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The Phytoestrogens, Calcitonin and Thyroid Hormones: Effects on Bone Tissue

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1. Introduction

The skeleton is a metabolically active organ that undergoes remodeling throughout life. This involves a complex process by which old bone is continuously replaced by new tissue. Bone remodeling refers to the sequential, coupled actions of osteoclasts and osteoblasts. In conditions of sex hormone deficiency during advancing age, after the menopause or andropause, the rate of remodeling increases and bone formation is reduced relative to resorption. These alterations can cause microarchitectural deterioration of bone tissues, which increases bone loss as a predisposition to the occurrence of osteoporosis (Rehman et al., 2005). However, in contrast to postmenopausal osteoporosis in women, age-related bone loss in men is less well defined.

Numerous studies attest to the importance of estrogen in bone remodeling, evident from the finding that hormone replacement therapy (HRT) administered in a dose-dependent manner effectively prevented bone loss in postmenopausal women (Lindsay et al., 1976, 1984). However, in addition to protective effects on bone, HRT is associated with an increased risk for breast, endometrial, ovarian or prostate cancers (Davison & Davis, 2003; Loughlin & Richie, 1997; Nelson et al., 2002). Therefore, it is important to examine alternative approaches for prevention and treatment of osteoporosis without side effects. It is well known that the incidence of osteoporosis-related fractures is significantly lower in Southern and Eastern Asian women than in Western women (Tham et al., 1998). One possible reason for this difference is a high intake of phytoestrogen-rich plants, which Asian people eat more often than Western people (Ho et al., 2003). As a result, over the past decade a number of clinical trials for prevention of bone loss have assessed the effectiveness of plant derived non-steroidal phytoestrogens found in a wide variety of foods, most notably soybean. Isoflavones, which include daidzein and genistein are a class of phytoestrogens that act like estrogens. Since these compounds bind to estrogen receptors (ERs) and have estrogen-like activity (Branca, 2003), they have attracted much attention because of their potential benefit in the prevention and treatment of osteoporosis.

In addition to the phytoestrogen-mediated protective mechanisms against bone loss, recent evidence suggests that daidzein may also act on rat bone tissue through enhancement of thyroid C cell activity (Filipović et al., 2010). Namely, thyroid C cells produce the hormone, calcitonin (CT), which lowers plasma calcium concentration by suppressing osteoclast activity. Synthesis of CT and its release from C cells were decreased in conditions of gonadal hormone

deficiency (Filipović et al., 2003, 2007; Isaia et al., 1989; Lu et al., 2000; Sakai et al., 2000). Due to its osteoprotective properties, CT is widely applied in the therapy of osteoporosis.
It is known that parathyroid hormone (PTH) is a major factor involved in the systemic regulation of bone resorption. Phytoestrogens may affect the parathyroid gland and reduce PTH secretion (Wong et al., 2002), suggesting that one way in which these compounds inhibit bone loss may be through reducing PTH levels.
Thyroid hormones are essential for normal bone maturation *in utero* and during early life. In adults an excess of thyroid hormones in the body affects the remodeling system in cortical and trabecular bone and may contribute to the development of osteoporosis (Kung, 1994). Receptors for these hormones are present in bone cells and they may directly increase bone resorption (Abu et al., 1997; Rizzoli et al., 1986). Additionally, thyroid-stimulating hormone (TSH), which stimulates the release of thyroid hormones, positively influences bone remodeling. Therefore, demonstrating both anabolic and antiresorptive effects, TSH may represent a promising candidate for the treatment of osteoporosis (Sendak et al., 2007).
In this chapter we will describe the known effects of phytoestrogens on bone. In addition to the direct action of these plant compounds, special attention will be paid to their influence on thyroid C and follicular cells, as producers of CT and thyroid hormones, using the latest data in the literature and our own results. These hormones, together with PTH may be involved in the indirect effects of phytoestrogens on bone tissue.

2. Bone cells and bone remodeling

Bone is a dynamic organ that undergoes remodeling throughout life. This process results from the separate action of bone forming cells called osteoblasts and bone resorbing cells called osteoclasts. Osteoblasts are responsible for the production of bone matrix constituents and are found in clusters on bone surfaces (Fig 1). They originate from multipotent mesenchymal stem cells, which have the capacity to differentiate into osteoblasts or other cells, such as adipocytes, chondrocytes, myoblasts and fibroblasts (Bianco et al., 2001). A mature osteoblast that is trapped in the bone matrix and remains isolated in lacunae becomes an osteocyte. (Fig.1). Bone formation involves production and maturation of the osteoid matrix, followed by mineralization of the matrix. Osteoblasts produce growth factors, such as insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF- β) and bone morphometric protein (BMP) (Canalis et al., 1993, 1993a; Chen et al., 2004; Globus et al., 1989; Rydzel et al., 1994). These factors regulate osteoblast activity in an autocrine and paracrine manner.
Osteoclasts are large multinucleate cells responsible for bone resorption. They are derived from hematopoetic cells of the mononuclear lineage (Teitelbaum, 2000) (Fig.1). Osteoclasts have an abundant Golgi complex, mitochondria and transport vesicles loaded with lysosomal enzymes, such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K. These enzymes are secreted via the specialized (ruffler border) plasma membrane of osteoclasts into the bone-resorbing compartment (Väänänen et al., 2000). The process of osteoclast attachment to the bone is complex and involves binding of integrins expressed in osteoclasts with specific amino acid sequences within proteins at the surface of the bone matrix and cytoskeleton activation (Davies et al., 1989; Reinholt et al., 1990). Dynamic structures, called podozomes allow movement of osteoclasts across the bone surface. Bone resorption occurs due to acidification and proteolysis of the bone matrix. As a result of this resorptive activity in contact

with the surface of calcified bone, osteoclasts create resorptive lacunae. Osteoclast function is regulated both by locally acting cytokines and by systemic hormones.

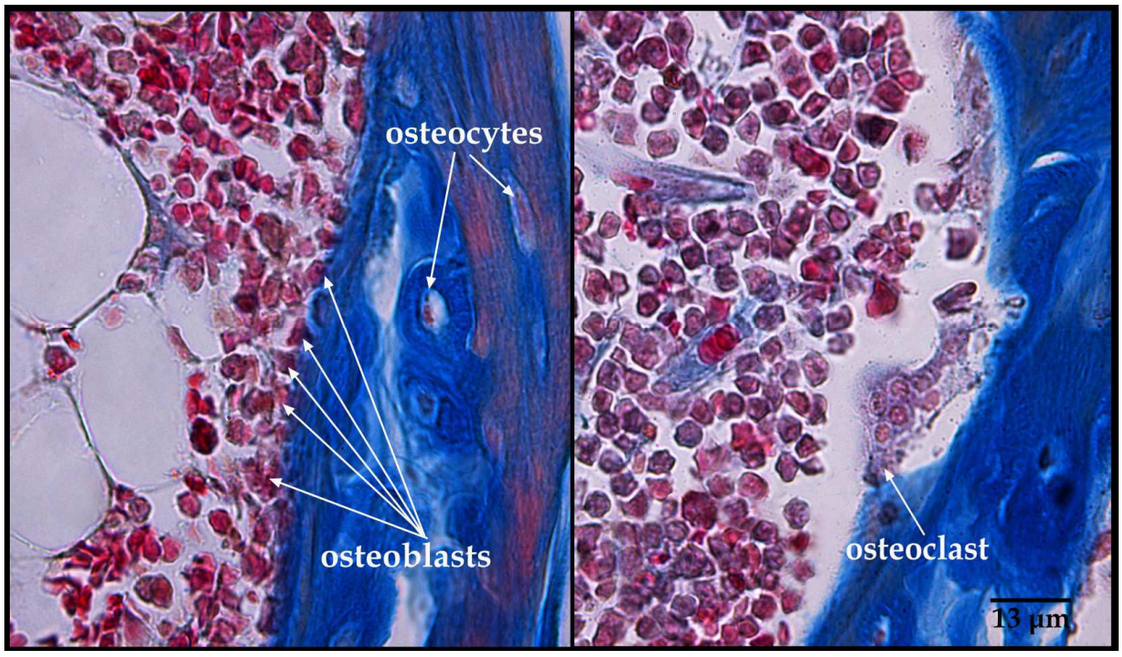

Fig. 1. Bone cells – osteoblasts, osteocytes and osteoclasts; unpublished image of Filipović et al.

In homeostatic equilibrium, bone resorption and formation are balanced. It appears that osteoclasts and osteoblasts closely collaborate in the remodeling process in what is called a "Basic Multicellular Unit", or BMU. This indicates that a coupling mechanism must exist between formation and resorption (Frost, 1964), although its nature is not known. Organization of the BMU in cortical and trabecular bone differs. Between 2% and 5% of cortical bone is remodeled each year. The remodeling process in trabecular bone is mainly a surface event. Due to the much larger surface to volume ratio, it is more actively remodeled than cortical bone, with remodeling rates that can be up to 10 times higher (Lee & Einhorn, 2001).

The remodeling cycle consists of three consecutive phases: resorption, reversal and formation. Resorption begins with the migration of partially differentiated preosteoclasts, which form multinucleated osteoclasts on the bone surface. During the reversal phase, mononuclear cells prepare the resorption lacunae for bone formation and provide signals for osteoblast differentiation and migration (Eriksen et al., 1990). Bone formation starts with activation of preosteoblasts to differentiate into osteoblasts. They secrete bone-matrix proteins to form the organic matrix, which is later mineralized. During this period, osteoblasts completely replace the resorbed bone by new tissue. After this phase, the surface is covered with flattened lining cells and a prolonged resting period ensues until a new remodeling cycle is initiated. Duration of the resorption phase is about 2 weeks, the reversal phase lasts for up to 4 or 5 weeks, while the formation phase can continue for 4 months.

At each remodeling site, bone resorption is coupled with bone formation, locally released growth factors and cytokines acting as mediators of this process (Canalis et al., 1988; Mundy, 1995). The decrease of bone mass, which may be due to different causes, is a

consequence of an imbalance between the amount of mineral and matrix removed and that subsequently incorporated into each resorption cavity (Kanis et al., 1990).

3. Phytoestrogens in bone protection

Phytoestrogens are structurally and functionally similar to estrogens and their estrogenic activity may occur through ERs. There are three main classes of phytoestrogens: isoflavonoids, coumestans and lignans (Fig. 2). Due to their estrogenic and anti-estrogenic activity, they are termed - natural selective ER modulators (SERMs). Therefore, soybean isoflavones have received great attention as alternatives to HRT for the prevention of postmenopausal osteoporosis. Genistein and daidzein, the main isoflavones in soybean, may protect against osteoporosis, because they can affect both types of bone cells.

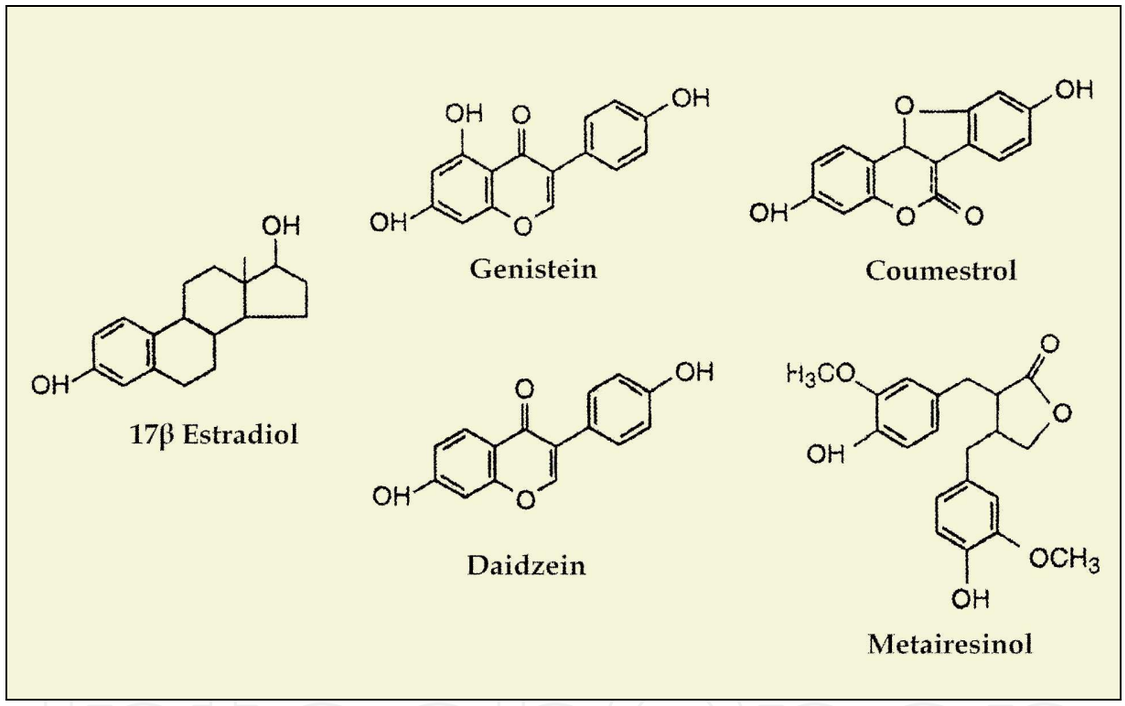

Fig. 2. Structure of 17β estradiol, isoflavones (genistein and daidzein), coumestan (coumestrol) and lignans (metairesinol); Filipović et al.

Isoflavones can stimulate the proliferation and differentiation of osteoblasts. Thus, the presence of genistein or daidzein led to a significant increase in protein synthesis, alkaline phosphatase activity, and DNA content in cultures of osteoblastic MC3T3-E1 cells (Sugimoto & Yamaguchi, 2000, 2000a; Yamaguchi & Sugimoto, 2000).

In addition to a stimulating effect on bone formation, these plant compounds may also suppress osteoclastic bone resorption in vitro. Thus, genistein was found to induce apoptosis of osteoclasts isolated from rat femoral tissues. Daidzein also decreased the number of these bone resorbing cells in rats (Gao & Yamaguchi, 1999) and their development in cultures of porcine bone marrow (Rassi et al., 2002). Osteoclast activity is regulated by phosphorylation of cell membrane constituents, involving tyrosine kinases. As a naturally tyrosine kinase inhibitor, genistein was found to suppress avian osteoclastic

activity through inhibition of tyrosine kinase (Blair et al., 1996). Genistein also caused a significant increase in tyrosine phosphatase activity, which is a negative regulator of osteoclastogenesis and osteoclast-resorbing activity in mutant mice (Aoki et al., 1999; Gao &Yamaguchi, 2000) (Fig 3).

While investigations in vitro give clues about the effects of isoflavones on individual bone cells, studies in vivo provide knowledge about their influence in intact systems. Aged gonadectomized female and male rodents are suitable animal models for studying osteoporosis (Comelekoglu et al., 2007; Filipović et al., 2007; Pantelić et al., 2010; Vanderschueren et al., 1992.) Using them it has been demonstrated that isoflavones can prevent bone loss in female rats and mice after ovariectomy (Ovx) (Blum et al., 2003; Erlandsson et al., 2005; Fonseca & Ward, 2004; Ishimi et al., 1999; Lee et al., 2004; Om & Shim, 2007; Ren et al., 2007; Wu et al., 2004). The bone-preventing effects of isoflavones were also confirmed in male orchidectomized (Orx) rats and mice (Filipović et al., 2010; Ishimi et al., 2002; Khalil et al., 2005; Soung et al., 2006; Wu et al., 2003). On the contrary, some studies showed that isoflavones had minimal or no effects on bone loss in animal models (Bahr et al., 2005; Nakai et al., 2005; Picherit et al., 2001). Moreover, in the monkey, a nonhuman primate, dietary isoflavones do not effectively prevent ovariectomy-induced bone loss (Register et al., 2003). However, others suggested that soy phytoestrogens were protective against loss of bone volume (Ham et al., 2004).

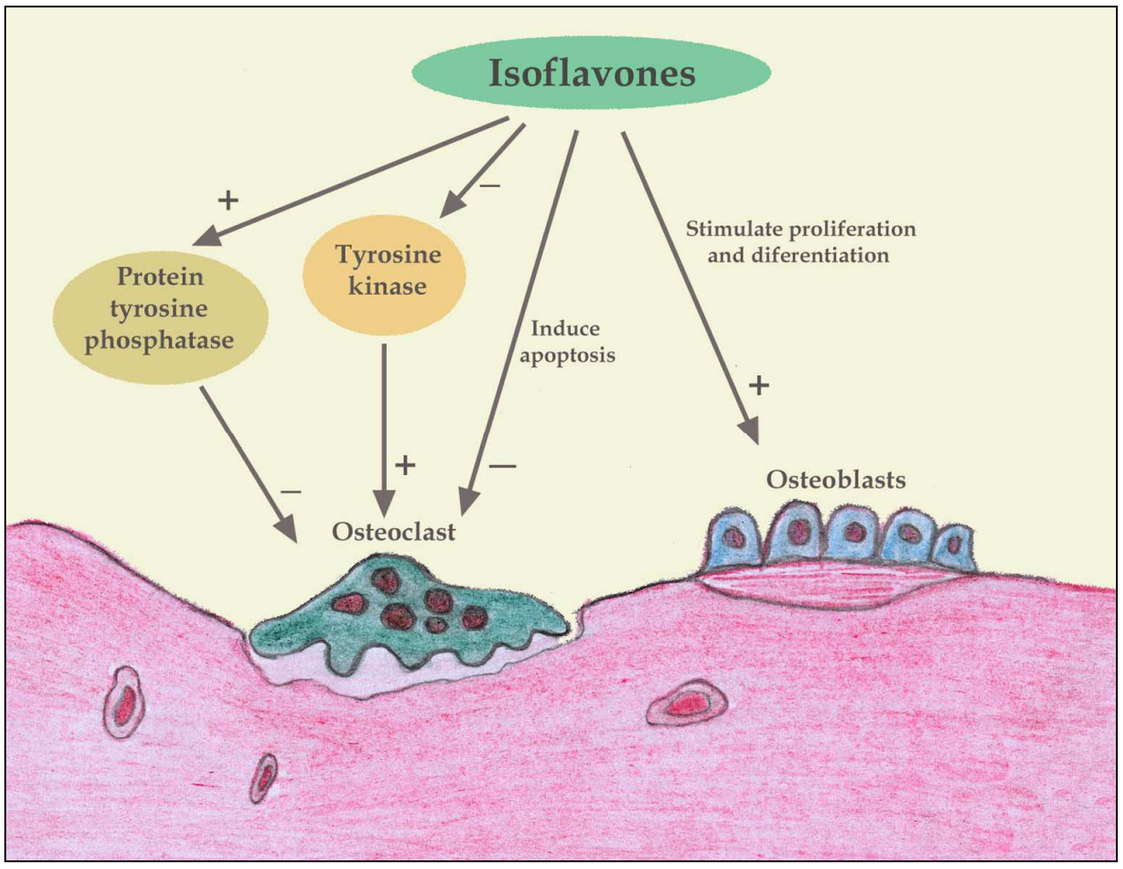

Fig. 3. Influence of isoflavones on bone cells; Filipović et al.

During recent years, numerous human studies have evaluated the effect of soy protein-containing isoflavones or pure isoflavones on bone mass. However, the results of these observational and dietary interventional investigations have been variable and conflicting. In general, isoflavone supplementation studies indicate a beneficial effect on bone mass (Huang et al., 2006; Lydeking-Olsen et al., 2004; Newton et al., 2006), no effect (Anderson et al., 2002; Arjmandi et al., 2005; Brink et al., 2008; Wu et al., 2006) or a possible negative effect in terms of increased circulating concentrations of biochemical markers associated with bone resorption (Geppert et al., 2004; Wanger et al., 2000).

The large heterogeneity of these results may be due to study design, differences regarding hormonal status of the subjects, together with the duration, type and dose of isoflavone supplementation. In addition, bone sparing benefits may depend on the extent of conversion of isoflavones to metabolites. Thus, equol binds with greater affinity to ERs than daidzein from which it is derived (Setchell et al., 2002). Equol production is dependent on the intestinal microflora and there are large interindividual differences in this metabolism. Some people produce more equol than others. Also, production of this metabolite may at least partially explain why the beneficial effects of isoflavones observed in laboratory rodents, which consistently produce high levels of equol, have not been easily recapitulated in humans, where this is not the case. Generally, the relative importance of phytoestrogens in human health must be resolved and longer-term studies are needed to determine their effects on human bone tissue.

4. Phytoestrogens – Mechanisms of action in bone

Although the mechanisms by which soy phytoestrogens may alter bone remodeling are still not completely known, Atmaca et al. (2008) state that they act on both osteoblasts and osteoclasts through genomic and nongenomic pathways.

Due to their low molecular weight these plant compounds can pass through cell membranes and interact with receptors and enzymes (Adlercreutz et al., 1998). Phytoestrogens possess estrogenic activity and act as natural SERMs. This suggests that their effect on bone can be achieved by binding to ERs. Both α and β subtypes of ERs have been identified in bone (Arts et al., 1997; Onoe et al., 1997). The protective effect of phytoestrogens is probably achieved mainly through binding to ER-β, the expression of which is increased during bone mineralization (Arts et al., 1997; Kuiper et al., 1998). In addition to ERs, phytoestrogens can bind to androgenic receptors and act as phytoandrogens (Chen & Chang, 2007).

Both genistein and daidzein stimulate osteoblast proliferation, differentiation and activation by an ER-dependent mechanism (De Wilde et al., 2004; Pan et al., 2005). These isoflavones regulated the synthesis of core binding factor-1 (Cbfa-1) and bone morphogenic protein-2 (BMP-2), which is involved in the differentiation of osteoblasts (De Wilde et al., 2004; Jia et al., 2003; Pan et al., 2005). Genistein and daidzein activate peroxisome proliferator activator receptors (PPARs). The balance between PPAR and ER activation may govern the balance between adipogenesis and osteoblastogenesis (Dang et al., 2003, 2004).

Osteoclasts express the receptor activator of nuclear factor kappa B (RANK) (Hsu et al., 1999), while the receptor activator of nuclear factor kappa B ligand (RANK-L) and osteoprotegerin (OPG) is expressed by osteoblasts (Udagawa et al., 1999). Binding of RANKL to RANK stimulates osteoclastogenesis, whereas binding of RANK-L to OPG prevents RANK-L – RANK binding and indirectly inhibits osteoclastogenesis (Fuller et al., 1998; Theoleyre et al., 2004). The relative levels this triad of proteins are important for

controlling osteoclastogenesis. It was shown that isoflavones may increase the activity of osteoblasts by stimulating the secretion of OPG and RANK-L (De Wilde et al., 2004; Yamagishi et al., 2001) (Fig. 4).

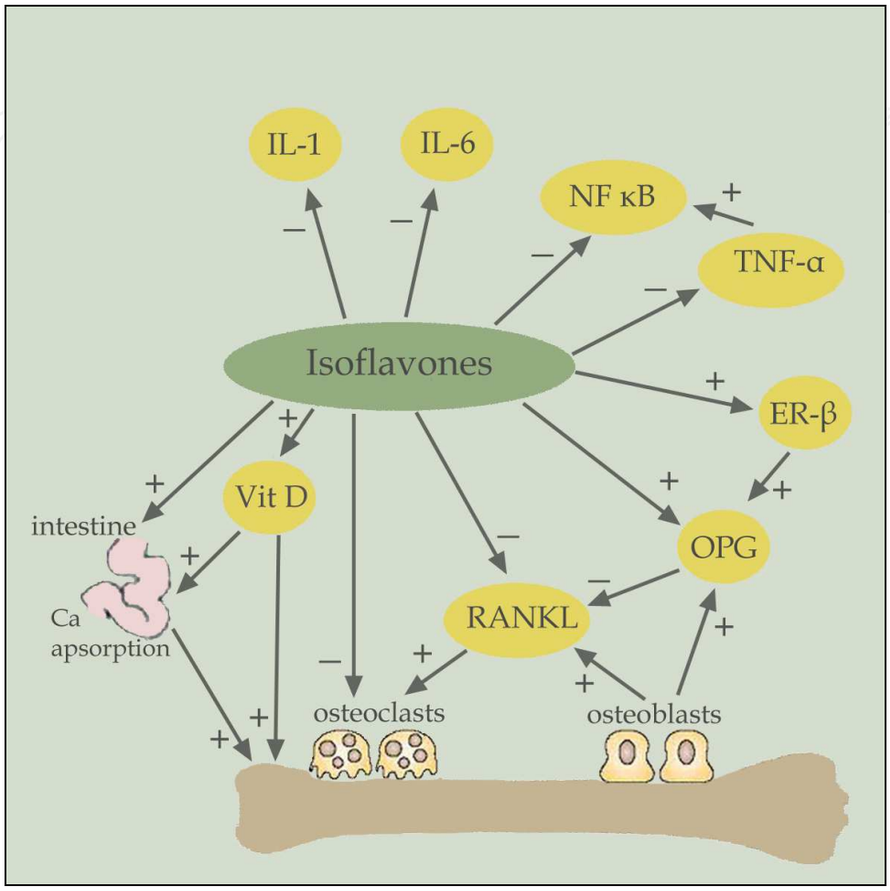

Fig. 4. Mechanisms of isoflavone action in bone; Filipović et al.

Proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α), stimulate osteoclastogenesis and bone resorption. These effects can be achieved by both RANK-L dependent and RANK-L-independent mechanisms (Collin-Osbody et al., 2001; Katagiri et al., 2002). Isoflavones have been shown to inhibit IL-6 synthesis by MC3T3-E1/4 osteoblast-like cells in vitro (Chen et al., 2003; Suh et al., 2003) and to reduce serum IL-1β and TNF-α concentrations in Ovx rats (Li, 2003). Also, a soy supplemented diet may inhibit serum concentrations of proinflammatory cytokines in postmenopausal women (Huang et al., 2005).
In addition to osteoclastogenesis, isoflavones appear to influence osteoclast activity through inhibition of inward rectifier K+channels in osteoclasts. This leads to membrane depolarization, intracellular influx of Ca2+ and inhibition of bone resorption (Okamoto et al., 2001). One beneficial effect of isoflavones on bone is increased intestinal calcium absorption (Fig. 4). However, it is not known whether the mechanism(s) by which isoflavones influence calcium absorption include interactions with intestinal ER and/or vitamin D receptor-mediated calcium transport or not (Arjmandi et al., 2002).

Nongenomic effects do not involve ERs. These effects of phytoestrogens include inhibition of tyrosine kinase which directly modulate osteoclastic acid secretion (Blair et al., 1996; Williams et al., 1998) or topoisomerase I and II, which helps to regulate cell differentiation and the cell replication cycle (Okura et al., 1998; Yamagishi et al., 2001).

5. Indirect effects of phytoestrogens on bone – The role of calcitonin, parathyroid and thyroid hormones

5.1 Effects of phytoestrogens on thyroid C cells and calcitonin production

Thyroid C cells are dispersed neuroendocrine cells that produce many bioregulatory peptides, among which CT is considered the most important. This calcium regulating hormone lowers plasma calcium concentration by inhibiting osteoclast activity. In addition to sex steroids, a voluminous literature has accumulated for therapeutic use of CT in treating osteoporosis. Thus, C cells may also be very important in the pathogenesis of osteoporosis.

The C cells are mostly located in the middle of the thyroid lobes and appear in clusters or as solitary cells between follicular cells and the capillary wall. They have a round, elliptical or polygonal shape and never face the follicular lumen. The nucleus is located in the center of the cell. The most salient ultrastructural feature of C cells is the numerous round secretory granules that fill extensive areas of the cytoplasm. The Golgi complex and endoplasmic reticulum are well developed. There is a moderate number of mitochondria, which are mostly round to elongate in shape and not uniformly distributed. Lysosomes are large and contain acid phosphatase and other lysosomal enzymes (Fig. 5).

CT suppresses the number and motility of osteoclasts (Gao & Yamaguchi, 1999; Zaidi et al., 1990) and induces a change in their contractile elements (Hunter et al., 1989). Also, CT increases osteoblast proliferation by acting on components of the insulin-like growth factor system (Farley et al., 2000) and enhancing alkaline phosphatase activity, which is associated with increased synthesis and deposition of bone matrix collagen (Farley et al., 1988, 1992; Ito et al., 1987). The action of CT bone formation is at least in part, mediated via CT receptors located on osteoblasts, through the cAMP second messenger system (Farley et al., 1992; Villa et al., 2003).

It was shown that gonadal hormone deficiency affects thyroid C cell activity. Thus, synthesis of CT and its release from rat C cells were decreased after Ovx due to lack of estrogens (Filipović et al., 2002, 2003; Sakai et al. 2000). Also, the decline in testosterone level induced by Orx altered thyroid C cell structure and reduced the synthesis and release of CT (Filipović et al., 2007; Lu et al., 2000). The same effects were noticed after Orx or the natural menopause in women (Isaia et al. 1989). On the other hand, estrogen treatment was found to have a stimulatory effect on CT secretory activity of C cells in Ovx rats (Grauer et al., 1993; Filipović et al., 2003), Orx rats (Filipović et al., 2010a) and women (Isaia et al., 1992). In addition to estrogen, chronic calcium administration after Ovx increased the release of CT from C cells without affecting CT synthesis, suggesting that estrogen plays an important role in CT synthesis (Filipović et al., 2005). On the other hand, CT administration, which may be useful for treatment of osteoporosis, negatively affected rat thyroid C cells by a negative feedback mechanism (Sekulić et al., 2005).

Among the few studies concerning the potential effects of phytoestrogens on CT production, the influence of ipriflavone, a derivative of isoflavone, on CT synthesis and secretion was

investigated. Administration of ipriflavone to intact rats had a gender-related effect on serum CT, which increased in females, but no significant change was seen in male rats (Watanabe et al., 1992). With regard to the inhibitory effect of testosterone on the synthesis of some enzymes it is possible that testosterone inhibited ipriflavone-stimulated CT synthesis (Weiner & Dias, 1990).

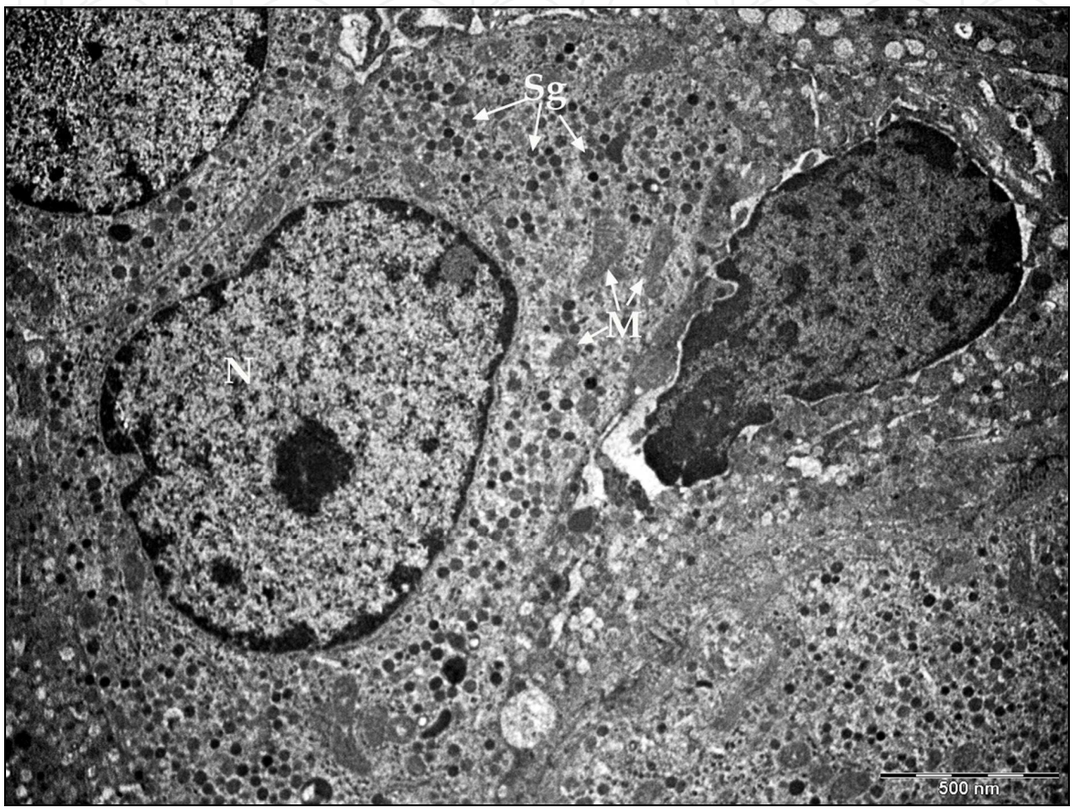

Fig. 5. Ultrastructure of a thyroid C cell; nucleus (N), mitochondria (M), secretory granules (Sg); unpublished image of Filipović et al.

Recently the first experimental data suggesting that daidzein affects thyroid C cells and stimulates CT secretory activity in Orx middle-aged rats were presented (Filipović et al., 2010). The androgen deficiency after Orx strongly affected thyroid C cell structure and reduced the synthesis and release of CT. Daidzein treatment decreased immunoreactivity for CT, significantly increased C cell volume (Fig. 6) and slightly raised serum CT concentration.

Daidzein administration also decreased bone turnover, prevented loss of cancellous bone and the plate-like structure was recovered after trabecular bone destruction caused by Orx (Fig. 7). Based on these results, the authors suggested that, besides direct action on the skeleton, daidzein may affect bone structure indirectly through enhancement of thyroid C cell activity (Filipović et al., 2010).

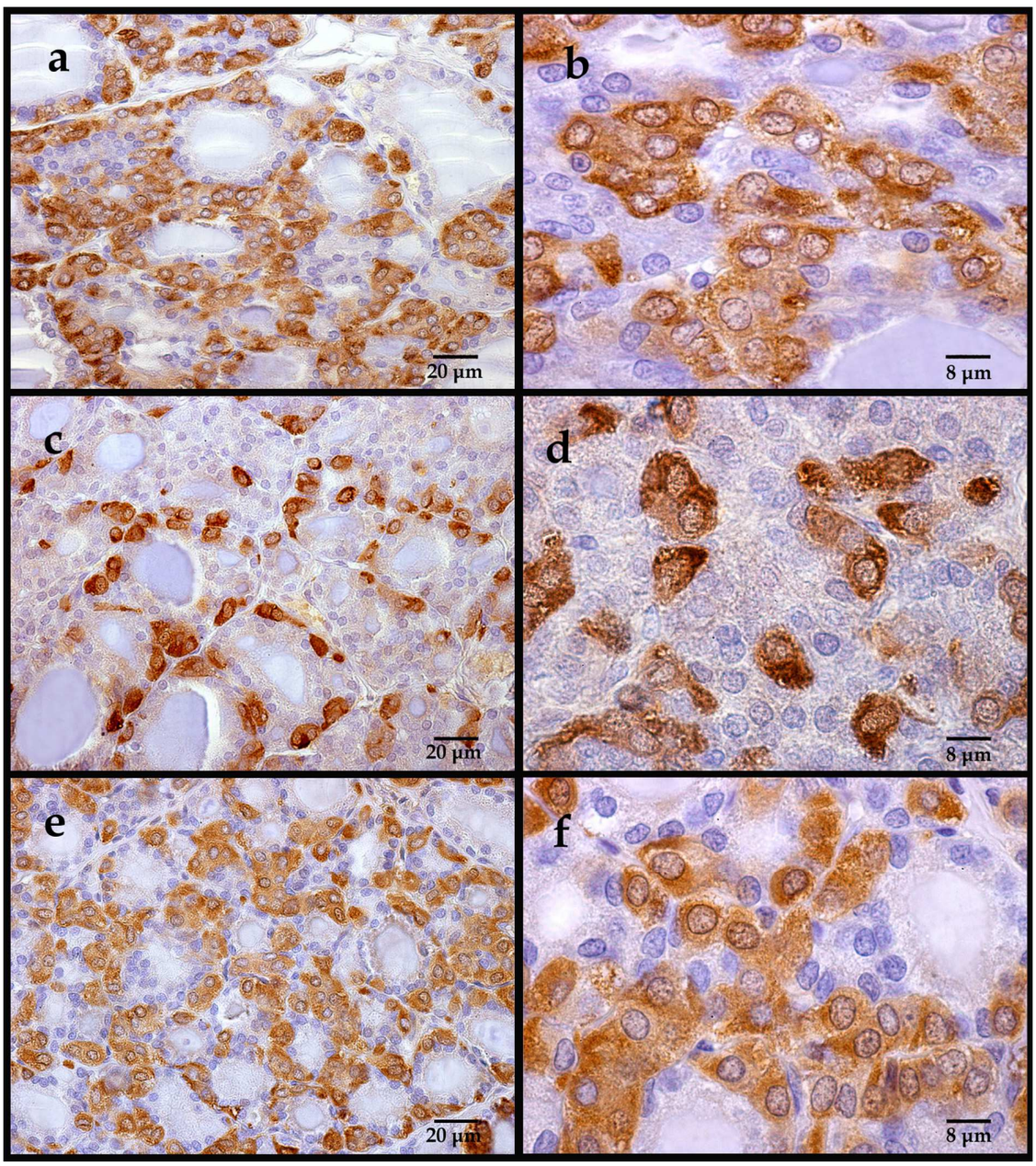

Fig. 6. Calcitonin producing thyroid C cells in control (a, b), orchidectomized (c, d) and orchidectomized rats treated with daidzein (e, f); immuno-staining for calcitonin; unpublished image of Filipović et al.

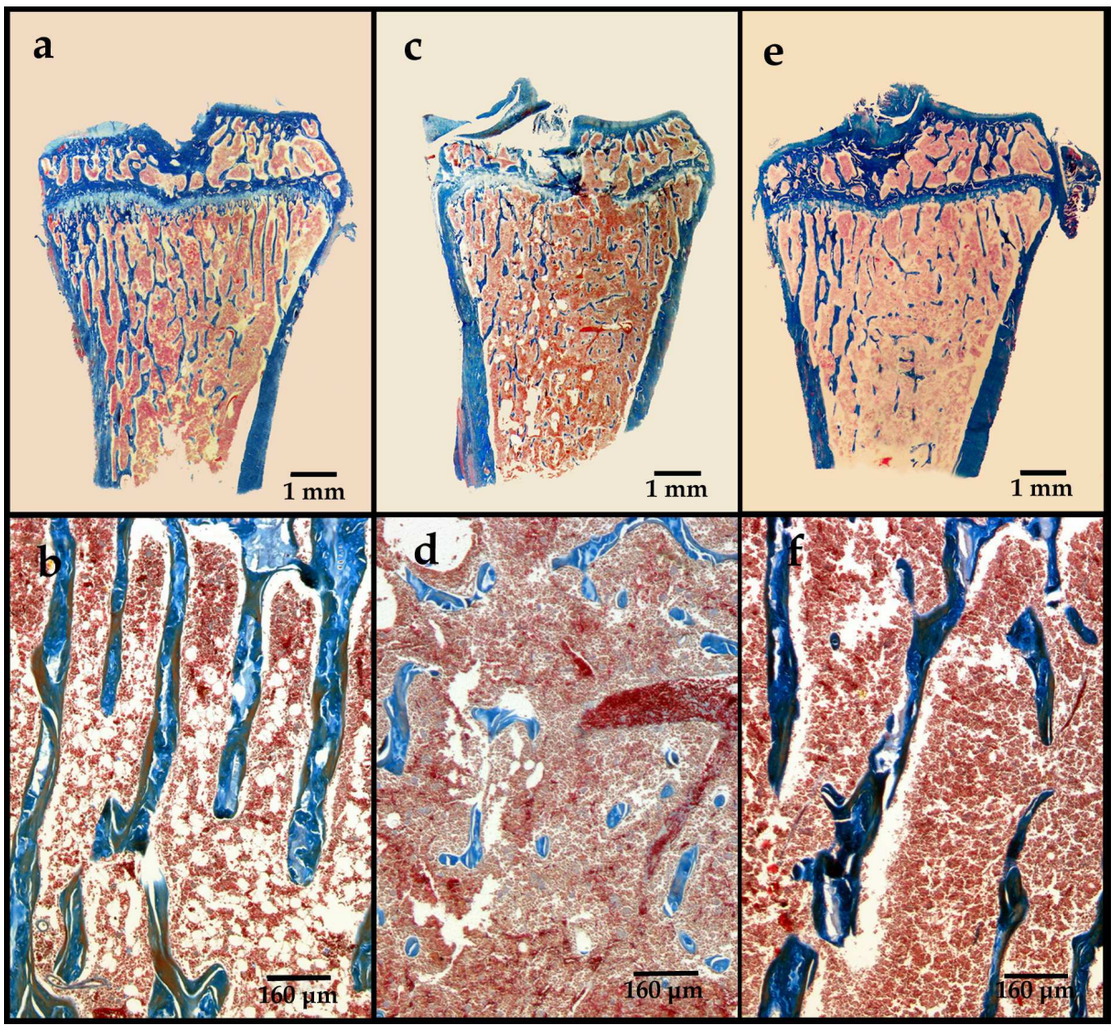

Fig. 7. Trabecular microarchitecture of the proximal tibial metaphysis in control (a, b), orchidectomized (c, d) and orchidectomized rats treated with daidzein (e, f); azan staining method; unpublished image of Filipović et al.

5.2 Effects of phytoestrogens on parathyroid hormone production

Parathyroid glands are constituted of chief, clear and oxyphilous cells. The chief cells synthesize and secrete PTH and are arranged in rather dense cords or nests around abundant capillaries. These cells are oval or polygonal in shape. The nucleus is irregularly shaped, with a few spots of chromatin located in the margin, and the nuclear membrane is infolded. The plasma membrane shows interdigitations. Mitochondria are dispersed throughout the cytoplasm. The cisternae of the rough-surfaced endoplasmic reticulum are arranged in parallel arrays or randomly distributed in the cytoplasm. The Golgi complexes are well developed. Storage granules are filled with finely particulate electron-dense material (Fig. 8).

PTH plays an important role in calcium homeostasis and has a critical role in bone turnover. It antagonizes CT produced by thyroid C cells and acts directly on bone and kidney to increase Ca influx into the blood circulation. This hormone increases the tubular re-

absorption of calcium and induces increased conversion of 25(OH)-D to 1,25(OH)2-D, which enhances intestinal calcium absorption and increases skeletal calcium mobilization.

PTH has a biphasic effect on bone, as it stimulates bone formation when given intermittently, whereas continuous infusion reduces bone mass (Kim et al., 2003). Treatment with PTH significantly increases ALP activity, which suggests that this hormone modulates SaOS-2 osteoblastic cell differentiation and has an anabolic effect on bone. However, increases in RANKL mRNA and decreased OPG mRNA expression in SaOS-2 cells due to PTH indicates induction of bone resorption (Chen & Wong, 2006).

Elevated PTH secretion contributes to the greater bone resorption in osteoporosis which is related to estrogen deficiency. Estrogen therapy prevented the increase in PTH levels associated with the menopause (Khosla et al., 1997). Similarly, phytoestrogens behave as estrogen and may prevent the bone loss caused by estrogen deficiency in female animals and women through reduction of PTH levels. It was shown that phytoestrogens from medical plants can lower serum PTH levels in aged menopausal monkeys (Trisomboon et al. 2004). Also, postmenopausal women with habitually high intakes of dietary isoflavones had significantly lower levels of serum PTH and higher BMD (Mei et al., 2001). These plant compounds bind to ERs in the kidney, gastrointestinal tract and bone and improve calcium absorption resulting in a secondary decrease in the PTH level. Moreover, phytoestrogens may directly reduce PTH secretion from the parathyroid gland (Wong et al., 2002).

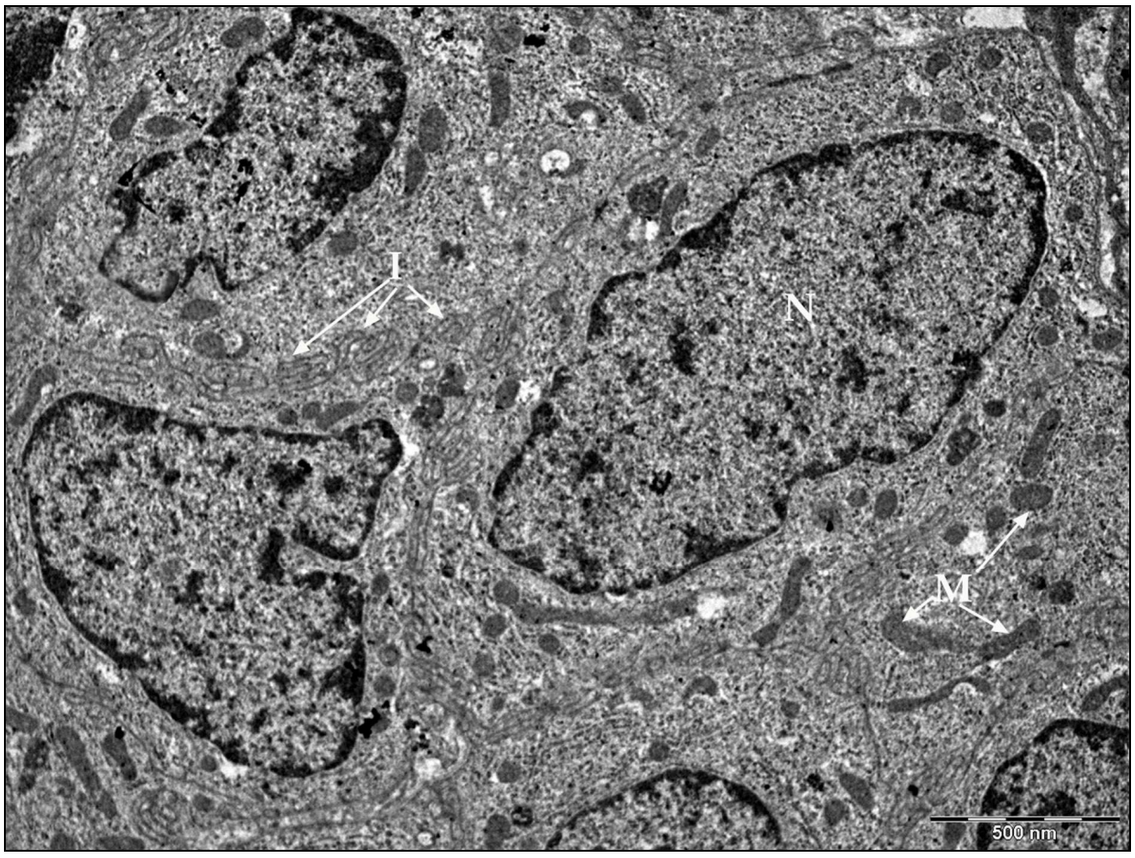

Fig. 8. Ultrastructure of parathyroid chief cells; nucleus (N), mitochondria (M), interdigitations of the plasma membrane (I); unpublished image of Pantelić et al.

Mimicking the effect of estrogen, phytoestrogens can modulate the action of PTH on bone. Thus, one study in vitro showed that pre-treatment of SaOS-2 osteoblastic cells with genistein enhanced PTH-induced ALP activity and attenuated PTH up regulation of RANKL mRNA expression and PTH down regulation of OPG mRNA expression (Chen & Wong, 2006).

5.3 Effects of phytoestrogens on thyroid glands and thyroid hormones production

Hypothalamic–pituitary–thyroid axis (HPT) plays a key role in skeletal development, attainment of peak bone mass and regulation of adult bone turnover (Gogakos et al., 2010; Roef et al., 2011). Additionally, thyroid disorders are associated with alterations in bone metabolism (Lakatos, 2003).

Soy-food, soy-based infant formula, as well as dietary supplements containing purified soybean isoflavones, genistein and daidzein, are increasingly consumed in typical "Western" diet in the recent years. Commonly cited reasons for using soy infant formula are to feed infants who are allergic to dairy products or are intolerant of lactose, galactose, or cow-milk protein (Tuohy, 2003). In elderly, reason is potential health benefit of soybean isoflavones in protection of age-related diseases, including osteoporosis (Setchell, 1998).

Structurally, soybean isoflavones genistein and daidzein are polyphenolic compounds, similar to estradiol-17β and bind with a weaker potency to both types of ERs, with higher affinity for ERβ (Kuiper et al., 1998). Despite the numerous beneficial effects of soy isoflavones, epidemiological and experimental data also exist showing an adverse effect on human health, namely on reproductive and thyroid axis. The association between high soy isoflavones intake and goitrogenesis, as well as protective effect of adequate iodine intake, was reported both in humans (Chorazy et al.1995; Van Wyk et al., 1959) and in different animal models (Ikeda et al., 2000; Kimura et al., 1976; McCarrison, 1933).

Therefore, besides the direct beneficial effect of soybean phytoestrogens on bone tissue, isoflavones may also act indirectly, through endocrine disruption and interference with HPT axis. Most researchers who examined osteoprotective potential of isoflavones did not include in their research examining of the thyroid status. We will address that aspect in this subchapter.

5.3.1 Phytoestrogens, thyroid hormones and skeletal development

Normal thyroid function in childhood is essential for development of endochondral and intramembranous bone, for normal linear growth, as well as for establishing peak bone mass. Hypothyroidism in children causes growth arrest, delayed bone maturation, and epiphyseal dysgenesis, while T_4 replacement results in rapid catch-up growth (Basset & Williams, 2003). Exposure to soybean isoflavones during development may alter thyroid hormone concentrations and disturb feedback regulation of HPT axis, and these effects can be more serious than in the adults.

Soy infant formula is fed to infants as a replacement for human milk, or as an alternative to cow milk formula. Genistein is the predominant isoflavone found in soy infant formula (58-67%), followed by daidzein (29-34%) and glycitein (5-8%) and infants fed soy infant formula have higher daily intakes of genistein and other isoflavones than other populations (Patisaul & Jefferson, 2010). The question of whether or not soy infant formula is safe has been widely debated for more than a decade, and early epidemiological studies demonstrated that infants fed adapted soy formula without iodine supply were hypothyroid (Van Wyk et al., 1959). This effect was eliminated by supplementing commercial soy infant formulas with iodine, or by

switching to cow milk (Chorazy et al., 1995). Today, soy formula is regularly supplied with iodine and a more recent study demonstrated no significant changes in the serum level of bone alkaline phosphatase, osteocalcin, intact PTH, and the urinary levels of the markers of bone metabolism in children (mean age of 37 months) fed with soy formula (Giampietro et al., 2004). However, infants with congenital hypothyroidism fed with iodine supplemented diet still need higher doses of L-thyroxine (Jabbar et al., 1997). This finding is of particular importance, keeping in mind that the consequence of congenital and juvenile acquired hypothyroidism is retardation of skeletal development and that the effects of T_4 replacement (achievement of predicted adult height) strongly depend on the duration of untreated hypothyroidism (Rivkees et al., 1988).

Soybean isoflavones may functionally disrupt the thyroid hormone (TH) system by influencing different steps such as synthesis, transport, action and metabolism of TH. Genistein and daidzein inhibit the activity of thyroid peroxidase (TPO), the key enzyme in the synthesis of thyroid hormones, both in vitro and in vivo (Chang & Doerge, 2000; Divi et al., 1997; Doerge & Chang, 2002). Besides the inhibitory effects of isoflavones on TPO, iodine deficiency is important risk factor for thyroid dysfunction and goiter development, both in humans and in rats. An adequate iodine supply is a way to prevent goitrogenic effects of soy bean isoflavones, especially in the high-risk group of patients with congenital hypothyroidism.

Besides the serum concentrations of TH, biological activity of T_3 on bone tissue is determined by the membrane transporters of TH, local expression and activity of deiodinase enzymes and receptors for TSH and TH. Polymorphisms in above mentioned genes are associated with important chronic skeletal diseases, including osteoporosis and osteoarthritis (Andersen et al., 2002, 2003; Peeters et al., 2006).

Entry of T_3 and T_4 into target cells is determined by the active uptake of free hormones by specific cell membrane transporters: monocarboxylate transporter-8 (MCT8), MCT10 and organic acid transporter protein-1c1 (OATP1c1) (van der Deure et al., 2010). MCT8 is expressed in growth plate chondrocytes, bone forming osteoblasts and bone resorbing osteoclasts at all stages of cell differentiation, and its expression is regulated by thyroid status (Capelo et al., 2009), although its functional importance is still unclear. It seems that OATP1c1 is not expressed in the mouse skeleton (Capelo et al., 2009), but there are still no data regarding expression of MCT10. Tyrosine kinase inhibitors sunitinib and imatinib inhibit MCT8 – mediated iodothyroinine transport (Schweizer et al., 2010), but there are still no data regarding possible effects of genistein, which is a potent thyrosine kinase inhibitor as well, on cellular transport of TH.

Deiodinase (Dio) enzymes determine the intracellular levels of bioactive T3 and thus cell-specific gene expression. Expression of deiodinases is tissue specific: Dio 1 enzyme is not expressed in bone, while Dio 2 plays an important role in local regulation of thyroid hormone signaling during fetal bone development. In the adult skeleton Dio 2 activity is restricted to osteoblasts (Williams et al., 2008). Dio 2 expression and activity are inhibited by high concentrations of substrate (T_4) and thus are maximal in hypothyroidism and suppressed in thyrotoxicosis. Locally regulated activity of Dio 2 in osteoblasts maintains intra-cellular T_3 concentrations constant over the euthyroid range and preserves optimal bone mineralization. Inactivating deiodinase type 3 (Dio 3) is expressed in the skeleton, although the highest levels of enzyme activity occur in growth plate chondrocytes prior to weaning (Yen, 2001). Genistein inhibit both Dio 1 and Dio 2 activity in vitro (Mori et al., 1996), but the physiological importance of this mechanism is still unclear.

Based on analyses of rare monogenic diseases and the results of animal studies, it was proposed that T_3 play a key role in bone development, while TSH is not required for normal skeletal development (Bassett et al., 2008). T_3 enters the nucleus and binds to its nuclear receptors (TR). There are three functional TRs: TRα1, TRβ1 and TRβ2, encoded by the THRA and THRB genes. These receptors act as hormone inducible transcription factors that regulate expression of T_3-responsive target genes (Yen, 2001). Both TRα1 and TRβ1 isoforms are expressed in bone and TRα1 levels are at least 10-fold greater than TRβ1. These findings support the opinion that TRα1 is the principal mediator of T_3 action in bone (Bassett & Williams, 2009; O'Shea et al., 2003).

In vitro experiments demonstrated that effects of T_3 in osteoblastic cell lines and primary osteoblast cultures depend on species, cell type, anatomic origin, differentiation phase and duration of the treatment. T_3 was reported to increase expression of osteocalcin, osteopontin, type I collagen, alkaline phosphatase, IGF-I and its regulatory binding proteins IGF1BP-2 and -4 (Milne et al., 2001; Pereira et al., 1999; Varga et al., 2004). Therefore, T_3 may exert its stimulatory effect on osteoblasts via complex pathways involving many growth factors and cytokines.

5.3.2 Phytoestrogens, thyroid hormones and osteoporosis prevention

Similar to osteoporosis, thyroid diseases are much more common in elderly women than in men and is associated with significant morbidity if left untreated (Schindler 2003; Suchartwatnachai et al., 2002). Still, this fact does not imply a causal relationship between the two diseases and many patients may independently develop both. Hypothyroidism occurs in 10% of females and 2% of males in patients older than 60 years. The prevalence of hyperthyroidism in the elderly is approximately 2% (Maugeri et al., 1996), though other authors reported that 10 to 15% of elderly patients were hyperthyroid (Kennedy & Caro, 1996). Thyrotoxicosis increase risk in developing secondary osteoporosis (Amashukeli et al., 2010; Lakatos, 2003).

Thyroid hormones play a significant role in maintaining adult bone homeostasis. Results of clinical and experimental studies are consistent and demonstrate that hypothyroid state slows down bone turnover and affect overall gain in bone mass and mineralization. By contrast, bone resorption and formation are accelerated in hyperthyroidism, while the remodeling cycle is shortened (Davies et al., 2005). Increased bone turnover and osteoporosis in thyrotoxicosis are attributed to the thyroid hormone excess and are not a consequence of deficient TSH receptor (TSHR) signaling. However, TSH may play a direct role in regulation of bone turnover, since TSH receptor was identified in osteoblasts. The experiment with ovariectomized rats, which were treated with low doses of TSH (insufficient to alter serum T_3, T_4 or TSH levels), demonstrated that TSH treatment prevented bone loss and increased bone mass (Sampath et al., 2007; Sun et al., 2008). Although the TSHR is expressed in osteoblasts, current data from in vitro studies are contradictory and suggest that TSH may enhance, inhibit or have no effect on osteoblast differentiation and function (Bassett et al., 2008).

Prevention and treatment of osteoporosis involve Ca and vitamin D supplementation, as well as different drug therapy approaches, which include bisphosphonate, salmon CT and estrogen or androgen replacement therapy for menopausal women and andropausal men, respectively. In addition, in recent years, numerous discussions on safety and benefit of synthetic steroids (both estrogens and androgens) favor the trend towards consumption of

"green" natural "phytosteroids" or "phyto-selective modulator of ERs". That is why nutritional supplements and concentrated extracts containing purified soybean phyto-SERMs genistein and daidzein are increasingly used as alternative therapy for osteoporosis and other age-related diseases in both sexes (Ramos, 2007; Setchell, 1998; Tham et al., 1998). However, all these treatments may affect thyroid function as well.

Not so many researchers have tried to link effects of supplementation or drug treatment on bone metabolism with modulation of thyroid hormone levels. Rodents are considered useful models for thyroid studies, even though significant differences between rodent and human thyroid physiology have been reported (Choksi et al., 2003; Poirier et al., 1999). Rat thyrocytes are characterized by abundant granular endoplasmic reticulum, well developed Golgi, prominent lysosomes, luminal (apical) microvilli, small mitochondria, and round nuclei with homogeneous chromatin (Fig. 9).

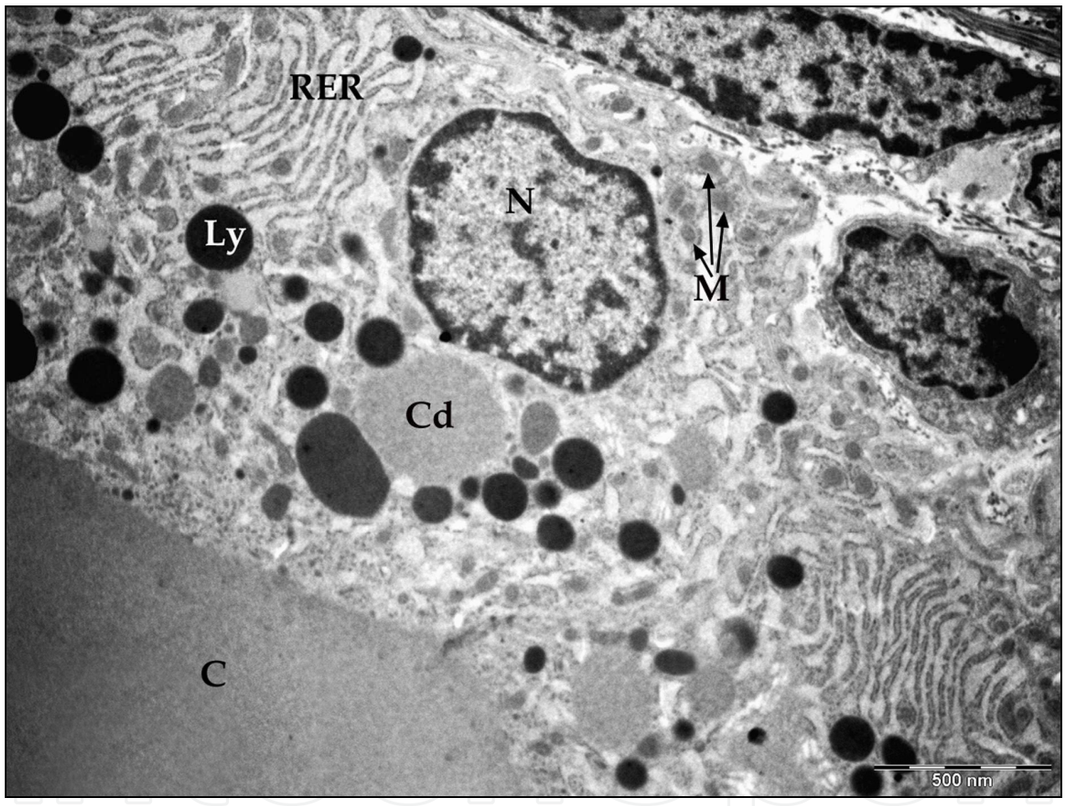

Fig. 9. Ultrastructure of thyroid follicular cell; nucleus (N), mitochondria (M), rough endoplasmatic reticulum (RER), lysosomes (Ly), colloidal droplets (Cd), colloid (C); unpublished image of Šošić-Jurjević et al.

In our laboratory we demonstrated that chronic Ca administration to middle-aged female rats significantly decreased the volume density of the thyroid follicular epithelium, epithelium's height and the index of activation rate, which are morphometric parameters of TH synthetic and secretory potential of thyrocytes (Šošić -Jurjević et al., 2002). Consistent with histomorphometric changes, reduced serum levels of total T_4 and T_3 were detected (Šošić -Jurjević et al., 2006). At the same time, we determined significant decrease of serum osteocalcin and urinary Ca, as biochemical parameters of reduced bone turnover after Ca

treatment (unpublished data). In vitro studies with FTRL-5 cells demonstrated that Ca did not affect the morphology of these cells, but when administered together with TSH, it acted directly, by reducing the thyrotropin stimulatory effect (Gaberscek et al., 1998). Isoform VI of adenilyl cyclase, the enzyme crucial for TSH-induced activation of thyroid follicular cells, was found negatively modulated by Ca in human and dog thyroids (Vanvooren et al., 2000). Doses of Ca were chosen to mimic human exposure to high doses of Ca in treatment of osteoporosis. We can speculate that slowing down of thyroid hormone synthesis may be an indirect mechanism, which lead to decreased bone turnover detected after Ca treatment under our experimental conditions.

Sex steroids, estrogen and testosterone, play an important role in bone physiology and pathology. Endogenous estrogens are regularly produced in bone via aromatase enzyme activity, and exert their effects through ER, which are also detected in male bones (Carani et al., 1997; Grumbach & Auchus, 1999; Korach, 1994). Bone cells are sensitive to both estrogens and androgens, and aromatase inhibition causes similar degree of osteoporosis in male animals as orchidectomy (Vanderschueren et al., 1998).

There is a close relationship between sex steroids and thyroid function. Epidemiological studies suggest that the use of estrogens may contribute to the pathogenesis of thyroid tumors (Ron et al., 1987). Experimental studies on rodents demonstrated numerous sex-related differences in thyroid function and, in general, adult male rodents have higher levels of TSH than females associated with lower T_4 and higher plasma levels of T_3 (Capen, 1997). The results related to treatment effects of sex steroids on different set points of thyroid function are inconsistent and depend on experimental conditions: type of experimental animal, animal's age and applied dose (Chen & Wallfish, 1978; Henderson et al., 1982; Sekulić et al., 2007). Our previous results demonstrated an inhibitory effect of pharmacologic doses of estradiol (previously used in human studies for treatment of osteoporosis) on thyroid follicular cells in ovariectomized young adult and ovarium-intact young and middle-aged rats, (Sekulić et al., 2006; Šošić -Jurjević et al., 2005, 2006a), as well as after treatments of orchidectomized 16-month-old rat males with 10 times lesser dose of estradiol dipropionate (Sekulić et al., 2010). We choose the dose of estradiol in the experiment which was previously reported to prevent bone loss in males (Fitts et al., 2001; Vanderput et al., 2001). Consistent with literature data, we also detected decreased serum osteocalcin levels, accompanied by decreased urinary Ca concentration in Orx rats treated with EDP (unpublished data). Contrary to effects of estradiol, testosterone treatment of castrated middle-aged males moderately increased serum TSH and total T_4 levels (Sekulić et al., 2010), but similarly to estradiol treatment, decreased both serum osteocalcin levels and urinary Ca concentration (unpublished data). Therefore, it seems that the direct effect of sex steroids on bone tissue is more relevant for the net result of replacement therapy on bone protection then the indirect effect, mediated through modulation of thyroid function.

Direct negative effect of isoflavones on thyroid hormone synthesis, by significant blocking of TPO activity (more than 60%), has been well described. Genistein and daidzein were demonstrated to block both TPO-catalyzed reactions: iodination of thyrosine residues of Tg, and T_4 formation by coupling reactions, but this effect was eliminated by iodine (Chang & Doerge, 2000; Divi et al., 1997; Doerge & Chang 2002). Despite significant inactivation of this enzyme, serum thyroid hormone levels were unaffected by isoflavone treatments in young adult rats of both sexes. The authors supposed that soy could cause goiter, but only in animals or humans consuming diets marginally adequate in iodine, or who were predisposed to develop goiter. Most other authors, who performed their studies on young adult animals of both sexes, also reported that soy or isoflavones alone, in the absence of

other goitrogenic stimulus, did not affect thyroid weights, histopathology and the serum levels of TSH and thyroid hormones (Chang & Doerge, 2000; Schmutzler et al., 2004). The thyroid function becomes impaired with aging in rodents, and the number of thyroid dysfunction increase in elderly population (Donda & Lemarchand-Béraud, 1989; Reymond et al., 1992). We were the first who demonstrated that therapeutic doses of both genistein and daidzein induce hypertrophy of Tg-immunopositive follicular epithelium and colloid depletion (Fig. 10), and reduce the level of serum thyroid hormones, accompanied by

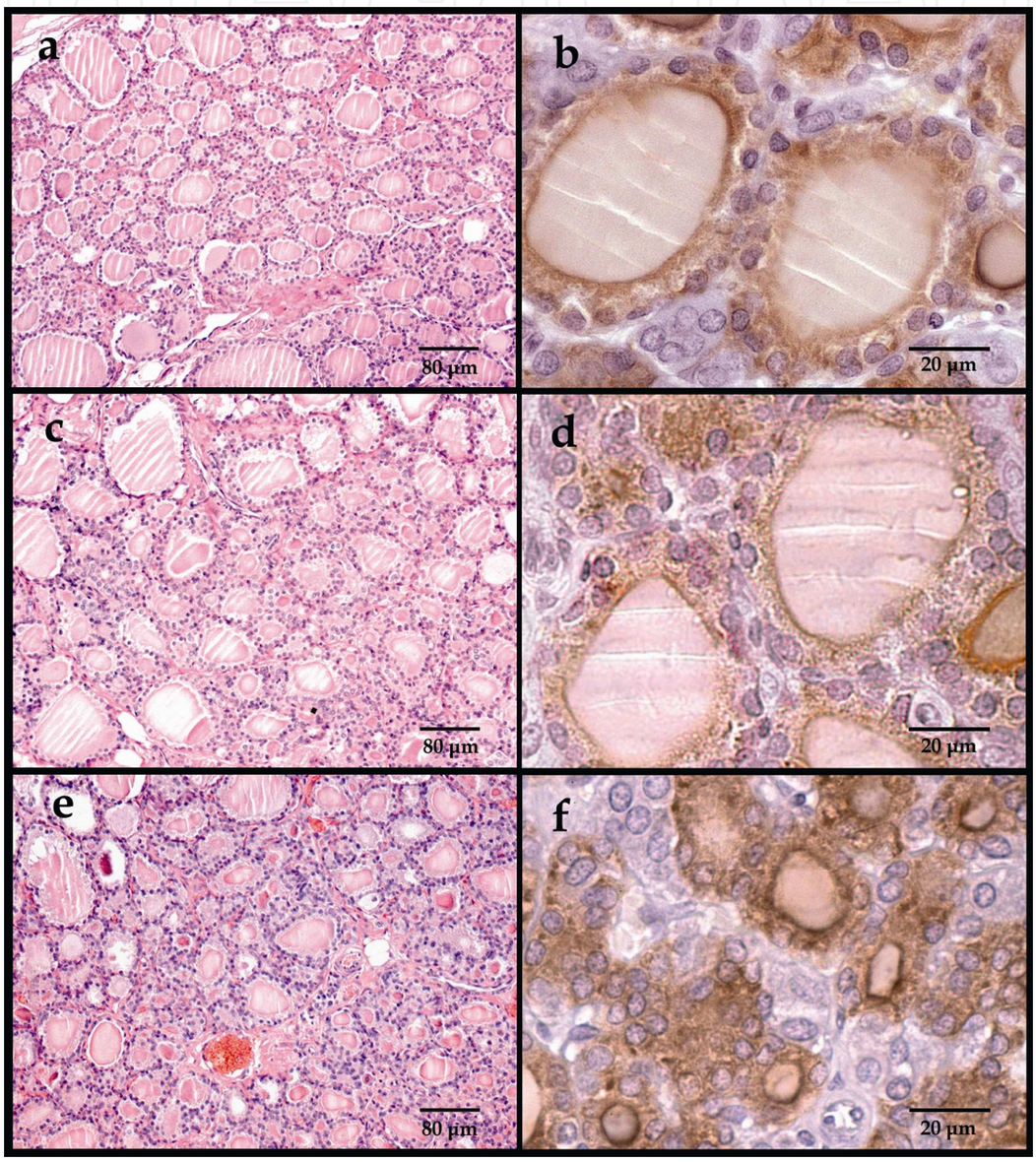

Fig. 10. Thyroid gland tissue of control (a, b), orchidectomized (c, d) and orchidectomized rats treated with daidzein (e, f); hematoxylin-eosin and immuno-staining for thyroglobulin; unpublished image of Šošić-Jurjević et al.

increased serum TSH, in orchidectomized (Orx) middle-aged male rats fed a iodine-sufficient soy-free diet (Šošić -Jurjević et al., 2010). Our research team obtained that both genistein and daidzein increased bone mass following orchidectomy of middle-aged males (Filipovic et al., 2010 and unpublished data). Therefore, decreased serum level of TH might contribute to the detected increase in trabecular bone mass, and decrease in bone turnover in aged male orchidectomized rat model.

6. Conclusion

Phytoestrogens have the potential to maintain bone health. Owing to their properties, these plant-derived non-steroidal compounds have a potential beneficial role in delaying or preventing osteoporosis. Therefore, they have attracted much attention as alternatives to HRT. As SERM, phytoestrogens may generate a bone protective effect via stimulation of osteoblastic bone formation and inhibition of osteoclastic bone resorption. Proposed molecular mechanisms are based on their ER-mediated effects. In addition to direct action, phytoestrogens can affect bone structure indirectly, by stimulating or inhibiting the synthesis of certain hormones, i.e. through increased synthesis of CT from thyroid C cells, as well as reduction of PTH and thyroid hormone levels.

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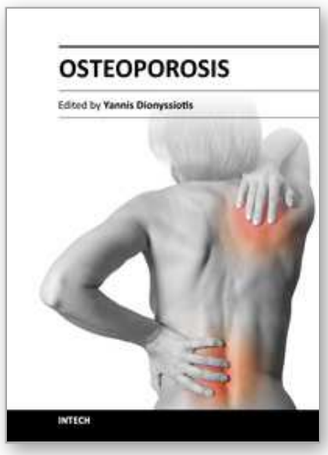

Osteoporosis

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Osteoporosis is a public health issue worldwide. During the last few years, progress has been made concerning the knowledge of the pathophysiological mechanism of the disease. Sophisticated technologies have added important information in bone mineral density measurements and, additionally, geometrical and mechanical properties of bone. New bone indices have been developed from biochemical and hormonal measurements in order to investigate bone metabolism. Although it is clear that drugs are an essential element of the therapy, beyond medication there are other interventions in the management of the disease. Prevention of osteoporosis starts in young ages and continues during aging in order to prevent fractures associated with impaired quality of life, physical decline, mortality, and high cost for the health system. A number of different specialties are holding the scientific knowledge in osteoporosis. For this reason, we have collected papers from scientific departments all over the world for this book. The book includes up-to-date information about basics of bones, epidemiological data, diagnosis and assessment of osteoporosis, secondary osteoporosis, pediatric issues, prevention and treatment strategies, and research papers from osteoporotic fields.

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