial biopsies prior to starting therapy. Two women in the series were diagnosed as having endometrial hyperplasia in an endometrial polyp, whilst the rest of the endometrium was atrophic or reported as normal.

Earlier studies state that vaginal bleeding does occur in women taking 2.5 mg/d tibolone, but no incidence was given (Genazzani *et al.*, 1991; Lindsay *et al.*, 1980). In 1994, Rymer (Rymer *et al.*, 1994) reported for the first time the incidence of bleeding during a 2 year non-randomised study in one hundred early postmenopausal women. They found a 20% incidence in the tibolone group compared to 9,4% in the group which did not receive medication. Furthermore, women who bled in the tibolone group were younger at menopause, were recently menopausal and may have had remaining endogenous estrogen production. This was not confirmed by a recent double-blind placebo controlled study (Berning *et al.*, 2000). In a report in which the majority of women were more than 5 years past their menopause (Ginsburg *et al.*, 1996), the incidence of vaginal bleeding was 13%, suggesting that vaginal bleeding may be less in late postmenopausal women. In the recently reported preliminary data from 2 double-blind placebo-controlled dose finding (0,3–2,5 mg/d tibolone) studies, involving 770 early postmenopausal women (Gallagher *et al.*, 1999), vaginal bleeding/spotting ranged from 27-42% in the first year in the active treatment groups. Berning et al. reported an incidence of vaginal bleeding of 51% and 44% for 2,5 mg/d and 1.25 mg/d tibolone, respectively (Berning *et al.*, 2000). About 50% of the bleeding will occur within 3 months; by 9 months 90% of all first bleedings have occurred (Berning *et al.*, 2000). Furthermore, bleeding episodes is dose related and statistical significant for 1,25mg/d and 2,5mg/d tibolone compared to placebo (Berning *et al.*, 2000). In late postmenopausal women the incidence of vaginal bleeding is less (20%) (Bjarnason *et al.*, 1996).

Two observational studies on postmenopausal women who had vaginal bleeding while taking tibolone showed that in about 50% uterine pathology could be found (Habiba *et al.*, 1996; Ginsburg *et al.*, 1996). Therefore it is concluded that, as with any "bleed-free" HRT regimen, investigation of the endometrium is mandatory if late onset vaginal bleeding occurs.

Clinical studies that directly compare tibolone to sequential HRT or continuous combined HRT are listed in Table 5. Again tibolone showed no stimulatory effect on the endometrium as judged by endometrial thickness and/or endometrial biopsies. Interestingly, when tibolone was compared to continuous combined HRT the incidence of vaginal bleeding in the first three months and during the total study period was less for tibolone. Furthermore, discontinuation from both studies due to vaginal bleeding was less in women using tibolone

Table 5 Comparative studies of tibolone with sequential or continuous combined HRT in respect to data on endometrium and/or vaginal bleeding.

Reference	study design	duration	population	treatment	vaginal bleeding (incidence)	endometrial thickness or histology	pathology or other measurements
Ergarter et al.,1996	randomized, open, parallel group	26 wks	129 postmenopausal women, mean age 53 yrs	2,5mg/d tibolone or conjugated estrogens	Occurrence of vaginal bleeding was significantly lower with tibolone than with conjugated estrogens after one month. At 6 mths rates were 4% with tibolone and 27% with conjugated estrogens.		
Hänggi et al., 1997	randomized, open parallel group	104 wks	85 postmenopausal women, mean age 52 yrs	2,5mg/d tibolone, no treatment, oral or transdermal estrogen		No difference between tibolone and no treatment at 12 and 24 mths for endometrial thickness. All tibolone patients had inactive endometrial samples at 24 mths.	No change in uterine dimensions with tibolone or controls
Botsis et al., 1997	randomized, open parallel group	24 wks	72 postmenopausal women with atrophic vaginitis	2,5mg/d tibolone or intravaginal estrogens	One woman experienced vaginal bleeding with tibolone.	No change in endometrial thickness with tibolone.	No change in uterine dimensions with tibolone.
Hammar et al., 1998	randomized, db-bl, parallel group	48 wks	315 postmenopausal women > 53 yrs	2,5mg/d tibolone or estradiol-norethisterone	(34%) (58%) Drop outs for bleeding 2% with tibolone, 12% with estradiol-norethisterone		
Al Azzawi et al., 1999	randomized, open parallel group	48 wks	235 postmenopausal women, mean age 53.8 yrs .	2,5mg/d tibolone or estradiol-norethisterone	(15%, after 3 mths) (20%, after 3 mths)		

Wks: weeks; mths: months
N.A.: not applicable
db-bl-pc: double-blind placebo-controlled.

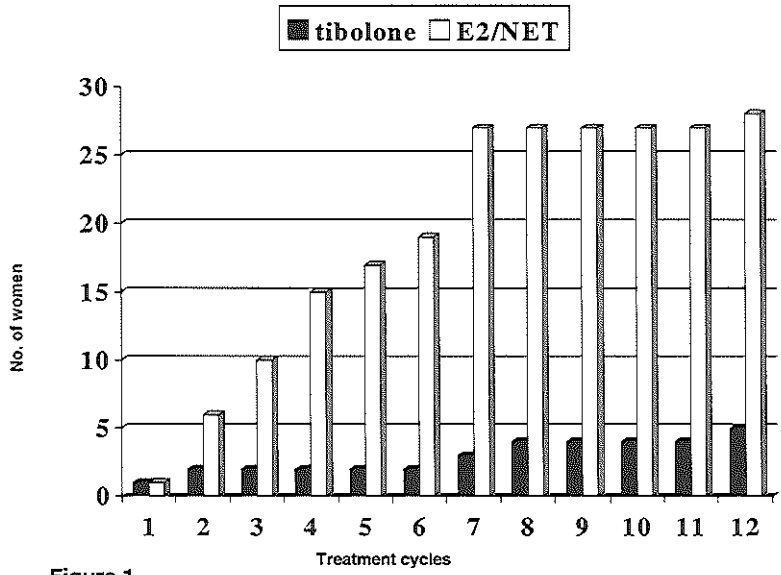

Figure 1
Cumulative number of women who discontinued treatment due to unacceptable bleeding or spotting in postmenopausal women treated with tibolone or continuous combined estradiol (E2)/norethisterone (NET).

(Figure 1), suggesting that in terms of compliance tibolone is superior to continuous combined HRT regimens (Hammar *et al.*, 1998).

The SERM, raloxifene is chemically distinct from tamoxifen and estradiol. It binds to ERs to completely block estrogen-induced DNA transcription in the breast and endometrium (Grese *et al.*, 1997; Brzozowski *et al.*, 1997). In animal studies, raloxifene antagonises the mitogenic effects of both estrogen and tamoxifen in the uterus (Sato *et al.*, 1996; Kleinman *et al.*, 1996). A double-blind placebo-controlled study with raloxifene (30, 60 or 150 mg/d) involving over 600 women (Delmas *et al.*, 1996) showed no difference in endometrial thickness in any of the four study groups. Furthermore, vaginal bleeding occurred in 2-3% of the women taking raloxifene, which was not different from the placebo group. All women who bled while taking raloxifene had an endometrial thickness of less than 5 mm. When adverse experiences of raloxifene were compared to placebo or HRT, the incidence of vaginal bleeding in women on raloxifene was comparable with the placebo group and significantly less than in women using other HRT regimens (Davies *et al.*, 1999). In the MORE study involving 7705 women, there were 10 cases with endometrial cancer, 4 in the placebo group

and 6 in the raloxifene group within the first 3 years of the trial (Ettinger *et al.*, 1999). For those women who had transvaginal ultrasonography, there was a slight increase in endometrial thickness of 0.3 mm. Endometrial thickness exceeded 5 mm in 4% more of those in the raloxifene group than in those in the placebo group. Fluid in the endometrial cavity was seen in 2% more women on raloxifene. The latter is assumed to be a benign finding that sometimes occurs in healthy postmenopausal women (Vuento *et al.*, 1996; Goldstein, 1994). Lastly, in women who had an endometrial biopsy, there were a total of 6 cases of hyperplasia of which 3 were in the placebo group.

In summary, data from clinical studies show that various continuous combined HRT regimens (with doses of progestins as low as 2.5mg/d MPA or 0.5mg/d NET), tibolone and raloxifene do not stimulate the endometrium in postmenopausal women. For continuous combined HRT and tibolone breakthrough bleeding during the first months of treatment is common. After one year of treatment amenorrhoea is achieved in the majority of the patients. Data from direct comparative studies between tibolone and continuous combined HRT suggest that the incidence and severity of vaginal bleeding is less with tibolone than with continuous combined HRT. Furthermore, in women using tibolone or continuous combined HRT, the incidence of vaginal bleeding lessens the further a woman is beyond the onset of menopause. As might be expected, the incidence of vaginal bleeding in women using raloxifene is low and comparable to placebo. One should bear in mind that with any "bleed free" HRT regimen, investigation of the endometrium is mandatory if late onset vaginal bleeding occurs.

1.6.2 Breast.

Breast cancer is the most common cancer in women in developed countries, affecting up to 1 in 10 women. One major concern among both women and physicians is the fact that the use of HRT may increase the risk of developing breast cancer. Out of approximately 50 observational studies only four have shown significant increases in relative risk, of between 1.6 and 1.9 (Bergkvist, 1997). Similarly, out of six meta-analyses that have been published only one (Colditz et al., 1993) found an increased risk of 1.4 for ever users (Bergkvist, 1997). However, the best evidence of the effect of HRT on breast cancer comes from a collaborative re-analysis of individual patient data from 51 epidemiological studies covering about 90% of the worldwide epidemiological data on this topic (Beral, 1997). The results demonstrated that a 35% increase in risk for the diagnosis of breast cancer in postmenopausal women using

HRT for 5 years or more. There was an increased risk of about 2% for each year of HRT use, which was found to be comparable with the effect on breast cancer of delayed menopause. Five years after stopping HRT there was no longer an increased risk of breast cancer. Although the increased risk for breast cancer was highly significant, the excess number of women with breast cancer after 5, 10 or 15 years of use was small: 1-3, 3-9 and 5-20 cases, per 1000 women who began HRT between 50-70 years of age. Finally, the overall risk was similar when estrogen plus progestins was used.

Because the number of women receiving tibolone compared with conventional HRT regimens worldwide is relatively small, no epidemiological studies have directly compared the effect of tibolone on the incidence of breast cancer. However, some evidence can be sought from experimental studies in tumour models of breast cancer. In vitro, the effect of tibolone on the growth of MCF-7 tumor cell lines and subclones A and A of T47-D cells was studied (Kloosterboer *et al.*, 1994). In general, the effect of tibolone on cell proliferation was substantially less than that of estrogens.

In vivo, the influence of tibolone on dimethylbenzanthracene (DMBA)-induced mammary tumor in rats was studied (Kloosterboer *et al.*, 1994). As preventive treatment in rats that received DMBA, there was a marked reduction in tumour load when tibolone or one of its metabolites was given for 10 weeks. As therapeutic treatment in rats with DMBA-induced mammary tumours, tibolone stabilised the tumour load to a similar extent as tamoxifen, while tumour load increased markedly in control rats. In humans, estrogen is one of the most important factors supporting the growth and evolution of breast cancer. In postmenopausal women with breast cancer, breast tissue contains high levels of estrogens (Pasqualini *et al.*, 1997). Two main pathways are involved in the local production of estrogens. Firstly, the aromatase pathway which converts androgens into estrogens. Secondly, the sulphatase pathway that transforms estron-sulphate into estron. The latter is assumed to be the most important source of estrogens in breast cancer tissue (Pasqualini *et al.*, 1997). Tibolone and its metabolites have been shown to have an intense inhibitory effect on the sulphatase pathway in hormone-dependent breast cancer cells (Chetrite *et al.*, 1997).

Tissue homeostasis is the result of a balance between proliferation, differentiation and apoptosis, or programmed cell death, and is thought to play a major role in the growth of both normal and tumor tissue. Tibolone decreases cell proliferation, promotes 17β hydroxysteroid dehydrogenase activity (a marker of epithelial cell differentiation) and increases apoptosis in normal as well as in breast cancer cells (Gompel *et al.*, 1997). These finding suggest that tibolone may have potential for the treatment of breast cancer. A small study of 14 women

with terminal stage breast cancer who received tibolone after appropriate treatment for their cancer, noted that one woman developed cancer in the other breast, one woman improved and the other women showed no change. Therefore, the significance of this, if any, is as yet unclear (O'Brien *et al.*, 1996).

Finally, density on mammography is a marker for increased risk: in several studies increased breast density predicted a two-fold increased risk of breast cancer (Boyd *et al.*, 1995). A small prospective study of mammographic changes in women taking tibolone showed minor changes in two out of 25 women: one of increased parenchymal density and one of microcalcifications. The authors quote a frequency of increases in mammographic density with estrogens of 11-27% (Erel *et al.*, 1998).

Tamoxifen, a SERM that inhibits the action of oestrogen on breast tissue, improves disease-free survival among women with hormone-dependent breast cancer (Fisher *et al.*, 1989) and reduces the risk of contralateral breast cancer (Early Breast Cancer Trialists Collaborative Group, 1998). The largest study on preventive treatment for breast cancer showed a reduction in risk of about 50% for women using tamoxifen (Fisher *et al.*, 1998). Raloxifene, a SERM that is chemically distinct from tamoxifen, binds to ERs to competitively block estrogen-induced DNA transcription in the breast and endometrium (Grese *et al.*, 1997; Brzozowski *et al.*, 1997). In animal studies, raloxifene inhibits the estrogen-stimulated growth of mammary cancers (Anzano *et al.*, 1996). In the MORE study, in which 7705 postmenopausal women received raloxifene 60 or 120 mg or placebo, the raloxifene group showed a significant reduction in breast cancer risk after 3 years of treatment (Cummings *et al.*, 1999). There was an overall relative risk of 0.24 (95% CI: 0.13-0.44). Consequently, to prevent one case of breast cancer, 126 women would need to be treated. The reduction in risk for ER positive cancer was 90%, whereas no significant reduction in risk was found for ER negative breast cancer. As noted by Cummings et al., breast cancer requires several years to grow to a clinically detectable stage. Therefore, the reduction of 40 months of treatment with raloxifene probably represents suppression or regression of subclinical cancer. Consequently, it is important to assess the long-term effects of raloxifene (and other SERM's) on breast cancer risk. Only if treatment continues to safely reduce the risk of breast cancer as long as it is taken, would it be worthwhile to use raloxifene in a broader spectrum of women than is currently practised (namely women at high risk for osteoporotic fractures).

In summary, the use of unopposed estrogens for more than 5 years is associated with an increased risk for the diagnosis of breast cancer (RR: 1.35). Use of estrogens in combination with progestins (HRT) has a similar overall risk as compared to estrogens alone. This

increased risk disappears within 5 years after cessation of therapy. No epidemiological data on the risk of breast cancer with the use of tibolone are available. The preclinical effects on breast epithelium cells and breast cancer cells give no cause for concern and are in fact encouraging. Treatment for 40 months with raloxifene showed a marked reduction in risk for the diagnosis breast cancer in a large prospective randomised placebo-controlled trial. However, before raloxifene can be used for primary prevention of breast cancer, the long-term effects on risk reduction need to be established.

1.6.3 Cardiovascular disease

Cardiovascular disease (CVD) is the most common cause of death in women above the age of 60 in western countries (Wenger *et al.*, 1993). However, the risk of CVD in women is low until the age of sixty (Tunstal-Pedoe, 1998). The lower risk of women compared with age-matched men, and the disparity in the incidence of CVD between pre- and postmenopausal women, suggests a protective effect for estrogens. However, the factors that influence the risk of CVD are many; they include geographic, ethnic, dietary, lifestyle, environmental and biochemical factors, any of which may also interact with each other.

Nearly every observational study has found a decreased risk of CVD in women who ever used estrogen. A recent meta-analysis found a summary relative risk of 0.70 for CVD in women who used unopposed estrogens. In studies that separately assessed HRT, the risk estimate was 0.66 (Barret-Connor *et al.*, 1998). Cardioprotection by estrogens is plausible since a growing number of studies on the surrogate markers of CVD risk (see Table 6) showed beneficial effects from HRT. In sharp contrast, the recently published Heart and Estrogen/progestin Replacement Study (HERS), the first randomised placebo controlled trial with hard cardiovascular end-points among women with a history of CVD, did not show a favourable effect, with a trend towards an increased risk of CVD in the first year of treatment. However, after the first year of treatment, there is a decreased risk of events (Hulley *et al.*, 1998). Summarising, epidemiological and various experimental data gave biological plausibility to a protective cardiovascular impact of HRT, whereas the first randomised clinical trial did not support this.

Baal (Baal van *et al.*, 1999) and Moore (Moore, 1999) recently reviewed the effects of continuous combined HRT and tibolone on various surrogate markers of risk for CVD. Table 7 summarises their conclusions. The effects of raloxifene on serum lipids and coagulation factors (Walsh *et al.*, 1998; Delmas *et al.*, 1997) are also summarised in Table 7.

Table 6 Surrogate marker for risk of cardiovascular disease.

Classical cardiovascular risk factors:		
Lipids:	cholesterol:	Low Density Lipoprotein ↑ High Density Lipoprotein ↓
	triglycerides ↑	
	Lipoproteine (a) ↑	
Bloodpressure ↑		
Glucose metabolism:		insulin resistance ↑ glucose tolerance ↓
Newly established cardiovascular risk factors:		
Factor VII ↑		
Fibrinogen ↑		
Plasminogen activator inhibitor-1 ↑		
Tissue-type plasminogen activator ↑		
Homocysteine ↑		

For continuous combined HRT there is an overall beneficial effect on serum lipids. However, in continuous combined HRT with norethisterone, high-density lipoprotein (HDL) is lowered and triglycerides are increased in continuous combined HRT regimens with medroxyprogesteronacetate as well as norethisterone. For tibolone, the potentially deleterious effect of reduced HDL may be balanced by the beneficial effects of reduced triglycerides and lipoprotein(a). Furthermore, in cholesterol-fed ovariectomised rabbits, the arterial wall was completely protected against atherosclerotic processes by tibolone (Zandberg et al., 1998). For raloxifene changes in serum lipids are beneficial although the magnitude of changes seem somewhat less. Raloxifene was shown to prevent atherosclerosis in cholesterol-fed ovariectomised rabbits (Bjarnason *et al.*, 1997) but not in postmenopausal monkeys (Clarkson *et al.*, 1998).

In conclusion, data presented in Table 7 and animal studies give an incomplete view of the overall picture. Only large-scale clinical trials and epidemiological surveys can illuminate the effect of continuous combined HRT, tibolone and raloxifene on the incidence of

Table 7 Surrogate makers of risk of cardiovascular disease: influence of continuous combined (cc) hormonal replacement therapy (HRT) with medroxyprogesteron acetate (MPA) or norethisteron acetate (NET), tibolone and raloxifene.

Surrogate maker	cc-HRT MPA/NET [a]	tibolone[b]	Raloxifene[c,d]
Blood pressure	-/-	-	-
Glucose metabolism	-?/-?	-?	-?
Lipids:			
Total Cholesterol	↓	↓-	↓
High Density Lipoprotein	- ↑/↓	↓	-
Low Density Lipoprotein	↓/↓	-	↓
Triglycerides	↑/↑	↓	-
Lipoprotein (a)	↓↓/↓↓	↓↓	↓
Fibrinogen	↓- ?/?	↓- ?	↓?
Fibrinolysis/coagulation balance	→	←	- ?
Homocysteine	?/?	?	↓

Reference
a: Baal van et al., 1999
b: Moore, 1999
c: Delmas et al., 1997
d: Wlash et al., 1998

cardiovascular disease. For continuous combined HRT (Women Health Initiative) and raloxifene (Raloxifene Use for The Heart) such large-scale clinical trials are now ongoing.

1.6.4 Venous thromboembolism

Exogenous estrogens used in the combined oral contraceptive pill have been recognised as factors in the pathogenesis of venous thromboembolism (VTE) (Spitzer et al., 1996). HRT also exposes women to exogenous estrogen, but was not considered to be associated with VTE (Devor *et al.*, 1992; Lobo, 1992). The difference between these preparations was attributed to "physiological" doses of natural estrogens, which contrasted, with the

"pharmacological" doses of synthetic estrogens of high potency in the pill (Lobo, 1992). However, recent case control studies have shown a modest increase in the relative risk (range: 2-4) of VTE in women on unopposed estrogens or HRT (Daly et al., 1996; Grodstein et al., 1996; Jick et al., Gutthann et al., 1997). Furthermore, the HERS study, involving 2763 women with coronary heart disease, showed a relative risk for VTE of 2.89 (95% confidence interval between 1.5 and 5.6) in women using HRT compared to placebo (Hulley *et al.*, 1998).

The mechanism whereby replacement therapy provokes an increased risk of VTE is unclear. Unopposed estrogens and HRT appear to be associated with a shift in the procoagulant-anticoagulant balance towards a procoagulant state. An effective increase in fibrinolytic activity is not consistently observed in randomised trials (Baal van *et al.*, 1999). This may be relevant in explaining the increased risk of VTE associated with unopposed estrogens and HRT. However, the frequency of VTE might be expected to be higher when these haemostatic changes would appear to be the sole or even the major mechanism for replacement therapy associated VTE, since these changes occur in virtually all women on medication. The potential role of the progestin component was highlighted when an increased risk of VTE was associated with certain third generation progestins (Spitzer *et al.*, 1996), but there are no clues as to the exact mechanism of the thrombosis. At present, there are no data from randomised trials concerning the role of progestins and whether a difference exists between oral and transdermal administration in respect to the balance of the procoagulant and anti-coagulant state (Baal van *et al.*, 1999). The epidemiological data relate VTE to the first year of unopposed estrogens or HRT exposure. This raises the possibility that replacement therapy unmasks one of the congenital thrombophilias, namely anti-thrombin III deficiency, protein S/C deficiency, Factor V Leiden or acquired thrombophilic defect such as anti-phospholipid antibody syndrome, lupus anti-coagulant or hyperhomocysteinemia. Finally, in absence of recognised congenital thrombophilic defects, a combination of risk factors for VTE (THRIFT Consensus Group, 1992) may exist in a women, which, when coupled with unopposed estrogens or HRT, leads to thrombosis.

No data are available on the incidence of VTE in women using tibolone. Short-term studies on haemostatic factors found decreases in fibrinogen, increases in anti-thrombin III (ATIII), plasminogen (Walker *et al.*, 1985) and increased fibrinolytic activity on fibrin plates (Walker *et al.*, 1985; Cortes-Prieto, 1987). More recently, studies showed a significant fall in tissue plasminogen activator and plasminogen activator inhibitor-1 and an increase in plasmin-anti-plasmine complexes over 1 to 2 years (Bjarnason *et al.*, 1997; Wersch *et al.*, 1994) indicating increased fibrinolysis, without affecting other clotting factors (fibrinogen, ATIII, thrombin-

anti-thrombin complexes, Factor VII and Factor X) (Parkin *et al.*, 1987; Wersch *et al.*, 1994; Bjarnason *et al.*, 1997; Hänggi *et al.*, 1997). Comparison of tibolone with transdermal or oral E2 showed little difference for fibrinogen and ATIII (Hänggi *et al.*, 1997). A separate comparison with oral estrogens with cyproterone acetate showed changes indicative of enhanced fibrinolysis in women using tibolone. In contrast, estrogens with cyproterone acetate had no effect on markers of fibrinolysis (Wersch *et al.*, 1994). These studies of haemostatic factors show a trend for tibolone towards increased fibrinolysis without affecting coagulation. Therefore, in contrast to unopposed estrogens or HRT, tibolone may alter the balance between pro-coagulant and anti-coagulant state in the direction of the anti-coagulant state. Although interpretation with respect to clinical relevance of various changes in haemostatic factors is difficult, in general the effect of tibolone on the balance of coagulation and fibrinolysis can be considered to be advantageous. Eventually, only large scale trials and epidemiological surveys can illuminate the effect of tibolone on VTE. As for estrogen containing HRT, the incidence of VTE is increased in women using raloxifene. Data from the MORE-study (Ettinger *et al.*, 1999) involving 7705 postmenopausal osteoporotic women showed a RR 3.1 (CI: 1.5-6.2), which is comparable to the increased risk seen with estrogens (Hulley *et al.*, 1998). At present data regarding the effect of raloxifene on coagulation factors is limited.

In summary, estrogen-containing HRT and raloxifene are associated with a moderate increased risk of VTE. No data on the association between tibolone and VTE exists. However, in contrast to HRT, the balance between the pro-coagulant and the anti-coagulant states in women using tibolone seems to be altered in the direction of the anti-coagulant state. Women starting HRT or raloxifene should have a personal and family history taken for VTE as well as an assessment of additional risk factors for VTE. Women with a personal or family history of VTE should undergo thrombophilia screening before starting treatment (Greer *et al.*, 1999).

2

Clinical studies

2.1 DETERMINANTS OF LUMBAR BONE MINERAL DENSITY IN NORMAL WEIGHT, NON-SMOKING WOMEN SOON AFTER MENOPAUSE. A STUDY USING CLINICAL DATA AND QUANTITATIVE COMPUTED TOMOGRAPHY.

2.1.1 Introduction

Postmenopausal bone mass depends on the amount of peak bone mass and the subsequent bone loss. Peak bone mass is influenced by many hereditary and environmental factors. In the literature there is controversy about the effect of oral contraceptive use, parity and lactation on bone mass. This is partly due to differences in study populations and in the skeletal sites measured.

The primary cause of postmenopausal bone loss of bone mass is the cessation of ovarian reproductive function resulting in low serum estrogen levels. It has been documented that hormonal replacement therapy can reduce the incidence of both hip and vertebral fractures (Weis *et al.*, 1980; Lindsay *et al.*, 1980; Krieger *et al.*, 1982; Naessén *et al.*, 1990) by preserving bone mass (Lindsay *et al.*, 1976; Christiansen *et al.*, 1980). Since there is, at present, no simple and effective method to restore lost bone mass, prevention of bone loss is the most rational approach in reducing the incidence of fractures related to osteoporosis.

The major problem in prevention of osteoporosis is the selection of women who need preventive medication. At present bone mass measurements provide the best prediction of fracture risk (Hui *et al.*, 1989; Wasnich *et al.*, 1989; Gärdsell *et al.*, 1991) . It could be claimed that medication should be restricted to women with an elevated fracture risk for the following reasons: at first, uncertainty remains in respect to the incidence of breast cancer and

cardiovascular disease during combined hormonal replacement therapy. Secondly, information about fracture risk is mandatory in case women are only willing to accept hormonal replacement therapy if their fracture risk is augmented. Finally, prediction of fracture risk could be useful to enhance both the cost-effective use and compliance of preventive therapy.

This cross-sectional study was designed to discuss the following questions: Is there an influence of oral contraceptive use, parity and lactation on early postmenopausal bone mass? Is assessment of reproductive history, body weight in combination with routine biochemical markers of bone metabolism suitable to predict lumbar bone mass soon after menopause in women without any obvious risk factors? In order to answer these questions non-smoking, normal weight, healthy postmenopausal women within three years after their natural menopause - a period in which a decision concerning preventive medication should be made - were selected. To our knowledge, this is the first cross-sectional study using Single energy Quantitative Computer Tomography (seQCT) which exclusively measures trabecular bone mineral density (BMD) of the spine in a homogeneous population of early postmenopausal women.

2.1.2 Materials and Methods

Subjects and study protocol: The study protocol was approved by the Ethics Review Committee of the Erasmus University Rotterdam, and informed consent was obtained from each participant. Ninety-four healthy white women were recruited through announcements in the local media. All women were amenorrheic, due to spontaneous menopause, for at least 12 months with a maximum of 36 months. All participants were between 45 and 60 yrs of age and had a body mass index (BMI) between 18 and 27 kg/m^2. See Table 1 for further clinical characteristics. None of the participants smoked, abused alcohol (more than 4 units/day), or used any medication considered to affect bone/calcium metabolism (such as gonadal steroids, calcitonin, thyroxin, biphosphanates, fluorides, corticosteroids, vitamin D, insulin, anticonvulsants, heparin, diuretics, antacids, calcium supplements and long-term use of antibiotics).

Gynaecological parameters and exclusion criteria used in this study were based on factors suggested by various reviews (Riggs et al., 1986; Lam et al., 1988). Age at menarche, months after menopause, oral contraceptive (OAC) use (years), number of pregnancies, parity (number of births), lactation (episodes exceeding 2 weeks) and its duration (weeks) was assessed through interview performed by the same physician (BB). Height was measured using a wall-mounted headboard. Weight was measured with a mechanic weightscale. The BMI was calculated as

weight (kg) divided by height^2 (m). Venous blood samples were taken between 12:00 and 16:00 h. After overnight fasting, urine samples of second morning voids were collected two hours after drinking 300 ml of water.

Bone Measurements: SeQCT (Somatom Plus, Siemens AG, Erlangen, Germany) was used for estimation of trabecular and cortical Bone Mineral Density (BMD) of the lumbar vertebral body (L1-L3). Midvertebral slices were determined by an automated procedure as published previously (Kalender *et al.*, 1988). For measurement within the vertebrae a computerised selection of the trabecular and cortical area was used (Sandor *et al.*, 1985). BMD was expressed as milligram calcium (Ca) hydroxyapatite per cm^3, using a standard reference device (Kalender *et al.*, 1987). Scanning parameters were: 80 kVp 125 mAs with 10 mm slice thickness. Coefficient of variation is less than 1 percent as measured with COMAC-BME phantom (Kalender *et al.*, 1991). The in vivo precision is generally reported to be less than 2% in clinical trials (Faulkner *et al.*, 1991).

Laboratory Measurements: For biochemical estimation of bone metabolism the following markers were used. Albumin (ALB), Alkaline Phosphatase (AP) and serum Ca were measured with a Bayer Technicon Chem-1 system according to the manufacturers instructions. Urinary Ca, creatinine and hydroxyproline were measured with commercial kits: creatinine and Ca with Merek kits (creatinine Mercko-test A and Ca ERIS-test respectively) and hydroxyproline with a Hypronosticon kit (Organon Teknika, The Netherlands). Urinary ratios of hydroxyproline-creatinine (OH-index) and Ca-creatinine (Ca-index) were calculated in mmol per mol. Corrected serum Ca (Ca-corr) was calculated by the equation: Ca-corr = Ca - 0.025 ALB + 1 mmol.

Statistical analysis: All variables were tested for normal distribution. If appropriate (i.e. AP and OH-index) logarithmic transformation was performed. Only for continuous normally distributed variables, Pearson's correlation coefficient and the Student-t test was used. For all other variables Spearman's correlation coefficient and the Mann-Whitney test were performed. Analysis of OAC-use, parity and duration of lactation was done by dividing the study population according to these parameters (Table 2). Further analysis of the gynaecological parameters, age, BMI, biochemical markers was performed by dividing the study population in a group with low trabecular and low cortical bone mineral density (defined as bone mass measurements within the lower tertile) and the remainder of the study population. Correlations were calculated between these variables and bone mass measurements (Table 3). Multiple regression analysis (Table 4)

was performed to determine the extend of interrelationships among variables and to calculate a linear model for the best prediction of trabecular and cortical BMD. All values are presented as median and range with exception of table 2. A *P*-value below 0.05 was considered significant. All presented *P*-values are two tailed. Statistical calculations were performed in Statgraphics version 4.0 (STSC Inc., Rockville, Md.).

2.1.3 Results

The study population characteristics are shown in Table 1. The measurements of trabecular and cortical lumbar BMD were highly correlated (r=0.77).

The values of trabecular and cortical BMD after dividing the study population according OAC-use, parity or duration of lactation are shown in Table 2. The trabecular and cortical BMD of women who used OAC did not differ from that of non-users. The nulliparous women did not differ in respect to trabecular and cortical BMD from the parous women studied. Women who breast fed more than 2 weeks had no significantly different trabecular and cortical BMD compared to parous women who had not breast fed. However, trabecular BMD was significantly (*P*=0.02) higher in women who had lactated more than 24 weeks compared with parous women who lactated 24 weeks or less (Table 2).

Only AP was significantly higher in women with low trabecular ($\leq$ 107 mg/cm^3) and low cortical ($\leq$ 267 mg/cm^3) BMD (*P*=0.02) as compared with the remainder of the study population. OH-index was slightly lower in the low trabecular BMD group but this difference did not reach the level of statistical significance (*P*=0.06). In respect to the other variables (as mentioned in Table 1), women with low trabecular and cortical BMD did not differ significantly from the remainder of the study population.

Most of the variables showed no individually significant correlation with trabecular or cortical BMD (Table 3). A significant correlation with trabecular lumbar BMD existed for AP (r=-0.31, *P*=0.003), parity (r=0.26, *P*=0.01) and duration of lactation (r=0.29, *P*=0.005). AP (r=-0.30, *P*=0.004) and parity (r=0.22, *P*=0.04) correlated significantly with cortical lumbar BMD. The correlation between duration of lactation and cortical BMD (r=0.20) was of borderline significance (*P*=0.05). Between variables no significant correlations were found, with exception of AP and Ca-index (r=-0.29, *P*=0.006) and between pregnancies, parity, duration of lactation and number of breast-fed children. Multiple regression analysis showed that parity does not

Table 1 Clinical and biochemical characteristics of 94 women 1-3 yrs after spontaneous menopause.

Variables	median (range)
Age (yrs)	52.9 (45.7-60.0)
Height (m)	1.64 (1.48-1.77)
Weight (kg)	64.5 (47.0-80.5)
BMI (kg/m^2)	24.3 (18.2-27.0)
Gynecological parameters:	
Age at Menarche (yrs)	14 (10-18)
OAC-use (yrs) (n=73)	10 (1-30)
Parity[a] (n=85)	2 (1-4)
Lactation (n=62)	
(Number of children)	1 (1-4)
(weeks)	12 (2-120)
Months since Menopause	20 (12-36)
Biochemical markers:	
OH-index (mmol/mol)	28 (10-190)
Ca-index (mmol/mol)	338 (39-1186)
AP (U/L)	54 (27-141)
Ca-corr (mmol/l)	2.21 (2.00-2.40)
Lumbar BMD:	
Trabecular (mg/cm^3)	120 (55-230)
Cortical (mg/cm^3)	288 (186-409)

[a]number of births, only parous women included

relate to trabecular (P=0.52) or cortical (P=0.52) BMD when duration of lactation was taken into account (Table 4: model 2). Furthermore, inclusion of nulliparous women did not change the slope or significance of the simple regression of duration of lactation with lumbar BMD. The regression of AP and duration of lactation with trabecular BMD and cortical BMD was independent of each other (Table 4 models 2 and 3) as well as of the variables that weren't significant determinants in the simple regressions. The third regression model from Table 4 could only account for 17 % of the variability of trabecular BMD (R^2) and 11 % of the variability of the cortical BMD (R^2). Comparing the predicted values of BMD with the obser ved, it was

Table 2 Trabecular and Cortical BMD[a] (mean ± SD) of the lumbar spine according to: use of OAC, parity and duration of lactation; in 94 women 1-3 yrs after spontaneous menopause.

	subject number	trabecular BMD	cortical BMD
All subjects	94	124 ± 33	290 ± 48
Use of OAC:			
non-users	21	125 ± 32	275 ± 48
users	73	122 ± 36	294 ± 48
P-value		0.71	0.11
Parity:			
nullipara	9	116 ± 17	270 ± 34
multipara	85	125 ± 34	292 ± 49
P-value		0.43	0.20
Duration of lactation (wks):			
duration = 0	24	122 ± 28	301 ± 46
duration > 2	61	126 ± 36	288 ± 50
P-value		0.65	0.30
duration = 24	58	119 ± 28	289 ± 47
duration > 24	27	138 ± 41	299 ± 53
P-value		0.02	0.35

[a]mg/cm^3.

found that only 17% of the women with low trabecular and 10% of the women with low cortical BMD were correctly identified by these regression models.

2.1.4 Discussion

Early postmenopausal bone mass depends on the amount of peak bone mass and subsequent bone loss. Therefore, early postmenopausal bone mass is influenced by many hereditary and environmental factors. The present study addresses to a selection of these environmental factors. The CA-intake of the subjects in this study was not estimated. Low CA-intake may have an independent influence on premenopausal bone mass (Elders *et al.*, 1989; Hansen *et al.*, 1991;

Table 3 Correlation coefficients between clinical, biochemical characteristics and trabecular, cortical BMD in 94 women 1-3 yrs after spontaneous menopause.

Variables	Lumbar BMD	
	Trabecular	Cortical
Age (yrs)	-0.06	-0.01
Height (m)	-0.13	-0.16
Weight (kg)	-0.14	-0.18
BMI (kg/m^2)	-0.04	-0.04
Gynecological parameters:		
Age at Menarche (yrs)	0.10	0.10
OAC-use (yrs)	-0.06	0.11
Pregnancies (Number)	0.14	0.11
Parity (Number of births)	0.26**	0.22*
Lactation (weeks)	0.29**	0.20
(Number of children)	0.18	0.05
Months since Menopause	-0.12	-0.04
Biochemical markers:		
OH-index (mmol/mol)	-0.06	-0.02
Ca-index (mmol/mol)	-0.08	-0.15
AP (U/L)	-0.31**	-0.30**
Ca-corr (mmol/l)	-0.08	-0.15

* $P < 0.05$
** $P < 0.01$

Heany, 1991). We assumed that the CA-intake of the study population is representative for the Dutch population. In the Netherlands CA-intake is relatively high compared to other countries (i.e. USA)(Heany, 1991). Furthermore, a dutch study (Elders *et al.*, 1989) with a similar study population found no relationship between CA-intake and peri- or postmenopausal bone mass, BMI or other life-style factors. Therefore we believe that CA-intake has not substantially influenced our results.

Influence of OAC on early postmenopausal bone mass: When non-users of OAC were compared with users, no significant difference in trabecular and cortical BMD was found. The finding that OAC-use has no effect on early postmenopausal BMD is in agreement with observations in premenopausal women (Rodin *et al.*, 1987; Hreshchyshyn *et al.*, 1988; Llyod *et*

al., 1989; Stevenson *et al.*, 1989; Mazess *et al.*, 1991; Rodin *et al.*, 1991). Goldsmith and Johnston (Goldsmith *et al.*, 1975) found a higher amount of peripheral bone mass, measured by single photon absortiometry (SPA) of the distal radius, only in women using high dosages (> 100 µg) of mestranol. Stevenson and colleagues (Stevenson *et al.*, 1989) found a positive effect of OAC on postmenopausal lumbar BMD, using DPA. However, the age of postmenopausal women in this study ranged from 28 to 69. Lindsay (Lindsay *et al.*, 1986) suggested that the positive effect of OAC on premenopausal bone mass, measured by DPA, was still present in postmenopausal women within two years following their last menstruation. This suggestion could not be confirmed by the present observations.

Influence of parity and lactation on early postmenopausal bone mass: There was no significant difference in BMD when nulliparous women were compared with parous women. Parity had a weak but significant positive correlation with trabecular and cortical BMD. However, this association disappeared when duration of lactation was taken into account (Table 4, model 2). Total duration of breast-feeding rather then number of children that had been breast-fed showed a positive association with early postmenopausal trabecular BMD (r=0.29, P=0.002). Furthermore, trabecular BMD in women who lactated more than 24 weeks was significantly higher compared with parous women who lactated 24 weeks or less. Although the number of nulliparous women in the present study was small (n=9), this suggests that parity per se has no influence on postmenopausal lumbar BMD. This is in agreement with most (Lindquist *et al.*, 1981; Aloia *et al.*, 1983; Hreshchyshyn *et al.*, 1988; Lissner *et al.*, 1991; Hansen *et al.*, 1991), but not all previous studies (Stevenson *et al.*, 1989; Goldsmith *et al.*, 1975; Rodin *et al.*, 1987). When premenopausal bone mass is concerned, previous studies observed a negative (Wardlaw *et al.*, 1986; Lissner *et al.*, 1991), or no (Stevenson *et al.*, 1989; Goldsmith *et al.*, 1975; Koetting *et al.*, 1988) association with duration of lactation. Recently, longitudinal studies observed a decrease in BMD during lactation (Lamke *et al.*, 1977; Hayslip *et al.*, 1989; Kent *et al.*, 1990; Drinkwater *et al.*, 1991). The initial deficit of BMD due to lactation may disappear within 6 months after weaning (Lamke *et al.*, 1977; Kent *et al.*, 1990). According to literature, the effect of lactation on postmenopausal bone mass is negative (Goldsmith *et al.*, 1975; Caraceni et al., 1987; Lissner *et al.*, 1991), absent (Wasnich *et al.*, 1983) or positive (Aloia *et al.*, 1983; Hreshchyshyn *et al.*, 1988; Hansen *et al.*, 1991). Studies focusing on fracture risk (Kreiger *et al.*, 1982; Aloia *et al.*, 1983) showed that osteoporotic women breast-fed less often then their aged matched controls.

Table 4 Interrelationships between determinants of Lumbar BMD in 94 women 1-3 yrs after spontaneous menopause: selected regression models (1-3), with partial coefficients (and significance level below).

	Trabecular BMD				Cortical BMD			
Model	AP[a]	Parity[b]	Lactation[c]	R^2	AP	Parity	Lactation	R^2
1	-32.6	6.2	N.A[d].	0.11	-46.4	7.3	N.A.	0.09
	(0.005)	(0.06)			(0.008)	(0.14)		
2	-35.8	2.3	0.36	0.16	-49.8	3.6	0.35	0.10
	(0.002)	(0.52)	(0.01)		(0.005)	(0.52)	(0.12)	
3	-37.1	N.A.	0.40	0.17	-51.7	N.A.	0.41	0.11
	(0.001)		(0.002)		(0.003)		(0.04)	

[a]Log U/l.
[b]Number of births.
[c]Duration in weeks.
[d]N.A.: Variables that are not applicable because excluded from that particular model.

Little is known about the possible mechanisms responsible for the positive influence of lactation on postmenopausal BMD. Experiments in beagles (Miller *et al.*, 1989) have shown some evidence for a "reversible mineral deficit" hypothesis. Based on these observations it may be postulated that increased Ca mobilisation is followed by an increased formation of a larger volume of osteoïd, which may result in an increased BMD after weaning, when the osteoïd is calcified. Furthermore, in humans there is evidence of an increased bone turnover and augmented renal Ca conservation during lactation (Kent *et al.*, 1990). Even after recovery of BMD within 6 months, elevated Parathyroid Hormone (PTH) and 1,25 hydroxy vitamin D levels as well as enhanced renal conservation of CA persists (Kent *et al.*, 1990; Specker *et al.*, 1991). In addition, PTH may induce bone formation through Insulin-like Growth Factor 1 (Canalis *et al.*, 1989). Therefore we speculate that a "rebound effect" after lactation may be a mechanism that could explain the association between duration of lactation and trabecular BMD seen in the present study.

Prediction of early postmenopausal bone mass: In the present study an attempt was made to select predictors for spinal bone mass in healthy early postmenopausal women without any

obvious risk factors for osteoporosis. Like in clinical practice, possible determinants were used which could be easily obtained by interview, physical examination and laboratory measurements. With the exception of the influence of lactation, only AP showed a clear negative correlation with early postmenopausal BMD. Interpretation of this finding in the present cross-sectional study is difficult. However, a high turnover seems unlikely because OH-index tended to be lower in our group of low BMD. Furthermore AP showed a significant negative correlation with Ca-index. Since the accelerated phase of bone loss after menopause (Block *et al.*, 1989) in this study is relatively short (1-3 years), peak bone mass is more likely to be the main determinant of early postmenopausal bone mass. This may explain why the OH-index and CA-index did not correlate with lumbar BMD in this study. There is evidence that after menopause OH-index and Ca-index in combination with AP may be useful in discriminating "fast-losers" of bone from "slow-losers" (Christiansen *et al.*, 1987; Hansen *et al.*, 1991). The meaning of these markers in respect to the subsequent rate of bone loss in the lumbar spine is now subject of further investigation in a longitudinal study.

Present observations show that lumbar trabecular and cortical BMD cannot be predicted by assessment of reproductive history, BMI and measurements of routine markers of bone metabolism. This is in agreement with data obtained by others (Citron *et al.*, 1987; Slemenda *et al.*, 1990). In a similar study (Slemenda *et al.*, 1990), the linear model for prediction of lumbar spine bone mass, measured with dual photon absorptiometry (DPA), correctly predicted 61% of the perimenopausal women with low BMD, compared with only 17% in our study. Reason for this may be the fact that the study population in this study was more homogeneous. All women were early postmenopausal with a BMI in the normal range and none of them smoked, which suggests that prediction of lumbar trabecular BMD in this selected group of women is even more difficult. Furthermore, in this study seQCT was used which is capable of measuring trabecular BMD exclusively. Previously, it has been suggested that the bone mineral determination in the cortical envelope of the vertebral body with seQCT could potentially give additional diagnostic information (Sandor *et al.*, 1989). In this study a correlation of r=0.77 was found between the trabecular and cortical measurements. The prediction of the BMD in both compartments using the clinical data was equally disappointing. The meaning of BMD of the cortical envelope in fracture risk prediction is at present unknown. Long-term follow-up studies are necessary to resolve this issue. The meaning of the present finding is clear: the assessment of clinical risk factors is unsuccessful in distinguishing women with low bone mass at a time when, most likely, a decision about hormone replacement therapy should be made. The present study addresses only low bone mass, not fracture risk. Therefore, the possibility that risk factors provide an additional

(independent of low bone mass) contribution to the prediction of fracture risk cannot be excluded. Further research is needed to clarify this matter.

2.2 EFFECTS OF TWO DOSES OF TIBOLONE ON TRABECULAR AND CORTICAL BONE LOSS IN EARLY POSTMENOPAUSAL WOMEN: A TWO-YEAR RANDOMIZED, PLACEBO-CONTROLLED STUDY.

2.2.1 Introduction

It is well documented that oestrogens alone or in combination with progestagens are effective in preventing early postmenopausal bone loss (Ettinger *et al.*, 1987; Riis *et al.*, 1987; Munk-Jensen *et al.*, 1988; Ribot *et al.*, 1990; Stevenson *et al.*, 1990; Marslew *et al.*, 1992). Effects of progestagens alone are less clear. Some observations suggest that Medroxy Progesterone Acetate could reduce cortical but not trabecular bone loss (DeCherney *et al.*, 1993), whereas Norethisterone has been shown to prevent early postmenopausal cortical bone loss (Abadalla et al., 1985).

Tibolone is a synthetic C-19 steroid with weak oestrogenic, progestagenic and androgenic properties (Visser *et al.*, 1984). It has been demonstrated that tibolone at a daily dose of 2,5 mg effectively alleviates vasomotor climacteric symptoms (Tax *et al.*, 1987). Preliminary data in early postmenopausal women suggest that this dose may also be capable of preventing metacarpal and spinal bone loss (Lindsay *et al.*, 1980; Rymer *et al.*, 1994) by suppressing skeletal metabolism (Fogelman *et al.*, 1981). In addition, tibolone prevented bone loss in premenopausal women treated with gonadotrophin-releasing hormone agonist medication (Lindsay *et al.*, 1996), as well as after oophorectomy (Lyritis et al., 1995). The present randomised double-blind placebo-controlled study is the first to evaluate the effect of two doses of tibolone (1,25 and 2,5 mg daily) on trabecular and phalangeal bone density in early postmenopausal women. Single energy quantitative computed tomography was used to measure exclusively trabecular bone density in the lumbar spine (Sandor *et al.*, 1985), whereas micro densitometry of the mid phalangeal shaft, which consists of 80% cortical bone (Trouerbach et al., 1988), was used for estimation of cortical bone density.

2.2.2 Materials and Methods

Subjects: Approximately 500 women responded after the study was announced in local media. After information was collected by telephone about race, height, weight, earlier gynaecological history, smoking habits and drug intake, 120 were invited for an interview. In this interview

medical history was taken and thorough information about the trial was given. Ninety six women gave written consent to participate. After a general and gynaecological examination 94 women entered the trial. All were white non-smoking women, 1 to 3 years following spontaneous menopause, with an Body Mass Index (weight divided by square of height) ≤ 27 kg/m^2, free of past or present diseases or medication known to influence calcium metabolism or contra-indicate the trial medication.

Study protocol: The study protocol was approved by the Ethics Review Committee of the Dijkzigt Hospital/Erasmus University. The 94 participants were allocated by random medication numbers to a placebo group (n=23), tibolone (Org OD 14, Livial^R, NV Organon, Oss, The Netherlands) 1.25 mg/d treatment group (n=36) or tibolone 2,5 mg/d treatment group (n=35) according to a 2:3:3 ratio. This was done to increase the power of the study in respect to the comparison between treatment groups. Medication was provided as identical looking tablets and the daily dose was one tablet. Patients were seen at three-month interval for two years (9 visits total). Blood and urine sampling was performed at each assessment. Trabecular and phalangeal bone density was assessed at six-month intervals (five total). Medical examination was repeated after one year and at the end of the trial.

Bone measurements: Single energy quantitative computed tomography (Somatom Plus, Siemens AG, Erlangen, Germany) was used for estimation of trabecular bone density of the lumbar vertebral bodies (L1-L3) as described previously (Berning *et al.*, 1993). Midvertebral slices were determined using an automated procedure as described previously. For measurement within the vertebrae a computerised selection of the trabecular area was used (Sandor *et al.*, 1985). Trabecular bone density was expressed as milligram calcium hydroxyapatite per cm^3, using a standard reference device. Scanning parameters were: 80 kVp 125 mAs with 10 mm slice thickness. Coefficient of variation was less than 1 percent, as measured with COMAC-BME phantom (Kalender *et al.*, 1995). The in vivo precision is reported to be less than 2%.

Bone density of the phalanx was measured by radiographic absorption densitometry, as described previously (Trouerbach *et al.*, 1988). In short: using a standard radiographic technique (50 kV, 20 mS; 3M GT X-ray films and 3M Trimax alpha2 intensifying screens) radiographs of the right hand were exposed together with a linear Aluminium wedge. Radiographic absorption densitometry was performed of the mid-phalanx of the index finger at mid-length. The coefficient of variation was found to be less than 2% (Trouerbach *et al.*, 1988). Results were expressed as Aluminium equivalents/mm^3 (Al eq/mm^3).

Laboratory measurements: Venous blood samples were taken between 12:00 and 16:00 hrs at 3-month intervals. For biochemical estimation of bone metabolism the following markers were used. Alkaline Phosphatase, Phosphorus and serum Calcium were measured with a Bayer Technicon Chem-1 system according to the manufacturers instructions. After overnight fasting, urine samples of second morning voids were collected 2 hours after drinking 300 ml of water. Measurement of urinary Hydroxyproline, Calcium and Creatinine were determined by standard procedures in our laboratory, as has described previously (Berning *et al.*, 1993). Urinary ratios of hydroxyproline/creatinine and calcium/creatinine were calculated in mmol per mol.

Statistical analysis: For trabecular and phalangeal bone density of the spine, analysis of covariance was applied on the percentage change from baseline (with the baseline bone density as covariate). This adjusted analysis was required due to differences between the 3 groups with regard to the baseline values. For biochemical parameters of bone metabolism analysis of variance was applied. Results are expressed as the *P*-value of the overall F-test and the 3 paired group comparisons in *P*-values and 95%-confidence intervals (CI). The analysis was performed on all subjects who participated in this trial and had at least one post-baseline assessment of bone density.

2.2.3 Results

Baseline data and drop-outs: Clinical and biochemical data and bone density at baseline are shown in Table 5. In respect to parity or oral contraceptive use the 3 groups were comparable. Initial bone density of the two treatment groups were similar. However, women in the placebo group had a significantly higher trabecular bone density (P=0.03) compared to the tibolone 1.25 mg group.

Of the 94 women 84 (89%) completed the two-year study. Four women of the placebo group discontinued prematurely due to intercurrent illness (two after 12 months and two after 18 months). There were three drop-outs in each treatment group. In the tibolone 1,25 mg group, one woman started oestrogens without consulting the investigator and two women discontinued due to adverse experiences (i.e. vaginal bleeding, allergic skin reaction). In the tibolone 2,5 mg group, one woman had hypercholesterolaemia, one participant experienced a for her unacceptable weight gain of 3 kg, the other had enlargement of myoma uteri. Two women of

Table 5 Clinical, biochemical data and bone mineral mass (mean ± SD) at baseline of the placebo group and the two treatment groups.

Variables	placebo (n=23)	tibolone1,25 mg/d (n=36)	tibolone2,5 mg/d (n=35)
Age (yrs)	51.9 ± 2.5	52.9 ± 3.1	51.6 ± 2.9
Body mass index (kg/m^2)	24.5 ± 2.2	23.8 ± 2.3	23.6 ± 2.4
Gynaecological parameters:			
Age at menarche (yrs)	14 ± 1.7	14 ± 1.9	13 ± 1.5
Age at menopause (yrs)	50.5 ± 2.4	51.1 ± 2.8	49.9 ± 2.8
Months since menopause	19 ± 7	23 ± 7	23 ± 8
Biochemical markers:			
urinary Hydroxyproline/ creatinine ratios[a]	0.031±0.008	0.028±0.008	0.029±0.01
urinary Calcium/ creatinine ratios[a]	0.326±0.201	0.411±0.242	0.349±0.175
Alkaline Phosphatase (IU/l)	58 ± 20	57 ± 20	58 ± 17
Calcium (mmol/l)	2.44 ± 0.07	2.42 ± 0.11	2.44 ± 0.12
Phosphorus (mmol/l)	1.24 ± 0.16	1.23 ± 0.14	1.25 ± 0.16
Bone density:			
Trabecular (mg/cm^3)	138 ± 40[b]	118 ± 28	121 ± 29
Phalangeal (Al eq/mm^3)	0.530±0.035	0.513±0.046	0.514±0.036

[a](mmol/mmol)
[b]P < 0.05 compared to tibolone 1,25 mg/d group

each treatment group were excluded from the analysis because there was no post-baseline assessment of bone density. Hence, the final statistical analysis was performed on 90 women. The remaining drop-out in each treatment group discontinued after 18 months. None of the subjects missed more than 10 % of the tablets over the 2 year treatment period, as determined by the record of dispensed and returned tablets.

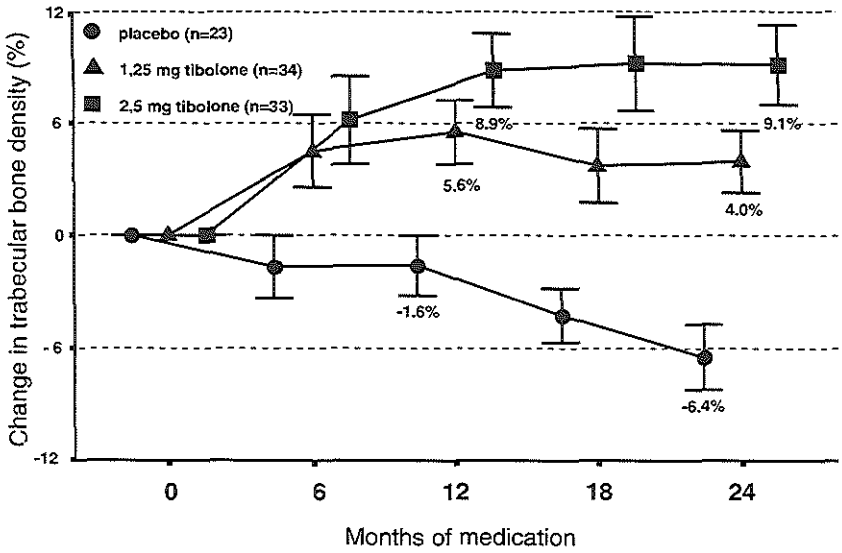

Figure 1
Unadjusted percent change in trabecular bone density of the lumbar spine (mean percentage initial value with standard error of the estimate) in 90 healthy postmenopausal women, using placebo, 1.25 or 2.5 mg/day tibolone.

Trabecular bone density: The results on trabecular bone density are illustrated in Figure 1. In the 1,25 mg tibolone group 24 of the 32 women had a higher trabecular bone density at the end of the trial compared to baseline, whereas 8 women exhibited a lower trabecular bone density. Analysis of the trend in the second year showed no significant change for either 2,5 mg or the 1,25 mg tibolone groups. The baseline trabecular bone density was significantly negatively related to the response expressed as percent change from baseline. An adjusted analysis was required to give unbiased estimates for comparisons between the placebo and the treatment groups (Table 6). When compared to the placebo group both treatment groups showed a significantly positive response at 12 and 24 months. Furthermore, the response to 2,5 mg tibolone was significantly greater compared to the response to 1,25 mg tibolone at 12 and 24 months (Table 6).

Phalangeal bone density: Results on phalangeal bone density are illustrated in Figure 2. Phalangeal bone density of the placebo group did not show a significant decrease over the study period (mean -1.7%, 95% CI [-6.7% - +4.0%]). Both treatment groups showed a linear increase

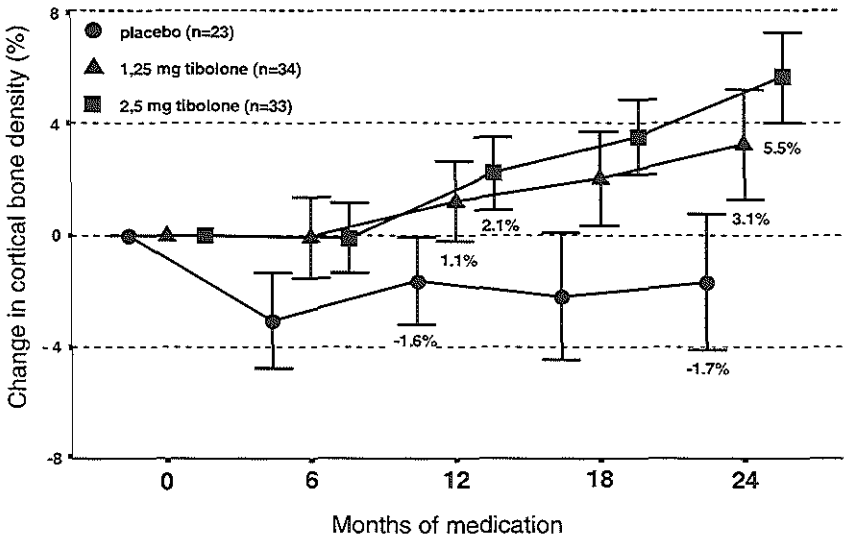

Figure 2
Unadjusted percent change in cortical bone density of the phalanx spine (mean percentage initial value with standard error of the estimate) in 90 healthy postmenopausal women, using placebo, 1.25 or 2.5 mg/day tibolone.

in phalangeal bone density, starting 6 months after initiation, continuing throughout the study period (Figure 2). Although this increase seems higher in the 2,5 mg tibolone group as compared to the 1.25 mg this difference did not reach statistical significance. The baseline phalangeal bone density was significantly negatively related to the response expressed as percent change from baseline. An adjusted analysis was required to give unbiased estimates for comparisons between the placebo and the treatment groups (Table 6). Both tibolone treatment groups had a significantly higher phalangeal bone density compared to the placebo group at 12 and 24 months (Table 6).

Body weight and biochemical parameters of bone metabolism: There was a significant weight gain in the placebo group as well as in the 1.25mg/d and 2.5 mg/d tibolone treatment group (2.6 kg, 2.4 kg and 2.8 kg, respectively). Group comparison showed no significant differences.

Results of serum and urinary parameters of bone metabolism are summarized in Table 7. Both treatment groups showed a significant reduction of alkaline phosphatase and phosphorus

Table 6 Group comparison of percental change from baseline to 12 or 24 months and from 12 to 24 months for trabecular and phalangeal bone density, expressed as mean and 95% confidential interval (95% CI).

		Trabecular bone density			Phalangeal bone density		
Period	Group comparison	mean	[95% CI]	P[a]	mean	[95% CI]	P[a]
0-12 months	2,5 mg[b] vs. placebo	9.5	[7.0 - 12.0]	<0.001	3.3	[1.2 - 5.3]	0.002
	1,25 mg[b] vs. placebo	6.0	[3.5 - 8.5]	<0.001	2.3	[0.3 - 4.3]	0.026
	2,5 mg vs. 1,25 mg	3.5	[1.3 - 5.7]	0.003	1.0	[-0.8 - 2.8]	0.28
0-24 months	2,5 mg vs. placebo	14.7	[11.8 - 17.5]	<0.001	6.8	[3.8 - 9.8]	<0.001
	1,25 mg vs. placebo	9.4	[6.6 - 12.2]	<0.001	4.4	[1.5 - 7.4]	0.004
	2,5 mg vs. 1,25 mg	5.3	[2.9 - 7.6]	<0.001	2.3	[-0.1 - 4.8]	0.064
12-24 months	2,5 mg vs. placebo	6.0	[3.3 - 8.8]	<0.001	2.5	[-0.6 - 5.5]	0.11
	1,25 mg vs. placebo	4.2	[1.4 - 6.9]	0.003	0.7	[-2.3 - 3.7]	0.63
	2,5 mg vs. 1,25 mg	1.9	[-0.5 - 4.2]	0.12	1.8	[-0.8 - 4.4]	0.18

[a] ANCOVA,
[b] daily dosage of tibolone.

compared to the placebo group. There was no significant difference between tibolone treatment groups. Although there were a dose-related decreases in urinary calcium/creatinine ratios and hydroxyproline/creatinine ratios in both treatment groups, these decreases were statistically only in the 2,5 mg tibolone group.

2.2.4 Discussion

Postmenopausal bone mass - which is the best predictor of fracture risk at present (Gardsel *et al.*, 1991) - depends on the amount of peak bone mass and subsequent loss. From the onset of perimenopause and in the initial years following menopause, an increase in bone remodelling (Recker *et al.*, 1988) and an imbalance between bone resorption and formation is observed (Nordin *et al.*, 1985). This results in an accelerated loss of primarily trabecular bone (Riggs *et al.*, 1986). Oestrogen deficiency plays a crucial role in this accelerated bone loss. Several studies have shown that oestrogen replacement therapy can effectively prevent trabecular as well as cortical bone loss by reducing bone resorption.

The present study, focusing on early postmenopausal bone mass during 2 years of tibolone medication, included measurements of both phalangeal (which comprises mainly cortical bone) and trabecular bone density at 6-month intervals. Measurement of trabecular bone density of the lumbar spine by quantitative computed tomography may be less precise than Dual X-ray Absorptiometry (Reinbold *et al.*, 1986). However, this technique allows earlier detection of changes in response to treatment, since trabecular bone (which is measured exclusively) has a much faster turnover rate. Moreover, trabecular bone is much more responsive to hormonal as well pharmacological intervention as compared to cortical bone (Genant *et al.*, 1982; Nilas *et al.*, 1985). This is in agreement with observations in the present study. Loss of trabecular bone in the placebo group was -6.4 %, whereas cortical bone showed no significant loss during the study period. Furthermore in the treatment groups a more pronounced dose-related increase was observed in trabecular bone density as compared to phalangeal bone density. In both treatment groups trabecular bone density showed an increase in the first year with a plateau phase in the second year. Cortical bone density increased after 6 months and showed a linear pattern.

Comparison with similar trials using oestrogens is hampered by differences in treatment regimens (combination with progestagens and/or calcium supplements), types of oestrogens used, routes of administration and applied methods for bone mass measurement. Earlier studies using quantitative computed tomography showed conservation of trabecular bone density with

Table 7 Biochemical parameters of bone metabolism.

variable	treatment group	mean	[95% CI]	P-value
Hydroxyproline/ creatinine ratios (mmol/mmol)	2,5 mg[a]	-0.006	[-0.012 to -0.0003]	0.041
	1,25 mg[a]	-0.003	[-0.009 to +0.003]	0.4
Calcium/ creatinine ratios (mmol/mmol)	2,5 mg	-0.208	[-0.334 to -0.082]	0.002
	1,25 mg	-0.102	[-0.229 to +0.025]	0.12
Alkaline Phosphatase (IU/l)	2,5 mg	-15	[-23 to -7]	<0.001
	1,25 mg	-11	[-19 to -4]	0.004
Calcium (mmol/l)	2,5 mg	-0.03	[-0.11 to +0.04]	0.37
	1,25 mg	-0.02	[-0.09 to +0.06]	0.67
Phosphorus (mmol/l)	2,5 mg	-0.20	[-0.29 to -0.10]	<0.001
	1,25 mg	-0.17	[-0.26 to -0.08]	<0.001

[a]Group comparison of treatment groups versus placebo group at 24 months, expressed as mean difference and 95% confidence interval [95% CI].
[b]daily dosage of tibolone.

0.625 mg conjugated oestrogens after oophorectomy (Genant *et al.*,1982) and in osteoporotic women (Pacifici *et al.*, 1998). Early postmenopausal women treated with low dose (0.3 mg) conjugated oestrogens combined with calcium supplements showed no significant loss of trabecular bone density (Ettinger *et al.*, 1987). Studies using dual photon absorptiometry or dual x-ray absorptiometry for estimation of spinal bone mass in early postmenopausal women using various oestrogen replacement regimens showed changes between 0 and 6.4% (Riis *et al.*, 1987; Munk-Jensen *et al.*, 1988; Stevenson *et al.*, 1990; Ribot *et al.*, 1990; Marslew *et al.*, 1992). A recent study in early postmenopausal women treated for 2 years with 2,5 mg/d tibolone showed an increase of 2.5% in spinal bone density measured with dual x-ray absorptiometry (Rymer *et al.*, 1994), which is somewhat lower as compared to the 9.1% increase observed with the same dose of tibolone in the present study. The most probable explanation for this discrepancy is

measurement of exclusively trabecular bone density of the spine in the present study, as previously mentioned. A study of tibolone 2,5 mg/d in women with established osteoporosis (Geussens *et al.*, 1991) showed an increase of spinal bone mass (measured with dual photon absorptiometry) of 8%, which is similar to the increase induced by fluoride (Hansson *et al.*, 1987) or biphosphonates (Storm *et al.*, 1990).

Biochemical parameters showed a doses-related response following tibolone medication, indicating a decrease in bone resorption and turnover rate.

In summary the present study shows that tibolone induces a dose-related increase in early postmenopausal trabecular and cortical bone mass. We therefore conclude that 2,5 mg tibolone effectively prevents early postmenopausal bone loss. The 1,25 mg dose can prevent bone loss in the majority of early postmenopausal women.

2.3 INCREASED LOSS OF TRABECULAR BUT NOT CORTICAL BONE DENSITY, 1 YEAR AFTER DISCONTINUATION OF 2 YEAR HORMONE REPLACEMENT THERAPY WITH TIBOLONE.

2.3.1 Introduction

Several studies have documented that hormone replacement therapy (HRT) with estrogens alone, or in combination with progestagens are effective in preventing early postmenopausal bone loss (Ettinger *et al.*, 1987; Riis *et al.*, 1987; Stevenson *et al.*, 1990; Marslew *et al.*, 1992). Effects of cessation of HRT on bone metabolism and bone loss are less clear. Some (Lindsay *et al.*, 1978; Horsman *et al.*, 1979; Fogelman *et al.*, 1980) but not all (Christiansen *et al.*, 1981; Thomsen *et al.*, 1987) studies in women after oophorectomy or following natural menopause suggested a rebound phenomenon on markers of bone metabolism or accelerated bone loss following cessation of HRT. Further doubts regarding long-term beneficial effect of temporary HRT used in early postmenopause on eventual bone mass at advanced age have arisen from data of The Framingham Osteoporosis Study (Felson et al., 1993) and The Rancho Bernardo Study (Schneider *et al.*, 1997).

Tibolone is a synthetic C-19 steroid with weak estrogenic, progestagenic and androgenic properties (Visser de *et al.*, 1984). It has been previously documented that tibolone is capable of preventing metacarpal and spinal bone loss (Lindsay *et al.*, 1980; Rymer *et al.*, 1994; Berning *et al.*, 1996; Bjarnason *et al.*, 1996) in postmenopausal women by suppressing skeletal metabolism (Fogelman *et al.*, 1981). In addition, tibolone prevented bone loss in premenopausal women treated with gonadotropin-releasing hormone agonist medication (Lindsay *et al.*, 1996), as well as after oophorectomy (Lyritis *et al.*, 1995).

The present study is an extension of an earlier, randomized, double-blind, placebo-controlled study (Berning *et al.*, 1996) evaluating the effect of tibolone on early postmenopausal bone loss. This study reported, for the first time, a dose dependent increase in trabecular and cortical bone density in early postmenopausal women using 1,25 mg/d or 2,5 mg/d tibolone during a 2 year study period. The present study reports on trabecular and cortical bone density one year after completing this initial study. Single energy quantitative computed tomography was used to measure exclusively trabecular bone density in the lumbar spine (Sandor *et al.*, 1985), whereas micro densitometry of the mid phalangeal shaft (which

consists of 80% cortical bone) (Trouerbach *et al.*, 1988), was used for the estimation of cortical bone density. (Berning *et al.*, 1996).

2.3.2 Materials and Methods

Subjects: Sixty-four out of 84 subjects who completed a 2 year randomised placebo controlled study regarding the effects of two doses of tibolone (NV Organon, Oss, The Netherlands) on early postmenopausal bone loss entered the present study. Selection of subjects and inclusion criteria for the initial study have been described earlier (Berning *et al.*, 1996). In brief; 94 healthy Caucasian non-smoking women, 1-3 years following spontaneous menopause, with a body mass index (BMI: weight divided by square length) < 27 kg/m^2, free of diseases or medication known to influence calcium metabolism or contraindicate the trial medication entered the initial trial. Subjects were allocated by random medication number, according to a 2:3:3 ratio, to a placebo (n=23), a tibolone 1.25 mg/d (n=36) or a tibolone 2.5 mg/d (n=35) treatment group. Ten subjects dropped out of the initial trial due to adverse experiences or intercurrent illness. As described earlier, they were distributed equally among the 3 groups (Berning *et al.*, 1996). Of the remaining 84 subjects, 4 wanted to continue the use of HRT for relieve of climacteric symptoms. Five subjects refused to participate further for no particular medical reason. Fifteen subjects of the original group of 84 participants had a trabecular bone mineral density below 1 standard deviation (SD) of the mean value of the study group at baseline. Eleven of these women started or continued HRT after the initial trial and were therefore excluded from study. They were distributed equally among the three groups (placebo: 2 , tibolone 1,25 mg: 4 and tibolone 2.5 mg: 5). The remaining four women did not use HRT in the 1 year follow-up, one was a drop out in the initial study and 3 enrolled in the present study. Subjects who did not participate regardless the reason, were also distributed equally among the three groups, 7 in the placebo group, 11 in the 1,25 mg tibolone group and 12 in the 2.5 mg tibolone group, regarding the 2:3:3 ratio of randomisation. Therefore, sixty-four subjects who were free of disease or medication known to influence calcium metabolism were included in this 1 year follow-up period.

Study protocol: The study protocol was approved by the Ethics Review committee of the Dijkzigt Hospital/Erasmus University. Sixty-four participants were assessed 1 year after

completing the initial 2 year medication period. Blood and urine sampling was performed as well as measurement of trabecular and phalangeal bone density.

Bone measurements: Single energy Quantitative Computed Tomography (QCT) (Somatom plus, Siemens AG, Erlangen, Germany) was used for estimation of trabecular bone density of the lumbar vertebral bodies (L1-L3). Trabecular bone density was expressed as milligram hydroxyapatite per cm^3, using a standard reference device. Bone density of the mid-phalanx of the index finger was measured by radiographic absorption densitometry. Results were expressed as aluminium equivalents per mm^3 (Al eq/mm^3). Both techniques have been described in detail previously (Berning *et al.*, 1993).

Laboratory measurements: For biochemical estimation of bone metabolism the following markers were used. Alkaline Phosphatase, Phosphorus and serum Calcium were measured with a Bayer Technicon Chem-1 system (Bayer AG, Leverkusen, Germany) according to the manufacturers instructions. After overnight fasting, urine samples of second morning voids were collected 2 hours after drinking 300 ml of water. Measurement of urinary Hydroxyproline, Calcium and Creatinine were determined by standard procedures in our laboratory, as has been described previously (Berning *et al.*, 1993). Urinary ratios of hydroxyproline/creatinine and calcium/creatinine were calculated in mmol per mmol.

Statistical analysis: For trabecular and phalangeal bone density, analysis was applied regarding changes expressed as percentage from baseline. For parameters of bone metabolism comparison of means was performed within the study group as well as between groups. All variables were tested for normal distribution. Only for normally distributed variables student t test for independent sample, was used for group comparison. For comparison within groups the t-test for paired samples was applied. For all others variables the Mann-Whitney test was used for group comparison, and for comparison within groups the Wilcoxon Rank Sum W Test was used. The upper limit for statistical significance was 0.05. All p-values are two-tailed. A difference between treatment groups of 5,5% in trabecular bone density could be detected with a power of 80% with the conventional 0,05 level for alpha. Statistical calculations were performed in SPSS for windows, release 6.1.

2.3.3 Results

Study group: Initial clinical and biochemical characteristics and bone density of the 64 participants are shown in Table 8. There were no significant differences. With respect to parity or oral contraceptive pill use the 3 groups were also comparable (data not shown).

Trabecular bone density: Results in relative changes of trabecular bone density are depicted in Figure 3. During the 2 year medication period trabecular bone density increased significantly in both treatment groups as compared to baseline, whereas during the same time period a significant loss was observed in the placebo group. During the 1 year follow-up period a significant loss of bone mass occurred in both treatment groups, whereas the placebo group showed a further loss of 1,4%. Compared to baseline, at 36 months trabecular bone density was significantly lower in the placebo and 1.25 mg tibolone group, whereas the reduction of 2% trabecular bone density in the 2.5 mg tibolone group reached borderline significance (P=0.05). Group comparison of the percent change during the 2 year medication period and 1 year follow-up period are presented in Table 9. When compared to the placebo group both treatment groups showed a significantly greater decrease in trabecular bone density ($P<0.03$ and $P<0.001$) during the one year follow-up period. The decrease in trabecular bone density in both treatment groups during the 1 year follow-up period was not significantly different.

Phalangeal bone density: Results of relative changes in phalangeal bone density are also depicted in Figure 3. During the 2 year medication period phalangeal bone density increased significantly in both treatment groups as compared to baseline. A continuous decrease in phalangeal bone density was observed in the placebo group during 3 years of monitoring. Compared to baseline, at 36 months phalangeal bone density was significantly higher in the 2.5 mg treatment group. In contrast there was a significantly lower phalangeal bone density in the placebo group at 36 months compared to baseline, whereas phalangeal bone density at 36 months in the 1.25 mg treatment group did not differ significantly from baseline. Group comparison of the percent change during the 2 year medication period and 1 year follow-up period are presented in Table 9. When compared to the placebo group both treatment groups showed no significantly different change in phalangeal bone density during the 1 year follow-up period.

Table 8 Clinical, biochemical data and bone mineral mass (mean ± SD) of the 64 postmenopausal women at baseline of the initial study divided in placebo group and the two treatment groups.

Variables	placebo (n=16)	tibolone 1,25 mg/d (n=25)	tibolone 2,5 mg/d (n=23)
Age (yrs)	52.9 ± 2.6	53.6 ± 3.3	52.3 ± 3.0
Body mass index (kg/m^2)	24.3 ± 2.4	23.6 ± 2.5	23.4 ± 2.7
Gynaecological parameters:			
Age at menarche (yrs)	13 ± 1.9	14 ± 1.7	13 ± 1.4
Age at menopause (yrs)	50.9 ± 2.5	51.2 ± 3.0	52.2 ± 3.1
Months since menopause	20 ± 7	24 ± 8	23 ± 8
Biochemical markers:			
urinary Hydroxyproline/creatinine ratios[a]	0.030 ± 0.008	0.030 ± 0.009	0.030 ± 0.01
urinary Calcium/creatinine ratios[a]	0.331 ± 0.217	0.457 ± 0.248	0.367 ± 0.189
Alkaline Phosphatase (IU/l)	53 ± 14	52 ± 13	57 ± 14
Calcium (mmol/l)	2.42 ± 0.07	2.42 ± 0.11	2.47 ± 0.11
Phosphorus (mmol/l)	1.23 ± 0.17	1.23 ± 0.12	1.26 ± 0.17
Bone density:			
Trabecular (mg/cm^3)	148 ± 39	127 ± 26	129 ± 26
Phalangeal (Al eq/mm^3)	0.539 ± 0.035	0.519 ± 0.042	0.511 ± 0.0354

[a](mmol/mmol)

Biochemical parameters of bone metabolism: There was no change in serum calcium throughout the 2 year medication period as well as in the 1 year follow-up. Changes in alkaline phosphatase, phosphate, urinary calcium/creatinine ratio and urinary hydroxyproline/creatinine ratio are illustrated in Figure 4. For alkaline phosphatase, phosphorus and urinary calcium/creatinine ratio a significant decrease during the 2 year medication period was observed in both treatment groups, resulting in significantly lower values in the group comparison at the end of the 2 year period. During the 1 year follow-up these values increased to pre-treatment levels. Therefore, group comparison for these parameters after 1 year of follow up showed no significant differences. A significant reduction in urinary hydroxyproline/creatinine ratio during the 3 years of monitoring was observed in all groups. Group comparison after 1 year follow-up showed no significant differences.

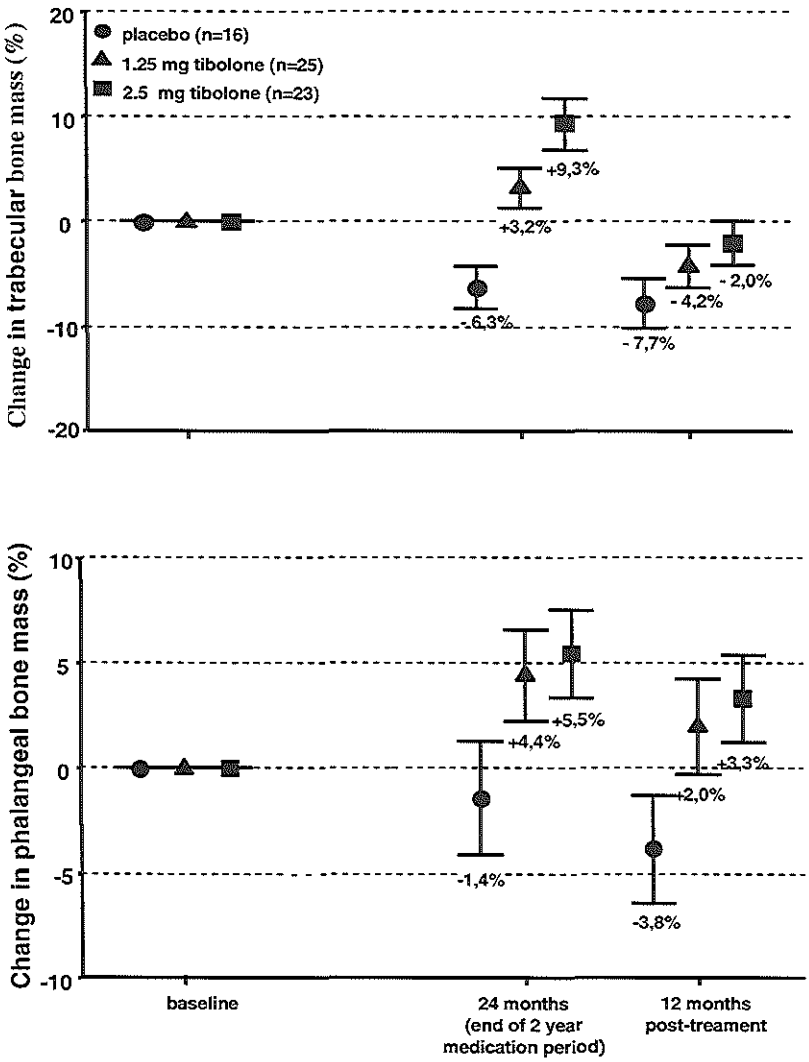

Figure 3
Percent change as compared to baseline in trabecular bone density of the lumbar spine (mean and 95% confidence interval) (upper panel) and phalangeal bone density (mean and 95% confidence interval) (lower panel) in 64 healthy early postmenopausal women having used placebo, 1.25 or 2.5 mg/day tibolone for 2 years (24 months), followed for 1 additional year after discontinuation of medication (36 months).

2.3.4 Discussion

The skeleton consists of two different types of bone. Cortical bone forms the shafts of long bones and most effectively resists bending forces. The internal structure of metaphyses of long bones and vertebral bodies consists of trabecular bone and is best designed to resist compressive stress (Dempster, 1992). Trabecular bone has a much higher surface to volume ratio. Since the processes of bone remodelling are primarily surface phenomena (Parfitt, 1979) trabecular bone is considerably more metabolically active as compared to cortical bone. Therefore, trabecular bone is much more responsive to hormonal and pharmacological intervention as compared to cortical bone (Genant *et al.*, 1982; Nilas *et al.*, 1985). From the onset of perimenopause and during initial years after menopause an accelerated loss of primarily trabecular bone takes place due to decremental estrogen production.

It has been clearly documented in the literature that HRT can effectively prevent trabecular as well as cortical bone loss. In contrast, there are few data on bone loss after cessation of HRT. Histomorphometry in oophorectomised rats showed an accelerated bone turnover after discontinuation of estrogen therapy (Wornski *et al.*, 1993; Takahashi *et al.*, 1994). Studies in oophorectomised (Lindsay *et al.*, 1978) and postmenopausal women (Horsman *et al.*, 1979; Davis *et al.*, 1995) showed an accelerated bone loss after withdrawal of HRT. Lindsay and colleagues, (Lindsay *et al.*, 1978) who used single photon absorptiometry (SPA) at the mid-point third metacarpal of the right hand for determination of bone density, found no benefit of 4 years of treatment with estrogens 4 years after discontinuation of treatment. A population based study showed that women who stopped HRT had a greater than average loss of bone mass in the calcaneus, distal and proximal radius using SPA (Davis *et al.*, 1995). One study (Christiansen *et al.*, 1981) using SPA at the distal forearm in postmenopausal women found no accelerated bone loss after withdrawal of HRT. However, women in this study used supplementary calcium tablets, which may have influenced their results. When skeletal uptake of diphosphonate (a sensitive measure of skeletal metabolism) is measured in postmenopausal women, one study suggested a period of accelerated bone turnover (Fogelman *et al.*, 1980), whereas Thomsen (Thomsen *et al.*, 1987) found a bone turnover similar to the placebo group after withdrawal of HRT.

The present study evaluated for the first time both trabecular bone density at the lumbar spine and mid-phalangeal bone density (which comprises mainly cortical bone), 1 year after cessation of 2 years of HRT with tibolone. Due to ethical considerations the present study

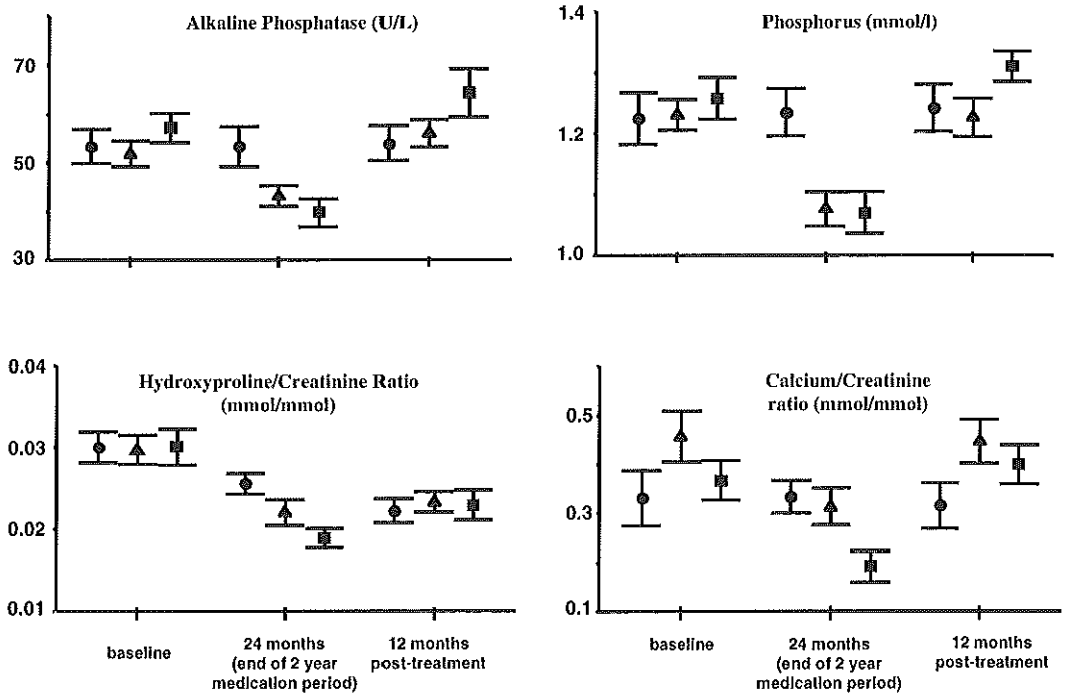

Figure 4
Biochemical parameters of bone metabolism (mean and SE) at baseline, after a 2 year period of tibolone medication or placebo (24 months) and 1 additional year after discontinuation (36 months) in 64 healthy early postmenopausal women, having used placebo (n=16) (●), 1.25 mg/day (n=25) (▲), 2.5 mg/day (n=23) (■) tibolone.

population did not include 11 out of 15 subjects with low bone mass at baseline of the initial study since these patients started or continued to use HRT during the 1 year follow-up period. However, these subjects where distributed equally among the three groups. Hence, most likely group comparison is not affected. Furthermore, in the initial study bone density at baseline was negatively related to the response during the 2 year medication period. This means that low initial bone mass was associated with a greater increase in both treatment groups and a smaller decrease in the placebo group. Since there is a strong negative correlation between changes during the 2 year medication period and in the 1 year follow-up period, it is unlikely that any selection avoiding low bone mass would have lead to the observations in this study. The present observations show a significantly greater decrease in trabecular bone density in both tibolone regimens as compared to the placebo group. The decrease in trabecular bone density during the 1 year follow-up period in both treatment groups (7-11%) is comparable with the decrease in trabecular bone density (also measured with QCT) seen after bilateral oophorectomy (7-9%) (Genant *et al.*, 1982) but is substantially greater than the annual decrease of 3.2% seen after spontaneous menopause (Berning *et al.*, 1996). In the placebo group the decrease in trabecuar bone density during the 1 year follow-up period is approximately half the rate during the two year study period. The exact reason is not known but it could be hypothesised that the increased loss of trabecular bone density in the years immediately after menopause decreases in the fourth to sixth year since menopause. In contrast to trabecular bone density, the decrease in phalangeal bone density during the 1 year follow-up, in both tibolone regimens did not differ from the placebo group. After 1 year follow-up following 2 years of tibolone medication, phalangeal bone density was still significantly greater compared to the placebo group. However, since cortical bone may react slower than trabecular bone, the decreasing trend seen in cortical bone density during the first year after HRT is likely to continue. The decrease of phalangeal bone density during the 1 year follow-up is greater than during the two year study period, the reason for this is not known. Only long-term follow-up of healthy early postmenopausal women gives insight in the annual rate of phalangeal bone density during the years following menopause. Reduction in serum Alkaline Phosphatase, Phosphorus as well as decrease in urinary excretion of calcium and hydroxyproline indicated a reduction in bone resorption during tibolone medication with a return to pre treatment values 1 year after discontinuation of tibolone. Unfortunately biochemical markers of bone metabolism were not assessed immediately after the wash-out period of the tibolone medication, therefore

Table 9 Group comparison of percent change from baseline to end of study (24 months) and in post study year (24-36 months) for trabecular and phalangeal bone density, expressed as mean difference and 95% confidence interval (CI).

		Trabecular bone density			Phalangeal bone density		
Period	Group comparison	Mean	(95% CI)	P	Mean	(95% CI)	P
study period: 0-24 months	2.5 mg[a] vs. placebo	15,6	(12,5 - 18,7)	<0,001	6,9	(3,6 - 10,2)	<0,001
	1,25 mg[a] vs. Placebo	9,5	(6,8 - 12,2)	<0,001	5,8	(2,5 - 9,2)	0,001
	2,5 mg vs. 1,25 mg	6,1	(3,0 - 9,2)	<0,001	1,0	(-1,9 - 4,0)	0,47
post study year: 24-36 months.	2.5 mg vs. placebo	-10,0	(-12,9 - -6,9)	0,001	-0,3	(-4,5 - 4,0)	0,9
	1,25 mg vs. Placebo	-6,0	(-8,4 - -3,5)	0,03	0,02	(-3,5 - 3,5)	1,0
	2,5 mg vs. 1,25 mg	-3,9	(-6,7 - 1,1)	0,12	-0,3	(-4,2 - 3,6)	0,9

a :Daily dosage of tibolone

a rebound phenomenon normalising within one year can not be excluded. At the end of the follow-up year these markers did not indicate an increased bone resorption compared to the placebo group or to baseline values of the initial study.

Observations from the present study suggest that there is an accelerated loss of more metabolically active trabecular bone, but not of cortical bone following cessation of 2 year tibolone intake. It should be realised, however, that techniques of bone mass measurements used in the present study as well as skeletal sites measured may have influenced results, and may attribute to the present controversy in the literature. Although our follow-up period is short, it seems that HRT with tibolone like with estrogens should be continued into late life in order to maintain high bone density. Data concerning early HRT use and bone density later in live are available from the Framingham Study (Felson *et al.*, 1993). Only early HRT longer than 7 years resulted in a higher bone density up to 75 years of age. Even this duration of HRT in early postmenopause may have little residual effect on bone density among women older than 75 years of age, who are at the highest risk for hip fractures. Data from Schneider and colleagues (Schneider *et al.*, 1997) also suggests that past estrogen use provides little or no long-term benefit for preservation of bone density. Therefore, the possibility of an alternative strategy to start HRT many years after menopause and to continue treatment for the rest of a women lifetime, in order to prevent osteoporotic fractures, has been proposed (Ettinger *et al.*, 1996; Schneider *et al.*, 1997). Since a daily dose of 1.25 mg or 2.5 mg tibolone has been shown to prevent bone loss in late menopause in a similar way (Bjarnason *et al.*, 1996), this medication may represent an alternative strategy for the prevention of postmenopausal osteoporosis in late postmenopausal women (Riggs, 1996).

In summary, the present study suggests that in contrast to cortical bone density there is an increased loss of trabecular bone density in the first year after discontinuation of treatment with tibolone.

2.4 ABSENT CORRELATION BETWEEN VAGINAL BLEEDING AND OESTRADIOL LEVELS OR ENDOMETRIAL MORPHOLOGY DURING TIBOLONE USE IN EARLY POSTMENOPAUSAL WOMEN.

2.4.1 Introduction:

Despite the well documented beneficial long-term effects of hormone replacement therapy (HRT) on postmenopausal osteoporosis and cardiovascular disease, compliance of HRT is poor. In the Netherlands, more than 60% of women withdraw from HRT within 6 months and only 8% of the women remained on HRT for more than 2 years (Groeneveld *et al.*, 1998). The two most common reasons for stopping oestrogen's or failing to initiate HRT in postmenopausal women are fear for cancer and the withdrawal bleeding induced by cyclical progesterone given to prevent endometrial hyperplasia and subsequent cancer.

Tibolone is a synthetic steroid with an unique pharmacological profile. Studies in animals showed oestrogenic, progestagenic and weak androgenic properties (Visser de *et al.*, 1987). Tibolone relieves vasomotor symptoms (Trevoux *et al.*, 1983; Tax *et al.*, 1987) and maintains skeletal integrity in early (Berning *et al.*, 1996) and late (Bjarnason *et al.*, 1996) postmenopausal women comparable to estrogens. Several clinical studies established that tibolone 2,5 mg/d does not stimulate the endometrium (Genazzani *et al.*, 1991; Rymer *et al.*, 1994; Ginsburg *et al.*, 1995, Habiba *et al.*, 1996) thus eliminating the need for women to have a progesterone induced withdrawal bleed. However, some postmenopausal women do bleed while taking tibolone (Lindsay *et al.*, 1980; Trevoux *et al.*, 1983; Genazzani *et al.*, 1991; Rymer *et al.*, 1994; Ginsburg *et al.*, 1995, Habiba *et al.*, 1996). In the present study we compare baseline characteristics and serum levels of oestradiol (E2) in women who bled and those who did not, in an attempt to determine factors that are associated with bleeding in early postmenopausal women using two doses (1.25 mg/d and 2.5 mg/d) of tibolone in a two year randomised, placebo-controlled study. Furthermore, endometrial histology is studied in women with vaginal bleeding.

2.4.2 Subjects and Methods:

Subjects: Ninety four healthy women were recruited for a 2 year randomised placebo-controlled study evaluating two doses of tibolone (Livial®,NV Organon, Oss, The Netherlands) on early postmenopausal bone loss. Selection of subjects and inclusion criteria

for the initial study have been described earlier (Berning et al., 1996). In brief; 94 healthy Caucasian non-smoking women, 1-3 years following spontaneous menopause, with a body mass index (BMI: weight divided by square length) < 27 kg/m^2, free of diseases or medication known to influence calcium metabolism or contraindicate the trial medication. At the initial visit information was collected regarding medical history, gynaecological history and drug intake. Detailed description of baseline characteristics has been published earlier (Berning *et al.*, 1993).

Study protocol: The study protocol was approved by the Ethics Review committee of the Dijkzigt Hospital/Erasmus University. Following thorough information 96 women gave written consent to participate. After a general and gynaecological examination 94 women entered the study. Subjects were allocated by random medication number, according to a 2:3:3 ratio, to a placebo, tibolone 1.25 mg/d or a tibolone 2.5 mg/d treatment group. Medication was provided as identically looking tablets. The daily dose was one tablet. Patients were asked to make a note of any vaginal bleeding or other side effect. Patients were seen at three-months intervals for 2 years (9 visits total) at which they were asked about vaginal bleeding or any other side effect. In case of vaginal bleeding women underwent a Vabra curettage (Alberico *et al.*, 1986). Assessment of BMI was performed at each visit.

Laboratory measurements: The serum concentrations of E2 and Follicle-Stimulating Hormone (FSH) were measured at each assessment (i.e. every 3 months) by a commercially available fluoro-immunoassay (Delfia®, Wallac Oy, Turku, Finland). For E2 the assay sensitivity was 50 pmol/l. The intra- and inter-assay variability was 4,5-7,8% and 6,3-10,3% for E2 and FSH, respectively.

Statistical analysis: Baseline characteristics of normally distributed variables were expressed as mean and standard deviation (SD). For not normally distributed variables median and range was used. Baseline characteristics of women who bled were compared to those who did not within each group as well as both treatment groups together. The Student t test for independent samples was used for comparison of means of normally distributed variables. For comparison of medians of not normally distributed variables the Mann-Whitney U test was used. The number of women who bled as well as the number bleeding episodes in each group were compared using the χ^2 test. To determine whether the E2 serum level prior to bleeding was higher than the overall E2 serum level of women who did not bleed in each group, the

median E2 serum concentration prior to the bleeding episode was compared to the median of the median of the 9 E2 levels measured during the entire study period of women who did not bleed. Since E2 levels were not normally distributed the Mann-Whitney U test was used. Furthermore, the number of women who had E2 serum levels > 70 pmol/l at any occasion during the study period were also compared using the χ^2 test. The analysis included all women randomised to the study (i.e. intent to treat subjects) to rule out bias as a result of selection caused by leaving out patients who did not complete the study.

2.4.3 Results:

During a 18 months recruitment period 94 women were allocated (according to the 2:3:3 ratio) to a placebo (n=23), a 1,25 mg (n=36) and a 2,5 mg tibolone treatment group (n=35). Their mean age was 52.7 ± 2,9 years, the median time since menopause was 20 months (range: 12-36). The mean age at menopause was 50,5 ± 2,8 years. The median BMI at baseline was 24.3 kg/m^2 (range 18,2-27,0) The median serum E2 level and the mean concentration FSH at baseline was 54 pmol/l (range: 50-447 pmol/l) and 69 ± 26 IU/l, respectively.

Eighty-four (89%) women completed the 2 year study. In the placebo group there were 4 drop-outs due to intercurrent illness. In the 1,25 mg tibolone group one women started estrogen use without consulting the investigator. Two women discontinued due to adverse experiences (i.e. vaginal bleeding, allergic skin reaction). In the 2,5 mg tibolone group one woman had hypercholesterolemia, one participant experienced an unacceptable weight gain of 3 kg, and the other had enlargement of uterine fibroids.

As shown in Table 10, the number of bleeders in the 2,5 mg tibolone group were significantly higher compared to placebo. Also in the 1,25 mg tibolone group percentage of women who bled was also higher (44% compared to 22% in the placebo group) but this difference failed to reach statistical significance. When both treatment groups together were compared to placebo, percentages of bleeders was significantly higher 48% versus 22% (χ^2 test $P<0.05$). When bleeding episodes were expressed in months of usage of trial-medication, in the placebo group there were 8 in nearly 500 months. For 1,25 mg tibolone this was 20 in approximately 770 months of usage. In the 2,5 mg tibolone group there were 42 episodes of bleeding in nearly 740 months which was significantly more compared to placebo and 1,25 mg tibolone (χ^2 test

Table 10 Number of women who experienced bleeding, number of bleeding episodes and distribution among 94 early postmenopausal women receiving placebo, 1,25 mg/d or 2,5 mg/d tibolone during the two year study period.

	Group		
	placebo (n=23)	1,25 mg tibolone (n=36)	2,5 mg tibolone (n=35)
No. of bleeders	5 (22%)	16 (44%)	18 (51%)[a]
No. of bleeding episodes			
0	18 (79%)	20 (56%)	17 (49%)
1	3 (13%)	13 (36%)	12 (34%)
2	1 (4%)	1 (3%)	1 (3%)
3	1 (4%)	2 (6%)	1 (3%)
4			1 (3%)
5			1 (3%)
8			2 (6%)
Total no. of bleeding episodes	8	20	42[b,c]

[a] : significantly different from placebo P<0.05
[b] : significantly different from placebo P<0.01
[c] : significantly different from 1,25 mg/d tibolone P<0.05

P<0.001 and P<0.05, respectively).

Table 11 compares bleeders to non-bleeders with respect to baseline characteristics and serum E2 levels during the study period. In the placebo group bleeders were younger, had their menopause earlier and months since menopause was less compared to the non-bleeders (Table 11). In the 2,5 mg tibolone group, non-bleeders were younger and had their menopause at an younger age. In contrast, months since menopause was less for bleeders although this failed to be statistical significant (Table 11). In the 1,25 mg tibolone group as well as both treatment groups as a whole, baseline characteristics were comparable to non-bleeders (Table 11). Table 11 also compares bleeders to non-bleeders with respect to serum E2 levels. In the placebo group the incidence of E2 levels > 70 pmol/l during the two year study period were comparable. However, when the median E2 level prior to bleeding was compared to the overall serum E2 level of non-bleeders during the study period, bleeders had a higher median serum E2 level (Table 11). In both treatment groups (separately as well as together), bleeders were comparable to non-bleeders with respect to serum E2 levels (Table 11).

Table 11 Comparison of age, age at menopause, months (mths) since menopause and Body Mass Index (BMI) at baseline between bleeders and non-bleeders (mean ± SD or median and range). Comparison of the median oestradiol (E2) level prior to bleeding to the median of the median E2 level of the non-bleeders during the two year study period. Comparison of number of women with detectable (E2 level > 70 pmol/l) or premenopausal (E2 levels >100 pmol/l) E2 levels at any occasion, during the two year study period, between bleeders and non-bleeders, for placebo group, 1,25 mg tibolone group, 2,5 mg tibolone group and both tibolone groups.

	Bleeders	Non-bleeders	P
Placebo group (n=23)	n=5	n=18	
Age (yrs)	50.0 ± 3.3	53.2 ± 1.8	<0.01*
Age at menopause (yrs)	48.6 ± 3.3	51.0 ± 1.9	<0.05*
Mths since menopause (yrs)	13 (12-18 0)	19 (12-36)	<0.05†
BMI (kg/m^2)	24.3 (23.0-26.8)	25.3 (19.1-26.9)	
E2 level (pmol/l)	56 (50-285)	50 (50-62)	<0.05†
E2 levels > 70 pmol/l (n)	4	10	
E2 levels > 100 pmol/l (n)	3	8	
1,25 mg tibolone group (n=36)	n=16	n=20	
Age (yrs)	53.1 ± 3.1	53.6 ± 3.2	
Age at menopause (yrs)	50.9 ± 3.0	51.2 ± 2.9	
Mths since menopause (yrs)	23 (12-18)	22 (12-36)	
BMI (kg/m^2)	25.2 (18.2-26.9)	23.7 (20.3-27.0)	
E2 level (pmol/l)	51 (50-197)	53 (50-69)	
E2 levels > 70 pmol/l (n)	7	8	
E2 levels > 100 pmol/l (n)	6	3	
2,5 mg tibolone group (n=35)	n=18	n=17	
Age (yrs)	53.2 ± 2.0	51.2 ± 3.2	<0.05*
Age at menopause (yrs)	51.2 ± 2.0	48.9 ± 3.3	<0.05*
Mths since menopause (yrs)	18 (12-36)	24 (12-36)	
BMI (kg/m^2)	23.4 (19.8-26.9)	23.8 (18.3-26.8)	
E2 levels (pmol/l)	56 (50-447)	51 (50-70)	
E2 levels > 70 pmol/l (n)	6	5	
E2 levels > 100 pmol/l (n)	5	2	
Both tibolone groups (n=71)	n=34	n=37	
Age (yrs)	53.1 ± 2.5	52.5 ± 3.3	
Age at menopause (yrs)	51.1 ± 2.5	50.1 ± 3.3	
Mths since menopause (yrs)	20 (12-36)	24 (12-36)	
BMI (kg/m^2)	24.1 (18.2-26.9)	23.7 (18.3-27)	
E2 level (pmol/l)	55 (50-447)	51 (50-70)	
E2 levels > 70 pmol/l (n)	13	13	
E2 levels > 100 pmol/l (n)	11	5	

* : Student-t test
† : Mann-Whitney *U* test

Figure 5 shows the cumulative percentage of women presenting with a first bleeding during the study period. Ninety percent of the first bleeding episodes in the 1,25 mg and 2,5 mg tibolone group were within 41 and 36 weeks, respectively. Within 12 weeks about 50% of the first bleeding episodes occurred in both treatment groups.

Table 12 shows the endometrial findings of women who experienced bleeding. No hyperplasia was found. One woman, who bled 8 times, had uterine fibroids diagnosed with vaginal ultrasound. At hysteroscopy no abnormal findings were seen. All women who bled after the endometrial sampling had normal findings at vaginal ultrasound with a regular endometrium and a endometrial thickness of less than 4 mm.

2.4.4 Discussion:

Tibolone is a synthetic C-19 steroid ((7α,17α)-17-hydroxy-7-methyl-19 norpregn-5(10)-en-20-yn-3-one) which effectively alleviates climacteric vasomotor symptoms (Trevoux *et al.*, 1983; Tax *et al.*, 1987). Furthermore, randomised placebo-controlled trials showed that it is capable of preventing bone loss in early (Berning *et al.*, 1996) and late (Bjarnason *et al.*, 1996) postmenopausal women. When tibolone is used for long-term HRT it is important to assess its effects on the endometrium as well as on the incidence of vaginal bleeding.
Tibolone has a mixed hormonal profile. The estrogenic potency of tibolone is about 1/50 that of ethinyl oestradiol, the progestagenic potency is 1/8 that of norethisterone, and the androgenic potency is about 1/3 that of norethisterone (Vies van der *et al.*, 1987). These actions are receptor mediated, as shown by the inhibition of anti-oestrogens and anti-progestins (Markiewicz *et al.*, 1994). Tibolone is extensively metabolised in 3α hydroxy tibolone, 3β hydroxy tibolone and its Δ4 isomer (Sandker *et al.*, 1994). These metabolites exhibit different oestrogenic and progestagenic capabilities in vitro. Furthermore, concentrations of metabolites differ depending on the target tissue. For instance, in the human endometrium tibolone is predominately metabolised to its Δ4 isomer, which has more progestagenic activity (Tang *et al.*, 1993). This finding suggests a metabolic regulation of hormonal activity of tibolone at the target tissue level (Markiewicz *et al.*, 1990).

Several clinical trials showed that tibolone does not stimulate the endometrium (Genazzani *et al.*, 1991; Rymer *et al.*, 1994; Habiba *et al.*, 1996; Ginsburg *et al.*, 1996). Even during long-term use up to 5 years no stimulatory effects on the endometrium could be found (Genazzani

Table 12 Endometrial histological findings after Vabra curettage in 39 women who experienced first vaginal bleeding during the 2 year study period (number of subjects).

Histological finding	placebo	1,25 mg tibolone	2,5 mg tibolone	total.
Missing/not performed	0	2	1	3
Insufficient amount of material	3	8	9	20
Atrophic/inactive	0	4	8	12
Proliferative	2	2	0	4

et al., 1991; Ginsburg *et al.*, 1996). Earlier studies state that vaginal bleeding does occur in women taking 2,5 mg/d tibolone, but no incidence was given (Genazzani *et al.*, 1991; Lindsay *et al.*, 1980). In 1994 Rymer et al. (Rymer *et al.*, 1994) reported for the first time the incidence of bleeding during a 2 year non-randomised study in one hundred early postmenopausal women. They found a 20% incidence in the tibolone group compared to 9,4% in the group which did not receive medication. Furthermore women who bled in the tibolone group were younger at menopause, were recently menopausal and may have remaining endogenous oestrogen production. Ginsburg et al. (Ginsburg *et al.*, 1995) published their experience with long-term use of tibolone and calculated an incidence of vaginal bleeding of 12,6%. The majority of women in this report were 5 years or more after menopause, suggesting that the incidence of vaginal bleeding may be less in late postmenopausal women.

The present randomised placebo-controlled study is the first reporting on the incidence of bleeding using two doses of tibolone in early postmenopausal women. Vaginal bleeding occurred in 22% of the placebo group, and in 44% (1,25 mg/d) and 51% (2,5 mg/d) in tibolone users which is substantially higher than the incidence reported by Rymer (Rymer *et al.*, 1994) involving a similar population of early postmenopausal women. Taking the incidence of the placebo-group into account, like Rymer, we found that the percentage of bleeding is about twice as high in tibolone group. We found that women who bled in the placebo group were younger, had their menopause at an younger age and may had endogenous oestrogen production. In contrast to Rymer (Rymer *et al.*, 1994), in the tibolone group we could not determine factors that are associated with bleeding. With respect to the incidence of bleeders, the 1,25 mg/d tibolone group was comparable to 2,5 mg/d tibolone group. However, looking at bleeding episodes, recurrence of bleeding was significantly less in the 1,25 mg tibolone group compared to the 2,5 mg/d tibolone group. The use of 1,25 mg/d

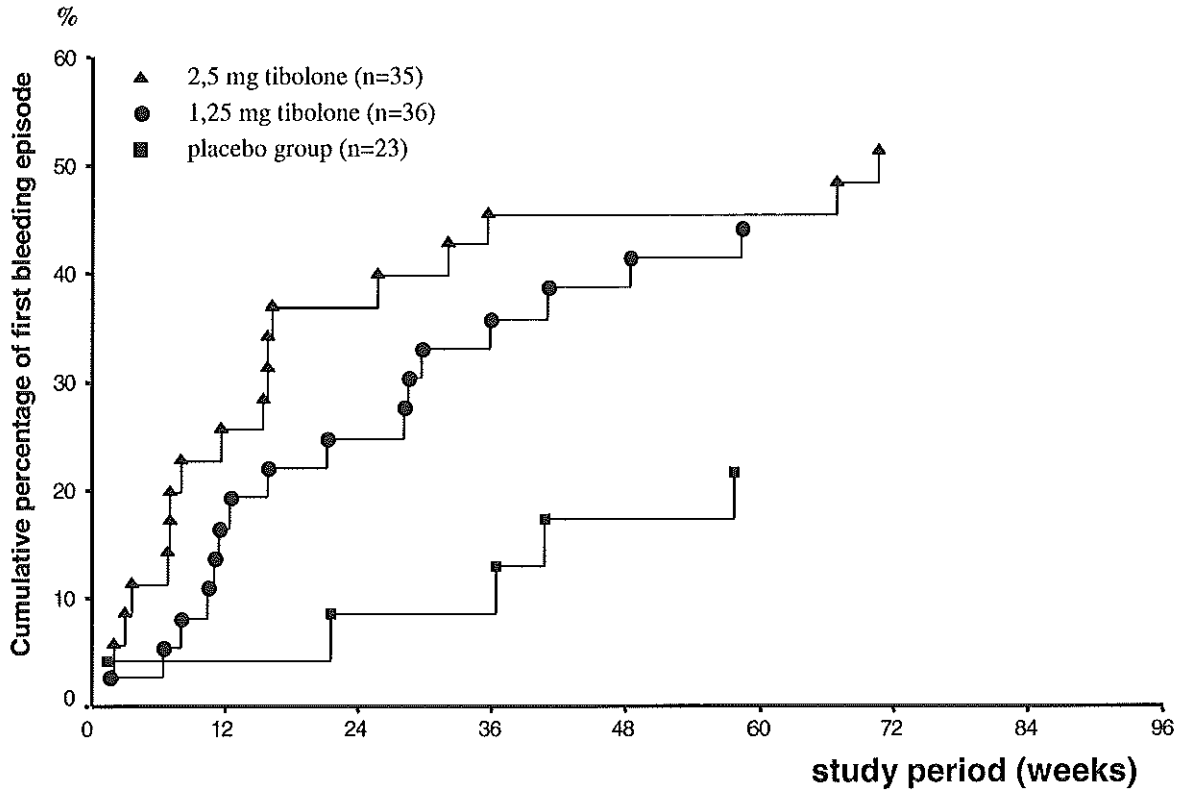

Figure 5
Cumulative percentage of first bleeding episode in early postmenopausal women during the 2 year study period, using placebo, 1.25 mg/d or 2.5 mg/day tibolone.

and 2,5 mg/d tibolone in women who had their menopause at least 10 years ago (Bjarnason *et al.*, 1996), resulted in an incidence of bleeding of, 11% and 20%, respectively. These findings are in agreement with the contention that the incidence of bleeding is less in late postmenopausal women.

Like other clinical trials (Genazzani et al., 1991; Rymer *et al.*, 1994, Ginsburg *et al.*, 1995; Bjarnason *et al.*, 1996; Habiba *et al.*, 1996) we found no stimulatory effect on the endometrium in women who bled during tibolone treatment, using Vabra endometrium sampling. This seems in agreement with present observations that no relationship exists between E2 levels and the occurrence of bleeding in tibolone users. In the present study one woman with recurrent bleeding showed uterine fibroids on vaginal ultrasound. Hysteroscopy in this patient showed no abnormalities. All other women with recurrent bleeding had normal findings on vaginal ultrasound at the end of the study. Therefore, no further diagnostic evaluation was performed.

Hysteroscopic evaluation was not performed routinely in the present study and therefore the possible role of intracavitary anomalies could not be addressed. Recently Ginsburg et al. (Ginsburg *et al.*, 1996) reported on the cause of vaginal bleeding in 47 out of 434 women using 2,5 mg/d tibolone. They found endometrial polyps in 11 cases and uterine fibroids in 7. In 3 out of 6 women with an thickened endometrium on vaginal ultrasound no histological abnormality could be found. Two women had simple hyperplasia, one had used tamoxifen for years, the other consumed large quantities of Chinese vegetables raising the possible role of phyto-oestrogens. There were two cases of carcinoma in situ, which is less than the presumed occurrence of 2-3 in 300 women (Daldeszen *et al.*, 1994). Despite full investigation, no morphological abnormality was found in over half the women who bled during tibolone. Habiba et al. found in 17 women (39%) benign intracavitary abnormalities and one case of simple hyperplasia. In addition, immunohistochemical makers for proliferation and for oestrogenic effect did not demonstrate any differences with a matched control group of women who did not bleed during tibolone treatment.

In conclusion, in the present study occasional bleeding in the first 9 months of treatment with tibolone in early postmenopausal women is approximately 2-2,5 times as likely as compared to placebo. The majority of the first bleedings occurred within 9 months of treatment. No correlation with E2 serum levels or stimulatory effect of tibolone on the endometrium was seen.

3

Tibolone and its effects on bone, a review.

3.1 Introduction

Osteoporosis is defined as a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to an increase in bone fragility with susceptibility to fracture (anonymous Am J Med; 1993). In contrast with earlier consensus definitions this definition does no longer state the presence of a non-traumatic fracture necessary for the diagnosis of osteoporosis.

The skeleton consists of two different types of bone. Cortical bone forms the shaft of long bones and most effectively resists bending forces. The internal structure of metaphyses of long bones and vertebral bodies consists of trabecular bone and is best designed to resist compressive stress (Dempster, 1992). Over their lifetimes, women lose about 35 percent of their cortical bone and 50 percent of their trabecular bone (Riggs *et al.*, 1981; Mazess, 1982). Trabecular bone, with its greater surface area, is metabolically much more active than cortical bone and thus more responsive to changes in mineral homeostasis (Parfitt, 1979; Genant *et al.*, 1982; Nilas *et al.*, 1985). From the onset of perimenopause and during initial years after menopause decremental estrogen production leads to increased bone resorption which exceeds bone formation, resulting in an accelerated loss of primarily trabecular bone. These differences in the rate and timing of cortical bone and trabecular bone loss determine the age at which specific osteoporotic fractures occur. The incidence of fracture of trabecular bone dominant sites (such as the distal radius, or vertebral bodies) starts to increase within a few years after menopause, while at sites predominantly consisting of cortical bone (for example the hip) the incidence of fractures increases after the age of 70 (Riggs *et al.*, 1986). It should be mentioned that 90% of the hip fracture are the result of a fall (Sattin *et al.*, 1990). Furthermore, several risk factors for falling have been identified (Grisso *et al.*, 1991). Prevention of falling may considerably reduce the risk for fracture in osteoporotic elderly. As

yet no, specific intervention which has been proved to be widely effective exists. Therefore, the first goal for therapy of osteoporosis should be the restoration of bone turnover to premenopausal levels.

Hormone replacement therapy (HRT) of traditional dose regimens has been shown to maintain or increase bone mass in healthy as well as in osteoporotic postmenopausal women (Ettinger *et al.*, 1987; Munk-Jensen *et al.*, 1988; Christiansen *et al.*, 1990; Stevenson *et al.*, 1990). Subsequently, numerous epidemiological and observational studies of traditional HRT dose regimens (Weiss *et al.*, 1980; Krieger *et al.*, 1982; Ettinger *et al.*, 1985; Kiel *et al.*, 1987; Naessén *et al.*, 1990; Kanis *et al.*, 1992; Cauley *et al.*, 1995) and three randomized prospective trials (Lufkin *et al.*, 1992; Komulainen *et al.*, 1998; Mosekilde *et al.*, 2000) showed a reduction in fracture risk. To maximize the preventive effect on osteoporosis, HRT should preferably be started soon after menopause and be continued for at least 7 years, but probably for life (Felson *et al.*, 1993; Schneider *et al.*, 1997). Current use of HRT in various countries ranges from 3% in Southeast Asia (Boulet *et al.*, 1994) to 34% in the United States of America (Utian *et al.*, 1994) and France (Limouzin-Lamothe, 1996). In addition, despite well-known advantages of long-term HRT, at present continuation of treatment is poor. In the Netherlands, the mean duration of use is only 7 months. Only 8% of women on HRT continue beyond 2 years (Groeneveld *et al.*, 1998). Other studies show similar results (Barlow *et al.*, 1991; Speroff *et al.*, 1991) Fear of breast cancer, and return of vaginal bleedings with the addition of progestins to protect the endometrium, are important factors in refusal or discontinuation of HRT. Therefore, avoiding or reducing bleedings could improve continuation with long-term HRT.

This review examines the evidence for the effects of tibolone (Org Od 14, Livial®) on bone. Therefore a sensitive search for published information used Medline and the words Livial, tibolone and Org Od 14. The date of the last search was January 2000. The intention was to use full journal publications or abstracts not yet published in full. Furthermore, data from a formal meta-analysis performed by Organon International (The Netherlands) on the efficacy of tibolone in prevention and treatment of osteoporosis are presented in this review. Tibolone was first introduced in the Netherlands in 1988 as treatment for vasomotor symptoms without the need for scheduled withdrawal bleedings (Kicovic *et al.*, 1982). Tibolone is a C19 synthetic steroid with a mixed hormonal profile, showing estrogenic, progestagenic, and weak androgenic properties (Vies *et al.*, 1987). Although vaginal bleeding may occur in the first months of tibolone use, the incidence of bleeding or spotting episodes during the first 6 months is lower compared to a continuous combined regimen (Hammar *et*

al., 1998). The standard available dose is 2.5 mg daily. Although lower doses (1.25 mg, 0.625 mg and 0.03 mg) are not yet clinically available, data on their effects on bone will be also discussed in the present review.

3.2 Mixed hormonal profile of tibolone

In classical in vivo bioassays the estrogenic potency of tibolone is about one-tenth that of ethinyl oestradiol, a progestagenic potency less than one-eighth that of norethisterone, and the androgenic potency is about one-fiftieth that of 17 α-methyltestosterone (Visser *et al.*, 1984). Endocrine studies in rats and rabbits also showed that tibolone had weak progestagenic estrogenic and very weak androgenic effects (Vies *et al.*, 1987). These actions are receptor mediated, as evidenced by the inhibitory effects of anti-estrogens and anti-progestogens. The effects of tibolone at the receptor level is complex, since progestogens reduce estrogen receptor levels, while estrogens increase progesterone receptor concentrations (Markiewicz *et al.*, 1994).

The complexity is increased by the fact that tibolone is rapidly converted after administration into three metabolites: a 3α(OH)-, 3β(OH) metabolite and a Δ4 isomer metabolite. These three metabolites of tibolone show different estrogenic and progestogenic properties. In vitro, these metabolites were found to exhibit different relative binding affinities to human progesterone, estrogen and androgen receptors. Tibolone bound weakly to all these receptors: progesterone receptor 1.1% (pure progestagenic agonist: Org 2058 = 100%), estrogen receptor 0.5% (estrogen = 100%), androgen receptor 2.4% (dihydrotestosterone = 100%). The Δ4 isomer only bound to the progesterone (10.4%) and the androgen receptor (29.1%). The 3α(OH) metabolite and 3β(OH) metabolite bound only to the estrogen receptor (2.5% and 1.9% respectively). Similar results were seen in Chinese hamster ovary cells containing progesterone, estrogen and androgen receptors (Kloosterboer, 1997).

Furthermore, the concentrations of metabolites differ depending on the metabolism of the target tissue. It is therefore likely that the estrogenic or progestagenic properties are different depending on the target tissue. For example, tibolone and its Δ4 isomer stimulate 17β dehydrogenase activity and glycogen accumulation in proliferative endometrium comparable to progesterone. In contrast, the 3α(OH)- and 3β (OH) metabolite increases prostaglandin output in proliferative endometrium (i.e. estrogenic effect). Furthermore, it was shown that

tibolone was extensively metabolised to its Δ4 isomer in the endometrium by 3βhydroxylase/isomerase. (Tang *et al.*, 1993). These findings suggest a complex receptor mediated as well as metabolic regulation of the activity of tibolone at the target tissue level, which probably accounts for tissue specificity in relative estrogenic/progestagenic activities of tibolone (Markiewicz *et al.*, 1990). To what extent tibolone is metabolised in other tissues is unknown. However, clinical effects clearly demonstrate estrogenic, progestogenic and androgenic properties in different tissues. For instance, tibolone has estrogenic effects on bone and in the brain judged by its effects on bone mineral density (BMD) and vasomotor symptoms. In contrast, the progestogenic effect predominates in the endometrium. As far as the breast is concerned, all preclinical data suggest that tibolone exhibits no estrogenicity in breast tissue (Kloosterboer *et al.*, 1994; Pasqualini *et al.*, 1997; Chetrite *et al.*, 1997; Gompel *et al.*, 1997), which is clinically confirmed by lack of effect on breast density assessed by mammography (Erel *et al.*, 1998) as well as the significantly lower incidence of mastalgia in postmenopausal women using tibolone compared to conventional HRT (Mol-Arts *et al.*, 1999).

3.3 Studies in animals

The effects of tibolone on bone mass, bone metabolism and bone quality have been studied extensively in rats. Recently, the effect of varying doses of tibolone administrated once or in two portions on trabecular BMD and biochemical markers of bone metabolism in 3-months-old ovariectomised rats were compared to varying doses of EE (Ederveen *et al.*, 1999). Trabecular BMD of the distal metaphysis of the femur was measured with peripheral Quantitative Computed Tomography or by quantitative x-ray densitometry on defatted bone. It was concluded that all doses of tibolone and EE significantly prevent trabecular bone loss. In contrast to EE, the effect of tibolone was independent from the frequency of dosing. This may be due to differences in plasma half-life of tibolone versus EE. Both tibolone and EE showed inhibition of bone resorption judged by the reduction of urinary deoxypyridinoline/creatinine excretion. Osteocalcin was dose dependently reduced by tibolone as well as EE. There was a significant increase in serum alkaline phosphatase in the tibolone treatment group, indicating that tibolone may reduce bone resorption more than bone formation. However, since the source of alkaline phosphatase is unknown (bone, liver or intestinal isoenzyme) no firm conclusions could be drawn from this observation. Unlike

androgens, tibolone significantly reduced length growth of the femur, equalling the effect of EE. Furthermore, they found that in the rat, tibolone and EE decreased body weight, which indicates that tibolone behaves also in this respect as an estrogenic compound, since androgens increase body weight. In addition, a dose dependent prevention of ovariectomy-induced bone loss of the appendicular as well as axial skeleton was seen in 3-months-old ovariectomized rats, treated with varying doses of tibolone. This was comparable with the effect seen in rats treated with 0.024 mg of EE (Ederveen *et al.*, 1996).

Since rats of 3 months of age show skeletal growth in addition to remodelling, the effect of ovariectomy-induced bone loss was also tested in 20 months old rats treated for 16 weeks. As in young rats, tibolone prevented bone loss in the axial and peripheral skeleton of these senescent, skeletal mature ovariectomized rats (Ederveen *et al.*, 1993).

Bending strength of the femoral diaphysis and compression strength of the vertebral body was tested in this rat model to evaluate the effect upon bone quality upon 16 months treatment (Ederveen *et al.*, 1996). Tibolone 1 mg and EE 0,024 mg resulted in a 10% increase in bending strength of the femoral diaphysis compared to placebo-treated ovariectomized rats. Compression strength of the vertebral body was increased by 60% and 23% for tibolone (0.5mg/d) and EE (0.024 mg/d), respectively. These data indicate that tibolone is as effective as EE in maintaining strength of the cortical bone. Furthermore tibolone was significantly more effective in maintaining trabecular bone strength. In mature ovariectomized rats on low calcium diet treated with tibolone similar results were seen in respect to femoral and vertebral BMD, as well as compression strength of the lumbar vertebra (Kasugai *et al.*, 1998). In mature ovariectomized rats on low calcium diet with established osteopenia, tibolone, EE and 1 alpha(OH) D3 showed increase in trabecular as well as cortical BMD, resulting in an increased compressive strength of the vertebral body in the treated group compared to the control group. In contrast to 1alpha(OH)D3, the effect of tibolone was similar to EE, showing a decrease in bone turnover as apparent from biochemical makers of bone metabolism (osteocalcin, bone specific alkaline phosphatase, urinary levels of deoxypyridinoline and pyridinoline). Accordingly histomorphometric indices like mineralising surface, bone formation rate, osteoclast surface and osteoclast numbers were decreased by tibolone and EE (Yoshitake *et al.*, 1999).

Interestingly, the protective effects of tibolone and 17β estradiol on BMD and bone metabolism in ovariectomized rats were blocked by a pure anti estrogen (ICI 164.384). The anti-androgen (flutamide) however could not block the protective effects of tibolone treatment on BMD and markers of bone metabolism. The fact that the anti-estrogen blocks the

protective effects of tibolone (and 17β estradiol) on bone confirms an action through the estrogen receptor (Ederveen *et al.*, 1997).

In summary, tibolone has been tested exclusively in the rat model. It has been shown that tibolone can prevent axial and appendicular bone loss induced by ovariectomy and/or low calcium diet in young and mature rats. In addition, tibolone increases trabecular and cortical BMD in rats with established osteopenia. Similar to estrogens this effect is due to reduction in bone turnover and tibolone seems to be as effective as estrogens. The protective effect on bone can be blocked by anti-estrogens indicating that the effect is estrogen-receptor mediated. Furthermore, tibolone acts as an estrogen rather than androgen in the rat model. Studies on bone quality show that the positive effect of tibolone is comparable to that of estrogens. In respect to compressive strength of the vertebra tibolone even may be more effective.

3.4 Studies in postmenopausal women

At present, the best available secondary endpoint to estimate fracture risk the assessment of bone mass (Wasnich *et al.*, 1985; Gärdsell *et al.*, 1993;Cummings *et al.*, 1995). Therefore, most clinical studies evaluating the efficacy of medications for prevention or treatment of osteoporosis have focused on bone mass.

Various techniques have been developed for the assessment of bone mass. At first single photon absorptiometry and X-ray micro-densitometry were used to determine BMD of the appendicular skeleton (radius, metacarpal and phalanx). Later dual photon absorptiometry made it possible to determine BMD of the axial skeleton (lumber spine and hip). Dual energy X-ray absorptiometry (DEXA) is the modern upgraded version of dual photon absorptiometry, it is the current "gold standard" method for assessment of BMD (Bracker *et al.*, 1998). None of these measurement techniques can differentiate between cortical and trabecular bone. Single energy Computed Tomography is the only method that can estimate BMD separately in the trabecular and cortical compartment (Sandor *et al.*, 1985), which, as mentioned above, differ in metabolic activity (Parfitt 1979). Correlation between different techniques is modest (between 0.6-0.7). This is due to technical differences between techniques and to differences in measurement sites, with different compositions (ratio cortical/trabecular bone). However, all of the above mentioned techniques have predictive power for future osteoporotic fractures (Kuijk *et al.*, 1994). Results of BMD measurement can be expressed as T-scores, defined as the difference of the individual bone mass, compared to

the mean peak bone mass of a young reference population expressed in standard deviations. For each standard deviation reduction in BMD there is about a two-fold increased risk of fracture (Marshall *et al.*, 1996). For clinical trials it is advised to measure BMD at least at two different sites. In addition, priority should be given to those sites of biological relevance (such as spine and hip) (Kuijk *et al.*, 1994).

Estimation of bone turnover is another accepted means for evaluation of medications for prevention or treatment of osteoporosis. Biochemical markers of bone turnover can be divided in markers of bone formation (serum osteocalcin or serum (bone specific) alkaline phosphatase) and markers of bone resorption (urinary hydroxyproline, urinary calcium corrected for urinary creatinine and Cross Laps™).

In 1980 Lindsay and collegues (Lindsay *et al.*, 1980) were the first to report the effects of tibolone 2.5 mg on postmenopausal BMD of the appendicular skeleton. This randomised, double-blind placebo-controlled study showed that tibolone 2.5 mg/d prevented postmenopausal metacarpal bone loss, whereas the placebo-group lost 3.6% annually (Table 1). Twenty-four of these patients (ten oophorectomized and 14 non-oophorectomized) who used tibolone, received Tc99m hydroxyethylidene diphosphonate. The 24-hrs whole body retention of Tc99m hydroxyethylidene diphosphonate (a sensitive measure of skeletal metabolism), was compared to 23 women receiving a placebo. Tibolone was found to have a powerful suppressive effect on skeletal metabolism in both oophorectomized and non-oophorectomized women when compared with control subjects (Fogelman *et al.*,1981). The degree of suppression was similar to that found with estrogen therapy (Fogelman *et al.*, 1980).

The first report on the effects of tibolone on the lumbar spine was published in 1994 by Rymer and colleagues (Rymer *et al.*, 1994). This open controlled study versus none use of HRT showed increase of spinal and femoral BMD in postmenopausal women who used tibolone 2.5 mg/d for 2 yrs of 2.5% and 3.5%, respectively. In the group of women who did not use HRT there was a decrease of 2.9% in spinal and 3.7% in femoral BMD during the study period (Table 1). Biochemical markers of bone turnover (serum alkaline phosphatase, osteocalcin, and urinary OH proline and Calcium excretion) indicated a suppression of bone turnover (Table 2). These data suggest that tibolone behaves similarly to estrogen with regard to its effect on biochemistry and bone density measurements. Lyritis co-workers (Lyritis *et al.*, 1995) studied the effect of 2.5mg tibolone on appendicular BMD, in women immediately after a hysterectomy and bilateral oophorectomy because of uterine fibroids and uterine bleeding (Table 1). In this open randomized trial 15 women were treated with tibolone 2.5 mg

Table 1 Effects of tibolone on bone mineral density in studies for prevention of postmenopausal osteoporosis.

Reference	medication	subjects	subject number*	yrs since menopause	design	duration	technique	site	therapy	placebo
Lindsay et al., 1980	2,5mg	pmp ovx	33/30	0,5-3	db-bl-pc	2 yrs	SPA	metacarpal	no change[#]	-3.6%
Rymer et al., 1994	2,5mg	pmp	46/45	0,5-3	controlled	2 yrs	DPA	hip spine L2-4	+3.5% +2.5%	-3.7% -2.9%
Lyritis et al., 1995	2,5mg 1g Ca all	ovx	15/10	-	randomized controlled	1 yr	SPA	distal radius proximal radius	no change no change	-12.4% -15.2%
Berning et al., 1996	2,5mg	pmp	35/23	1-3	db-bl-pc	2 yrs	MDM seQCT	phalanx spine L2-4	+5.5% +9.1%	no change -6.4%
	1,25mg		36/23				- -	phalanx spine L2-4	+3.1% +4.0%	- -
Bjarnason et al., 1996	2,5mg	pmp	28/13	>10	db-bl-pc	2 yrs	SPA DEXA	distal radius spine L2-4	+1.9% +5.1%	-2.1% no change
	1,25mg		29/13				- -	distal radius spine L2-4	+2.2% +5.9%	- -
Gallagher et al., 1999	2,5mg 500mg Ca all	pmp	total of 770	? mean age 52	db-bl-pc	2yrs	DEXA	spine study I/II[1] hip study I/II	2.6%[3]/2.6%[3] 0.7%[3]/1.3%[3]	-1.6%/-3.0% -2.0%/-3.2%
	1,25mg 500mg Ca all						-	spine study I/II hip study I/II	2.1%[3]/1.8%[3] 0.4%[2]/0.6%[3]	- -
	0,625mg 500mg Ca all						-	spine study I/II hip study I/II	0.7%[2]/1.5%[3] 0.8%[3]/-1.4%	- -
	0,3mg 500mg Ca all						-	spine study I/II hip study I/II	-0.6%/-0.2%[3] 0.5%[2]/-1.0%[1]	- -
Breadsworth et al., 1999	2,5mg	pmp	22/20	1-?	randomized controlled	2 yrs	DEXA	hip spine L2-4	no change +3.7%	-3.9% no change

*: treatment/placebo; [#] non-significant change from baseline; [1] 2 identical independent studies; [1]P<0.05; [2]P<0.01; [3]P<0.001, treatment versus placebo. Pmp, postmenopausal; ovx, oophorectomy; db-bl-pc, double-blind placebo-controlled; SPA, single Photon Absorptiometry; DPA, dual Photon Absorptiometry; seQCT, single energy Quantitative Computed Tomography; DEXA, Dual Energy X-ray Absorptiometry.

Table 2 Effects of tibolone on bone markers in studies for prevention of postmenopausal osteoporosis.

Reference	medication	subjects	subject number*	yrs since menopause	design	duration	bone marker	therapy	placebo
Lindsay et al., 1980	2,5mg	pmp ovx	33/30	0,5-3	db-bl-pc	2 yrs	Ca/Cr	↓	no change
							OH-P/Cr	↓	no change
							Alk Phosph	no change	no change
Rymer et al., 1994	2,5mg	pmp	46/45	0,5-3	controlled	2 yrs	Ca/Cr	↓	no change
							OH-P/Cr	↓	no change
							Alk Phosph	↓	no change
							Osteocalcin	↓	↑
Lyritis et al., 1995	2,5mg 1g Ca all	ovx	15/10	-	randomized controlled	1 yr	OH-P/Cr	↓	↑
							osteocalcin	—	↑
Berning et al., 1996	2,5mg	pmp	35/23	1-3	db-bl-pc	2 yrs	Ca/Cr	↓	↓
							OH-P/Cr	↓	no change
							Alk Phosph	↓	no change
	1,25mg		36/23				Ca/Cr	↓	↓
							OH-P/C	↓	no change
							Alk Phosph	↓	no change
Bjarnason et al., 1996	2,5mg	pmp	28/13	>10	db-bl-pc	2 yrs	OH-P/Cr.	no change	↑
							CrossLaps	↓	no change
							Osteocalcin	↓	↑
	1,25mg		29/13				OH-P/Cr.	↓	↑
							CrossLaps	↓	no change
							Osteocalcin	↓	↑

*: treatment/placebo

Pmp, postmenopausal; ovx, oophorectomy; db-bl-pc, double-blind placebo-controlled; Ca/Cr, urinary calcium creatinin ratio; OH-P/Cr, urinary hydroxyprolonin creatinin ratio; Alk Phosph, serum alkaline phosphatase.

↓: significant decrease compared to baseline

↑: significant increase compared to baseline

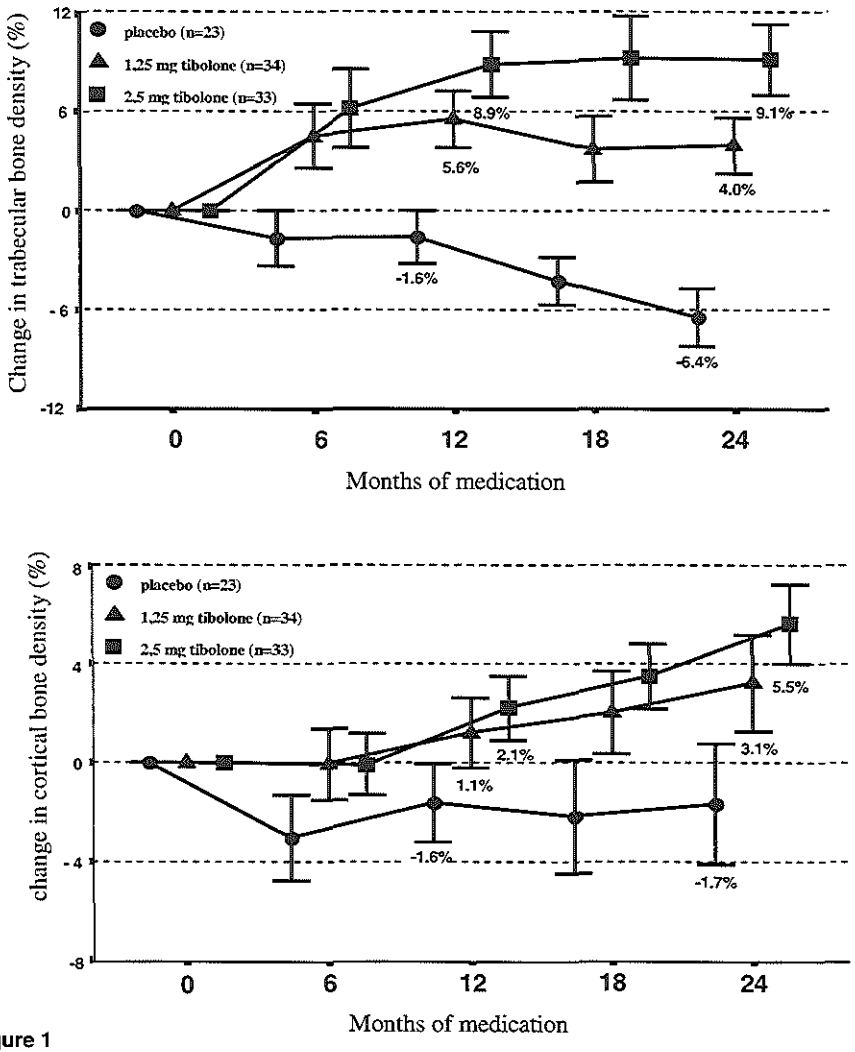

Figure 1
Percent change from baseline in trabecular bone density of the lumbar spine measured with single energy Computed tomography (mean and 95% confidence interval) and cortical bone density of the phalanx measured with X-ray microdensitometry (mean and 95% confidence interval), in early postmenopausal women treated with placebo, 1,25 mg/d or 2,5 mg/d tibolone for 24 months.

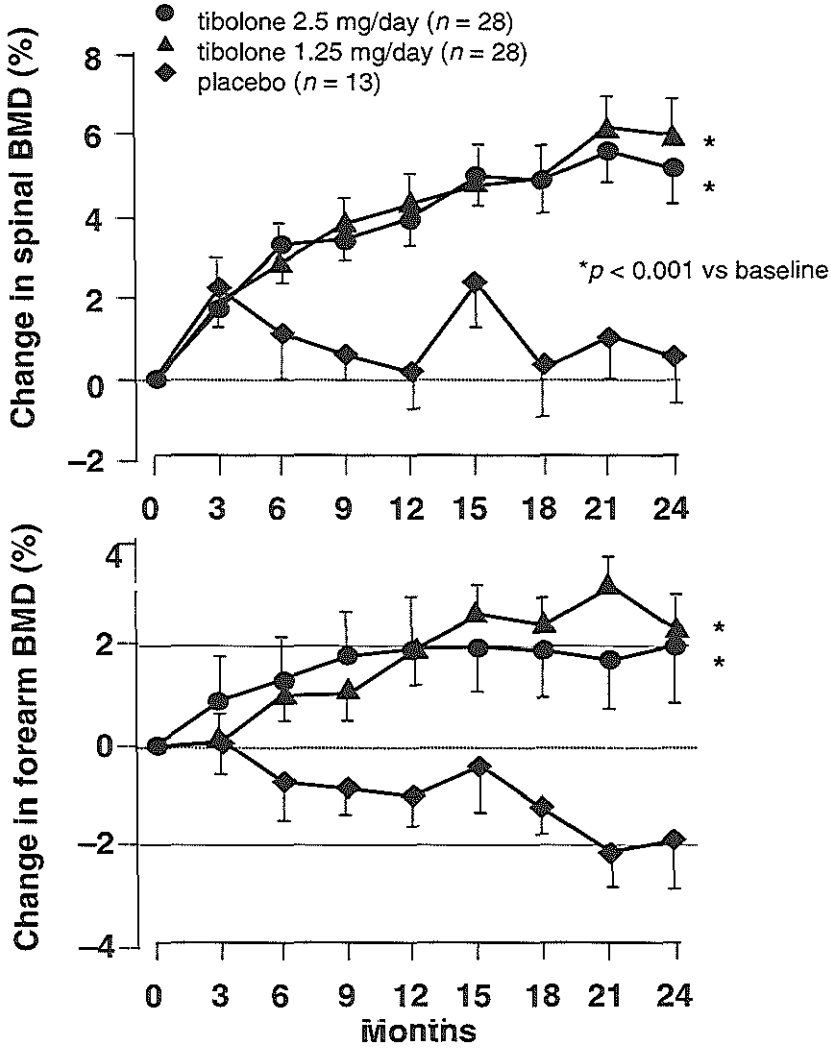

Figure 2
Densitometric measurements at 3-month intervals, expressed as percentages of initial values (mean ± SEM), 2,5 mg tibolone; 1,25 mg tibolone; placebo. BMD_{spine}, BMD of the spine (L_2—L_4)); BMD_{arm}, BMD of the distal forearm.

and calcium 1 g or calcium alone. Women treated with calcium alone had a decrease of BMD in the distal radius (12.4%) and radial shaft (15.2%).In contrast, BMD of the distal radius and radial shaft was unchanged in women treated with tibolone and calcium. Furthermore there was a decrease in urinary OH proline/creatinine ratio and serum osteocalcin levels were unchanged in the tibolone group indicating a decrease in bone turnover rate (Table 2).

Recently two randomized double-blind placebo-controlled 2-year studies with very similar study design evaluated the effect of two doses (1.25mg/d and 2.5 mg/d) of tibolone on the appendicular and axial skeleton in early (Berning *et al.*, 1996) and late (Bjarnason *et al.*, 1996) postmenopausal women (Table 1). In women 1-3 years after menopause there was a dose related increase in spinal trabecular BMD measured with single energy Quantitative Computed Tomography as well as cortical phalangeal BMD measured with Micro Densitometry (Berning *et al.*, 1996). As shown in Figure 1, spinal BMD showed an increase in the first year of the study with a plateau phase in the second study year. In contrast, the phalangeal BMD showed, after 6months, a more linear increase throughout the rest of the study period. In late postmenopausal women, who were at least 10 years after their natural menopause, the effect of tibolone 1.25 mg and 2.5 mg on BMD in the spine and distal forearm were studied in a randomized, double-blind, placebo-controlled study (Bjarnason *et al.*, 1996). Data from this study are shown in Figure 2. Both doses of tibolone showed a similar increase in BMD of the spine and distal forearm. This suggests that in contrast to early postmenopausal women, 1.25 mg tibolone is as effective as 2.5 mg in late postmenopausal women. The reason for this is unknown. As judged from the clinical observation of an increased incidence of estrogenic side-effects (such as breast tenderness) in late-postmenopausal women using HRT, it could be speculated that women long-deprived of estrogens are more sensitive to its effects. Nevertheless, evaluation of a lower dose could be interesting in this age group. In both studies, the effect of tibolone on bone turnover in early and late postmenopausal women showed a decrease in markers of bone turnover compared, to the placebo-group (Table 2).

As 1.25 mg tibolone was also effective in preventing postmenopausal bone loss, it is of interest to determine the minimum effective dose of tibolone in this regard. Recently, preliminary data from two large, identical, multi-center, randomized, placebo-controlled, studies to evaluate the efficacy and minimum effective dose of tibolone in the prevention of postmenopausal bone loss be came available (Gallagher *et al.*, 1999). A total of 770 early postmenopausal women were

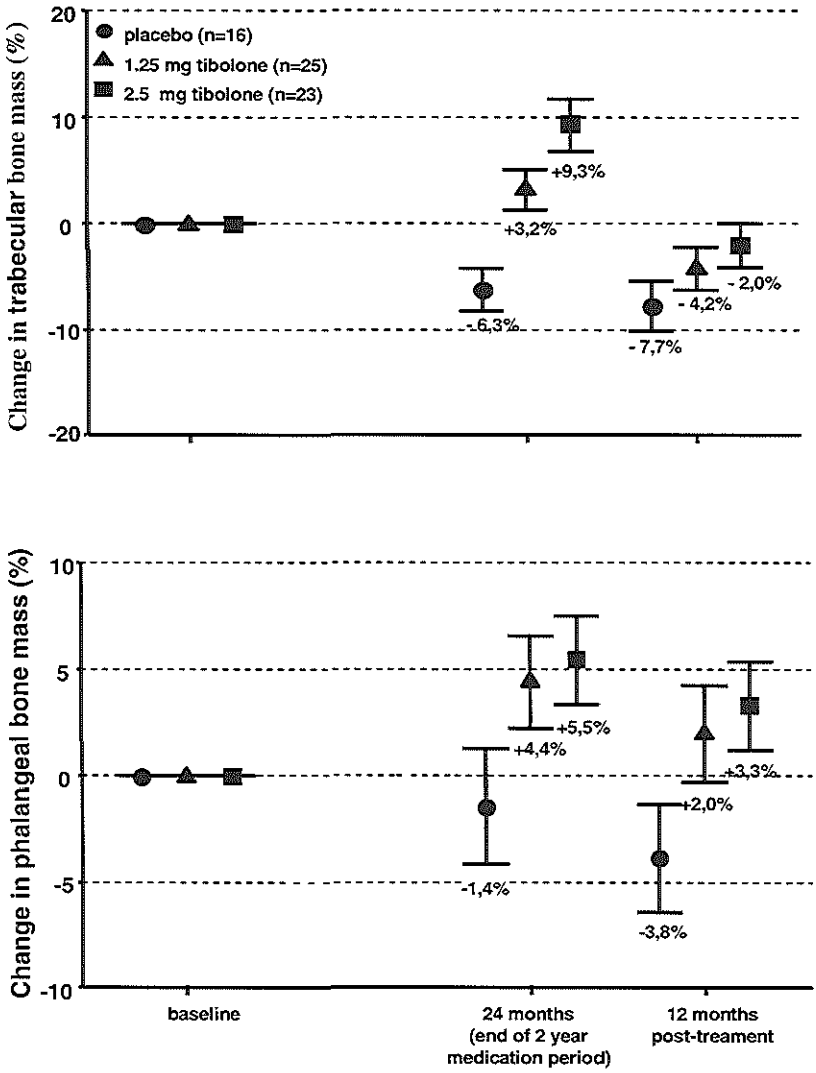

Figure 3

Percent change as compared to baseline in trabecular bone density of the lumbar spine (mean and 95% confidence interval) (upper panel) and phalangeal bone density (mean and 95% confidence interval) (lower panel) in 64 healthy early postmenopausal women having used placebo, 1.25 or 2.5 mg/day tibolone for 2 years (24 months), followed for 1 additional year after discontinuation of medication (36 months).

randomized to four tibolone treatment groups (0,3 mg/d, 0.625 mg/d, 1,25 mg/d and 2,5 mg/d) and a placebo group. Published data on BMD of both studies are also presented in Table 1. All doses tested were statistically significantly effective in preventing bone loss compared to placebo. However, the 1.25 mg/d and 2.5 mg/d doses gave more consistent results with more positive changes (increase of BMD above baseline) at both sites in both studies. The dose of 0.625 mg/d appears marginally effective. The preferred dose of tibolone for the indication prevention of postmenopausal bone loss seems to be 1.25 mg/d.

Only one study has evaluated the effect of cessation of 2 years' treatment with tibolone on trabecular and cortical BMD (Berning *et al.*, 1999). Sixty-four out of 84 women who completed the 2-year treatment period evaluating the effect of two doses of tibolone on early postmenopausal bone loss (Berning *et al.*, 1996) took part in this follow-up study. Interestingly, there was an increased loss of spinal (trabecular) BMD but not phalangeal (cortical) BMD as compared to the placebo group in one year after cessation of a 2 yrs of tibolone treatment (Figure 3). This observation suggests that metabolically active trabecular bone is lost more rapidly compared to the metabolic less active cortical bone. Although this follow-up period is short it seems that tibolone like estrogens (Felson *et al.*, 1993; Schneider *et al.*, 1997) should be continued into late life in order to maintain bone density.

3.5 Studies in women with established osteoporosis

In 1991, the first report (Geusens *et al.*, 1991) of the effect of tibolone on spinal and radial BMD in women with established osteoporosis was published by Geusens and colleagues (Table 3). Radial BMD did not change significantly in either group. A non-linear increase of 8% in 2 years was seen in spinal BMD. The placebo-group lost 2% annually and, probably due to small numbers of patients treated, there was no statistically significant reduction in fracture risk. More recently, two double-blind, placebo-controlled studies of women with established osteoporosis (Pavlov *et al.*, 1996; Studd *et al.*, 1996), with and without fractures, clearly showed significant increases of BMD in spine and hip compared to the placebo group (Table 3). These studies did not demonstrate the non-linear increase seen by Geusens and colleagues (Geusens *et al.*, 1991) and reduction in subsequent fractures was not assessed.

Table 3 Effects of tibolone on bone mineral density and markers of bone metabolism, in studies for treatment of postmenopausal osteoporosis.

Reference	medication	concurrent treatment	subject number*	duration	design	technique	site/type	therapy	placebo	Fracture risk
Geusens et al., 1990	2,5mg	-	14/17	2 yrs	db-bl-pc	SPA/DPA	distal radius	no change	no change	NS
							proximalradius	no change	no change	
							spine L2-4/	+8.0%	-4.0%	
						bone marker	Ca/Cr	no change	no change	
							OH-P/Cr	no change	no change	
							Alk Phosph	no change	no change	
Pavlov et al., 1996	2,5mg	-	63/43	2 yrs	db-bl-pc	DEXA	hip	+7.2%	no change	not done
							spine L2-4/	+2.6%	no change	
						bone marker	osteocalcin	↓	no change	
Studd et al., 1996	2,5mg	Ca 1g	31/36	2 yrs	db-bl-pc	DEXA	hip	+2.7%	+1.4%	not done
							spine L2-4/	+6.9%	+2.7%	
						bone marker	Ca/Cr	↓	no change	
							OH-P/Cr	↓	no change	
							Alk Phosph	↓	no change	

*: treatment/placebo

Db-bl-pc, double blind placebo controlled; Ca, elementary calcium; N.S., not significant; SPA, single Photon Absorptiometry; DPA, dual Photon Absorptiometry; DEXA: Dual Energy X-ray Absorptiometry; Ca/Cr, urinary calcium creatinin ratio; OH-P/Cr, urinary hydroxyprolonin creatinin ratio; Alk Phosph, serum alkaline phosphatase..

↓: significant decrease compared to baseline.

3.6 Comparative studies between tibolone and conventional HRT regimens

Comparison of placebo-controlled trials using estrogens is hampered by differences in treatment regimens (combination with progestogens and/or calcium supplements), types of estrogens used, routes of administration and applied methods for bone mass measurement. Earlier studies using dual photon absorptiometry or DEXA for estimation of spinal bone mass in postmenopausal women using various HRT regimens showed changes between 0 and +15.9% (Riis *et al.*, 1987; Munk-Jensen *et al.*, 1988; Stevenson *et al.*, 1990; Ribot *et al.*, 1990; Marslew *et al.*, 1992; Eiken *et al.*, 1996; Bush (PEPI trial) *et al.*, 1996; Hart *et al.*, 1998; Recker *et al.*, 1999). Studies with tibolone, using the same techniques showed comparable changes (between +2.5 and +8.0%) in spinal BMD (Geussens *et al.*, 1990; Rymer *et al.*, 1994; Bjarnason *et al.*, 1996, Pavlov *et al.*, 1996; Studd *et al.*, 1996; Breadsworth *et al.*, 1999).

Three open, non-randomized comparative head to head studies between tibolone 2.5 mg/d and conventional HRT regimens have been carried out. Stephán and Schreiber (Stephán *et al.*, 1995) compared tibolone 2.5 mg/d with transdermal estrogens 50µg/d in 38 women after hysterectomy and bilateral oophorectomy. Therapy was started 3 months after surgery; 9 patients were not treated. BMD of the lumbar spine was measured with DEXA. Bone loss of the lumbar spine was 7% in the first year. Women using tibolone showed a significant increase in spinal BMD with a return to pre-surgery values after 6 months of treatment, whereas women using transdermal, estradiol maintained their spinal BMD. Prelevic and colleagues (Prelevic *et al.*, 1996) compared tibolone to conjugated equine estrogens with sequential norgestrel for 12 days and transdermal 17β oestradiol in 82 postmenopausal women with an mean age of 64 years. Measurement of spinal and femoral BMD was performed using DEXA. Women taking tibolone showed a significant increase in spinal BMD of 10.3% over 3 years. In contrast, women using conjugated equine estrogens or transdermal estrogen showed a non-significant increase over 3 years in spinal BMD of 8.1% and 6.6% respectively. There was no significant change of BMD of the hip over the 3 yrs, irrespective of the therapy taken, although there was a tendency towards a progressive increase in women using tibolone.

Lippuner and co-workers (Lippuner *et al.*, 1997) compared tibolone 2.5 mg/d with oral 17β oestradiol 2 mg/d or transdermal 17β oestradiol 50µg/d in combination with dydrogestron during 14 days. Measurement of spinal, femoral and whole body BMD was performed using DEXA. Of

the 140 early postmenopausal women, 30 chose no treatment. Over the 2 years bone preservation in spine and hip was observed in all three treatment groups, compared with controls, without significant differences among treatment regimens.

3.7 Meta-Analysis of clinical studies in postmenopausal women

To describe the overall efficacy of tibolone in the prevention and treatment of osteoporosis, a formal meta-analysis (Organon NV January 1997, unpublished) has been carried out, with respect to relative change from baseline in BMD measurements of femoral neck and lumbar spine after 2 years of treatment with 2.5 mg/d tibolone. Included were all long-term clinical trials using dual photon absorptiometry or DEXA, in early postmenopausal (Rymer *et al.*, 1994; Lippuner *et al.*, 1997) and osteoporotic women (Geusens *et al.*, 1991; Pavlov *et al.*, 1996; Studd *et al.*, 1996). The meta-analysis results for prevention and treatment of osteoporosis trials and for both parameters are graphically displayed in figures 4 and 5. Early postmenopausal women (72 treated subjects versus 75 subjects using placebo) had an increase in femoral BMD of 4,6% (95% confidence interval: 3,0-6,2%) and an increase in spinal BMD of 5,5% (95% confidence interval: 4,4%-6,7%). Osteoporotic women (80 treated subjects versus 68 subjects using placebo) had an increase of femoral BMD of 3,9% (95% confidence interval: 2,4-5,5%) and an increase in spinal BMD of 6,3% (95% confidence interval: 3,5%-9,0%). Therefore it is concluded that tibolone is capable of increasing spinal and femoral BMD in early postmenopausal and osteoporotic women. However, prevention of fractures remains to be investigated.

3.8 Tibolone as add-back therapy

Long-term treatment with gonadotropin-releasing hormone (GnRH) agonist in case of endometriosis or uterine fibroids is complicated by a transient complete cessation of ovarian function. The estrogen deficiency results in vasomotor symptoms and increased bone loss. GnRH-agonist induced bone loss averages 1% per month during a 6-month, course (Pickersgill 1998), which is much greater than an average loss of 2-3% annually during the first years after

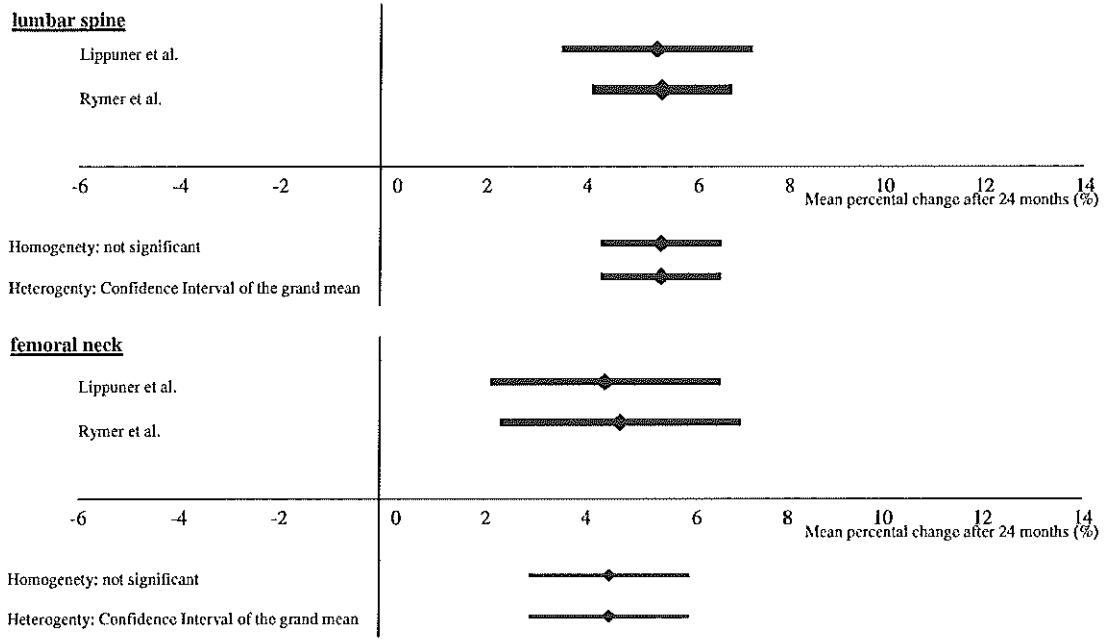

Figure 4
Meta-analysis results of percent change from baseline in bone mineral density of the lumbar spine (upper panel) and femoral neck (lower panel) in healthy early postmenopausal women treated with tibolone 2,5 mg/d for 2 years. The thickness of the lines is proportional to the weight of that study-centre in the meta-analysis.

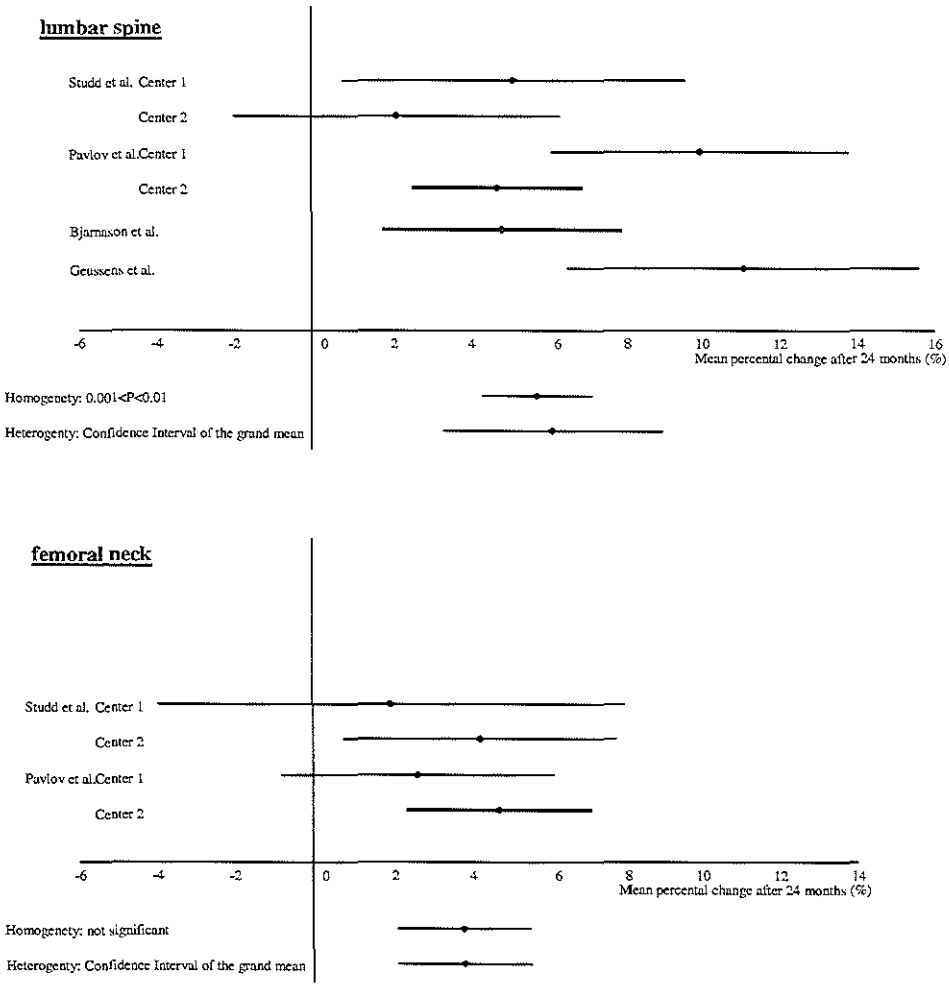

Figure 5
Meta-analysis results of percent change from baseline in bone mineral density of the lumbar spine (upper panel) and femoral neck (lower panel) in osteoporotic women treated with tibolone 2,5 mg/d for 2 years. The thickness of the lines is proportional to the weight of that study-centre in the meta-analysis.

natural menopause (Riggs *et al.*, 1986). This limits the duration for which treatment with a GnRH-agonist can be given. However, many of the conditions for which GnRH-agonists are indicated are chronic or recurrent in nature. The mixed hormonal profile of tibolone seems suitable to counteract these unwanted effects of GnRH-agonist treatment. Such concomitant therapy is known as add-back therapy.

A double-blind, placebo-controlled trial (Lindsay *et al.*, 1996) carried out in 29 women using triptorelin (Decapeptyl®) for 6 months showed a decrease in spinal BMD measured with DEXA of 5.1% in women using a placebo versus a 1.1% decrease in women using additional tibolone 2.5 mg/d. Bone loss in the femoral neck was not significantly different from the baseline values in both groups, reflecting the higher levels of cortical bone at this skeletal site. Furthermore, tibolone 2.5 mg significantly reduced vasomotor symptoms without affecting the efficacy of triptorelin on the treatment of endometriosis. Another randomized placebo-controlled trial (Taskin *et al.*, 1997) found, like Lindsay and colleagues, a reduction in urinary calcium/creatinine ratios indicating decrease in bone resorption with tibolone during GnRH-agonist treatment.

Although most studies indicate that bone mass is partially or completely restored after cessation of GnRH-agonist therapy it could be argued that adverse changes in cancellous bone structure may occur during bone loss. This could lead to permanently reduced bone strength, even when the total bone mass has been restored. Histomorphometric studies in cancellous bone (Compston *et al.*, 1995) from a subset of patients from the study of Lindsay (Lindsay *et al.*, 1996), showed reductions in connectivity of cancellous bone structure during GnRH-agonist treatment. No such changes were seen when tibolone was used as add back therapy. Since these adverse structural changes are probably irreversible, the importance of preventing bone loss during GnRH-agonist treatment is emphasised. Histomorphometric studies of cortical bone (Bell *et al.*, 1997) showed, like cancellous bone, an increased bone turnover during GnRH-agonist treatment, which can be counteracted by tibolone 2.5 mg.

3.9 Conclusion

From data in rats it can be concluded that tibolone is as effective as estrogens in preventing oophorectomy-induced bone loss of the appendicular and peripheral skeleton. Even in osteopenic

rats, an increase in BMD is observed. In the rat, administration of tibolone results in an increased femoral bending strength, similar to the effect with estrogens. Importantly, the beneficial effect of tibolone on bone metabolism is estrogen-receptor mediated since only anti-estrogens block the effect of tibolone on bone. Furthermore, in the rat tibolone acts as an estrogen-agonist rather than an androgen-agonist.

In humans, clinical trials have clearly shown that loss of bone in the spine and proximal hip can be prevented with tibolone 2.5 mg/d in early- and late postmenopausal women. In addition, a dose of 1.25 mg/d seems to be effective as well, especially in late-postmenopausal women. In two large randomized placebo-controlled, dose-finding, studies 1.25 mg/d of tibolone seems to be the preferred dose. In accordance with data from the animal studies, clinical trials indicate that the effect of tibolone, based on measurements of makers of bone turnover, is an estrogen-like decrease in bone turnover. Furthermore, effects of tibolone, on trabecular dominant sites are greater compared to cortical bone dominated sites. In women with established osteoporosis, bone density of the axial and appendicular skeleton increases with tibolone. In comparative studies, tibolone 2.5mg/d seems as effective as conventional HRT regimens. However, it should be noted that the effects of tibolone on postmenopausal bone mass have yet not been compared with the effects of biphosphanates and raloxifene. Furthermore, studies on the magnitude of reduction in fracture risk remain to be conducted.

In premenopausal women, tibolone seems to be effective in preserving bone density in patients treated with Gonadotropin releasing hormone agonist. Histomorphometric studies in humans indicate that possible irreversible adverse changes in the structure of trabecular bone can be prevented by tibolone 2.5 mg/d. The effect of tibolone on osteoporosis resulting from other causes than postmenopausal and gonadotropin-releasing hormone agonist induced bone loss, have not been investigated.

Finally, tibolone with its mixed hormonal profile seems to have a generally favorable clinical profile. It is capable of preventing bone loss in estrogen-deprived women and, in a dose of 2.5 mg/d it alleviates postmenopausal complaints. As with other "bleed-free" HRT regimens, vaginal bleeding, especially at the beginning of treatment, does occur. However, data suggests that the frequency and severity of vaginal bleeding during tibolone treatment is less, compared with continuous combined HRT. Late-onset bleeding during tibolone treatment, as with all "bleed-free" HRT regimens, merits investigation. Regarding cardiovascular effects, it is yet unknown

whether favourable metabolic changes with tibolone such as increased fibrinolysis, unchanged coagulation and lowering of lipoprotein (a) will counterbalance the potentially negative effect of the decrease in high-density lipoprotein cholesterol. As far as the breast is concerned, all preclinical data suggest that tibolone exhibits no estrogenicity in breast tissue. However, effects of long-term use of tibolone on incidence of breast cancer or cardiovascular events are yet unknown.

4

General Discussion and Conclusions

First objective of this thesis was to evaluate the effect of tibolone on postmenopausal bone loss. We studied this issue during a 2-year placebo-controlled randomised trial in healthy women, 1-3 yrs after spontaneous menopause. In a group of 94 healthy early postmenopausal women, tibolone 2.5 mg/d significantly increased trabecular lumbar bone density measured with single energy Computed Tomography. After an increase of 9% in the first year of treatment there is a plateau phase in the second year, a pattern comparable with estrogens. With 1.25 mg/d of tibolone the increase was less pronounced (5,6%) but the same pattern of increase in trabecular bone density was seen. Cortical phalangeal bone density, measured with radiographic absorption densitometry, also showed a significant increase with both doses of tibolone, though the pattern of increase was quite different. Cortical bone density started to increase after 6 months and then showed a linear increase throughout the study-period. Therefore it is concluded that tibolone in a dose of 2.5 mg/d and 1.25 mg/d is capable of preventing early postmenopausal bone loss. Preliminary data from two large dose-finding studies confirm this observation. Furthermore these studies seem to indicate that 1.25mg/d tibolone is the preferred dose for prevention of early postmenopausal bone loss. However, one should bear in mind that 2.5mg/d and not 1.25mg/d of tibolone is effective in alleviating vasomotor symptoms. Although it is clearly shown from the present, and other studies that tibolone effectively prevents postmenopausal bone loss, data on reduction of fracture risks are lacking. In order to establish the effectiveness of tibolone in prevention and treatment of postmenopausal osteoporosis, data from a large-scale randomised clinical trial focussing on fracture risk are mandatory. Recently, such a trial has been started so data on magnitude of fracture risk reduction will become available within a few years.

The second objective was to determine the rate of bone loss after discontinuation of tibolone treatment. We addressed this issue during a one-year follow-up study in a group of

64 postmenopausal women, who completed the initial study. Like in the initial 2-year study, the pattern in change of trabecular and cortical bone density is quite different. In contrast to the decrease of cortical bone density, there was a greater decrease of trabecular bone density, compared to the placebo group. These observations suggest that there is an accelerated loss of metabolically more active trabecular bone, but not of cortical bone following cessation of 2-year tibolone intake. Although our follow-up period is short, most of the increase of trabecular bone density is lost within one year after cessation of tibolone treatment. It seems therefore that replacement therapy with tibolone like with estrogens should be continued into late life in order to maintain high bone density. In literature there is controversy about whether or not there is an accelerated loss of bone density after HRT. This is partly due to the different techniques of bone mass measurements used, as well as skeletal sites measured. Data concerning early HRT use and bone density later in live are available from the Framingham Study. Only early HRT longer than 7 years resulted in a higher bone density up to 75 years of age. Even this duration of HRT in early postmenopause may have little residual effect on bone density among women older than 75 years of age, who are at the highest risk for hip fractures. Data from a cross-sectional study also suggests that past estrogen use provides little or no long-term benefit for preservation of bone density. It is therefore that the possibility of an alternative strategy to start HRT many years after menopause and to continue treatment for the rest of woman's lifetime, in order to prevent osteoporotic fractures has been proposed. Biphosphonates are the treatment of choice in osteoporotic postmenopausal women. However, at present treatment with biphosphonates is limited to a maximum duration of 5 years. For late postmenopausal women with low bone mass, preventive treatment with a tibolone 1.25 mg/d or raloxifene seems promising.

We also reported on the incidence of vaginal bleeding in early postmenopausal women and the effect of tibolone on the endometrium during the 2-year placebo-controlled randomised trial. The incidence of vaginal bleeding our study was 51% for 2.5 mg/d tibolone and 44% for 1.25 mg/d tibolone. In contrast to the incidence of vaginal bleeding the number of bleeding episodes was dose related. In women who bled we found no stimulatory effect on the endometrium nor could we confirm the earlier suggestion that endogenous estrogen levels were related to the occurrence of vaginal bleeding during tibolone treatment. Nevertheless, as with any "bleed-free" HRT regimen, late onset bleeding or recurrent bleeding merits full investigation.

In respect to the effect of tibolone on breast tissue and cardiovascular system there is uncertainty. This is due to lack of epidemiological data or other studies capable of measuring clinical events such as myocardial infarction, arteriosclerosis or breast cancer.

Preclinically data show that tibolone has favourable effects on breast tissue. Tibolone decreases cell proliferation, stimulates cell differentiation and apoptosis. In the rat its effect with respect to prevention and treatment of DMBA induced mammary cancer is comparable to tamoxifen. Regarding cardiovascular effects, it is yet unknown whether favourable metabolic changes with tibolone such as increased fibrinolysis, unchanged coagulation and lowering of lipoprotein (a) will counterbalance the potentially negative effect of the decrease in high-density lipoprotein cholesterol. These uncertainties need to be addressed by studies powered to detect relevant risk. Perhaps the recently started large-scale fracture study will provide some answers in the near future. Furthermore, the uncertainties of tibolone treatment have to be balanced with known problems of conventional HRT. These include increased risk of breast cancer, increased risk of deep venous thrombosis and lack of compliance due to bleeding problems, especially in late postmenopausal women. Therefore tibolone can be an attractive alternative for conventional HRT in patient-specific situations.

5

References

Abdalla HI, Hart DM, Lindsay R, Hooke A. Prevention of bone mineral loss in postmenopausal women by norethisterone. *Obstet Gynecol* **1985**;66:789-792.

AinMelk Y. Comparison of two continuous combined estrogen progestogen regimens in postmenopausal women: a randomized trial. *Fertil Steril* **1996**;66: 962-968.

Al-Azzawi F, Habiba M, Akkad A Mason T. Continuous combined hormone replacement therapy compared with tibolone. *Obstet Gynecol* **1999**;93:258-264.

Alberico S, Elia A, Dal Corso L, Mandruzzato GP, Di Bonito L, Patriarca S. Diagnostic validity of the Vabra Curettage of the uterine cavity. *Eur J Gynaecol Oncol* **1986**;7:135-138.

Aloia JF, Cohn SH, Vaswani AN, Yeh JK, Yuen K, Ellis K. Risk factors for postmenopausal osteoporosis. *Am J Med* **1983**;78:95-100.

Aloia JF, Vaswani AN, Yeh JK, Ross P, Ellis K, Cohn SH. Determinants of bone mass in postmenopausal women. *Arch Intern Med* **1985**;143:1700-1704.

Aloysio D, Fabiani AG, Mauloni M, Bottiglioni F. Use of Org OD 14 for the treatment of climacteric complaints. *Maturitas* **1987**;(Suppl 1):49-65.

Andersson K, Rybo G. Levonorgestrel-releasing intrauterine device in the treatment of menorraghia. *Br J Obstet Gynaecol* **1990**;97:690-694.

Andersson K; Mattsson LA; Rybo G; Stadberg E. Intrauterine release of levonorgestrel--a new way of adding progestogen in hormone replacement therapy. *Obstet Gynecol*, **1992**;79:963-967.

Anonymous. The ladies'physical directory or a treatise of all weaknesses, indispositions and diseases peculiar to the female sex, from eleven years of age to fifty and upwards. London **1727**.

Anonymous. Consensus Development Conference: Prophylaxis and treatment of osteoporosis. *Am J Med* **1993**;94:646-650.

Anonymous. Tibolone (Livial)-a new steroid for the menopause. *Drug Ther. Bull.* **1991**;29:77-78.

Anzano MA, Peer CW, Smith JM, et al.. Chemoprevention of mammary carcinogenesis in the rat: combined use of raloxifene and 9-cis-retnoic acid. *J Natl Cancer Inst* **1996**;88:123-125.

Archer DF, Pickar JH, Bottiglioni F, for The Menopause Study Group. Bleeding patterns in postmenopausal women taking continuous combined or sequential regimens of conjugated estrogens with medroxyprogesterone acetate. *Obstet Gynecol* **1994**;83:686-692.

Baal van WM, Kooistra T, Stehouwer CDA. Cardiovascular disease risk and hormone replacement therapy (HRT): A review based on randomised, controlled studies in postmenopausal women. *Current Med Chemistry* **2000**;7:499-517.

Barlow DH, Brockie JA, Rees CMP. Study of general practice consultations and menopausal problems. *Br Med J* **1991**;302:274-276.

Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson JA, Nilsson S. Differential response of estrogen receptor α and estrogen receptor β to partial estrogen agonist/antagonists. *Mol Pharmacol* **1998**;54:105-112.

Barret-Connor E, Grady D. Hormone replacement therapy, heart disease and other considerations. *Annu Rev Public Health* **1998**;19:55-72.

Bell KL, Loveridge N, Lindsay PC, et al. Cortical remodeling following suppression of endogenous estrogen with analogs of gonadotropin releasing hormone. *J Bone Miner Res* **1997**;12:1231-1240.

Benedek-Jaszmann LJ. Long-term placebo controlled efficacy and safety study of Org OD 14 in climacteric women. *Maturitas* **1987**;(suppl1):25-33.

Beral V, for the Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. *Lancet* **1997**;350:1047-1059.

Bergkvist L. Epidemiology of hormone replacement therapy and breast cancer. *Gynecol Endocrinol* **1997**;11(Suppl.1):13-16.

Berning B, van Kuijk C, Schütte HE, Kuiper JW, Drogendijk AC, Fauser BCJM. Determinants of lumbar bone mineral density in normal weight, non-smoking women soon after menopause. A study using clinical data and quantitative computed tomography. *Bone Miner* **1993**;21:129-139.

Berning B, van Kuijk C, Kuiper JW, Coelingh Bennink HJT, Kicovic PM, Fauser BCJM. Effects of two doses of Tibolone on trabecular and cortical bone loss in early postmenopausal women: A 2-year randomized, placebo-controlled study. *Bone* **1996**;19:395-399.

Berning B, van. Kuijk C, Kuiper JW, Coelingh Bennink HJT, Fauser BCJM. Increased loss of trabecular but not cortical bone density, 1 year after discontinuation of 2 year hormone replacement therapy with Tibolone. *Maturitas* **1999**;31:151-159.

Berning B, van. Kuijk C, Coelingh Bennink HJT, Fauser BCJM. Absent correlation between vaginal bleeding and oestradiol levels or endometrial morphology during tibolone use in early postmenopausal women. *Maturitas* **2000**;35(1):81-88.

Bjarnason NH, Bjarnason K, Haarbo J, Rosenquist C, Christiansen C. Tibolone: Prevention of bone loss in late postmenopausal women. *J Clin Endocrinol Metab* **1996**;81:2419-2422.

Bjarnason NH, Bjarnason K, Haarbo J, Coelingh-Bennnik HJ, Christiansen C. Tibolone markers of cardiovascular disease. *J Clin Endocrinol Metab* **1997**;82:1752-1756.

Bjarnason NH, Haarbo J, Byralsen I, Kauffman RF, Christiansen C. Raloxifene inhibits aortic accumulation of cholesterol in ovariectomized , cholesterol-fed rabbits. *Circulation* **1997**;96:1964-1969.

Black DM, Cummings SR, Karpf DB et al.. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. *Lancet* **1996**;348:1535-1541.

Block JE, Smith R, Glüer CC, Steiger P, Ettinger B, Genant HK. Models of spinal trabecular bone loss as determined by Quantitative Computed Tomography. *J Bone Miner Res* **1989**;4:249-257.

Boereboom FTJ, Raymakers JA, Groot de RRM, Duursma SA. Epidemiology of hip fractures in the Netherlands: women compared to men. *Osteoporosis Int* **1992**;2:279-284.

Boerrigter PJ, Weijer van de PHM, Baak JPA, Fox H, Haspels AA, Kenemans P. Endometrial response in estrogen replacement therapy quarterly combined with a progestogen. *Maturitas* **1996**;24:63-71.

Botsis D, Kassanos D, Kalogirou D, Antoniou G, Vitoratos N, Karakitsos P. Vaginal ultrasound of the endometrium in postmenopausal women with symptoms of urogenital atrophy on low dose estrogen or tibolone treatment: a comparison. *Maturitas* **1997**;26:57-62.

Boulet MJ, Oddens BJ, Lehert P, Vemer HM, Visser A. Climacteric and menopause in seven southeast Asian countries. *Maturitas* **1994**;19:157-176.

Boyd NF, Byng JW, Jong RA, et al.. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening study. *J Natl Cancer Inst* **1995**;87:670-675.

Bracker MD, Watts NB. How to get the most out of bone densitometry. *Postgrad Med* **1998**;104:77-86.

Breadsworth SA, Kearney CE, Purdie DW. Prevention of postmenopausal bone loss at lumbar spine and upper femur with tibolone: a two-year randomised controlled trial. *Br J Obstet Gynaecol* **1999**;106:678-683.

Brenner PF. The Menopausal Syndrome. *Obstet Gynecol* **1988**;72:6S-11S.

Brincat M, Moniz CJ, Studd JWW et al.. Long-term effects of the menopause and sex hormones on skin thickness. *Br J Ostet Gynaecol* 1985;92:256-259.

Brzozowski AM, Pike AC, Dauter Z et al.. Molecular basis of agonism and antagonism in the estrogen receptor. *Nature* **1997**;389:753-758.

Bungay GT, Vessey MP, McPherson CK. Study of symptoms in middle-life with special reference to the menopause. *Br Med J* **1980**;2:181-183.

Burger H, Hailes J, Nelson j, Menelaus M. Effects of combined implants of oestradiol and testosterone on libido in postmenopausal women. *Lancet* **1987**;294:936-937.

Bush TL, Wells HB, James MK (PEPI Trial), et al. Effects of Hormone Replacement Therapy on Bone Mineral Density. *JAMA* **1996**;276:1389-1396.

Butenandt A. Untersuchungen über das weibliche sexualhormon. Darstellung und eigenschaften der kristallisierter progynons. *Deutsh Med Wschr* **1929**:55:2171.

Canalis E, Centrella M, Burch W, McCarthy T. Insulin-like growth factor I mediates selective anabolic effects of parathyroid hormone in bone cultures. *J Clin Invest* **1989**;83:60-65.

Caraceni MP, Trevisan C, Bianchi ML, Ulivieri FM, Gandolini G, Dindelli M, et al. Negative influence of pregnancies and lactation on bone mineral density. In: Jensen J, Riis B, Christiansen C, eds. *International Symposium on Osteoporosis* Abstracts. Glostrup, Denmark: SK&F Pharmaceuticals, **1987**;144-145.

Casper RF, Yen SSC. Neuroendocrinology of the menopausal hot flushes: an hypothesis of flush mechanism. *Clin Endocrinol* **1985**:22:293-312.

Cauley JA, Seeley DG, Ensrud K, Ettinger B, Black D, Cummings SR, for the study of osteoporotic fractures Research group. Estrogen replacement therapy and fracture in older women. *Ann Intern Med* **1995**;122:9-16.

Chetrite G, Kloosterboer HJ, Pasqualini JR. Effect of tibolone (Org OD 14) and its metabolites on estrone sulphatase activity in MCF-7 and T47D mammary cancer cells. *Anticancer Res* **1997**;17:135-140.

Christiansen C, Christiansen MS, McNair PL, Stocklund KE, Transbol I. Prevention of early postmenopausal bone loss: controlled 2-year study in 315 normal females. *Eur J Clin Invest* **1980**;10:273-279.

Christiansen C, Christensen MS, Transbol IB. Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. *Lancet* **1981**;i:459-461.

Christiansen C, Riis BJ, Rodbro P. Prediction of rapid bone loss in postmenopausal women. *Lancet* **1987**;i:1105-1108.

Christiansen C, Riis BJ. Five years with continuous combined oestrogen/progestogen therapy. Effects on calcium metabolism, lipoproteins, and bleeding pattern. *Br J Obstet Gynaecol* **1990**;97:1087-1092.

Christiansen C, Riis BJ. 17 Beta estradiol and continuous norethisterone: a unique treatment for established osteoporosis in elderly women. *J Clin Endocrinol Metab* **1990**;71:836-841.

Citron JT, Ettinger B, Genant HK. Prediction of peak premenopausal bone mass using a scale of weighted clinical variables. In: Jensen J, Riis B, Christiansen C, eds. *International Symposium on Osteoporosis* Abstracts. Glostrup, Denmark: SK&F Pharmaceuticals, **1987**;146-149.

Clarkson TB, Anthony MS, Jerome CP. Lack of effect of raloxifene on coronary artery arteriosclerosis of postmenopausal monkeys. *J Clin Endocrinol Metab* **1998**;83:721-726.

Colditz GA, Egan KM, Stampfer MJ. Hormone replacement therapy and risk of breast cancer: results from epidemiological studies. *Am J Obstet Gynecol* **1993**;168:1473-1480.

Compston JE, Yamaguchi K, Croucher PI, Garrahan NJ, Lindsay PC, Shaw RW. The effects of Gonadotropin-Releasing Hormone Agonists on iliac crest cancellous bone structure in women with endometriosis. *Bone* **1995**;16:261-267.

Cooper C, Shah S, Hand DJ. Screening for vertebral osteoporosis using individual risk factors. *Osteoporosis Int* **1991**;2:48-53.

Cope E. Physical changes associated with the postmenopausal years. In: Campbell S, Editor. *The management of the menopause and postmenopausal years.* MTP press, Lancaster **1973**.

Cortes-Prieto J. Coagulation and fibrinolysis in postmenopausal women treated with Org OD 14. *Maturitas* **1987**;Suppl.1:67-72.

Crona N, Samsioe G, Lindberg UB, SilferstolpeG. Treatment of climacteric complaints with Org OD 14: A comparative study with oestradiol valerate and placebo. *Maturitas* **1988**;9:303-308.

Cummings SR, Nevitt MC, Browner WS, et al.. Risk factors for hip fracture in white women. *N Engl J Med* **1995**;332:767-773.

Cummings SR, Black DM, Thompson DE, et all.. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fracture: results from the Fracture Intervention Trial. *JAMA* **1998**;280:2077-2082.

Cummings SR, Eckert S, Krueger KA et al.. The effect of raloxifene on risk of breast cancer in postmenopausal women. *JAMA* **1999**;281:2189-2197.

Dadelszen Von P, Gillmer MDG, Gray MD, et al. Endometrial hyperplasia and adenocarcinoma during tibolone (Livial) therapy. *Br J Obstet Gynecol* **1994**;101:158-161.

Daly E, Vessey MP, Hawkins MM, Carson JL, Goug P, Marsh S. Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet* **1996**;348:977-980.

Davies GC, Huster WJ, Lu Y, Plouffe L, Lakshmanan M. Adverse events reported by postmenopausal women in controlled trials with raloxifene. *Obstet Gynacol* **1999**;93:558-565.

Davis JW, Ross PD, Johnson NE, Wasnich RD. Estrogen and calcium supplement use among Japanese-American women: Effects upon bone loss when used singly and in combination. *Bone* **1995**;17:369-373.

DeCherney A. Physiologic and pharmacologic effects of estrogen and progestins on bone. *J Reprod Med* **1993**;38:1007-1014.

Delmas PD, Bjarnason NH, Mitlak BH et al.. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. *N Engl J Med* **1997**;337:1641-1647.

Dempster DW. **1992** Bone remodelling. In: Cloe F, Favus MJ, eds. Disorders of bone and mineral metabolism. New York: Raven press; 355-382.

Devor M, Barret-Connor E, Renval M, Feigal D, Ramsdell J. Estrogen replacement therapy and the risk of venous thrombosis. *Am J Med* **1992**;92:275-282.

Drinkwater BL, Chesnut CH. Bone density changes during pregnancy and lactation in active women: a longitudinal study. *Bone Miner* **1991**;14:153-160.

Early Breast Cancer Trialists Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trails. *Lancet* **1998**;351:1451-1467.

Ederveen AGH, Schot LPC, Kloosterboer HJ. Effect of Org Od 14 on axial and peripheral bone mass in ovariectomised senescent rats. Presented at the 7th international congress on the Menopause, Stockholm, June 20-25,1993;55.

Ederveen AGH, Kloosterboer HJ. Effects of tibolone or EE on bone mass and bone turnover in mature ovariectomized rats. *J Bone Miner Res* **1996**;11: (S1), S350.

Ederveen AGH, Spanjers CPM, Quaijtaal JHM, Kloosterboer HJ. Biomechanical properties of cortical and trabecular bone after tibolone or EE treatment of mature ovariectomized rats. Amsterdam, May 18-23, 1996; *Osteoporosis Int* **1996**;6(Suppl 1):S210.

Ederveen AGH, Kloosterboer HJ. Tibolone, a tissue selective steroid, exerts its preventive action on trabecular bone loss in ovariectomised rats through the estrogen receptor. *J Bone Miner Res* **1997**;12(S1):S349.

Ederveen AGH, Kloosterboer HJ. Tibolone, a steroid with a tissue specific hormonal profile, completely prevents ovariectomy-induced bone loss in sexually mature rats. *J Bone Miner Res* **1999**;14:1963-1870.

Egarter C, Huber J, Leikermoser R et al. Tibolone versus conjugated estrogens and sequential progestogen in treatment of climacteric complaints. *Maturitas* **1996**;23:55-62.

Eiken P, Kolthoff N, Nielsen SP. Effect of 10 years' hormone replacement therapy on bone mineral content in postmenopausal women. *Bone* **1996**;19:191S-193S.

Elders PJM, Netelenbos JC, Lips P, et al.. Perimenopausal bone mass and risk factors *Bone Miner* **1989**;7:289-299.

Erel CT, Elter K, Akman C, et al... Mammographic changes in women receiving tibolone therapy. *Fertil Steril* **1998**;69:870-875.

Ettinger B, Genant HK, Cann CE. Long-term estrogen replacement therapy prevents bone loss and fractures. *Ann Intern Med* **1985**;102:319-324.

Ettinger B, Genant HK, Cann CE. Postmenopausal bone loss is prevented with low-dosage estrogen with calcium. *Ann Intern Med* **1987**;106:40-45.

Ettinger B, Grady D. The waning effect of postmenopausal estrogen therapy on osteoporosis. *N Engl J Med.* **1993**;329:1192-1193.

Ettinger B, Selby J, Citron JT, Vangessel A, Ettinger VM., Hendrickson MR. Cyclic hormone replacement therapy using quartely progestin. *Obstet Gynecol* **1994**;83:693-700.

Ettinger B, Black DM, Mitlak BH et al.. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with Raloxifene. *JAMA* **1999**;282:637-638.

Fantl JA, Cardozo L, McClish D. Estrogen therapy in the management of urinary incontinence in postmenopausal women: a meta-analysis. First report of the Hormones and Urogenital Therapy Committee. *Obstet Gynecol* **1994**;83:12.

Faulkner KG, Glüer CC, Majumdar, Lang P,Engelke K, Genant HK. Noninvasive measurements of bone mass, structure, and strength: current methods and experimental techniques. *Am J Radiol* **1991**;157:1229-1237.

Felson DT, Zhang MPHY, Hannan MT, Kiel DP, Wilson PWF, Anderson JJ. The effect of postmenopausal estrogen therapy on bone density in elderly women. *N Engl J Med.* **1993**;329:1141-1146.

Fisher B, Costantino J, Redmond C, et al.. A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positve tumors. *N Engl J Med* **1989**;320:479-484.

Fisher B, Costantino J, Wickerham L, et al.. Tamoxifen for the prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* **1998**;90:1371-1388.

Fogelman I, Bessant RG, Smith ML, Hart DM, Lindsay R. Assessment of synthetic steroid (Org OD 14): effect on skeletal metabolism by 24-H whole retention of diphosphonate. *Maturitas* **1981**;3:265-269.

Fogelman I, Bessent RG, Beastall G, Boyle IT. Skeletal uptake of diphosphonate. Method for prediction of postmenopausal osteoporosis. *Lancet* **1980**;ii:667-670.

Formosa M, Brincat MP, Cardozo LD, Studd JWW. Collagen: the significance in skin, bones and bladder. In: Lobo RA, editor. *Treatment of the postmenopausal woman.* Raven press. Ltd., New York **1994**.

Franke HR, Weijer van de PHM, Pennings TMM, Mooren van der MJ. Gonadotropin-releasing hormone agonist plus "add-back" hormone replacement therapy for treatment of endometriosis: a prospective randomised placebo-controlled, double-blind trial. *Fertil Steril* **2000**;74:534-539.

Fuleihan G; Brown EM; Curtis K et al. Effect of sequential and daily continuous hormone replacement therapy on indexes of mineral metabolism. *Arch Intern Med* **1992**;152:1904-1909.

Gallagher JC, Kable WT. Effect of progestin therapy on cortical and trabecular bone: comparison with estrogen. *Am J Med* **1991**;90:171-178.

Gallagher JC, Freeman R, Baylink DJ, et al. Tibolone prevents postmenopausal bone loss and is well tolerated: Phase III trial results. *J Bone Mineral Res* **1999**; 14 (Suppl 1): S158.

Gärdsell P, Johnell O, Nilsson BE, Gullberg. Fragillity fracture in women by forearm bone densitometry: a follow up study. *Calcif Tissue Int* **1993**;52:348-353.

Gärdsell P, Johnell O, Nilsson BE. The predictive value of bone loss for fragility fractures in women: a longitudinal study over 15 years. *Calcif Tissue Int* **1991**;49:90-94.

Geelen JM van, Weijer PHM van de, Arnolds HT. Urogenitale verschijnselen en hinder daarvan bij thuiswomende Nederlandse vrouwen van 50-75 jaar. [Urogenital symptoms and the resulting discomfort in non-institutionalized Dutch women aged 50-75 years. *Ned Tijdschr Geneeskunde* **1996**;140:713-716.

Genant HK, Cann CE, Ettinger B, Gordon GS. Quantitative computed tomography of vertebral spongiosa: a sensitive method for detecting early postmenopausal after oophorectomy. *Ann Intern Med* **1982**;97:699-705.

Genazzani AR, Petraglia F, Facchinetti F et al.. Effects of Org OD 14 on pituitary and peripheral β-endorphin in castrated rats and post-menopausal women. *Maturitas* **1987**;(Suppl 1): 35-48.

Genazzani AR, Benedek-Jaszman LJ, Hart DM, Andolsel L, Kicovic PM, Tax L. Org OD 14 and the endometrium. *Maturitas* **1991**;13:243-251.

Geusens P, Dequeker J, Gielen J, Schot LPC. Non-linear increase in vertebral density induced by a synthetic steroid (Org OD 14) in women with established osteoporosis. *Maturitas* **1991**;13:155-162.

Ginsburg J, Prelevic GM, Butler D, Okolo S. Clinical experience with tibolone (Livial) over 8 years. *Maturitas* **1995**;21:71-76.

Ginsburg J, Prelevic GM. Cause of vaginal bleeding in postmenopausal women taking tibolone. *Maturitas* **1996**;24:107-110.

Goldsmith NF, Johnston JO. Bone Mineral: Effects of oral contraceptives, pregnancy, and lactation. *J Bone Joint Surg* **1975**;57a:657-668.

Goldstein SR. Postmenopausal fluid collections revisted: look at the donut rather than the hole. *Obstet Gynecol* **1994**;83:738-740.

Gompel A, Kandouz M, Siromchkova M, et al.. The effect of tibolone on proliferation, differentiation and apoptosis in normal breast cells. *Gynecol Endocrinol* **1997**;11(Suppl..1):77-79.

Gnoth CH, Gödtke K, Freundl G, Godehardt E, Kienle E. Effects of add-back on bone mineral density and pyridinium crosslinks in patients with endometriosis treated with gonadotropin-releasing hormone agonists. *Gynecol Obstet Invest* **1999**;47:37-41.

Grady D Gebretsadik T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: A meta-analysis. Obstet Gynecol 1995;85:304-313.

Greer IA, Walker ID. Hormone replacement therapy and venous thromboembolism. Royale College of Obstricians and Gynaecologists: Guideline 19, April 1999. *Climacteric* **1999**;2:224-231.

Gregoriou O, Konidaris S, Vitoatos N, Papadias C, Chryssicopoulos A. Gonadotrophin-releasing hormone analogues plus hormone replacement therapy for the treatment of endometriosis: a randomized controlled trial. *Int J Fertil Womens Med* **1997**;42:406-411.

Grese TA, Sluka JP, Bryant HU et al.. Molecular determinants of tissue selectivity in estrogen receptor modulators. *Proc Natl Acad Sci USA* **1997**;94:14105-14110.

Grey AB; Cundy TF; Reid IR. Continuous combined oestrogen/progestin therapy is well tolerated and increases bone density at the hip and spine in post-menopausal osteoporosis. *Clin Endocrinol (Oxf)* **1994**;40:671-677.

Grisso AG, Kelsey JL, Storm BL et al.. Risk factors for falls as a cause of hip fracture in women. *N Engl J Med* **1991**;324:1326-1331.

Groeneveld FPMJ, Bareman FP, Barentsen R, Dokter HJ, Drogendijk AC, Hoes AW. Duration of hormonal replacement therapy in general practice; a follow-up study. *Maturitas* **1998**;29:125-131.

Grodstein F, Stampfer MJ, Goldhaber SZ et al.. Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet* **1996**;348:983-987.

Gutthann SP, Garcia Rodriguez LA, Castallsague J, Oliart AD. Hormone replacement therapy and risk of venous thromboembolism: population based case control study. *Br Med J* **1997**;314:796-800.

Habiba M, Ramsay J, Akkad A, Hart DM, Al-Azzawi F. Immunohistochemical and hysteroscopic assessment of postmenopausal women with uterine bleeding whilst taking tibolone. *Eur J Obstet Gynecol Reprod Biol* **1996**;66:45-49.

Hällström T, Samuelsson S. Mental health in the climacteric: the longitudinal study of women in Gothenburg. *Acta Obstet Gynecol Scand* **1985**;130:13-18.

Hammar M, Chritau S, Nathorst-Böös J, Rud T, Garre K. A doble blind randomised study comparing effects of Livail and Kliogest in postmenopausal women with climacteric complaints. *Acta Obstet Gynecol Scand* **1996**;75:52-53.

Hammar M, Chritau S, Nathorst-Böös J, Rud T, Garre K. A double-blind, randomised trial comparing the effects of tibolone and continuous combined hormone replacement therapy in postmenopausal women with menopausal symptoms. *Br J Obstet Gynaecol* **1998**;105:904-911.

Hänggi W, Bersinger N, Altermatt HJ, Birkhauser MH. Comparison of transvaginal ultrasonography and endometrial biopsy in endometrial surveillance in postmenopausal HRT users. *Maturitas* **1997**;27:133-143.

Hänggi W, Lippuner K, Riesen W, Jaeger P, Birkhäuser MH. Long-term influence of different postmenopausal hormone replacement regimens on serum lipids and lipoprotein(a): a randomised study. *Br J Obstet Gynaecol* **1997**;104:708-717.

Hansen AM, Overgaard K, Riis BJ, Christiansen C. Role of peak bone mass in postmenopausal osteoporosis: 12 year study. *Br Med J* **1991**;303:961-964.

Hansen MA, Overgaard K, Riis BJ, Christiansen C. Potential risk factors for development of postmenopausal osteoporosis. *Osteoporosis Int* **1991**;1[2]:95-102.

Hansson T, Roos HT. The effect of fluoride and calcium on spinal osteoporosis: a controlled prospective (three years) study. Calcif Tissue Int **1987**;40:315.

Hart DM, Farish E, Fletcher CD,et al.. Long-term effect of continuous combined HRT on bone turnover and lipid metabolism in postmenopausal women. *Osteoporosis Int* **1998**;8:326-332.

Hayslip CC, Klein TA, Wray LH, Duncan WE. The effects of lactation on bone mineral content in healthy postpartum women. *Obstet Gynecol* **1989**;74:588-592.

Heany RP. Effect of calcium on skeletal development, bone loss, and risk of fractures. *Am J Med* **1991**;91 (5B):23s-28s.

Herd RJM, Balena R, Blake GM, Ryan PJ, Fogelman I. The prevention of early postmenopausal bone loss by cyclical etidronate therapy: a 2 year, double blind, placebo-controlled Study. *Am J Med* **1997**;103:92-99.

Hirvonen E, Salmi T, Puolakka J et al. Can progestin be limited to every third month only in postmenopausal women taking estrogen? *Maturitas* **1995**;21:39-44.

Hilton P, Tweddel AL, Mayne C. Oral and intravaginal estrogens alone and in combination with alpha adrenergic stimulation in genuine stress incontinence. *Int Urogynecol J* **1990**;12:80-86.

Holst J, Backström T, Hammerbäch S, von Schoutz B. Progestin addiction during oestrogen replacement therapy. *Maturitas* **1989**;11:13-19.

Hornstein MD, Surrey ES, Weisberg GW, Casino LA. Leuprolide acetate depot and hormonal add-back in endometriosis: a 12 month study. *Obstet Gynecol* **1998**;91:16-24.

Horsman A, Nordin BEC, Crilly RG. Effect on bone of withdrawal of oestrogen therapy. *Lancet* **1979**;i:33.

Hosking DJ, Chilvers CE, Christiansen C et al.. Prevention of bone loss with alendronate in postmenopausal women under 60 years of age. Early Postmenopausal Intervention Cohort Study Group. *N Eng J Med* **1998**;338:485-492.

Howell R, Edmonds DK, Dowsett M, Crook D, Lees B, Stevenson JC. Gonadotrophin-releasing hormone analogues (goserelin) plus hormone replacement therapy for the treatment of endometriosis: a randomized controlled trial. *Fertil Steril* **1995**;64:474-481.

Hreshchyshyn MM, Hopkins A, Zylstra S, Anbar M. Associations of parity, breast-feeding, and birth control pills with lumbar spine and femoral neck bone densities. *Am J Obstet Gynecol* **1988**;159:318-322.

Hui SL, Slemenda CW, Johnston CC. Baseline measurement of bone mass predicts fracture in white Women. *Ann Intern Med* **1989**;111:355-361.

Hulley S, Grady D, Bush T et al.; for the Heart and Estrogen/progestin Replacement Study (HERS) Research Group. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA* **1998**;280:605-613.

Hunter M, Battersby R, Whitehead M. Realtionships between psychological symptoms, somatic complaints and menopausal status. *Maturitas* **1986**;8:217-228.

Jick H, Derby LE, Wald Meyers M, Vasilakis C, Newton KM. Risk of hospital admission for idiopathic venous thromboembolism among users postmenopausal oestrogens. *Lancet* **1996**;348:981-983.

Kalender WA, Brestowsky H, Felsenberg D. Bone mineral measurement: automated determination of midvertebral CT section. *Radiology* **1988**;168:219-221.

Kalender WA, Felsenberg D, Genant HK, Fisher M, Dequecker J, Reeve J. The European Spine Phantom- A tool for standardization and quality control in spinal bone mineral measurement by DXA and QCT. *Eur J Radiol* **1995**;20:83-92;.

Kalender WA, Felsenberg D. A phantom for standardization and cross-calibration of spinal bone mineral measurements by DXA and QCT (abstract). *Osteoporosis Int* **1991**;1:203.

Kalender WA, Suess C. A new calibration phantom for quantitative computed tomography. *Med Phys* **1987**;14:863-866.

Kanis JA, Johnell O, Gullberg B, et al.. Evidence for efficacy of drugs affecting bone metabolism in preventing hip fracture *Br Med J* **1992**; 305;1124-1128.

Kannus p, Parkkari J, Niemi S, et al.. Prevention of hip fracture in elderly people with the use of a hip protector. *N Engl J Med* **2000**; 343: 1506-1513.

Kasugai Y, Ikegami A, Matsuo K, et al Effects of tibolone (Org OD 14) treatment for 3 months on ovariectomy-induced osteopenia in 8 months old rats on a low calcium diet: preventive testing for 3 months. *Bone* **1998**;22(2);119-124.

Kent GN, Price RI, Gutteridge DH, Smith M, Allen JR, Bhagat CI, et al. Human lactation: forearm trabecular bone loss, increased bone turnover, and renal conservation of calcium and inorganic phosphate with recovery of bone mass following weaning. *J Bone Miner Res* **1990**;5:361-369.

Kicovic PM, Cortes-Prieto J, Luisi M, Milojevic S, Franchi F. Placebo-controlled cross-over study of effects of Org OD 14 in menopausal women. *Reproduction* **1982**;6:81-91.

Kiel D, Felson DT, Andersson JJ, Wilson PWF, Moskowitz MA. Hip fracture and the use of estrogens in postmenopausal women. The Framingham study. *N Engl J Med* **1987** 317; 1169-1174.

Kleinman D, Karas M, Danilenko M et al.. Stimulation of endometrial cancer cell growth by tamoxifen is associated with increased insulin-like growth factor (IGF)-I induced tyrosine phosphorylation and reduction in IGF binding proteins. *Endocrinology* **1996**;137:1089-1095.

Kloosterboer HJ, Schoonen WGEJ, Deckers GH, Klijn JGM. Effects of progestagens and Org OD 14 in *In vitro* and *In Vivo* tumor models. *J Steroid Biochem Mol Biol* **1994**;49:311-318.

Kloosterboer HJ. Tibolone and its metabolites: pharmacology, tissue specificity and effects in animal models of tumors. *Gynecol Endocrinol* **1997**;11:suppl (1), 63-68.

Koetting CA, Wardlaw GM. Wrist, spine, and hip bone densities in women with variable histories of lactation. *Am J Clin Nutr* **1988**;48:1479-1481.

Komulainen MH, Kroger H, Tuppurainen MT et al. HRT and Vit D in prevention of non-vertebral fractures in postmenopausal women; a 5 year randomized trial. *Maturitas* **1998**; 31:45-54.

Krieger N, Kelsey JL, Holford TR, O'Connor T. An epidemiologic study of hip fracture in postmenopausal women. *Am J Epidemiol* **1982**;116:141-148.

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel estrogen receptor.*Proc Natl Acad Sci USA* **1996**;93:5925-5930.

Kuijk van C, Genant HK. Detection of osteopenia and osteoporosis. In: Lobo RA, editor. *Treatment of the postmenopausal woman*. Raven press, Ltd., New York **1994.**

Lam SY, Baker HWG, Seeman E, Pepperell RJ. Gynaecological disorders and risk factors in premenopausal women predisposing to osteoporosis. A review. *Br J Obstet Gynaecol* **1988**;95:963-972.

Lamke B, Brundin J, Moberg P. Changes of bone mineral content during pregnancy and lactation. *Acta Obstet Gynecol Scand* **1977**;56:217-219.

Leather AT, Studd JWW, Watson NR, Holland EFN. The prevention of bone loss in young women treated with GnRH analogues with add back estrogen therapy. *Obstet Gynaecol* **1993**;81:104-107.

Liberman UA, Weiss SR, Broll J et al.. Effect of oral alendronate on bone density and the incidence of fractures in postmenopausal osteoporosis. *N Engl J Med* **1995**;333:1437-1443.

Limouzin-Lamothe MA. What women want from hormone replacement therapy: results of an international survey. *Eur J obstet Gynecol Reprod Biol* **1996**;64 S1: S21-S24.

Lindquist O, Bengtsson C, Hansson T, Roos B. Bone mineral content in relation to age and menopause in middle aged women. *Scand J Clin Lab Invest* **1981**;41:215-223.

Lindsay PC, Shaw RC, Coelingh Bennink HJ, Kicovic PM. The effect of add-back treatment with tibolone (Livial) on patients treated with the gonadotropin-releasing hormone agonist triptorelin (Decapeptyl). *Fertil Steril* **1996**;65:342-348.

Lindsay R, Hart DM, Aitken JM, MacDonald EB, Anderson JB, Clarke AC. Long-term prevention of postmenopausal osteoporosis by oestrogen. *Lancet* **1976**;i:1038-1041.

Lindsay R, Hart DM, Forrest C, Baird C. Prevention of spinal osteoporosis in oophorectomised women. *Lancet* **1980**;ii:1151-1154.

Lindsay R, Hart DM, Kraszewski A. Prospective double blind trial of synthetic steroid (Org OD 14) for preventing postmenopausal osteoporosis. *Br Med J* **1980**;280:1207-1209.

Lindsay R, Hart DM, MacLean A, Clark AC, Kraszewski A, Garwood J. Bone response to termination of estrogen treatment. *Lancet* **1978**;i:1325-1327.

Lindsay R, Tohme J, Kanders B. The effect of oral contraceptive use on vertebral bone mass in pre- and postmenopausal women. *Contraception* **1986**;102:333-340.

Lippuner K, Haenggi W, Birkhaeuser MH, Casez JP, Jaeger P. Prevention of postmenopausal bone loss using tibolone or conventional peroral or transdermal hormone replacement therapy with 17β estradiol and dydrogesterone. *J Bone Miner Res* **1997**;5:806-812.

Lissner L, Bengtsson C, Hansson T. Bone mineral content in relation to lactation history in pre- and postmenopausal women. *Calcif Tissue Int* **1991**;48:319-325.

Lloyd T, Buchanan JR, Ursino GR, Myers C, Wooward G, Halbert DR. Long-term oral contraceptive use does not affect trabecular bone density. *Am J Obstet Gynecol* **1989**;160:402-404.

Lobo RA. Editorial: Estrogen and the risk of coagulopathy. *Am J Med* **1992**;92:283-285.

Luciano AA; De Souza MJ; Roy MP; Schoenfeld MJ; Nulsen JC; Halvorson CV. Evaluation of low-dose estrogen and progestin therapy in postmenopausal women. A double-blind, prospective study of sequential versus continuous therapy. *J Reprod Med* **1993**;38:207-214.

Lufkin EG, Wahner HW, O'Fallon WM et all.. Treatment of postmenopausal osteoporosis with transdermal estrogen. *Ann Intern Med* **1992**;117:1-9.

Lyritis GP, Karpathios S, Basdekis K, Grigoriou O, Katostaras T, Paspati I, et al. Prevention of post-oophorectomy bone loss with Tibolone. *Maturitas* **1995**;22:247-253.

MacLennan AH; MacLennan A; Wenzel S; Chambers HM; Eckert K. Continuous low-dose oestrogen and progestogen hormone replacement therapy: a randomised trial. *Med J Aust* **1993**;159:102-106.

Maheux R, Lemay A, Blanchet P, Friede J, Pratt X. Maintained reduction of uterine leiyomyoma following addition of hormonal replacement therapy to a monthly luteinizing hormone-releasing hormone agonist implant: a pilot study. *Hum Reprod* **1991**;6:76:1439-1445.

Maheux R, Lemay A. Treatment of peri-menopausal women: potential long-term therapy with a depot GnRH agonist combined with hormone replacement therapy. *Br J Obstet Gynaecol* **1992**;99 (suppl 7):13-17.

Maintzer F. Vorschlag zur behandlung der ausfallerscheinungen nach kastration. *Deutsh Med Wschr* **1896**;12;199.

Makarainen L, Ronnberg L, Kauppila A. Medroxyprogesterone acetate supplementation diminishes the hypo-estrogenic side effects of gonadotrophin-releasing hormone agonist without changing its efficacy in endometriosis. *Fertil Steril* **1996**;65:29-34.

Markiewicz L, Gurpide E. Estrogenic and progestagenic activities coexisting in steroidal drugs: quantitative evaluation by in vitro bioassays with human cells. *J Steroid Biochem Molec Biol* **1994**;48;89-94.

Markiewicz L, Gurpide E. In vitro evaluation of estrogenic, estrogen antagonistic and progestagenic effects of a steroidal drug (Org OD-14) and its metabolites on human endometrium. *J Steroid Biochem* **1990**;35:535-541.

Marshall D, Johnell O, Wedel H. A meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *Br Med J* **1996**;312:1254-1259.

Marslew U, Overgaard K, Riis BJ, Christiansen C. Two new combinations of estrogen and progestagen for prevention of postmenopausal bone loss: long-term effects on bone, climacteric symptoms, and bleeding. *Obstet Gynecol* **1992**;79:202-210.

Mattsson LA; Bohnet HG; Gredmark T; Torhorst J; Hornig F; Hüls G. Continuous, combined hormone replacement: randomized comparison of transdermal and oral preparations. ***Obstet Gynecol*, 1999**;94:61-65.

Mazess RB. On aging bone loss. *Clin Orthop* **1982**;165:239-252.

Mazess RB, Barden HS. Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. *Am J Clin Nutr* **1991**;53:132-142.

McDonnell DP. Definition of the molecular mechanisms of action of tissue selective oestrogen-receptor modulators. *Biochem Soc Trans* **1998**;26:54-60.

McKinlay SM, Brambilla DJ, Pasner JG. The normal menopause transition. *Maturitas* **1992**;14:103-115.

McKinlay JB, McKinlay SM, Brambilla DJ. The relative contribution of endocrine changes and social circumstances to deprssion in mid-aged women. J Health Soc Behav 1987;28:345-363.

Metcalf MG, Donald RA, Livesey JH. Pituitary-ovarian function in normal women during the menopausal transition. *Clin Endocrinol* **1981**;14:245-255.

Miller MA, Omura TH, Miller SC. Increased cancellous bone remodeling during lactation in beagles. *Bone* **1989**;10:279-285.

Miller PD, Watts NB, Licata AA et al.. Cyclical etidronate in the treatment of postmenopausal osteoporosis: Efficacy and safety after 7 years of treatment. *Am J Med* **1997**;103:468-476.

Moghissi KS. Add-back therapy in treatment of endometriosis. The North America experience. *Br J Obstet Gynaecol* **1996**;103 (suppl 14);14.

Molnar GW. Body temperatures during menopausal hot flashes. *J Apll Physiol* **1975**;38:499-503.

Moll-Arts M, Helmond F, Stathopoulos V. Breast safety summary of tibolone. *Climacteric* **1999**; 2 (suppl 1):313.

Montgomery JC, Brincat M, Tapp A, et al.. Effects of oestrogens and testosterone-implants on psychological disorders in the climacteric. *Lancet* **1987**;I:297-299.

Moore RA. Livial: a review of clinical studies. *Br J Obstet Gynaecol* **1999**;106 (Suppl 19):1-22.

Moseklide L, Beck-Nielsen H, Sørensen OH, et al. Hormonal replacement therapy reduces forearm fracture incidence in recent postmenopausal women – results of Danish Osteoporosis Prevention Study. *Maturitas* **2000**;36:181-193.

Mosselman S, Polman J, Dijkema R. ERβ: identification and characterization of a novel human estrogen receptor. *FEBS Lett* **1996**; 392:49-53.

Munk-Jensen N, Pors Nielsen S, Obel EB, Bonne Eriksen P. Reversal of postmenopausal vertebral bone loss by oestrogen and progestagen: a double blind placebo controlled study. *Br Med J* **1988**;296:1150-1152.

Naessén T, Persson I, Adami H-O, Berström R, Bergkvist L. Hormone replacement therapy and the risk for first hip fracture: a prospective, population-based cohort study. *Ann Intern Med* **1990**;13:95-103.

Nathorst-Böös J, Hammar M. Effect on sexual life - a comparison between tibolone and a continuous estradiol-norethisterone acetate regimen. *Maturitas* **1997**;26:15-20.

Nevinny-Stickel J. Double-blind cross-over study with Org OD 14 and placebo in postmenopausal patients. *Arch Gynecol* **1983**;234:27-31.

Nickelsen T; Lufkin EG; Riggs BL; Cox DA; Crook TH. Raloxifene hydrochloride, a selective estrogen receptor modulator: safety assessment of effects on cognitive function and mood in postmenopausal women. *Psychoneuroendocrinology* **1999**;24:115-128.

Nilas L, Borg J, Gotfredsen A, Christiansen C. Comparison of single- and dual photon absorptiometry in postmenopausal bone mineral loss. *J Nucl Med* **1985**;26:1257-1262.

Nilsson S, Kuiper G, Gustafsson JA. ERβ: a novel estrogen receptor offers the potential for new drug development. *Trends Endocrinol Metab* **1998**;9:387-395.

Nordin BEC, Need AG, Morris HA, Horowitz H. Approaches to the problem of osteoporosis. *Clin Orthop Rel Res* **1985**;200:181-197.

O'Brien M, Montes A, Powles TJ. Hormone replacement as treatment of breast cancer-a phase II study of Org OD 14 (tibolone). *Br J Cancer* **1996**;73:1086-1088.

Oldenhave A, Jaszmann LJB, Haspels AA, Everaerd WTAM. Impact of climacteric on well-being: a survey based on 5213 women 39-60 years old. *Am J Obstet Gynecol* **1993**;186:772-780.

Pacifici R, McMurtry C, Vred I, Rupich R, Avioli L. Coherence therapy does not prevent axial bone loss in osteoporotic women: a preliminary comparative study. *J Clin Endocrinol Metab* **1988**;66:747-753.

Palacios S, Menendez C, Jurado AR, Castaño R, Vargas JC. Changes in sex behaviour after menopause: effects of tibolone. *Maturitas* **1995**;22:155-161.

Parfitt AM. The physiological an clinical significance of bone histomorphometric data. In: Recker R, ed. Bone histomorphometry: techniques and interpretations. Boca Raton: CRC Press, **1979**:143-223.

Parkin DE, Smith D, Al Azzawi F, Lindsay R, Hart DM. Effects of long-term Org OD 14 administration on blood coagulation in climacteric women. *Maturitas* **1987**;9:95-101.

Pasqualini JR, Chetrite G. Control of estron sulfatase activity in human breast cancer cells: effect of tibolone and its metabolites. *Gynecol Endocrinol* **1997**:11(Suppl.1):69-75.

Pavlov P, Ginsburg J, Tjon AHS, Prevelic G, Coelingh Bennink HJ, Kicovic PM. A double-blind placebo-controlled study of effects of Tibolone on bone mineral density in postmenopausal osteoporotic women with and without fractures. In: World Congress on Osteoporosis; Amsterdam, 18-23 May 1996. *Osteoporosis Int* **1996**;6(Suppl1):S231.

Pickersgill A. GnRH agonists and add-back therapy: is there a perfect combination? Br J Obstet Gynaecol **1998**;105:475-485.

Pols HAP, Felsenberg D, Hanley DA et al.. Multinational, placebo-controlled randomized trial of the effects of alendronate on bone density and fracture risk in postmenopausal women with low bone mass: results of the FOSIT Study. *Osteoporos Int* **1999**;9:461-468.

Prelevic GM, Bartram C, Wood J, Okolo S, Ginsburg J. Comparative effects on bone mineral density of tibolone, transdermal estrogen and oral estrogen/progestogen therapy in postmenopausal women. *Gynecol Endocrinol* **1996**;6:413-420.

Punnonen R, Liukko P, Cortes-Prieto J et al.. Multicentre study of effects of Org OD 14 on endometrium, vaginal cytology and cervical mucus in postmenopausal and oophorectomized women. *Maturitas* **1984**;5:281-286.

Ray NF, Chan JK, Thamer M, Melton LJ. Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995. *J Bone Miner Res* **1997**;12:24-35.

Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. *N Engl J Med* **1993**;329:753-756.

Recker RR, Kimmel DB, Parfitt AM. Static and tetracycline based bone histomorphometric data from 34 normal postmenopausal females. *J Bone Miner Res* **1988**;3:133-144.

Recker RR, Davies KM, Dowd RM, Heany RP. The effect of low-dose continuous estrogen and progesterone therapy with calcium and vit D on bone in elderly women. A randomized, controlled trial. *Ann Intern Med* **1999**;130:897-904.

Reinbold W-D, Genant HK, Reiser UJ, Harris ST, Ettinger B. Bone mineral content in early postmenopausal and postmenopausal women: comparison of measurement methods. *Radiology* **1986**;160:469-478.

Ribot C, Tremollieres F, Pouilles JM, Louvet JP, Peyron R. Preventive effects of transdermal administration of 17 B estradiol on postmenopausal bone loss: A 2-year prospective study. *Obstet Gynecol* **1990**;75(S):42-46.

Riggs BL, Wahner HW, Dunn WL, Mazess RB, Offord KP, Melton LJ III. Differential changes in bone mineral density of appendicular and axial skeleton with aging: relationship to spinal osteoporosis. *J Clin Invest* **1981**;67:328-335.

Riggs BL, Melton LJ. Involutional osteoporosis. *N Engl J Med* **1986**;314:1676-1686.

Riggs BL. Editorial: Tibolone as an alternative to estrogen for the prevention of postmenopausal osteoporosis in selected postmenopausal women. *J Clin Endocrinol Metab*. **1996**;81:2417-2418.

Riis BJ, Thomsen K, Strom V, Christiansen C. The effect of percutaneous estradiol and natural progesterone on postmenopausal bone loss. *Am J Obstet Gynecol* **1987**;156:61-65.

Riis BJ; Johansen J; Christiansen C. Continuous oestrogen-progestogen treatment and bone metabolism in post-menopausal women. *Maturitas* **1988**;10:51-58.

Rodin A, Chapman MG, Fogelman I. Bone density in users of combined contraception. Preliminary report of a pilot study. *Br J Fam Plan* **1991**;16:125-129.

Rodin A, Fogelman I, Chapman MG. Combined oral contraception as a determinant of bone mass. In: Jensen J, Riis B, Christiansen C, eds. *International Symposium on Osteoporosis* Abstracts. Glostrup, Denmark: SK&F Pharmaceuticals, **1987**;161-162.

Rymer J, Chapman MG, Fogelman I, Wilson POG. A study of the effect of tibolone on the vagina in postmenopausal women. *Maturitas* **1994**;18:127-133.

Rymer J, Chapman MG, Fogelman I. Effect of Tibolone on postmenopausal bone mass. *Osteoporosis Int* **1994**;4:314-319.

Rymer J, Fogelman I, Chapman MG. The incidence of vaginal bleeding with tibolone treatment. *Br J Obstet Gynecol* **1994**;101:53-56.

Sandker GW, Vos RME, Delbressine LPC, Slooff MJH, Meijer DKF, Groothuis GMM. Metabolism of three pharmacologically active drugs in isolated human and rat hepatocytes: analysis of interspecies variability and comparison with metabolism in vivo. *Xenobiotica* **1994**;24:143-155.

Sandor T, Kalender WA, Hanlon WB, Weissman BN, Rumbaugh C. Spinal bone determination using automated contour detection: application for single and dual energy CT. *SPIE* **1985**;555:188-194.

Sandor T, Weissman B, Brown E. Effect of intervertebral changes of the spinal trabecular and cortical mineral content on the precision requirements in longitudinal single- and dual-energy computed tomography examinations. *Med Phys* **1989**;16:218-224.

Sattin RW, Lamber-Huber DA, DeVito CA, et al.. The incidence of fall injury events among elderly in a defined population. *Am J Epidemiol* **1990**;131:1028-1037.

Sato M, Rippy MK, Bryant HU. Raloxifene, tamoxifen, nafoxidine, or estrogen effects on reproductive and non-reproductive tissues in ovariectomized rats. *FASEB J* **1996**;10:905-912.

Schneider DL, Barrett-Connor EL, Morton DJ. Timing of postmenopausal estrogen for optimal bone mineral density. *JAMA* **1997**;277:543-547.

Semmens JP, Wagner G. Estrogen deprivation and vaginal function in postmenopausal women. *JAMA* **1982**;248:445.

Semmens JP, Tsai CC, Curtis Semmens E, et al.. Effects of estrogen therapy on vaginal physiology during menopause. *Obstet Gynecol* **1985**;66:15-18.

Sherwin BB. Affective changes with estrogens and androgen replacement therapy in surgically menopausal women. J Affect Disord 1988;14:177-187.

Sherwin BB. The impact of different doses of estrogen and progestin on mood and sexual behavior in postmenopausal women. *J Clin Endocrinol Metab* **1991**;72:336-343.

Sherwin BB. Impact of changing hormonal milieu. In: Lobo RA, editor. *Treatment of the postmenopausal woman.* Raven press. Ltd., New York **1994.**

Simberg N, Tiitinen A, Silvast A, Viinikka L, Ylikokala O. High bone density in hyperandrogenic women: effect of gonadotrophin-releasing hormone agonist alone or in conjunction with estrogen-progestin replacement. *J Clin Endocrinol Metab* **1996**;81:646-651.

Siseles NO, Halperin H, Benecia HJ et al.. A comparative study of two hormone replacement therapy regimens on safety and efficacy variables. *Maturitas* **1995**;21:201-210.

Slemenda CW, Hui SL, Longcope C, Wellman H, Johnston CC. Predictors of bone mass in perimenopausal women. *Ann Intern Med* **1990**;112:96-101.

Smith DC, Prentice R, Thompson DJ, Hermann VL. Association of exogenous estrogen and endometrial carcinoma. *N Engl J Med* **1975**;293; 1164-1167.

Specker BL, Tsang RC, Ho ML. Changes in calcium homeostasis over the first year postpartum: effect of lactation and weaning. *Obstet Gynecol* **1991**;78:56-62.

Speroff L, Rowan J, Symons J, Genant H, Wilborn W; for the CHART study group. The comparative effect on bone Density, endometrium, and lipids of continuous hormones as replacement therapy. *JAMA* **1996**;276:1397-1403.

Speroff T, Dawson NV, Speroff L, Haber RJ. A risk-benefit analysis of elective bilateral oophorectomy: effect of changes in compliance with estrogen therapy on outcome. *Am J Obstet Gynecol* **1991**;164:165-174.

Spitzer WO, Lewis MA, Heineman LA, Thorogood M, Macrae KD. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case control study. *Br Med J* **1996**;312:83-88.

Stadberg E; Mattsson LA; Uvebrant M. 17 beta-estradiol and norethisterone acetate in low doses as continuous combined hormone replacement therapy. *Maturitas* **1996**;23:31-39.

Stephán JJ, Schreiber V. Anabolic effects of progestogens in bone remodeling. *Sborn Lék* **1995**;96:293-297.

Stevenson JC, Cust MP, Gangar KF, Hillard TC, Lees B, Whitehead MI. Effects of transdermal versus oral hormone replacement therapy on bone density in spine and proximal femur in postmenopausal women. *Lancet* **1990**;335:265-269.

Stevenson JC, Lees B, Devenport M, Cust MP, Ganger KF. Determinants of bone density in normal women: risk factors for future osteoporosis? *Br Med J* **1989**;298:924-928.

Storm T, Thamsborg G, Steiniche T, Genant HK, Sörensen OM. Effect of intermittent cyclical etidronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. *N Engl J Med* **1990**;322:1265-1271.

Studd J, Arnala I, Zamlera D, Kroger H, Holland N. Tibolone increases bone mass in women with previous fractures in a placebo-controlled bicentre study. In: World Congress on Osteoporosis; Amsterdam, 18-23 May 1996. *Osteoporosis Int* **1996**;6(Suppl1):S230.

Studd JWW, Zakaria FBP. The management of hormone replacement therapy, bleeding and compliance. *Gynecol Endocrinol* **1997**;11 (Suppl 1):5-10.

Suhonen SP; Allonen HO; Lähteenmäki P. Sustained-release estradiol implants and a levonorgestrel-releasing intrauterine device in hormone replacement therapy. *Am J Obstet Gynecol*, **1995**;172:562-567.

Suggimoto AK, Hodsman AB, Nisker JA. Long-term gonadotropin-releasing hormone agonist with standard postmenopausal oestrogen replacement failed to prevent bone loss in premenopausal women. *Fertil Steril* **1993**;60:672-674.

Suvanto Luukkonen E; Malinen H; Sundström H; Penttinen J; Kauppila A. Endometrial morphology during hormone replacement therapy with estradiol gel combined to levonorgestrel-releasing intrauterine device or natural progesterone. *Acta Obstet Gynecol Scand*, **1998**;77:758-763.

Takahashi K, Tsuboyama T, Matsushita M, et al. Effective intervention of peak bone mass and bone modelling in the spontaneous murine model of senile osteoporosis, sam-p/6, by Ca supplement and hormone treatment. *Bone* **1994**;15:209-215.

Tang B, Markiewicz L, Kloosterboer HJ, Gurpide E. Human endometrial 3 beta-hydroxysteroid dehydrogenase/isomerase can locally reduce intrinsic estrogenic/progestagenic activity ratios of a steroidal drug (Org OD 14). *J Steroid Biochem Molec Biol* **1993**;45:345-351.

Taskin O, Yalcinoglu AI, Kucuk S, Uryan I, Buhur A, Burak F. Effectiveness of tibolone on hypoestrogenic symptoms induced by goserelin treat in patients with endometriosis. *Fertil Steril* **1997**;67:40-45.

Tax L, Goorissen EM, Kicovic PM. Clinical profile of Org OD 14. *Maturitas* **1987**;suppl 1:3-13.

The writing group for the PEPI-Trial. Effects of hormone replacement therapy on endometrial histology in postmenopausal women. The Postmenopausal Estrogen/Progestin Intervention (PEPI) Trial. *JAMA* **1996**;275:370-375.

Thomsen K, Riis BJ, Johansen JS, Christiansen C, Rodbro P. Bone turnover in postmenopausal women after withdrawal of estrogen/gestagen replacement therapy. *Gynecol Endocrinol*. **1987**;1:169-175.

Thromboembolic Risk Factors (THRIFT) Consensus Group. Risk of and prophylaxis for venous thromboembolism in hospital patients. *Br Med J* **1992**;305:567-74.

Tilt EJ. The change of life in health and disease. A practical treatise on the nervous and other affections incidental to women after the decline of life. London, **1857**.

Titus SD. De fine mensium initiis morborum variorum opportune. Henckl, Magdeburg, **1710**.

Trevoux R, Dieulangard P, Blum A. Efficacy and safety of Org OD 14 in the treatment of climacteric complaints. *Maturitas* **1983**;5:89-96.

Trouerbach W Th, Birkenhäger JC, Schmitz PIM, van Hemert AM, van Saase JLCM, Collette HJA et al. A cross-sectional study of age related loss of mineral content of phalangeal bone in men and women. *Skeletal Radiol* **1988**;17:338-343.

Tunstall-Pedoe H. Myth and paradox of coronary risk and the menopause. *Lancet* **1998**;351:1425-1427.

Udolff L, Langenberg P, Adashi EY. Continuous combined hormone replacement therapy: A critical review. *Obstet Gynecol* **1995**;86:306-316.

Utian WH, Schiff I. NAMS-Gallup survey on women's knowledge, information sources, and attitudes to menopause and hormone replacement therapy. *Menopause* **1994**;1:39-48.

Vies van der J. Pharmacological studies with (7 alpha,17 alpha)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one (Org OD 14). *Maturitas* **1987**;suppl 1:15-24.

Visser de J, Coert A, Feenstra H, Vies van der J. Endocrinological studies with (7 alpha, 17 alpha)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one (Org OD 14). *Arzneimittelforschung* **1984**;34:1010-1017.

Volpe A, Facchinetti F, Grasso A, Petraglia F, Campanini D, Genazzani AR. Benefits and risks of different hormonal replacement therapies in postmenopausal women. *Maturitas* **1986**;8:327-334.

Vuento MH, Pirhone JP, Makinen JL et al.. Endometrail fluid accumulation in asymptomatic postmenopausal women. *Ultrasound Obstet Gynecol* **1996**;8:37-41.

Walker ID, Davidson, Richards A, Yates R, McEwan HP. The effect of the synthetic steroid Org OD 14 on fibrinolysis and blood lipids in postmenopausal women. *Thromb Haemost* **1985**;53:303-305.

Wardlaw GM,Pike AM. The effect of lactation on peak adult shaft and ultra distal forearm bone mass in women. Am J Clin Nutr **1986**;44:283-286.

Wasnich R, Yano K, Vogel J. Postmenopausal bone loss at multiple skeletal sites: relationship to estrogen use. *J Chron Dis* **1983**;36:781-790.

Wasnich RD, Ross PD, Davis JW, Vogel JM. A comparison of single and multi-site BMC measurements for assessment of spine fracture probability. *J Nucl Med* **1989**;30:1166-1171.

Watts NB, Harris ST, Genant HK et al.. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. *N Engl J Med* **1990**;323:73-79.

Weiss NS, Ure CL, Ballard HJ, Williams AR, Daling JR. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of oestrogen. *N Engl J Med* **1980**;303:1195-1198.

Weijer van de PHM, Barentsen R, Vries de M, Kenemans P. Relationship of estradiol levels to breakthrough bleeding during continuous combined hormone replacement therapy. *Obstet Gynecol* **1999**;93:551-557.

Wenger NK, Speroff L, Packard B. Cardiovascular health and disease in women. *N Engl J Med* **1993**;329:247-256.

Wersch van JWJ, Ubachs JMH, Ende van de A, Enk van A. Effects of two regimens of hormonal replacement therapy on the haemostatic profile in postmenopausal women. *Eur J Clin Chem Clin Biochem* **1994**;32:449-453.

Williams DB, Voigt BJ, Fu YS, Schoenfeld MJ, Judd HL. Assessment of less than monthly progestin therapy in postmenopausal women given estrogen replacement. *Obstet Gynecol* **1994**;84:787-793.

Williams SR; Frenchek B; Speroff T; Speroff L. A study of combined continuous ethinyl estradiol and norethindrone acetate for postmenopausal hormone replacement. *Am J Obstet Gynecol* **1990**;162:438-446.

Wollter Svensson LO; Stadberg E; Andersson K; Mattsson LA; Odlind V; Persson I. Intrauterine administration of levonorgestrel 5 and 10 microg/24 hours in perimenopausal hormone replacement therapy. A randomized clinical study during one year. Acta Obstet *Gynecol Scand*, **1997**;76:449-454.

Woodruff JD, Pickar JH, for The Menopause Study Group. Incidence of endometrial hyperplasia in postmenopausal women taking conjugated estrogens (Premarin) with medroxyprogesterone acetate or conjugated estrogens alone. Am J Obstet Gynecol **1994**;170:1213-1223.

Wornski TJ, Dann LM, Qi H, Yen CF. Skeletal effects of withdrawal of estrogen and diphosphonate treatment in ovariectomized Rats. *Calcif Tissue Int.* **1993**;53:210-216.

Yen SSC. The biology of menopause. *J Reprod Med* **1977**;18:287-296.

Yoshitake K, Yokota K, Kasugai Y, Sukamoto T, Nakamura T. Effects of 16 weeks of treatment with tibolone on bone bone mass and bone mechanical and histomorphometric indices in mature ovariectomized rats with established osteopenia on a low-calcium diet. *Bone* **1999**;25:311-319.

Zandberg P, Peters JLM, Demacker PNM, Smit MJ, Reeder de EG, Meuleman DG. Tibolone prevents atherosclerotic lesion formation in cholesterol-fed, ovariectomized rabbits. *Arterioscler Thromb Vasc Biol* **1998**:18:1844-1854.

Ziel HK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. *N Engl J Med* **1975**:293;1167-1170.

6

Summary / Samenvatting

Chapter 1

This chapter aims at providing the reader with a brief overview of the history and development of knowledge regarding climacteric complaints and hormonal replacement therapy. In more detail, it compares the use of tibolone as hormonal replacement therapy with continuous combined regimens of hormonal replacement and raloxifene.

Chapter 2

Section 2.1 Is there an influence of oral contraceptive use, parity and lactation on early postmenopausal bone mass? Is assessment of reproductive history, body weight in combination with biochemical markers of bone metabolism suitable to predict lumbar bone mass soon after menopause? The present study is a cross-sectional study in 94 healthy, normal weight, non-smoking women, 1-3 years after spontaneous menopause. Bone mineral density (BMD) of the lumbar spine was measured with quantitative computed tomography. Multiple regression analysis showed that only total duration of lactation and alkaline phosphatase (AP) levels are independently related to trabecular BMD (P=0.001, P=0.002 respectively). AP was also associated with cortical BMD (P=0.003). Assessment of reproductive history, body mass index and biochemical markers of bone metabolism could only account for 17% of the variation of trabecular BMD observed in the study population. This study suggests that total duration of lactation rather than parity is associated with trabecular BMD of the spine. Clinical assessment of risk factors unsuccessfully predicts lumbar BMD in healthy early postmenopausal women.

Section 2.2 This randomized, double-blind, placebo-controlled, two-year study is the first to evaluate the effect of 1.25 mg and 2.5 mg tibolone daily oral administration, on trabecular and cortical bone loss in early postmenopausal women. Ninety-four healthy, normal weight, non-smoking women, 1-3 years following spontaneous menopause, participated. Twenty-three subjects were randomised to the placebo group, 36 to the 1.25 mg/d tibolone group and 35 to the 2,5 mg/d tibolone group. Bone density was assessed at 6 months intervals. Spinal trabecular BMD was measured with quantitative computed tomography. Phalangeal cortical BMD was measured by radiographic absorptiometry. The 2-year change versus baseline in the placebo group for trabecular BMD was -6.4% (95% CI -8.1 to -4.7). Cortical BMD did not change significantly. At 24 months both tibolone groups showed a statistically significantly higher trabecular [9.4% (6.6 to 12.2) for the 1,25 mg group and 14.7% (95% CI 11.8 to 17.5%) for the 2.5 mg group] and phalangeal BMD [4.4% (95% CI 1.5 to 7.4) for the 1.25 mg group, and 6.8% (95% CI 3.8 to 9.8) for the 2.5 mg group] as compared to the placebo group. After 2 years of tibolone in both regimes trabecular and phalangeal BMD was significantly higher as compared to pre-treatment values. At 24 months the 2.5 mg group showed a significantly higher trabecular ($p<0.001$) but not phalangeal ($p=0.064$) BMD compared to the 1.25 mg group. Tibolone prevents early postmenopausal bone loss by inducing an increase in trabecular and phalangeal BMD.

Section 2.3 This study assessed the loss of BMD one year after a 2 yr period of hormone replacement therapy (HRT) with two doses of tibolone as compared to placebo in early postmenopausal women. Sixty four out of 84 women (1-3 yrs following spontaneous menopause) who completed a 2 yr randomised, placebo controlled study to evaluate effects of tibolone participated in this follow-up study. Quantitative computed tomography was used to exclusively measure trabecular BMD, micro densitometry of the mid phalangeal shaft was used for estimation of cortical BMD and biochemical markers of bone metabolism were assessed, one year after discontinuation of tibolone. The study group received either placebo (n=16), 1.25 mg/d tibolone (n=25) or 2.5 mg/d tibolone (n=23). Observations revealed a significantly greater decrease in trabecular BMD during the post-trial year in both treatment groups compared to the placebo group [for 1.25 mg/d tibolone: -6.0%, (*95% CI* -8.4 to -3.5); for 2.5 mg/d tibolone: -10.0%, (*95% CI*:-12,9 to -6.9)]. In contrast, there was no significant difference in loss of phalangeal BMD in both treatment groups compared to placebo. Biochemical markers (serum alkaline phosphatase, urinary excretion of hydroxyproline and calcium) do not suggest an increased bone turnover comparing tibolone groups to placebo, one year after cessation of tibolone. The present study suggests an increased loss of trabecular

but not cortical BMD as compared to the placebo group in the first year after cessation of HRT with tibolone in early postmenopausal women.

Section 2.4 This study investigates the potential correlation between vaginal bleeding and estradiol (E2) levels/endometrial morphology in early postmenopausal women using tibolone. A 2 year randomised placebo-controlled study of 94 healthy women, 1-3 years after spontaneous menopause, receiving either placebo (n=23), 1,25 mg/d (n=36) or 2,5 mg/d (n=35) tibolone. Episodes of vaginal bleeding throughout the 2 year study period were recorded. Age, age of menopause, months since menopause and Body Mass Index were recorded. Serum E2 levels were assessed at baseline and at 3 months intervals throughout the study period. In case of vaginal bleeding endometrium morphology was assessed by Vabra Curettage. At least one period of vaginal bleeding occurred in 51% (n=18, P<0.05) of women in the 2,5 mg/d tibolone group and in 44% (n=16, P=0.07) of the 1,25 mg/d tibolone group, compared with 22% (n=5) in the placebo-group. The women who bled in the placebo-group were younger (P<0.01), had menopause at an earlier age (P<0.05), had an shorter duration since menopause (P<0.05) and had a higher median E2 serum level prior to bleeding (P<0.05). In contrast, in both tibolone groups no determinants could be found for the vaginal bleeding. Ninety percent of the first bleedings occurred within 9 months after starting the treatment. At Vabra endometrium sampling there was no evidence of endometrial stimulation. In the present study, early postmenopausal women using 1,25 mg/d or 2,5 mg/d tibolone are 2-2,5 times more likely to present with vaginal bleeding compared to placebo (p<0.05) without evidence of higher serum E2 levels or endometrial stimulation.

Chapter 3

This chapter reviews the evidence for the effects of tibolone on bone. Tibolone is a synthetic steroid with a mixed (estrogenic/progestagenic/androgenic) hormonal profile. Data suggest a complex receptor mediated as well as metabolic regulation of the activity of tibolone at target tissue level. It has been shown that tibolone can prevent axial and appendicular bone loss induced by ovariectomy and/or low calcium diet in young and mature rats. In addition, tibolone increases trabecular and cortical BMD in rats with established osteopenia. In the rat, treatment with tibolone results in an increased strength of the femoral neck and of vertebral body similar to estrogens. The protective effect on bone can be blocked by anti-estrogens indicating that the effect is estrogen-receptor mediated.

Clinical trials have shown that loss of bone in the spine and proximal hip can be prevented with tibolone 2,5 mg/d in early and late postmenopausal women. In addition, a dose of 1,25 mg/d seems to be effective as well, especially in late postmenopausal women. In women with established osteoporosis, bone density of the axial and appendicular skeleton increases with tibolone. In comparative studies, tibolone 2,5mg/d seems as effective as conventional HRT regimens. There are no direct comparative studies between tibolone and biphosphonates or raloxifene. Furthermore, to establish the efficacy of tibolone for prevention osteoporotic fractures, studies on the magnitude of reduction in fracture risk remain to be conducted.

Finally, tibolone seems to be effective in preserving bone density in patients treated with gonadotropin-releasing hormone agonist.

Chapter 4

This chapter summarises the conclusions that can be drawn from this thesis and gives directions for future research.

Samenvatting (Dutch)

Hoofdstuk 1

Dit hoofdstuk geeft een overzicht van de historie en ontwikkeling van de kennis betreffende climacteriële klachten en hormonale substitutietherapie (HST). Tevens wordt het gebruik van tibolone als HST vergeleken met substitutietherapie door middel van continu gecombineerde oestrogeen-progestageen preparaten en raloxifene.

Hoofdstuk 2

Paragraaf 2.1 Beïnvloedt het gebruik van orale anticonceptiva, pariteit en lactatie de vroeg postmenopauzale botmassa? Voorspelt de gynaecologische/obstetrische voorgeschiedenis, lichaamsgewicht gecombineerd met biochemische markers van het botmetabolisme de botdichtheid van de wervelkolom vlak na de menopauze? Het betreft hier een cross-sectionele studie met 94 gezonde, niet rokende, postmenopauzale vrouwen, 1-3 jaar na de spontane

menopauze. Trabeculaire botdichtheid van de lumbale wervelkolom werd gemeten met behulp van quantitatieve computer tomografie. Uit de multipele regressie-analyse blijkt dat alleen totale duur van lactatie en serumwaarde van het alkalisch fosfatase onafhankelijk van elkaar geassocieerd zijn met de trabeculaire botdichtheid van de lumbale wervelkolom (respectievelijk: P=0.001 en P=0.002). Alkalisch fosfatase was ook geassocieerd met de corticale botdichtheid (P=0.003). Parameters van de gynaecologische en obstetrische voorgeschiedenis, alsmede lichaamsgewicht en markers van het botmetabolisme verklaren slechts 17% van de variatie in trabeculaire botdichtheid van de lumbale wervelkolom. De gevonden resultaten suggereren dat totale duur van lactatie en niet pariteit geassocieerd is met de botdichtheid van de lumbale wervelkolom. Tevens blijkt dat met evaluatie van klinische risicofactoren, in een gezonde populatie van vroeg postmenopauzale vrouwen, de lumbale botdichtheid van de wervelkolom niet voorspeld kan worden.

Paragraaf 2.2 Het effect van twee doseringen tibolone (1.25 mg - en 2.5 mg per dag) op het trabeculaire en corticale botverlies wordt geëvalueerd. Het betreft een tweejarig prospectief, gerandomiseerd, dubbelblind, placebo gecontroleerd onderzoek. Vierennegentig gezonde postmenopauzale vrouwen, 1 tot 3 jaar na de spontane menopauze worden in een 2:3:3 ratio gerandomiseerd in een placebogroep en twee behandelgroepen. Botdichtheid van het trabeculaire bot van de lumbale wervelkolom wordt gemeten met behulp van kwantitatieve computer tomografie. Het corticale bot van de phalanx wordt gemeten met behulp van radiologische absorptiometrie. In de placebogroep bedraagt het verlies aan trabeculaire botdichtheid 6.4 %, over een periode van 2 jaar. Er is geen significant verlies van corticale botdichtheid gedurende deze periode. Na 2 jaar is er sprake van een significant hogere botdichtheid in de beide behandelgroepen vergeleken met de placebogroep. Voor de trabeculaire botdichtheid bedraagt het verschil 9.4% (95% betrouwbaarheidsinterval: 6.6% tot 12.2%) en 14.7% (95% betrouwbaarheidsinterval: 11.8% tot 17.5%) in respectievelijk de 1.25 mg behandelgroep en de 2.5 mg behandelgroep. Voor de corticale botdichtheid bedraagt het verschil 4,4% (95% betrouwbaarheidsinterval: 1.5% tot 7.4%) en 6.8% (95% betrouwbaarheidsinterval: 3,8% tot 9,8%) in respectievelijk de 1.25 mg behandelgroep en de 2.5 mg behandelgroep. Na 2 jaar is zowel de trabeculaire als corticale botdichtheid in beide tibolone behandelgroepen significant gestegen. In tegenstelling tot de corticale botdichtheid is na 2 jaar de trabeculaire botdichtheid in de 2.5 mg behandelgroep significant hoger dan de trabeculaire botdichtheid in de 1.25 mg behandelgroep. Uit bovenstaande blijkt dat dagelijkse

inname van zowel 1.25 mg als 2.5 mg tibolone niet alleen vroeg postmenopauzaal verlies van botmassa voorkomt, maar de botmassa zelfs doet toenemen.

Paragraaf 2.3 Deze studie evalueert het verlies van trabeculaire en corticale botdichtheid na behandeling met 1.25 mg of 2.5 mg tibolone. Tevens wordt het verlies vergeleken met een placebogroep. Vierenzestig, van totaal vierentachtig vroeg postmenopauzale vrouwen, welke het in paragraaf 2.2 beschreven onderzoek voltooiden, nemen deel aan deze "follow-up"studie. Eén jaar na voltooiing van het eerder beschreven onderzoek wordt de trabeculaire en corticale botdichtheid gemeten (voor methode zie paragraaf 2.2). Ook worden markers van het botmetabolisme (serum alkalisch fosfatase, in de urine uitgescheiden hydroxyproline en calcium) bepaald. Er is sprake van een versneld verlies van trabeculaire botdichtheid, na 2-jarige behandeling met 1.25mg of 2.5mg tibolone, vergeleken met de placebogroep. Het trabeculaire verlies van botdichtheid bedroeg 6% (95% betrouwbaarheidsinterval: -8,4% tot –3,5%) in de 1.25 mg behandelgroep en 10% (95% betrouwbaarheidsinterval: -12,9 tot –6.9) in de 2.5 mg behandelgroep. In tegenstelling tot het verlies van trabeculaire botdichtheid kan geen significant verschillend verlies van corticale botdichtheid worden aangetoond. Eén jaar na behandeling met tibolone tonen biochemische markers voor botmetabolisme geen verhoogde botombouw in de behandelgroepen vergeleken met de placebogroep. De resultaten van deze studie wijzen in de richting van een verhoogd verlies van trabeculaire botdichtheid maar niet van corticale botdichtheid, na het staken van HST met tibolone.

Paragraaf 2.4 Deze studie onderzoekt de eventuele relatie tussen vaginaal bloedverlies tijdens gebruik van tibolone enerzijds en endogene oestrogeenspiegels en morfologie van het endometrium anderzijds. Het betreft een 2 jaar durende gerandomiseerde placebo gecontroleerde studie waarin 94 gezonde postmenopauzale vrouwen 1 tot 3 jaar na hun spontane menopauze deelnemen. Randomisatie resulteert in een placebogroep (n=23), 1.25 mg tibolone groep (n=36) en een 2.5 mg tibolone groep (n=35). Episoden van vaginaal bloedverlies gedurende studieperiodes worden geregistreerd evenals leeftijd, leeftijd ten tijde van de menopauze, aantal maanden na de menopauze en de Quetelet Index. Serum oestradiol spiegel wordt voor aanvang van het onderzoek en vervolgens elke drie maanden gedurende de studieperiode bepaald. Indien vaginaal bloedverlies optreedt wordt een Vabra Currettage uitgevoerd. Tenminste één episode van vaginaal bloedverlies treedt op bij 51% (n=18, P<0.005) van de vrouwen in de 2,5mg tibolone groep en in 44% (n=16, P=0.07) van de vrouwen in de 1.25 mg tibolone groep, vergeleken met 22% (N=5) van de vrouwen in de

placebogroep. De vrouwen bij wie vaginaal bloedverlies optreedt in de placebogroep, zijn jonger (P<0.01), hebben een jongere leeftijd ten tijde van de menopauze (P<0.05) en de mediane oestrogeenspiegel voorafgaand aan de bloeding was hoger (P<0.05). In tegenstelling tot de placebogroep, kan bij de tibolone behandelgroepen geen significante factoren voor het optreden van vaginaal bloedverlies worden gevonden. Negentig procent van de eerste vaginale bloedingen tijdens het gebruik van tibolone treedt op in de eerste 9 maanden. De verrichte Vabra Curettages lieten geen aanwijzingen zien voor stimulatie van het endometrium. In deze studie blijkt de kans op vaginaal bloedverlies, tijdens het gebruik van 1.25 mg of 2.5 mg tibolone, 2 tot 2.5 maal zo groot vergeleken met het gebruik van een placebo (p<0,05), zonder dat er aanwijzingen zijn voor een verhoogde endogene oestrogeenspiegel of stimulatie van het endometrium.

Hoofdstuk 3

Dit hoofdstuk geeft een overzicht van de tot nu toe gepubliceerde studieresultaten met betrekking tot het effect van tibolone op het bot. Tibolone is een synthetisch steroïd met gemengd (oestrogeen/progestageen/androgeen) hormoonprofiel. Studieresultaten over de weefselspecificiteit van tibolone wijzen in de richting van een receptor afhankelijk, alsmede metabool afhankelijk mechanisme dat de activiteit van tibolone op het niveau van het doelorgaan regelt. Uit dierstudies blijkt dat tibolone in staat is botverlies tengevolge van ovariëctomie of calcium arme voeding, te verhinderen in zowel jonge als volwassen ratten. Bovendien neemt zowel de trabeculaire als corticale botdichtheid toe indien osteopenische ratten worden behandeld met tibolone. Uit studies met ratten blijkt dat behandeling met tibolone leidt tot een toename in botsterkte van het femur en wervellichaam. Dit effect is vergelijkbaar met dat van oestrogenen. Toevoeging van een anti-oestrogeen blokkeert het beschermende effect van tibolone op het bot van de rat. Dit suggereert dat het effect van tibolone op bot plaatsvindt via de oestrogeenreceptor. Klinische studies tonen aan dat verlies van botdichtheid in heup en wervellichaam van vroeg postmenopauzale en oudere postmenopauzale vrouwen kan worden voorkomen door behandeling met 2.5 mg tibolone. Een dosering van 1.25 mg tibolone bliikt ook verlies van botdichtheid te kunnen verhinderen, met name bij oudere postmenopauzale vrouwen. In vrouwen met osteoporose wordt, tijdens de behandeling met tibolone, een toename van botdichtheid van zowel het perifere- als axiale skelet gezien. Uit vergelijkende studies blijkt tibolone even effectief als conventionele HST te

zijn. Er zijn geen vergelijkende studies tussen tibolone enerzijds en bifosfanaten en raloxifene anderzijds. Om de effectiviteit van tibolone met betrekking tot het verminderen van osteoporotische fracturen aan te tonen, moet nog gerandomiseerd onderzoek met osteoporotische fracturen als eindpunt, worden verricht. Tot slot lijkt tibolone effectief in het voorkomen van botdichtheidsverlies in patiënten die worden behandeld met een gonadotropin-releasing hormone agonist.

Hoofdstuk 4

Dit hoofdstuk geeft een overzicht van de studieresultaten van dit proefschrift en doet suggesties voor toekomstig onderzoek.

7

Publications

List of publications included in the present thesis
(numbers in bold refer to chapter sections)

2.1 Berning B, van Kuijk C, Schütte HE, Kuiper JW, Drogendijk AC, Fauser BCJM. Determinants of lumbar bone mineral density in normal weight, non-smoking women soon after menopause. A study using clinical data and quantitative computed tomography. *Bone Miner* **1993**;21:129-139.

2.2 Berning B, van Kuijk C, Kuiper JW, Coelingh Bennink HJT, Kicovic PM, Fauser BCJM. Effects of two doses of Tibolone on trabecular and cortical bone loss in early postmenopausal women: A 2-year randomized, placebo-controlled study. *Bone* **1996**;19:395-399.

2.3 Berning B, van. Kuijk C, Kuiper JW, Coelingh Bennink HJT, Fauser BCJM. Increased loss of trabecular but not cortical bone density, 1 year after discontinuation of 2 year hormone replacement therapy with Tibolone. *Maturitas* **1999**;31:151-159.

2.4 Berning B, van. Kuijk C, Coelingh Bennink HJT, Fauser BCJM. Absent correlation between vaginal bleeding and oestradiol levels or endometrial morphology during tibolone use in early postmenopausal women. *Maturitas* **2000**;35(1):81-88.

3 Berning B, Coelingh-Bennink HJT, Fauser BCJM. Tibolone and bone: a review. *Climacteric* **2001**;4:120-136.

Publications related to the present thesis

Smeets AJ, Kuiper JW, van Kuijk C, Berning B, Zwamborn AW. Skin thickness does not reflect bone mineral density in postmenopausal women. *Osteoporosis Int;* **1994**:4(1):32-35.

Abstracts/presentations related to the present thesis

van Kuijk C, Berning B, Kuiper JW, Zwamborn AW, Kicovic P, Coelingh Bennink HJT, Fauser BCJM. Prevention of early postmenopausal bone loss with Tibolone. *J Bone and Mineral Res* **1994**;9(Suppl 1):S325.

Berning B, Fauser BCJM. Effects of two doses of Org Od 14 on Lumbar Trabecular Bone Mineral Density in early postmenopausal women. **1994** XIV FIGO World Congress, Montreal, Canada.

Berning B, van Kuijk C, Kicovic P, Coelingh Bennink HJT, Fauser BCJM. Effects of withdrawal of HRT with Tibolone on bone mineral density in early postmenopausal women. **1996** World congress on osteoporosis 18-23 May Amsterdam, the Netherlands.

Berning B, Kicovic PM, Fauser BCJM, Coelingh Bennink HJT. Bleeding rates during a long-term placebo- controlled study with two doses of Tibolone. **1996** 8th International Congress on the Menopause 3-7 November Sydney, Australia.

Berning B. Fauser BCJM. Bot voor de massa? **1997** Gynaecongres november te Papendal.

Berning B. De rol van tibolone bij de preventie van osteoporose. **2001** IWO-bijeenkomst, 11april te Utrecht.

8

Curriculum vitae auctoris

De schrijver van dit proefschrift, Bernd Berning, werd geboren op 10 januari 1963 te Lüdenscheid, Duitsland. De studie Geneeskunde werd in december 1981 begonnen aan de Erasmus Universiteit te Rotterdam. Het doctoraal examen werd in mei 1987 behaald en op 19 mei 1989 was het artsexamen een feit. Al voor de co-schappen viel de keuze op het specialisme Gynaecologie / Verloskunde. Zowel het keuze-onderzoek op de afdeling Gynaecologie en Verloskunde van het Academisch Ziekenhuis Rotterdam, Dijkzigt, als het co-schap Gynaecologie/Verloskunde in het Elisabeth Hospitaal te Willemstad, Curaçao hebben de wens om gynaecoloog te worden versterkt. Van 1 september 1989 tot 1 april 1990 was eerdergenoemde werkzaam als arts-assistent Gynaecologie en Verloskunde in het Spaarne Ziekenhuis te Haarlem. Hierna werd onder toeziend oog van Prof. Dr. B.C.J.M. Fauser de basis gelegd voor dit proefschrift, als arts-onderzoeker verbonden aan de afdeling Gynaecologie en Verloskunde van het Academisch Ziekenhuis Rotterdam, Dijkzigt. Vanaf 1 september 1992 tot 1 maart 1993 was promovendus werkzaam op de afdeling Gynaecologie/Verloskunde van het Academisch Ziekenhuis Rotterdam, Dijkzigt. Vanaf 1 maart 1993 tot 1 maart 1999 was eerdergenoemde werkzaam als arts-assistent in opleiding op de afdeling Gynaecologie en Verloskunde van het Academisch Ziekenhuis Rotterdam, Dijkzigt (opleider: Prof. Dr. H.C.S. Wallenburg/Prof. Dr. Th J.M. Helmerhorst) en op de afdeling Gynaecologie en Verloskunde van het Reinier de Graaf Gasthuis te Delft (opleider: Dr. J.C. Kuypers). Vanaf 1 april 1999 werkt promovendus met veel plezier als gynaecoloog in het Ziekenhuis Leyenburg te 's-Gravenhage.

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