12

# Genistein Aglycone Demonstrates a Protective and Reversible Effect on the Development of Steroid-Induced Secondary Osteoporosis and Increases Bone Breaking Strength in Rats

Alessandra Bitto[1], Francesca Polito[2], Bruce P Burnett[3], Herbert Marini[2], Robert Levy[3], Domenica Altavilla[1] and Francesco Squadrito[1]
[1]*Department of Clinical and Experimental Medicine and Pharmacology Section of Pharmacology, University of Messina*
[2]*Department of Biochemical, Physiological and Nutritional Sciences Section of Physiology and Human Nutrition, University of Messina*
[3]*Primus Pharmaceuticals, Inc. Scottsdale, Arizona*
[1,2]*Italy*
[3]*USA*

## 1. Introduction

Glucocorticoids are used in the treatment of inflammatory and autoimmune diseases, cancers, and following organ transplantation. Glucocorticoid-induced osteoporosis is one of the primary side effects of glucocorticoid use resulting in increased risk of fractures. In a large meta-analysis study of glucocorticoid users, for example, van Staa et al (2002) found a relative risk increase of 1.91 for any fracture, 2.86 for vertebral fracture, 1.61 for hip fracture, and 1.13 for forearm fracture. Glucocorticoid-induced osteoporosis is characterized by low bone turnover and fractures, which occur in 30-50% of patients (van Staa et al., 2000). Glucocorticoids affect predominantly cancellous or trabecular bone, increasing the risk of vertebral fractures, which may be asymptomatic and occur early during the first months of glucocorticoid treatment (Laan et al., 1993; van Staa et al., 2000) and (Angeli et al., 2006). Trabecular bone accounts for approximately 20% of the total mass of bone (Bertazzo & Bertran, 2006) and also contributes to the ability of bone to tolerate stress to avoid fracture, especially in the spine. Long-term use of glucocorticoids also increases the risk of all osteoporotic fractures.

Published reports suggest no course of glucocorticoid therapy is safe for the skeleton. Regimens of daily prednisone at doses as low as 2.5 mg have been associated with an increased risk of hip and vertebral fractures. The risk increases by 5-fold, with prednisone doses above 7.5 mg daily. A dramatic 17-fold increase in vertebral fracture incidence was observed in subjects who used prednisone continuously more than 10 mg per day for longer than 3 months with increased fracture incidence in postmenopausal females and elderly males. The risk of osteoporotic fractures was also elevated in patients undergoing cyclic corticosteroid treatment at high doses (Van Staa et al., 2006) Although variable in onset,

fracture risk decreases after discontinuation of oral corticosteroids (Vestergaard et al., 2008). The risk of osteoporosis associated with inhaled glucocorticoids or with budesonide, a topical steroid used in inflammatory bowel disease, is small because their absorption is limited. Due to the high risk of fracture in glucocorticoid-administered patients, treatment guidelines exist.

Treatment guidelines for the use of glucocorticoids have been established which advise that if prednisolone is administered at ≥ 5 mg per day for three months or longer requires regular monitoring of bone mineral density (BMD) and treatment to prevent osteoporosis must be initiated (American college of Rheumatology, 2001; Adler & Hochberg, 2003; National Osteoporosis Society, 1998). Vitamin D and calcium are also recommended for the management of all patients treated with glucocorticoids. Bisphosphonates should be considered for the prevention and treatment of this disorder, because they can prevent the initial loss of bone mass from glucocoriticoids. Alendronate, risedronate, and zoledronic acid were shown to prevent and reverse the loss of BMD in glucocorticoid-induced osteoporosis with greater effects than those observed with vitamin D and calcium (Doga et al., 2008) and (Amin et al., 2002). In fact, bisphosphonates induce improvement of BMD that is 2-fold greater than that observed during vitamin D treatment alone (4.6% vs. 2.0%, respectively) (Amin et al., 2002). Anabolic therapy is also utilized for treatment of glucocorticoid-induced osteoporosis. Teriparatide causes a greater increase in BMD than alendronate and greater reduction in the risk of vertebral fractures (Saag et al., 2007). Even with these evidentiary clinical trials and guidelines, patient bone loss is, in general, poorly managed (Eastell et al., 1998; Walsh et al., 1996; Gudbjornsson et al., 2002). In glucocorticoid-induced osteoporosis, fractures also occur at higher BMDs than in postmenopausal osteoporosis in untreated women (van Staa et al., 2003). Consequently, guidelines for the treatment of postmenopausal osteoporosis are not applicable to glucocorticoid-induced osteoporosis, and patients should be treated at BMD T-scores of ≤−1.0 to −1.5 (Compston, 2004). In addition, vertebral fractures may be asymptomatic and often require radiological diagnosis before treatment. Since the incidence of fractures is higher in the spine compared to other areas of the body, glucocorticoid therapy appears to affect rapid bone remodelling.

During the initial phases of glucocorticoid exposure bone resorption is increased (van Staa et al., 2000). Glucocorticoids inhibit the formation of mature osteoblasts, but also activate apoptosis in these cell types (Jones & Sambrook, 1994; Canalis et al., 2007). Osteoprotegrin (OPG) expression, a key factor involved in modulating maturation of osteoclasts, is reduced also by glucocorticoids resulting in increased osteoclastogenesis (Orcel, 2005). Therefore, the combination of reduced osteoblast formation, increased osteoclast maturation leads to accelerated bone loss while on glucocorticoid therapy. Therapies are needed which modulate osteoclast as well as osteoblast activity to restore a more normal balance to the bone remodeling process in glucocorticoid treated patients.

## 2. Glucocorticoid-induced osteoporosis: Is there a role for genistein?

Among the anabolic compounds tested in recent years genistein aglycone seems a promising agent able to stimulate bone formation and to reduce bone resorption, acting *via* a genomic as well as a non-genomic pathways. Genistein is an isoflavone found in small quantities in certain legumes throughout the plant kingdom. Soybeans are a particularly rich source of genistin, the glucosidal precursor of genistein, although the concentration varies with the strain, location and environmental conditions of cultivation

of the plant. Another widely utilized source of genistin is *Sophora japonica* L (Tian et al., 2004). The glucosidal form and acetyl- and malonyl-glucosides are the major isoforms found in soybean derivates. The conversion from the glucosides to the aglycone form occurs in the gut, where the sugar residue is removed with the generation of genistein (Larkin et al., 2008).

Genistein is freely absorbed from the intestine and a large fraction is converted to the 7β-O-glucuronide as it crosses the brush border and ultimately enters the portal vein (Sfakianos et al., 1997), a process that is influenced by intestinal bacteria (Setchell et al., 2002; Day et al., 1998). Recent *ex vivo* data using isolated human gastrointestinal tract tissue also suggest that genistein may also undergo sulfonation in the small intestine, though the extent to which these sulfonates are absorbed following dietary intake is unknown (Ronis et al., 2006). The exact percentages of glucuronidated and sulfonated metabolites after crossing the lumen are also unknown, although it is clear that only a small percentage of the parent molecule remains as unconjugated genistein once it reaches the liver. Once in the liver, genistein undergoes additional biotransformation *via* CYP450-mediated hydroxylation (Hu et al., 2003) followed by glucuronidation and sulfonation by UDP-glucuronosyl transferase and sulphotransferases, respectively (Sfakianos et al. 1997). Genistein 7β-O-glucuronide can be recovered from bile after infusion of genistein into the small bowel of rats (Prasain et al., 2006). As much as 70% of the recovered genistein from bile is in the form of glucuronidated conjugates with smaller amounts reappearing in the distal duodenum and jejunum (Sfakianos et al., 1997). The vast majority of circulating genistein in serum has been found to be in the form of glucuronidated and sulfonated conjugates which represent excretion forms of the molecule (Setchell et al., 2002). Little is known about the bioactivity of conjugated isoflavones. Genistein is composed of two benzene rings (A and B) linked through a heterocyclic pyrane C ring. The hydroxyl group on the A ring confers to genistein a greater activity both *in vitro* and *in vivo*, compared to other isoflavones (Choi et al., 2008).

In addition to competing with endogenous estrogens for binding to the estrogen receptors at high concentrations, genistein may exert anti-estrogenic effects by several potential mechanisms (Tham et al., 1998). Genistein has both ER agonist and antagonist activity in different cell types and works in a promoter specific manner in gene activation *via* ERs. The ER domain structure is typical of nuclear receptors. The amino-terminal region is involved in trans-activation of gene expression and has been termed the activation function domain or AF1 domain. The middle region contains a two-zinc finger structure, which plays an important role in binding to specific DNA response elements and in receptor dimerization. The carboxyl-terminal region contains an activation domain, termed AF2. A ligand binding domain, lying within AF2, is crucial for binding to receptor specific ligands and also co-repressors and co-activators. This binding interaction affects receptor dimerization, nuclear translocation and modulation of target gene expression by AF2. The ligand-binding domain is structurally conserved among the nuclear receptor family and consists of between 10 and 12α helices folded in a globular domain. A central hydrophobic pocket accommodates the cognate ligand, which upon binding induces a conformational change in the ligand-binding domain, exposing a coactivator-docking site on ligand binding surface. However the binding of SERMs to ERs results in a different conformation change in which the coactivator-binding site is blocked from interaction with coactivator, thereby blocking AF2 mediated transcriptional activity (Liu et al., 2003). Several classes of nuclear receptor coactivators interact with ERs receptors and are specific for either ERα or ERβ. Using specifying coactivator recruitment and fluorescence resonance energy transfer, the genistein

interaction with ER receptors was investigated (Kuiper et al., 1998). Compounds with estrogen agonism induce agonist-mediated recruitment and allow fluorescence resonance energy transfer. Compounds with estrogen antagonism block the agonist-mediated recruitment of coactivators and prevents fluorescence resonance energy transfer. Genistein showed full agonism for ER α and only partial agonism for ER β, but higher affinity for ERβ than ERα (Kuiper et al., 1998).

A second action of genistein on membrane associated protein (membrane receptors) has been proposed, and remains still not fully elucidated. In this mechanism, several possible second messengers, including kinase enzymes, are activated and in turn stimulate or inhibit downstream pathways of metabolism and protein products. The duration of this effect *via* a membrane protein is short lasting and of limited efficacy. The positive effects of genistein on primary osteoporosis, due to estrogen fall, have been extensively investigated by our group as well as by others and have been recently summarized in a review article (Bitto et al., 2010); however pre-clinical evidence point out a role for genistein also in secondary osteoporosis induced by corticosteroid use.

### 2.1 Evidence from *in vitro* studies

Genistein has been most investigated for its effect on the proliferation and differentiation of a number of cell types. Through its effect on tyrosine kinase, genistein is able to modulate cell cycle progression in the S phase, to induce G2/M arrest and to induce apoptosis (Matsukawa et al., 1993). Numerous in vitro studies with human or animal osteoclast- or osteoblast-like cell lines have been carried out with consistent observations of direct effects of genistein on both cell types. Effects of genistein on bone metabolism derived from direct and indirect actions on bone cells and can be summarized in stimulation of osteoblastic bone formation and inhibition of osteoclastic bone resorption (Gao & Yamaguchi, 1999a; Sugimoto & Yamaguchi, 2000; Chen et al., 2003; Heim et al., 2004). In vitro studies indicate that genistein is able to stimulate osteoblastic activity and inhibit osteoclast formation and action at a range of concentrations ($10^{-5}$–$10^{-7}$ M) consistent with the levels observed in human subjects after ingestion of genistein. With regard to effects on osteoclast genistein inhibits their formation and function (Amano et al., 1998; Gao & Yamaguchi, 2000; Yamagishi et al., 2001; Albertazzi, 2002; Blair et al., 1996; Gao & Yamaguchi, 1999a; Kajiya et al, 2000; Williams et al, 1998). Genistein suppress osteoclast activity by a number of possible mechanisms, including induction of apoptosis, activation of protein tyrosine phosphatase, inhibition of cytokines, changes in intracellular $Ca^{2+}$, and membrane depolarization. More in details, in mouse marrow cultures genistein has a potent inhibitory effect on osteoclast-like cell formation, and this effect is similar to others anti-bone-resorbing agents, such as calcitonin and 17β-estradiol, and may involve cAMP signaling. Sliwiñski and coworkers (2005) compared the effects of genistein, estradiol and raloxifene, compounds affecting in different ways estrogen receptors, on formation and viability of osteoclast from neonatal rat bone marrow; the results shown that all treatments decrease the number of osteoclasts formed from bone marrow, particularly genistein at the lower concentration decreases the number of osteoclasts by induction cell death, while at higher concentration genistein prevents the formation of osteoclasts. These observations are consistent with the results of another study (Rickard et al. 2003), showing that genistein, acting as agonist of estrogen receptors, mimicked estradiol in affecting gene expression of progesterone receptor, the proteoglycan versican, alkaline phosphatase and osteopontin, and production of

interleukin-6 protein. However, actions of genistein at the cellular level depend on the target tissue, receptor status of the tissue, and the level of endogenous estrogen. Both estrogen receptors ERα and ERβ are present in bone tissue, although the expression of these subtypes varies considerably during differentiation. The greatly increased expression of ERβ during bone mineralization is particularly pertinent to the potential hormonal effects of isoflavones because compounds such as genistein show a much higher affinity for ERβ than for ERα (Arts et al., 1997).

One of the mechanism by which genistein induces apoptosis of mature osteoclasts involves the Ca2+ signaling, the inhibition of protein kinase and the activation of protein tyrosine phosphatase in osteoclasts. In this study the suppressive effect of genistein was completely abolished by the presence of inhibitors of $Ca^{2+}$-dependent protein kinases (Gao & Yamaguchi, 1999b). The most important cytokine machinery, which is involved in bone metabolism, is osteoprotegerin (OPG)/receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL) system. The molecular triad OPG/RANK/RANKL plays important roles in bone remodeling. RANKL is expressed by osteoblasts and is necessary and sufficient for osteoclastogenesis. RANKL binds to its receptor RANK, present at the surface of osteoclast precursors and mature osteoclasts, inducing osteoclast formation and activation. RANKL activity can be blocked by the soluble decoy receptor OPG, resulting in prevention of bone resorption. Studies, *in vitro*, have found that genistein induces apoptosis and inhibits RANKL signaling-related gene expression (Uchiyama & Yamaguchi, 2007).

Genistein was also reported to increase osteoblastic differentiation and affect osteoblast activity (Rickard et al., 2003; Pan et al., 2005; Morris et al., 2006; Okumura et al., 2006). In osteoblasts genistein stimulates a concentration-dependent increase in alkaline phosphatase activity. In osteoblastic MC3T3-E1 cells, genistein increases protein content, DNA content and alkaline phosphatase activity (Yamaguchi & Sugimoto, 2000; Sugimoto & Yamaguchi, 2000). This effect has also been demonstrated in tissue cultures. In femoral metaphyseal tissue from elderly female rats genistein increases $Ca^{2+}$ content and alkaline phosphatase activity. This effect is blocked by tamoxifen, indicating an ER-mediated pathway, and also by cycloheximide, suggesting that this isoflavone influence transcriptional or translational events (Yamaguchi & Gao, 1997, 1998). On the other hand, genistein also causes apoptosis of osteoblasts by activating caspase-3 and cleaving adhesion molecules such as cadherins and catenins (Hunter et al., 2001). In normal fetal osteoblast cells, genistein increases the progesterone receptor and alkaline phosphatase gene expression and inhibits osteopontin and interleukin-6 gene expression (Rickard et al. 2003).

### 2.2 Evidence from *in vivo* studies

The manner by which glucocorticoids induce bone loss is complex and incompletely understood (Patschan et al., 2001; Canalis et al., 2007), in part because there are no animal models absolutely comparable to humans. A major effect on the skeleton is a decrease in bone formation and unchanged or enhanced bone resorption (Lane et al., 2001). The main effect of glucocorticoids on bone is inhibition of osteoblastogenesis, augmented continued osteoclastogenesis and osteocyte apoptosis, leading to a decrease in bone formation, a rapid weakening of bone architecture and an increase in fracture risk (Manolagas & Weinstein, 1999). Chronic administration of steroids also causes avascular necrosis *via* an apoptotic mechanism of osteocytes and osteoblasts (Weinstein et al., 2000, Bekler et al., 2007). Once osteonecrosis occurs, glucocorticoids also cause inhibition of bone regeneration (Takano-

Murakami et al., 2009). In a rat model, we studied genistein preservative effects on methylprednisolone-induced bone loss and osteonecrosis of the femoral head (Bitto et al., 2009). In our study (Figure 1), genistein succeeded in preventing osteoporosis and osteonecrosis of the femoral head when co-administered with the glucocorticoid. The isoflavone statistically maintained bone mineral density (Figure 2) and content (Bitto et al., 2009) over the methylprednisolone-treated group and showed comparable efficacy with the vehicle group. Genistein co-administered with methylprednisolone also statistically maintained femoral bone's resistance to rupture compared with the methylprednisolone group (Figure 3C) and preserved the normal architecture of cartilage as well as both cortical and trabecular bones with a well-organized matrix in femoral head (Figure 3 A and B).

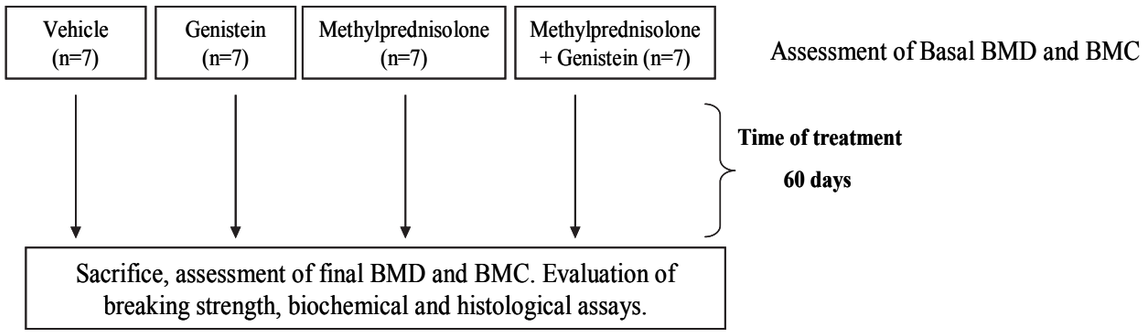



Fig. 1. Flow-chart of the experimental protocol.

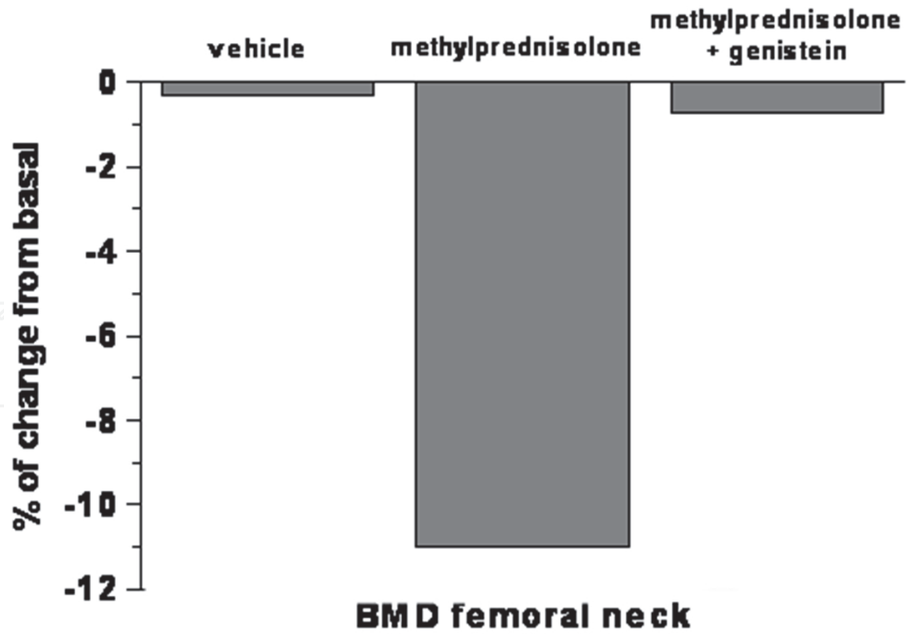



Fig. 2. Effects of aglycone genistein on femoral bone mineral density (BMD) in methylprednisolone-treated rats. Data are shown as % of variation from basal values in the same group of animals. * $p$<0.005 vs. methylprednisolone.

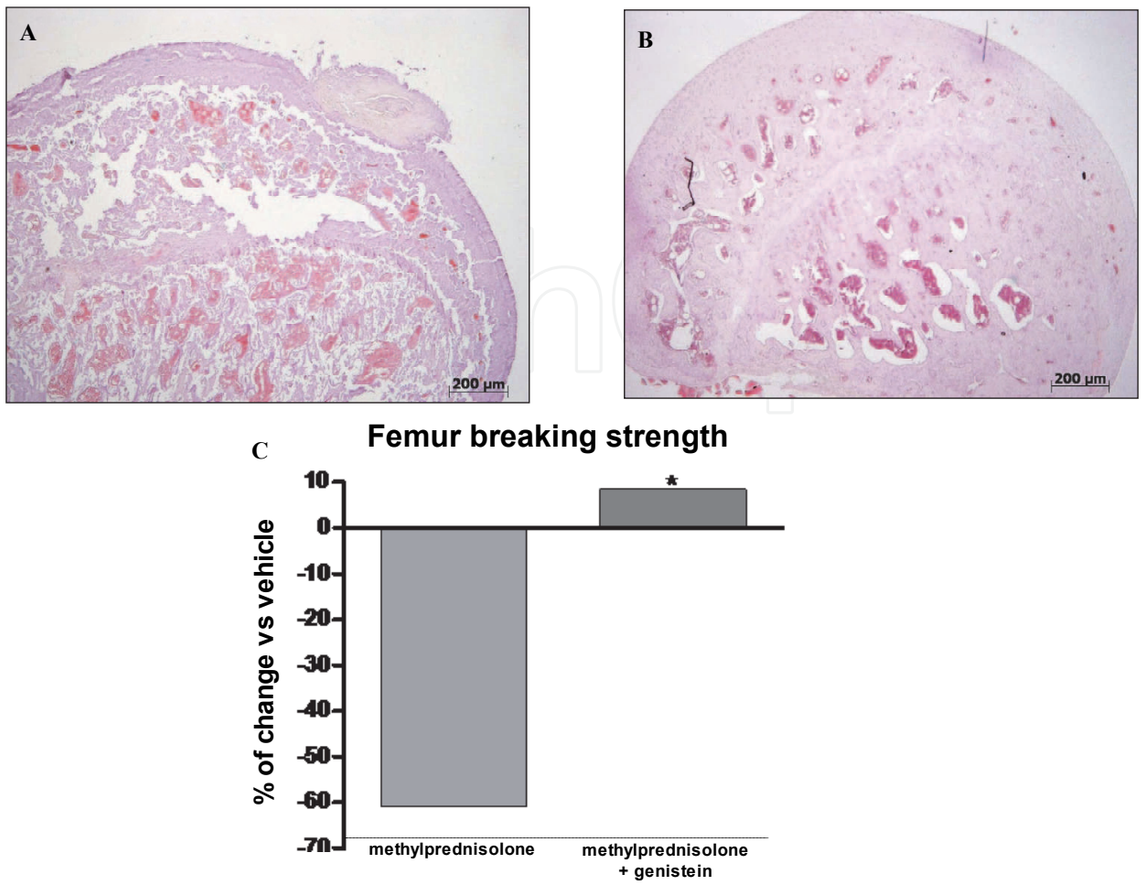



Fig. 3. A and B: Light microscopy of the bone structure of the femur head obtained from animals treated with methylprednisolone (A) or methylprednisolone plus genistein aglycone (B). (H&E original magnification X5). C: Femur breaking strength: *$p$<0.001 vs. methylprednisolone. Data are shown as % of variation from vehicle treated animals.

In addition, genistein caused a significant increase in b-ALP and OPG over methylprednisolone and vehicle confirming its role as an anabolic bone-forming agent (Figure 4A and B). Genistein administration also significantly reduced CTX circulating levels suggesting an anti-resorptive effect (Figure 4C).

Corticosteroid therapy has been shown to cause apoptosis in osteoblasts and inhibit the production of OPG required for bone formation, while RANKL accumulates resulting in further bone resorption (Bejar et al. 2005). Genistein has been shown to stimulate the production of osteoblasts *via* inhibition of a RANKL-resorptive mechanism by producing OPG (Viereck et al. 2002). This was also confirmed in our study where genistein increased OPG levels in glucocorticoid-treated animals. Osteoprotegrin is a glycoprotein secreted by osteoblasts in a differentiation-dependent manner and acts as a 'decoy receptor' (a soluble receptor that acts as antagonist) for RANKL regulating osteoclast functions and lifespan. In postmenopausal women with bone loss, genistein stimulated the production of OPG, down-regulated RANKL and decreased the RANKL/OPG ratio suggesting a direct effect on the RANK cytokine system (Marini et al., 2008). This work on genistein also suggests that increases in OPG in humans modulates the maturation process of osteoclast formation, thus

clarifying the putative antiresorptive effect being a non-apoptotic process, rather than an apoptotic process induced in antiresorptive therapy with bisphosphonates. Other mechanisms of genistein binding to glucocorticoid receptors have also been implicated.

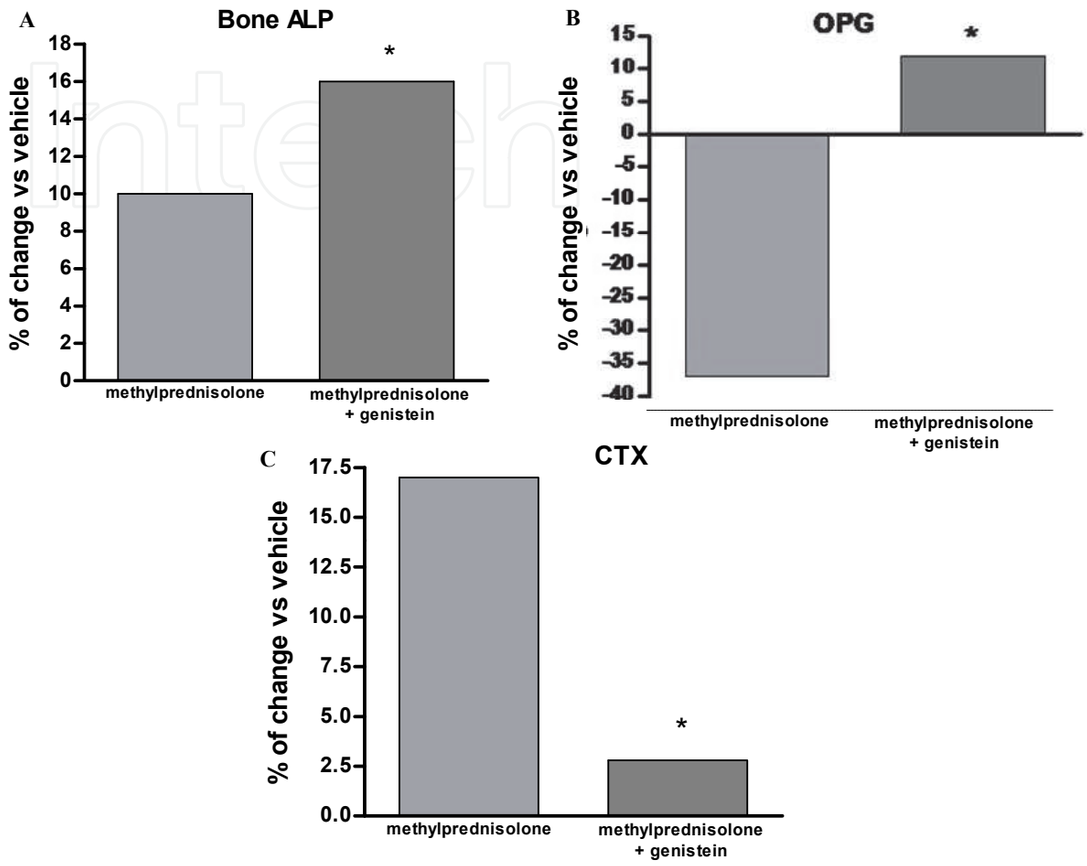



Fig. 4. A-C: Effects of aglycone genistein on serum bone-alkaline phosphatase (b-ALP), osteoprotegerin (OPG) and collagen C-telopeptides (CTX). Data are shown as % of variation from vehicle treated animals. b-ALP: * $p<0.005$ vs methylprednisolone. OPG: *$p<0.001$ vs. methylprednisolone. CTX: *$p<0.001$ vs. methylprednisolone.

It has been demonstrated that genistein inhibits glucocorticoid receptor transactivation and may also induce a proteosomal degradation of the glucocorticoid receptor complex *via* the p53 and ubiquitin pathways (Kinyamu & Archer 2003). Another mechanism might involve genistein activity as a tyrosine kinase inhibitor *via* the limitation of the subcellular nuclear transport and the recycling of the glucocorticoid receptors, blunting in turn the effects of glucocorticoids on bone (Yang et al., 1997).

## 3. Conclusions

Several studies and reports show a decrease in BMD and an increased risk of fractures during glucocorticoid use as well as an increase in osteonecrosis with chronic steroid use. Approximately 30% of all fractures of the hip and almost half of all fractures of the spine can

be attributed to chronic, high-dose glucocorticoid administration in humans (van Staa et al., 2001). Prior and current exposure to glucocorticoids increases the risk of fractures beyond that explained by values of BMD (Civitelli & Ziambaras, 2008). Pharmacological intervention for prevention of glucocorticoid-induced osteoporosis is needed depending on dose, expected duration of treatment, age and gender of the patient, and sometimes BMD at the start of the glucocorticoid therapy. At present, calcium and vitamin $D_3$ supplementation are considered as important support for the prevention of glucocorticoid-induced osteoporosis (Williams et al., 2004). Bisphosphonates are largely used to avoid bone loss and are cost effective in certain subgroups of patients depending on age, gender, glucocorticoid dose, and previous fracture history (Williams et al., 2004; Prinsloo & Hosking, 2006). Unfortunately, calcium and vitamin $D_3$ supplementation may not be enough to stave off bone deterioration and bisphosphonates have safety risks associated with long-term use, such as esophageal burns, bone and muscular pain (Ettinger et al., 1998; Wysowski & Chang, 2005). Osteonecrosis of the jaw, although rare and found primarily in cancer patients undergoing dental surgery, is a very serious and debilitating side effect of bisphosphonate use (Durie et al., 2005). Therefore, a safe and effective treatment for the prevention of bone loss and osteonecrosis of the femoral head in glucocorticoid-treated subjects is still needed.

Collectively, our results strongly suggest that genistein might be a new potential therapy for the prevention of glucocorticoid-induced osteoporosis, the most important secondary cause of osteoporosis in humans. And in the minority of cases, genistein may prevent necrotic deterioration of the femoral head. Usually, drugs used in management of osteoporosis have been classified as predominantly 'antiresorptive agents' or as 'bone-forming agents', but, on the basis of the present results, genistein might represent the first therapy to overcome this classification combining a powerful bone-forming as well as an anti-resorptive activity.

## 4. References

Adler, R.A. & Hochberg, M.C. Suggested guidelines for evaluation and treatment of glucocorticod-induced osteoporosis for the Department of Veterans Affairs. Arch Intern Med. 2003; 163:2619-24.

Albertazzi, P. Purified phytoestrogens in postmenopausal bone health: is there a role for genistein? Climacteric 2002; 5: 190–196

Amano, H., Yamada, S. & Felix, R. Colony-stimulating factor-1 stimulates the fusion process in osteoclasts. J Bone Miner Res, 1998; 13: 846–853.

American college of Rheumatology Ad Hoc Committee on glucocorticod-induced osteoporosis. Recommendation for the prevention and treatment of glucocorticod-induced osteoporosis. 2001 Update. Arthritis and Rheumat 2001; 44:1496-503.

Amin, S., Lavalley, M.P., Simms, R.W. & Felson, D.T. The comparative efficacy of drug therapies used for the management of corticosteroid induced osteoporosis: a meta-regression. J Bone Miner Res. 2002; 17: 1512-1526.

Angeli, A., Guglielmi, G., Dovio, A., Capelli, G., de Feo, D., Giannini, S., Giorgino, R., Moro, L. & Giustina, A. High prevalence of asymptomatic vertebral fractures in post-menopausal women receiving chronic glucocorticoid therapy: a cross-sectional outpatient study. Bone 2006; 39: 253–259

Arts, J., Kuiper, G.G., Janssen, J.M., Gustafsson, J.A., Löwik, C.W., Pols, H.A. & van Leeuwen, J.P. Differential expression of estrogen receptors alpha and beta mRNA during differentiation of human osteoblast SV-HFO cells. Endocrinology. 1997; 138: 5067-70

Bejar, J., Peled, E. & Boss, J.H. Vasculature deprivation – induced osteonecrosis of the rat femoral head as a model for therapeutic trials. Theor Biol Med Mod 2005; 2: 24.

Bekler, H., Uygur, A.M., Gökçe, A. & Beyzadeoğlu, T. The effect of steroid use on the pathogenesis of avascular necrosis of the femoral head: an animal model. Acta Orthop Traumatol Turc. 2007; 41: 58-63.

Bertazzo, S. & Bertran, C.A. Morphological and dimensional characteristics of bone mineral crystals. Bioceramics. 2006; 309-311:7-10

Bitto, A., Polito, F., Burnett, B., Levy, R., Di Stefano, V., Armbruster, M.A., Marini, H., Minutoli, L., Altavilla, D. & Squadrito, F. Protective effect of genistein aglycone on the development of osteonecrosis of the femoral head and secondary osteoporosis induced by methylprednisolone in rats. J Endocrinol. 2009; 201: 321-8.

Bitto, A., Polito, F., Squadrito, F., Marini, H., D'Anna, R., Irrera, N., Minutoli, L., Granese, R. & Altavilla, D. Genistein aglycone: a dual mode of action anti-osteoporotic soy isoflavone rebalancing bone turnover towards bone formation. Curr Med Chem. 2010; 17: 3007-18. Review.

Blair, H.C., Jordan, S.E., Peterson, T.G. & Barnes, S. Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. J Cell Biochem, 1996, 61, 629–637.,

Canalis, E., Mazziotti, G., Giustina A., & Bilezikian, J.P. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int 2007; 18: 1319–1328.

Chen, X., Garner, S.C., Quarles, L.D. & Anderson, J.J. Effects of genistein on expression of bone markers during MC3T3-E1 osteoblastic cell differentiation. J Nutr Biochem. 2003;14: 342-9

Choi, S.Y., Ha, T.Y., Ahn, J.Y., Kim, S.R., Kang, K.S., Hwang, I.K. & Kim, S. Estrogenic activities of isoflavones and flavones and their structure-activity relationships. Planta Med. 2008; 74: 25-32.

Civitelli, R. & Ziambaras, K. Epidemiology of glucocorticoid-induced osteoporosis. J Endocrinol Invest 2008; 31: 2–6.

Compston, J. US and UK guidelines for glucocorticoid-induced osteoporosis similarities and differences. Curr Rheumatol Rep. 2004; 6: 66-69.

Day, A.J., DuPont, M.S., Ridley, S., Rhodes, M., Rhodes, M.J., Morgan, M.R. & Williamson, G. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. FEBS Lett. 1998; 436: 71-5

Doga, M., Mazziotti, G., Bonadonna, S., Patelli, I., Bilezikian, J.P., Canalis, E. & Giustina, A. Prevention and treatment of glucocorticoid-induced osteoporosis. J Endocrinol Invest. 2008; 31: 53-88.

Durie, B.G., Katz, M. & Crowley, J. Osteonecrosis of the jaw and bisphosphonates. New Engl J Med 2005; 353: 99–102.

Eastell, R., Reid, D.M., Compston, J., Cooper, C., Fogelman, I., Francis, R.M., Hosking, D.J., Purdie, D.W., Ralston, S.H., Reeve, J., Russell, R.G., Stevenson, J.C. & Torgerson, D.J. A UK Consensus Group on management of glucocorticoid-induced osteoporosis: an update. J Intern Med 1998; 244:271-92.

Ettinger, B., Pressman, A. & Schein, J. Clinic visits and hospital admissions for care of acid-related upper gastrointestinal disorders in women using alendronate for osteoporosis. Am J Man Care 1998; 4: 1377–1382.

Gao, Y.H. & Yamaguchi, M. Inhibitory effect of genistein on osteoclast-like cell formation in mouse marrow cultures. Biochem Pharmacol, 1999a; 58: 767–772.

Gao, Y.H. & Yamaguchi, M. Suppressive effect of genistein on rat bone osteoclasts: apoptosis is induced through Ca2+ signaling. Biol Pharm Bull. 1999b; 22: 805-9.

Gao, Y.H. & Yamaguchi, M. Suppressive effect of genistein on rat bone osteoclasts: involvement of protein kinase inhibition and protein tyrosine phosphatase activation. Int J Mol Med 2000; 5: 261–267.

Gudbjornsson, B., Juliusson, U.I. & Gudjonsson, F.V. Prevalence of long term steroid treatment and the frequency of decision making to prevent steroid induced osteoporosis in daily clinical practice. Ann rheum Dis 2002; 61:32-6.

Heim, M., Frank, O., Kampmann, G., Sochocky, N., Pennimpede, T., Fuchs, P., Hunziker, W., Weber, P., Martin, I. & Bendik, I. The phytoestrogen genistein enhances osteogenesis and represses adipogenic differentiation of human primary bone marrow stromal cells. Endocrinology. 2004; 145: 848-59.

Hu, M., Krausz, K., Chen, J., Ge, X., Li, J., Gelboin, H.L. & Gonzalez, F.J. Identification of CYP1A2 as the main isoform for the phase I hydroxylated metabolism of genistein and a prodrug converting enzyme of methylated isoflavones. Drug Metab Dispos. 2003; 31: 924-31

Hunter, I., McGregor, D. & Robins SP. Caspase-dependent cleavage of cadherins and catenins during osteoblast apoptosis. J Bone Miner Res. 2001; 16: 466-77.

Jones, G. & Sambrook, P.H. Drug-induced disorders of bone metabolism. Drug Safety 1994; 10: 480-489

Kajiya, H., Okabe, K., Okamoto, F., Tsuzuki, T. & Soeda, H. Protein tyrosine kinase inhibitors increase cytosolic calcium and inhibit actin organization as resorbing activity in rat osteoclasts. J Cell Physiol 2000; 183: 83–90,

Kinyamu, H.K. & Archer, T.K. Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in Mdm2 protein expression. Mol Cell Biol 2003; 23: 5867–5881.

Kuiper, G.G., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B. & Gustafsson, J.A. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 1998; 139: 4252-63.

Laan, R.F., Buijs, W.C., van Erning, L.J., Lemmens, J.A., Corstens, F.H., Ruijs, S.H., van de Putte, L.B. & van Riel, P.L. Differential effects of glucocorticoids on cortical appendicular and cortical vertebral bone mineral content. Calcif Tissue Int 1993; 52:5-9.

Lane, N.E., Yao, W., Balooch, M., Nalla, R.K., Balooch, G., Habelitz, S., Kinney, J.H. & Bonewald, L.F. Glucocorticoid-treated mice have localized changes in trabecular bone material properties and osteocyte lacunar size that are not observed in placebo treated or estrogen-deficient mice. J Bone Miner Res. 2006; 21: 466–76

Larkin, T., Price, W.E. & Astheimer, L. The key importance of soy isoflavone bioavailability to understanding health benefits. Crit Rev Food Sci Nutr. 2008; 48: 538-52

Liu, J., Knappenberger, K.S., Kack, H., Andersson, G., Nilsson, E., Dartsch, C. & Scott, C.W. A homogeneous in vitro functional assay for estrogen receptors: coactivator recruitment. Mol Endocrinol. 2003; 17: 346-55.

Manolagas, S.C. & Weinstein, R.S. New developments in the pathogenesis and treatment of steroid-induced osteoporosis. J Bone Miner Res. 1999; 14: 1061-6.

Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., Atteritano, M., Gaudio, A., Mazzaferro, S., Frisina, A., Frisina, N., Lubrano, C., Bonaiuto, M., D'Anna, R., Cannata, M.L., Corrado, F., Cancellieri, F., Faraci, M., Marini, R., Adamo, E.B., Wilson, S. & Squadrito, F. OPG and sRANKL Serum Concentrations in Osteopenic, Postmenopausal Women After 2-Year Genistein Administration. J Bone Min Res. 2008; 23: 715-720

Matsukawa, Y., Marui, N., Sakai, T., Satomi, Y., Yoshida, M., Matsumoto, K., Nishino, H. & Aoike, A. Genistein arrests cell cycle progression at G2M. Cancer Research 1993; 53: 1328–1331.

Morris, C., Thorpe, J., Ambrosio, L. & Santin, M. The soybean isoflavone genistein induces differentiation of MG63 human osteosarcoma osteoblasts. J Nutr. 2006; 136: 1166-70.

National Osteoporosis Society. Guidelines on the prevention and management of corticosteroid-induced osteoporosis. Bath, England: National Osteoporosis Society; 1998.

Okumura, N., Yoshikawa, T., Iida, J., Nonomura, A. & Takakura, Y. Bone formation-promoting effect of genistein on marrow mesenchymal cell culture. Biomed Mater Eng. 2006; 16: 23-32.

Orcel, P. Prevention and treatment of glucocorticoidinduced osteoporosis in 2005. Joint Bone Spine 2005; 72: 461-465

Pan, W., Quarles, L.D., Song, L.H., Yu, Y.H., Jiao, C., Tang, H.B., Jiang, C.H., Deng, H.W., Li, Y.J., Zhou, H.H. & Xiao, Z.S. Genistein stimulates the osteoblastic differentiation via NO/cGMP in bone marrow culture. J Cell Biochem. 2005; 94: 307-16.

Patschan, D., Loddenkemper, K. & Buttgereit F. Molecular mechanisms of glucocorticoid-induced osteoporosis. Bone. 2001; 29: 498-505.

Prasain, J.K., Xu, J., Kirk, M., Smith Johnson, M., Sfakianos, J. & Barnes, S. Differential biliary excretion of genistein metabolites following intraduodenal and intravenous infusion of genistin in female rats. J Nutr. 2006; 136: 2975-9

Prinsloo, P.J.J. & Hosking, D.J. Alendronate sodium in the management of osteoporosis. J Therap Clin Risk Manag 2006; 2: 235–249.

Rickard, D.J., Monroe, D.G., Ruesink, T.J., Khosla, S., Riggs, B.L. & Spelsberg, T.C. Phytoestrogen genistein acts as an estrogen agonist on human osteoblastic cells through estrogen receptors alpha and beta. J Cell Biochem. 2003; 89: 633-46.

Ronis, M.J., Little, J.M., Barone, G.W., Chen, G., Radominska-Pandya, A. & Badger, T.M. Sulfation of the isoflavones genistein and daidzein in human and rat liver and gastrointestinal tract. J Med Food. 2006; 9: 348-55

Saag, K.G., Shane, E., Boonen, S., Marín, F., Donley, D.W., Taylor, K.A., Dalsky, G.P. & Marcus, R. Teriparatide or alendronate in glucocorticoid-induced osteoporosis. N Engl J Med. 2007;357:2028-2039.

Setchell, K.D., Brown, N.M. & Lydeking-Olsen, E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr. 2002; 132: 3577-84

Sfakianos, J., Coward, L., Kirk, M. & Barnes, S. Intestinal uptake and biliary excretion of the isoflavone genistein in rats. J Nutr. 1997; 127: 1260-8

Sliwiński, L., Folwarczna, J., Janiec, W., Grynkiewicz, G. & Kuzyk, K. Differential effects of genistein, estradiol and raloxifene on rat osteoclasts in vitro. Pharmacol Rep. 2005; 57: 352-9.

Sugimoto, E. & Yamaguchi, M. Anabolic effect of genistein in osteoblastic MC3T3-E1 cells. Int J Mol Med. 2000; 5: 515-20.

Takano-Murakami, R., Tokunaga, K., Kondo, N., Ito, T., Kitahara, H., Ito, M. & Endo, N. Glucocorticoid inhibits bone regeneration after osteonecrosis of the femoral head in aged female rats. Tohoku J Exp Med. 2009; 217:51-8.

Tham, D.M., Gardner, C.D. & Haskell, W.L. Clinical review 97: Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. J Clin Endocrinol Metab. 1998; 83: 2223-35.

Tian, Z,, Wan, M., Wang, Z. & Wang, B. The preparation of genistein and LC-MS/MS on-line analysis. Drug Dev Res. 2004; 61: 6-12

Uchiyama, S. & Yamaguchi, M. Genistein and zinc synergistically stimulate apoptotic cell death and suppress RANKL signaling-related gene expression in osteoclastic cells. J Cell Biochem. 2007; 101: 529-42.

van Staa, T.P. The pathogenesis, epidemiology and management of glucocorticoid-induced osteoporosis. Calcif Tissue Int. 2006; 79: 129-37.

van Staa, T.P., Laan, R.F., Barton, I.P, Cohen, S., Reid, D.M. & Cooper, C. Bone density threshold and other predictors of vertebral fracture in patients receiving oral glucocorticoid therapy. Arthritis Rheum. 2003; 48: 3224-3229.

van Staa, T.P., Leufkens, H.G. & Cooper, C. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. Osteoporos Int 2002; 13:777-87.

van Staa, T.P., Leufkens, H.G., Abenhaim, L., Zhang, B. & Cooper, C. Use of oral corticosteroids and risk of fractures, J Bone Miner Res 15 (2000), pp. 993–1000.

Vestergaard, P., Rejnmark, L. & Mosekilde, L. Fracture risk associated with different types of oral corticosteroids and effect of termination of corticosteroids on the risk of fractures. Calcif Tissue Int. 2008; 82: 249-257.

Viereck, V., Grundker, C., Blaschke, S., Siggelkow, H., Emons, G. & Hofbauer, L.C. Phytoestrogen genistein stimulates the production of osteoprotegrin by human trabecular osteoblasts. J Cell Biochem 2002; 84: 725–735.

Walsh, I.J., Wong, C.A., Pringle, M. & Tattersfield, A.E. Use of oral corticosteroids in community and the prevention of secondary osteoporosis: a cross sectional study. BMJ 1996; 313:344-6.

Weinstein, R.S., Nicholas, R.W. & Manolagas, S.C. Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. J Clin Endocrinol Metab. 2000; 85: 2907-12.

Williams, D., Bennett, K. & Feely, J. Prescribing for osteoporosis following the use of inhaled and oral glucocorticoids in general practice. Br J Clin Pharmacol 2004; 58: 665–672.

Williams, J.P., Jordan, S.E., Barnes, S. & Blair, H.C. Tyrosine kinase inhibitor effects on avian osteoclastic acid transport. Am J Clin Nutr 1998; 68: 1369S–1374S.

Wysowski, D.K. & Chang, J.T. Alendronate and risedronate: reports of severe bone, joint, and muscle pain. Arch Int Med 2005; 165: 346–347.

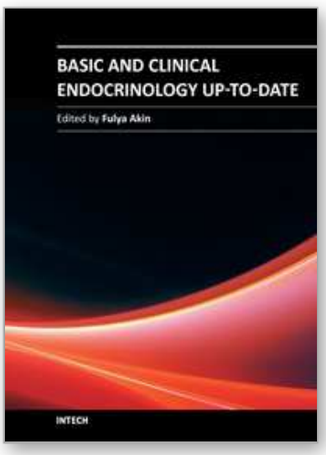
Yamagishi, T., Otsuka, E. & Hagiwara, H. Reciprocal control of expression of mRNAs for osteoclast differentiation factor and OPG in osteogenic stromal cells by genistein: evidence for the involvement of topoisomerase II in osteoclastogenesis. Endocrinology 2001; 142: 3632–3637.

Yamaguchi, M. & Gao, Y.H. Anabolic effect of genistein on bone metabolism in the femoral-metaphyseal tissues of elderly rats is inhibited by the anti-estrogen tamoxifen. Res Exp Med (Berl). 1997; 197: 101-7.

Yamaguchi, M. & Gao, Y.H. Inhibitory effect of genistein on bone resorption in tissue culture. Biochem Pharmacol. 1998; 55: 71-6.

Yamaguchi, M. & Sugimoto, E. stimulatory effect of genistein and daidzein on protein synthesis in osteoblastic MC3T3-E1 cells: activation of aminoacyl-t-RNA synthetase. Mol Cell Biochem 2000; 214: 97–102

Yang, J., Liu, J. & De Franco, D.B. Subnuclear trafficking of glucocorticoid receptors in vitro: chromatin recycling and nuclear export. J Cell Biol 1997; 137: 523–538.




**Basic and Clinical Endocrinology Up-to-Date**

Edited by Dr. Fulya Akin

ISBN 978-953-307-340-8
Hard cover, 350 pages
**Publisher** InTech
**Published online** 17, October, 2011
**Published in print edition** October, 2011

This book provides the most up-to-date information on the basic and clinical aspects of endocrinology. It offers both researchers and clinicians experts, gold-standard analysis of endocrine research and translation into the treatment of diseases such as insulinoma, endocrine disease in pregnancy and steroid induced osteoporosis. Investigates both the endocrine functions of the kidneys and how the kidney acts as a target for hormones from other organ systems. Presents a uniquely comprehensive look at all aspects of endocrine changes in pregnancy and cardiovascular effects of androgens.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Alessandra Bitto, Francesca Polito, Bruce P. Burnett, Herbert Marini, Robert Levy, Domenica Altavilla and Francesco Squadrito (2011). Genistein Aglycone Demonstrates a Protective and Reversible Effect on the Development of Steroid-Induced Secondary Osteoporosis and Increases Bone Breaking Strength in Rats, Basic and Clinical Endocrinology Up-to-Date, Dr. Fulya Akin (Ed.), ISBN: 978-953-307-340-8, InTech, Available from: http://www.intechopen.com/books/basic-and-clinical-endocrinology-up-to-date/genistein-aglycone-demonstrates-a-protective-and-reversible-effect-on-the-development-of-steroid-ind

INTECH
open science | open minds

**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

**InTech China**
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen