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Osteoporosis and Its Treatments

Monir Ali El-Ganzuri[1], *Rasha Rashad Ahmed[2], Eslam Muhammad Bastawy[1]

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Corresponding Author

Monir Ali El-Ganzuri

Abstract

INTRODUCTION

Bone diseases:

Bone disease refers to the medical conditions which affect the bone, A bone disease is also called an "osteopathy". Several diseases cause various abnormalities or deformities of bone. Examples of bone diseases include, rickets, osteomalacia, osteogenesis imperfecta, marble bone disease (osteopetrosis), Paget disease of bone, fibrous dysplasia and osteoporosis. In clinical terms, bone diseases may result in bone pain and loss of height (due to compression of vertebrae), and they predispose patients to fractures**.**

Osteoporosis is a more frequent disease in women than in men [(1)] although mortality due to osteoporotic fractures is higher in men than in women [(2)]. In addition, post-menopausal women suffer from osteoporosis and osteoporotic fractures at a higher frequency than pre-menopausal women [(3)]. Through adult life there is a dynamic progress which is called as we mentioned before "bone remodelling".

5.1- Types of osteoporosis:

Osteoporosis is mainly of two types. They are:

5.1.1- Primary osteoporosis [(4)]**:**

It occurs in both genders at all ages but often follows menopause in women and occurs late in life in men.

5.1.1. a- Primary Osteoporosis Type I (or) Postmenopausal Osteoporosis:

It is characterized by an increased bone resorption that primarily affects trabecular bone and is directly linked to the decreased production of estrogen that coincides with menopause. Rapid bone loss is osteoclast-mediated and occurs in women within the first 5 to 10 years after menopause.

5.1.1. b- Primary osteoporosis Type II:

Here, proportionate loss of trabecular and cortical bone occurs usually due to a decrease in bone cell activity accompanying aging. This type of osteoporosis predominately afflicts men and women over the age of 70 years and is called senile osteoporosis.

5.1.2- Secondary osteoporosis: [(5)]:

This is a condition where osteoporosis occurs as a result of an identifiable cause. There are several causes for secondary osteoporosis which include:

(i). **Endocrine disorders** like: Acromegaly, adrenal atrophy in Addison disease, cushing syndrome.
(ii). **Eating disorders** like: Endometriosis, gonadal insufficiency (primary or secondary) hyperparathyroidism, hyper-prolactinemia, hyperthyroidism, hypogonadism, Type 1 diabetes mellitus.
(iii). **Nutritional disorders** like: Tumour secretion of parathyroid hormone–related peptide, gastro-intestinal disease.
(iv). **Alcohol-related liver diseases** like: Celiac disease, chronic active hepatitis, chronic cholestatic diseases, gastrectomy, inflammatory bowel disease, jejunoileal bypass.
(v). **Malabsorpion syndromes** like: Pancreatic insufficiency, parenteral nutrition, primary biliary cirrhosis, severe liver disease.
(vi). **Marrow-related disorders like**: Amyloidosis, hemochromatosis, hemophilia, leukemia, lymphoma, mastocytosis, multiple myeloma, pernicious anemia, sarcoidosis, sickle cell anaemia, thalassemia.
(vii). **Organ transplantation** like: Bone marrow, heart, kidney, liver and lung.
(viii). **Miscellaneous causes** like: Ankylosing spondylitis, chronic obstructive pulmonary disease, congenital porphyria, epidermolysis bullosa, hemophilia, idiopathic hypercalciuria, idiopathic scoliosis, multiple sclerosis, rheumatoid arthritis.
(ix). **Genetic disorders** like: Hypophosphatasia, osteogenesis.

5.2- Epidemiology of osteoporosis:
Elderly people are the fastest growing population in the world and, as people age, bones mass declines and the risk of fractures increases [6]. Osteoporosis is a major public health problem throughout the world [7]. The social and economic burden of osteoporosis is increasing steadily because of the aging of the world population. Currently affecting more than 10 million people in the United States, osteoporosis is projected to impact approximately 14 million adults over the age of 50 by the year 2020 (National Osteoporosis Foundation, 2002). Worldwide, approximately 200 million women have osteoporosis (International Osteoporosis Foundation, 2005). Although the likelihood of developing osteoporosis currently is greatest in North America and Europe, it will increase in developing countries as population longevity in these countries continues to increase.

The annual incidence of osteoporotic fractures exceeds 1.5 million in the United States (National Osteoporosis Foundation, 2002). Hip fractures, long considered more devastating than any other type of osteoporotic fracture, are projected to increase from an estimated 1.7 million in 1990 to 6.3 million by the year 2050 [6]. Notably, 1 in 5 persons die during the first year after a hip fracture (National Institutes of Health, 2000), whereas nearly one third require nursing home placement after hospital discharge, and fewer than one third regain their prefracture level of physical function (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001).

5.3- Experimental models of osteoporosis (Fig. 7):
Experimental animal models play an important role in improving the knowledge of the etiology, pathophysiology, and diagnosis, as well as on preventive and therapeutical techniques, regarding osteoporosis. A large variety of animal species, including rodents, rabbits, dogs, and primates, have been used as animal models in osteoporosis research. Among these, the laboratory rat is the preferred animal for most researchers. Its skeleton has been studied extensively, and although there are several limitations to its similarity to the human condition, these can be overcome through detailed knowledge of its specific traits or with certain techniques. Rats are currently principal laboratory animals, used to investigate this disease, since they are inexpensive to maintain, grow rapidly, have a relatively short lifespan and are widely available [8, 9].
Animal models of osteoporosis can be classified in **two main categories**: 1- those in which increased bone resorption is the dominant mechanism (i.e., ovariectomy, limb immobilization, diet calcium restriction), and 2- models in which decreased formation reveals (i.e., senile rats, Senescence-Accelerated Mouse (SAM mouse), or glucocorticoid administration).

5.3.1- Immobilization:
Another method for inducing osteoporosis in rats is through immobilization. There are several methods of immobilization, which can be surgical, such as nerve [10], tendon [11] and spinal cord resection [12]. Because of the regional acceleratory phenomenon, the rate of bone loss is more rapid after surgical methods than conservative immobilization [13].

5.3.2- Pharmaceutical agents:
Alternative methods to surgical ovariectomy leading to osteoporosis are the administration of pharmaceutical agents, such as a gonadotropin releasing hormone agonist [14], estrogen receptor antagonist [15], and aromatase inhibitor [16], these pharmaceutical agents, which are used in humans for the endocrine treatment of endometriosis or breast cancer, are associated with accelerated bone loss [9]. The administration of gonadotropin releasing hormone agonists creates a hypogonadotrophic–hypogonadal model, whereas estrogen receptor antagonists and aromatase inhibitors produce a hypergonadotrophic–

hypogonadal model of osteoporosis. Other hormonal interventions resulting in osteopenia, including hypophysectomy [17], orchidectomy [18], and parathyroidectomy [19], also is well studied in the rat skeleton.

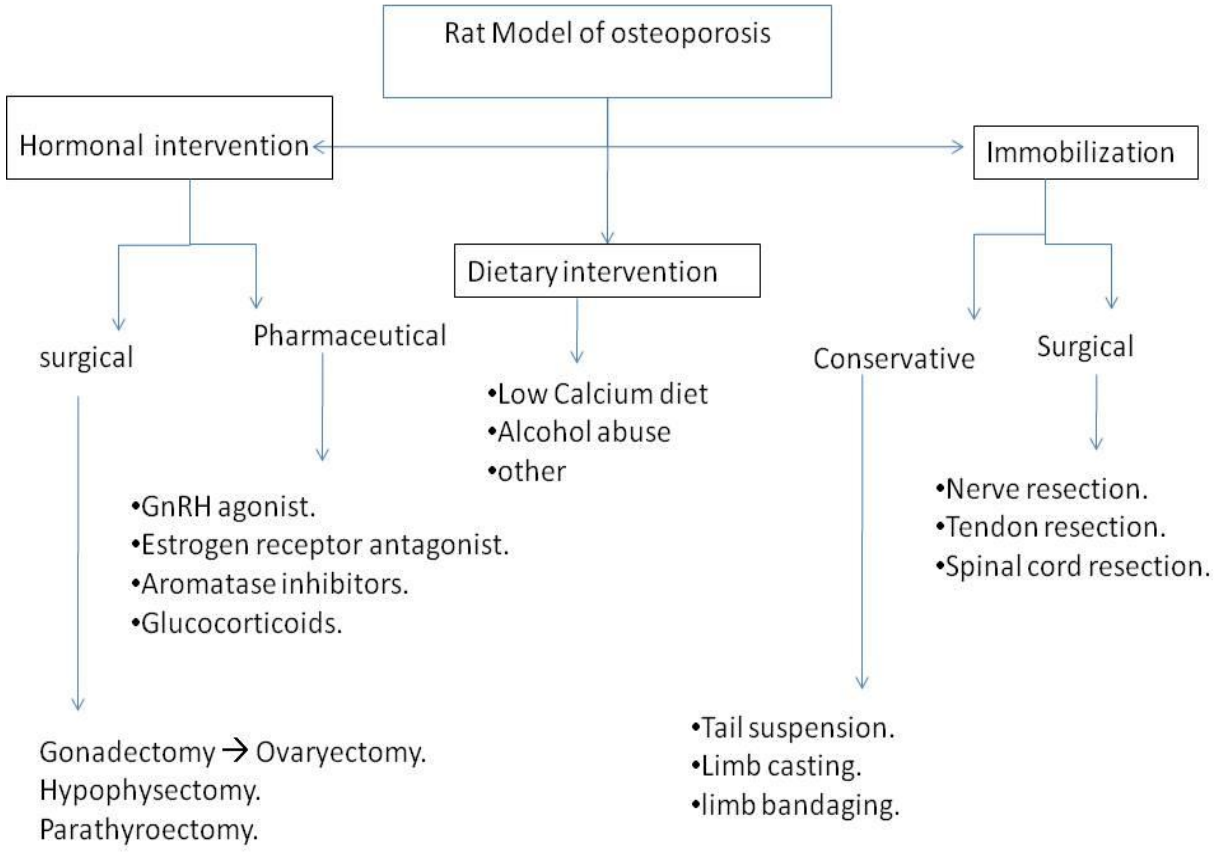

Fig. 7: Experimental models of osteoporosis (Pavlos *et al*., 2008).

5.3.2.1- Glucocorticoid-induced osteoporosis (GIO):

GIO is characterized by an early, transient phase of increased bone resorption, which results in an increased remodelling rate, in association with a reduction in bone formation at both the tissue and cellular levels that persists throughout the duration of glucocorticoid therapy [20]. These changes are similar in some respects to those seen in postmenopausal osteoporosis, but differ in others [21]. In postmenopausal osteoporosis, the increase in resorption at the tissue level is maintained and accompanied by increased tissue-level bone formation. Both GIO and postmenopausal osteoporosis are associated with a reduction in bone formation at the cellular level, this effect being quantitatively greater in GIO. Thus, increased remodelling rate and decreased formation at the level of the individual bone remodelling unit contribute to bone loss in both conditions, but with differences in the time course and magnitude of these changes. The early increase in bone resorption in association with reduced bone formation in GIO is likely to be a major contributor to bone loss and increased fracture risk within the first few months of initiating therapy, and is, therefore, an important therapeutic target. Disruption of cancellous bone microarchitecture, similar to that seen in postmenopausal osteoporosis, may be present, depending on the dose of glucocorticoids used [20].

The mechanisms underlying the glucocorticoid mediated transient increase in bone resorption have not been fully elucidated, but include increased production of macrophage colony stimulating factor and receptor activator of nuclear factor κB ligand (rANKl) by osteoblastic cells and down-regulation of osteoprotegerin (OPG), resulting in increased osteoclastogenesis and prolongation of osteoclast lifespan (Fig.8). This transient change may be limited by the potent suppression of

osteoblastogenesis by glucocorticoids, resulting in reduced *rANKl* production [22] In addition, there is evidence that long-term glucocorticoid use is associated with decreased osteoblastogenesis which, together with an increase in osteoblast apoptosis, explains the low bone turnover and reduced bone formation at cellular level that is characteristic of long-term glucocorticoid therapy.

Direct effects of glucocorticoids on bone formation include reduced osteoblastogenesis due to inhibition of the *Want* signalling pathway and up-regulation of peroxisome proliferator activated receptor *γ2* (*PPARγ2*), and increased osteoblast and osteocyte apoptosis due to activation of caspase (evidence for reduced angiogenesis and its consequent effects on the osteocyte–canalicular circulation has also been reported [23]. These changes, together with increased osteocyte apoptosis, may explain the impairment of biomechanical properties in surrounding cancellous bone. Hypogonadism, reduced production of insulin growth factor and increased losses of calcium from the kidney and intestine also contribute indirectly to glucocorticoid- induced bone loss, although evidence for secondary hyper parathyroidism is lacking (Fig.9).

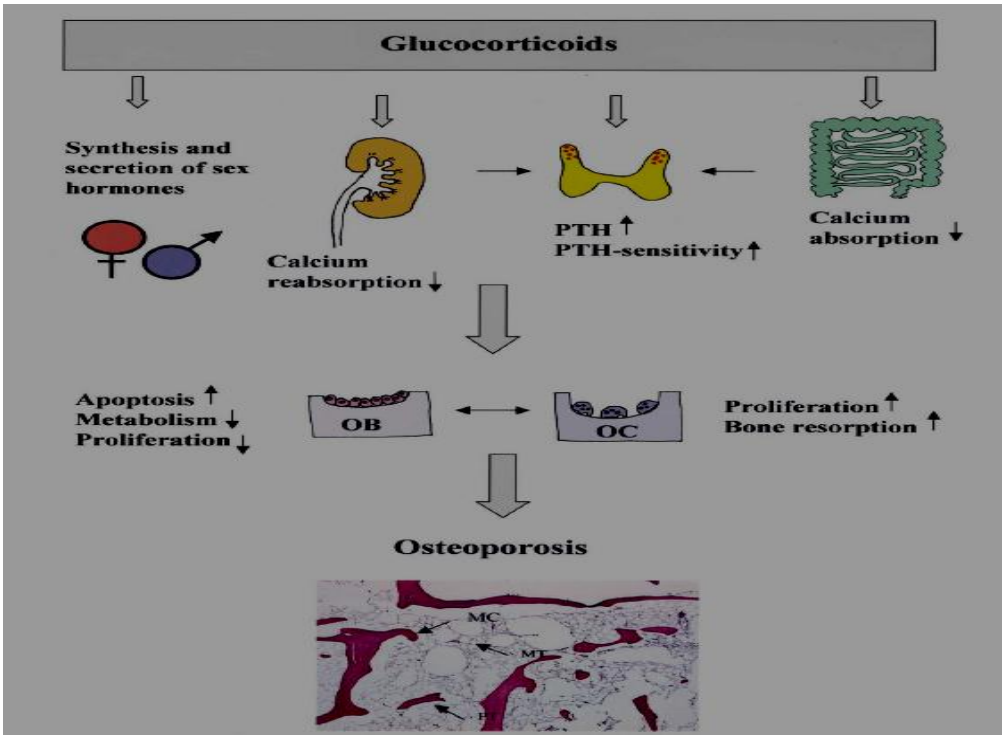

Fig. 8**:** Mechanisms of glucocorticoid-induced osteoporosis. In addition to their direct effects on bone cells, glucocorticoids have effects on the intestine, the kidneys, the gonads, and probably the parathyroid glands, which indirectly lead to osteoporosis. OB, osteoblasts; OC, osteoclasts; MC, microcallus; MT, missing trabecular structure; PTH, perforated trabecular structure (Kaneko *and Kawai*, 2011)

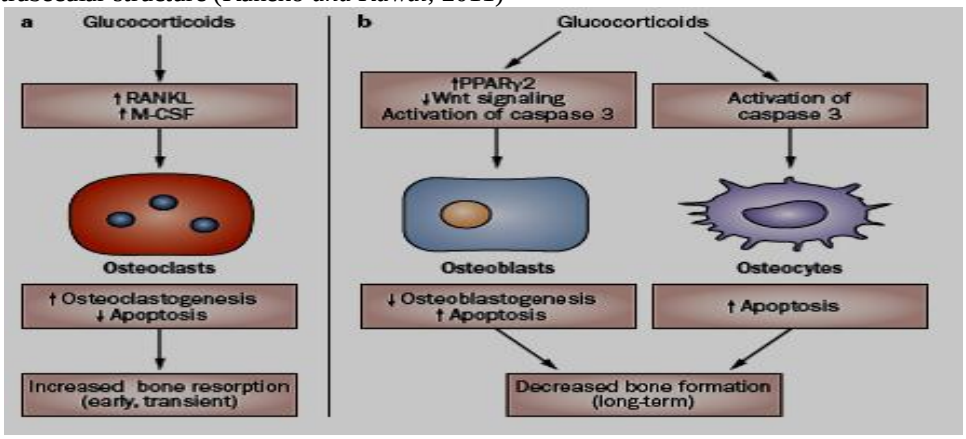

Fig. 9: Direct effects of glucocorticoids on bone. a) Glucocorticoids induce upregulation of expression of RANKL and M-CSF in osteoblasts and downregulation of osteoprotegrin in osteoclasts ,which leads to increased osteoclastogenesis and osteoclast life span. These effects on the number and activity of osteoclasts cause the transient increase in bone resorption observed early in the cousre of glucocorticoid therapy. b) In osteoblasts and osteocytes, increased PPAR *γ2* expression and decreased *Wnt* signalling contribute to decreased oseoblastogenesis and activation of caspase 3 results in increased osteoblast and osteocyte apoptosis. These glcocorticoid induced changes in osteoclast and osteocyte function result in the long-term state of decreased bone formation that is characteristic of GIO (Seibel *et al*., 2013).

abbreviations: M-CSF macrophage colony-stimulating factor, PPAR *γ2* : peroxisome proliferator activated receptor *γ2,*RANKL :receptor activator for nuclear factor K_B

- Glucocorticoids and bone formation (Osteoblast and Osteocytes):

Glucocorticoids also reduced serum markers of bone formation, such as osteocalcin [24, 25] and PICP (procollagen type I carboxy-propeptide) [26], glucocorticoids appear to inhibit production of bone matrix components, such as type I collagen, by osteoblasts [27], increase osteoblastic and osteocytic apoptosis, decrease expression of Runx2/Cbfa1 and tumour growth factor b(TGF-b) type 1 receptor , increase peroxisome proliferator-activated receptor-c2(PPARc2) expression [28], Runx2/Cbfa1 is a transcriptional factor that is essential to osteoblastic differentiation. TGF-b and IGF are local factors that are important for bone formation, and PPARc2 is an important transcriptional factor for differentiation from bone marrow mesenchymal cells to adipocytes, not to osteoblasts.

Osteocytes serve as mechanosensors, and play a role in the repair of bone microdamage [29]. Loss of osteocytes disrupts the osteocyte-canalicular network resulting in a failure to detect signals that normally stimulate processes associated with the replacement of damaged bone. Disruption of the osteocyte-canalicular network can disrupt fluid flow within the network adversely affecting the material properties of the surrounding bone, independent of changes in bone remodelling or architecture. Glucocorticoids affect the function of osteocytes, by modifying the elastic modulus surrounding osteocytic lacunae. Glucocorticoids induce the apoptosis of osteocytes. As a result, the normal maintenance of bone through this mechanism is impaired and the biomechanical properties of bone are compromised [30]

- Glucocorticoids and bone resorption (Osteoclasts):

Glucocorticoids decrease intestinal calcium absorption, and increase renal calcium excretion [31], and the secondary hyperparathyroidism caused by the negative calcium balance may increase osteoclastic activity and decrease bone mass. Glucocorticoids may cause hypogonadism by inhibiting gonadotropin secretion and inhibiting dehydroepiandrosterone sulphate (DHEA-S) secretion via adrenocorticotropic hormone (ACTH) suppression, and the decrease of sex hormone levels may increase bone resorption and decrease bone mass. In terms of local effects, glucocorticoids may directly promote osteoclastogenesis by increasing receptor activator of NF-kB ligand (RANK-L) and colony-stimulating factor-1 (CSF-1) and decreasing osteoprotegerin [32]. RANK-L is a molecule that stimulates osteoclast generation and differentiation, whereas osteoprotegerin is a decoy receptor of RANK-L and inhibits osteoclast generation and differentiation. CSF-1 stimulates osteoclastic differentiation. Glucocorticoids may also increase the number of osteoclasts by inhibiting osteoclast apoptosis [33].

Glucocorticoids decrease the replication of cells of the osteoblastic lineage, reducing the pool of cells that may differentiate into mature osteoblasts, In the presence of glucocorticoids, bone marrow stromal cells, the precursors of osteoblasts, do not differentiate or are directed, instead, towards cells of the adipocytic lineage.

An additional mechanism by which glucocorticoids inhibit osteoblast cell differentiation is by opposing Wnt/β-catenin signalling [22]. Wnt signaling has emerged as a key regulator of osteoblastogenesis. In this pathway, when Wnt is absent, β-catenin is phosphorylated by glycogen-synthase kinase-3β (GSK-3β), and then degraded by ubiquitination. When Wnt is present, it binds to specific receptors, called frizzled, and to co-receptors, low density lipoprotein receptor related proteins (LRP)-5 and (LRP)-6, leading to inhibition of GSK-3β activity. When GSK-3β is not active, stabilized β-catenin translocates to the nucleus, where it associates with transcription factors to regulate gene expression. Deletions of either Wnt or β-catenin result in the absence of osteoblastogenesis, and increased osteoclastogenesis [34]. The Wnt pathway can be inactivated by Dickkopf, an antagonist that prevents Wnt binding to its receptor complex. Glucocorticoids enhance Dickkopf expression and maintain GSK 3-β in an active state, leading ultimately to the inactivation of β-catenin [35].

Glucocorticoids have pro-apoptotic effects on osteoblasts and osteocytes due to activation of caspase 3, a common downstream effector of several apoptotic signalling pathways. Caspases are synthesized as proenzymes and are activated

through autocatalysis or a caspase cascade. Active caspases contribute to apoptosis by cleaving target cellular proteins. Caspase 3 is a key mediator of apoptosis and is a common downstream effector of multiple apoptotic signalling pathways [36].

The inhibitory effects of glucocorticoids on osteoblastic cell replication and differentiation and the increased apoptosis of mature osteoblasts, all contribute to the depletion of the osteoblastic cellular pool and decreased bone formation [37].

Osteoclasts are members of the monocyte/macrophage family of cells that differentiate under the influence of two requisite cytokines, namely macrophage colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANK-L). Glucocorticoids increase the expression of M-CSF and RANK-L, and decrease the expression of its soluble decoy receptor, osteoprotegerin, in stromal and osteoblastic cells.

Glucocorticoids also enhance the expression of Interleukin-6, an osteoclastogenic cytokine, and suppress the expression of interferon-beta, an inhibitor of osteoclastogenesis [38]. Glucocorticoids decrease the apoptosis of mature osteoclasts. Consequently, there is increased formation of osteoclasts with a prolonged life span explaining, at the cellular level, the enhanced and prolonged bone resorption observed in GIO. Glucocorticoids suppress IGF I gene transcription insulin-like growth factor (IGF) I. IGF-I increases bone formation and the synthesis of type I collagen, and decreases bone collagen degradation and osteoblast apoptosis (Fig.10) [39].

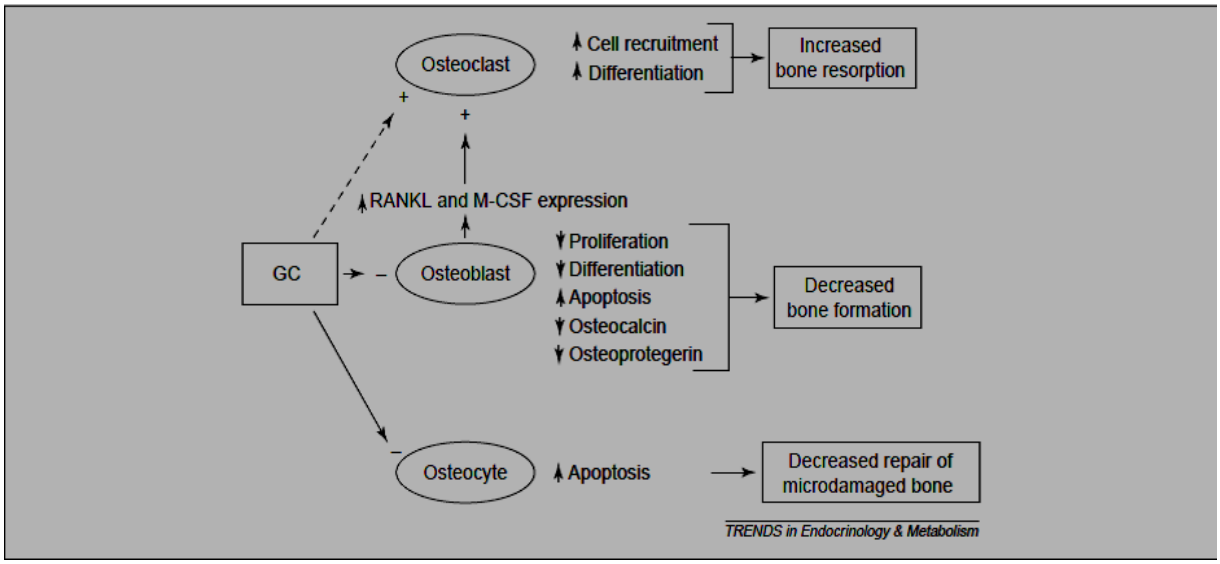

Fig. 10: Effect of Glucocorticoids on Bone cells (Gherardo *et al.*, 2006)

5.4- Examples of glucocorticoid-induced osteoporosis (PREDNISOLONE):

Prednisolone is a corticosteroid drug with glucocorticoid properties, making it useful for the treatment of a wide range of asthma, autoimmune diseases (systemic lupus erythematosus), skin conditions as pyoderma gangrenosum, and other inflammatory conditions such as asthma, uveitis, rheumatoid arthritis, ulcerative colitis . They can help to prevent or suppress inflammation and immune responses [40].

Prednisolone following systemic absorption diffuses across cell membranes and irreversibly binds with glucocorticoid receptors (GR) alpha and beta for which they have a high affinity. α GR and β GR are found in virtually all tissues with variable numbers between 3000 and 10000 per cell, depending on the tissue involved, And complex with specific cytoplasmic receptors. These complexes may enter the cell nucleus, bind to DNA and stimulate transcription of mRNA. Subsequent cellular responses result in a variety of local and systemic effects. Anti-inflammatory processes such as edema, fibrin deposition, decreased prostaglandin/thromboxane synthesis, capillary dilatation, migration of leukocytes, the phagocytosis stage of wound healing and cicatrization are inhibited. Immune reactions are suppressed [41].

Metabolically, protein catabolism and increased gluconeogenesis, along with decreased peripheral utilization of glucose, leads to glycogen storage in the liver, increased blood glucose concentration and insulin resistance (diabetogenic effect). During therapy lipolysis is enhanced and abnormal distribution of fat may result (Cushingoid effect). Skeletal calcium is mobilized and lost *via* renal excretion.

Prolonged use of prednisolone can lead to the development of osteoporosis which makes bones more fragile and susceptible to fractures.

5.4.1-Nomenclature and Structure:
Prednisolone (INN) is a synthetic steroid that is chemically defined as 11b, 17a,21-trihydroxypregna-1,4-diene-3,20-dione. Its structure is shown in (Fig.11) and its molecular weight =360.4

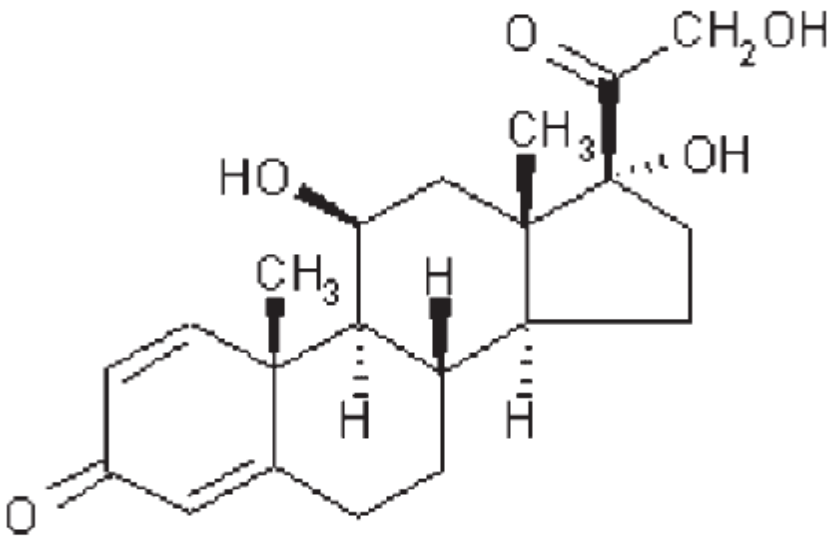

Fig. 11**:** Structure of Prednisolone $C_{21}H_{28}O_5$ (Vogt *et al*., 2006).

5.4.2-Pharmacokinetic properties:

- Absorption and Bioavailability:

Following oral intake prednisolone is rapidly absorbed from the GI tract. The systemic availability is almost complete and reported to range from 75% to 98% [(40)], with a concentration time profile that is very similar to that when prednisone is taken orally [(42)]. Maximum serum concentrations occur within 1–2 hr. after administration of a single dose [(40)].

- Distribution:

The volume of distribution of prednisolone was reported as 0.22–0.70 l/kg, With increasing dose, the volume of distribution of prednisolone increases, due to a shift in a larger fraction of the body burden from the plasma compartment to other body tissues or to sites of greater metabolic activity. The concentration-dependent binding of prednisolone to the plasma proteins (i.e., transcortin and albumin) results in the dose-dependent nonlinear pharmacokinetics observed for prednisolone [(44)]. Binding of prednisolone to plasma protein is independent of the route of administration.

- Metabolism and Excretion:

Prednisolone is pharmacologically active and may be metabolized in a variety of tissues, including liver, lung, kidney, and skin. Prednisolone is partially converted into prednisone, which is inactive. The serum half-life of prednisolone is known to be 2–4 hr. [(45)], and may be influenced by time of day, age, gender, physical exercise, pregnancy, drugs, and several diseases. Prednisolone is cleared from the body primarily by hepatic metabolism by hydroxylation and reduction forming metabolites which conjugate with glucuronic acid and sulphate [(46)].

5.5. Glucocorticoid and apoptosis:
Glucocorticoids are frequently prescribed for various inflammatory and/or life-threatening conditions, such as rheumatoid arthritis and chronic obstructive pulmonary disease. An estimated 0.2% to 0.5% of the general population use glucocorticoids for 3 months or longer.

Osteoclasts, terminally differentiated cells with a short life span, are multinucleated giant cells primarily responsible for bone resorption. They undergo rapid apoptosis in the absence of trophic factors such as macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL). However, the molecular events implicated in these processes still remain elusive [(47)].

Apoptosis is the genetically programmed cell death to remove the unwanted cells. The abnormalities of apoptosis regulation induce various sicknesses such as cancer, autoimmune diseases and degenerative disorders. There are two distinct apoptosis signal pathways in mammals. One pathway is initiated by death receptors, members of tumor necrosis factor receptor (TNF-R) family [(47)]. The other pathway is regulated by pro- and anti-apoptotic *Bcl-2* family member *via* mitochondrial release of cytochrome c and caspase-9 activation. The antiapoptotic Bcl-2 family members include mammalian Bcl-2 and Bcl-xL and they share similarity within three or four Bcl-2 homology (BH) domains. So far, more than 20 pro-apoptotic Bcl-2 family proteins have been identified in mammals. They can be further divided into 2 groups: multi-domain members possess homology in two or three BH regions, such as Bax and Bak, whereas the BH3 domain-only proteins, such as Bad, Bid and Bim/Bod, share only the short BH3 region. The pro-apoptotic activity of BH3-only proteins is strictly regulated at both the transcriptional and post-translational level to prevent inappropriate cell death [(47)].

The Fas/FasL system provides an important apoptotic mechanism for many cell types, especially those of the immune system. Fas, a transmembrane death receptor of the TNF receptor family, are activated by binding of Fas ligand (FasL) or Fas activating antibody. Trimerized Fas receptors recruit FADD (Fas Associated Death Domain) and initiate a downstream caspase activation cascade, which amplifies the death signal to downstream targets. The mitochondrial component of the apoptotic process is mediated by truncated BID translocation to the mitochondria from the cytosol and subsequent cytochrome c release. It has been reported that Fas appears to be up-regulated during the differentiation of haemopoietic cells, suggesting that the Fas system participates in regulation of haemopoietic differentiation. Moreover, it is implicated that *Fas* gene in alendronate-induced osteoclast apoptosis [(48)].

5.6- Management of glucocorticoid-induced osteoporosis:

Glucocorticoid-induced osteoporosis is a common condition that results in significant morbidity and mortality. The skeletal effects of glucocorticoids include both direct and indirect actions on bone that result in an early, transient increase in bone resorption accompanied by a decrease in bone formation, which is maintained for the duration of glucocorticoid therapy [(49)].

Rapid bone loss and increased fracture risk occur soon after the initiation of glucocorticoid therapy and are dose dependent. The increase in fracture risk is partly independent of bone mineral density, probably as a result of changes in bone material properties and an increased risk of falling [(50)].

Bisphosphonates are the front-line choice for prevention of fracture in glucocorticoid treated patients, with teriparatide as the second-line option; calcium and vitamin D supplements should be co-prescribed in the majority of individuals. Future guidelines for the management of glucocorticoid-induced osteoporosis should recognize the limitations of the Fracture Risk Assessment Tool (FRAX®) in assessing fracture risk in glucocorticoid-treated patients, and should include recently-approved interventions, such as zoledronate and teriparatide [(51)].

New concepts of antiresorptive therapy should therefore not trigger osteoclast apoptosis, but only block the resorptive activity.

5.7. Osteoporosis treatment:

Fracture repair in the compromised osteoporotic patient has not gained that much attention and the treatment options are currently rather conventional. Based on the knowledge of the local mechanisms of bone regeneration that basically recapitulate the program of bone development, new therapies have been developed that support the naturally-occurring progress and support the healing cascade in fractures that sometimes refuse to heal.

The BMU is the door to osteoporosis diagnosis and therapy and for the new recommended nomenclature of drugs used to treat osteoporosis: the new term “anticatabolic”. It is consistent not only with a decrease in resorption but also with a decrease in the total number of BMUs, hence a reduction of bone remodelling. Anabolic drugs increase formation more than resorption. The terms “anabolic” and “anticatabolic” not only describe the therapeutic effects more clearly but are semantically more appropriate because the literal definition of “anabolic” is to build up and that of “catabolic” is to break down.

The biologic activity of PTH resides in the N-terminus, with most clinical studies using the first 34-amino acids, now termed teriparatide [(52)]. After 1 year of anabolic therapy with teriparatide, bisphosphonates can further increase bone mineral density [(53)]. In the serum, however, C-terminal fragments are the major PTH species, which do not bind to the classical PTH receptor and the existence of a separate receptor for the C-terminus is postulated [(54)]. Intermittently administered PTH and N-terminal PTH peptide fragments or analogues also augment bone mass and currently are being introduced into clinical practice as therapies for osteoporosis. The mechanism of action can be based on an increase of the bone-remodelling rate by activating

previously quiescent lining cells and increased motility of osteogenic cells in their BMUs [55]. PTH can also prevent osteoblast apoptosis. Together, a larger osteoid-covered surface, an increase in trabecular connectivity and a greater radial outer dimension of the cortex characterize teriparatide treatment [56].

5.7.1.Anabolic Agents:

These drugs stimulate osteoblastic new bone formation and thereby increase bone density. Anabolic agents include fluoride salts, parathyroid hormone (PTH), and anabolic steroids.

Initial studies showed that sodium fluoride increased spine bone density by up to 35% over 4 years in women with vertebral osteoporosis [57]. PTH increases trabecular bone density by as much as 50% [58], but as with fluoride, this increase may be at the expense of cortical bone. Anabolic steroids such as stanozolol and nandrolone increase bone mass in patients with osteoporosis by 5% to 10% [59].

5.7.1.a. Statins:

The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are among the most prescribed drugs. About 25 million patients use them every day worldwide. Statins inhibit the synthesis of mevalonate by HMG-CoA reductase; the limiting step in the cholesterol synthesis pathway (Fig.12). The inhibition of mevalonate synthesis prevents the synthesis of isoprenoid precursors (geranyl and farnesyl pyrophosphates), which are substrates for prenylation of small GTP binding proteins; Rho, Rac, Rab [60]. This decrease in prenylation inhibits osteoclast activity because these small proteins cannot anchor to the membrane of osteoclasts by lack of a lipid chain. Statins share these effects on cholesterol pathway downstream to mevalonate with nitrogen containing bisphosphonates, which inhibit specifically farnesyl pyrophosphate synthase and act as bone anti-resorptive agents.

These effects of statins are part of their pleiotropic effects. Some statins are lipophilic (simvastatin and lovastatin, which are prodrugs); others are more hydrophilic (pravastatin, atorvastatin, fluvastatin) [60]. Due to differences in their polarity and bone availability, their individual bone effects might differ.

Many convincing *in vitro* and animal studies have established the beneficial effects of statins on bone [61], as both antiresorptive and anabolic agents. In 1999, statins were first shown to enhance expression of bone morphogenetic protein 2 (BMP-2), which stimulates osteoblast differentiation [62]. Statins have been shown to stimulate BMP-2 synthesis in cultured animal and human bone cells.

Recent attention has focused on the role of statins, widely prescribed for treatment of cardiovascular disease, as agents capable of promoting bone growth. Possible mechanisms of statins in bone formation involve stimulation of endothelial nitric oxide synthase (eNOS) [62, 63, 64]. eNOS is found sequestered in invaginations of the osteoblast membrane. Knockout mice lacking the eNOS gene demonstrate reduced bone formation. Statins have been shown to increase the expression and activity of the eNOS gene and to inhibit eNOS-induced osteogenesis in the mouse calvaria system. In studies with human osteoblasts, however, eNOS inhibition did not prevent the action of statins on bone formation [65].

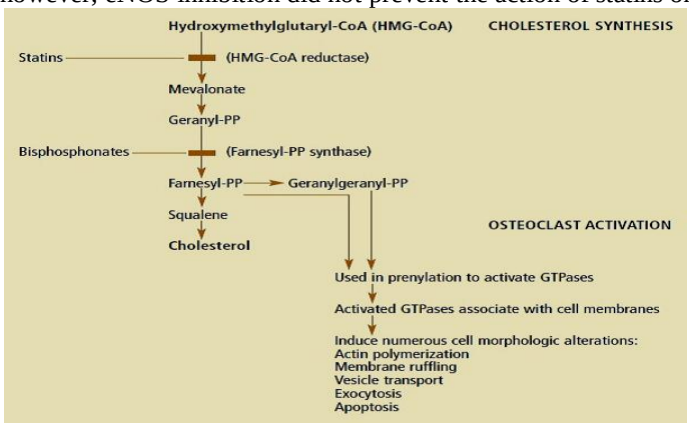

Fig. 12: Propable Mechanism of action of statins on osteoclast activation (Athanasios *et al*., 2012).

5.7.2. Antiresorptive agents:

There have been a number of studies implicating oxidative stress as one of the causes of bone degradation. Superoxide anion was reported to be an intermediary in the formation and activation of osteoclasts, causing bone degradation. In another study, hydrogen peroxide was reported to stimulate osteoclastic bone resorption [66].

Recently, it was reported that mature osteoclasts produce reactive oxygen species contributing to bone resorption. In the same study, the authors stated that an antioxidant, α- lipoic acid, had a therapeutic potential for prevention of bone loss [67]. Although the mode of action is not known, it appears that antioxidants have the ability to protect cells from oxidative damage by quenching free radicals. Removing free radicals supports the concept that increasing intracellular concentrations of antioxidants prevents oxidation-induced damage to bone cells and may even prevent bone loss [68].

Not much is known about associations between intake of antioxidants and bone health particularly in the area of the male skeleton. Two intervention trials involving vitamin supplementation have shown that vitamin C supplementation increased bone mineral density in post-menopausal women [69]. Several studies have reported bone protective effects of soy protein and soy isoflavones− a good source of antioxidants− on the female skeleton [70]. A recent study by [71] investigated antioxidant intake and the risk of osteoporotic hip fracture and found that increased intake of vitamin E; ß- carotene and selenium were associated with reduced risk of osteoporotic hip fracture in a dose-responsive manner among those who had never smoked in a Utah population.

The effect of citrus juice on enhancing serum antioxidant status and prevention of osteoporosis in orchidectomized rats was evaluated [72], their studies revealed that orchidectomy decreased total antioxidant capacity, femoral density and biochemical properties whereas orchidectomy plus orange juice and orchidectomy plus grapefruit juice reversed the orchidectomy induced antioxidant suppression and delayed time induced femoral fracture.

5.7.2.1. Grapefruit as a source of antioxidants:

Citrus fruits contain vitamin C, polyphenolic compounds, and limonoids with free radical scavenging activities, which may protect bone loss from occurring [73]. A flavonoid was shown to inhibit osteoclastic differentiation [74]. Limonoids have also been shown to possess antioxidant activity that may protect bone against resorption [75].

Certain studies have looked at the antioxidant property of grapefruit and its advantages for lowering plasma lipid and its interaction with lipid lowering drugs. A study found that naringin that was isolated from grapefruit had lipid lowering and plasma antioxidant capacity and grapefruit beneficially influenced plasma lipid levels and plasma antioxidant capacity [76].

Grapefruit contains compounds that may reduce atherosclerotic plaque formation and inhibit cancer cell proliferation [77]. Naringin is the most abundant flavonoid in grapefruit and can account for 40-70% of the dry weight of the small fruit [78]. It is one of the flavanones that is a consistent component of grapefruit and found in all varieties of grapefruit [76]. The properties of naringin were studied in rats fed cholesterol, and it was found to have a plasma lipid lowering effect, a mechanism that involved an increase in the bile flow, biliary cholesterol and bile acid concentrations. In addition, a significant increase in the plasma antioxidant capacity was found in the grapefruit diet group [76].

Naringin has also been reported to suppress cytotoxicity and apoptosis induced by H_2O_2, a proxidant in mouse leukemia cells [79].

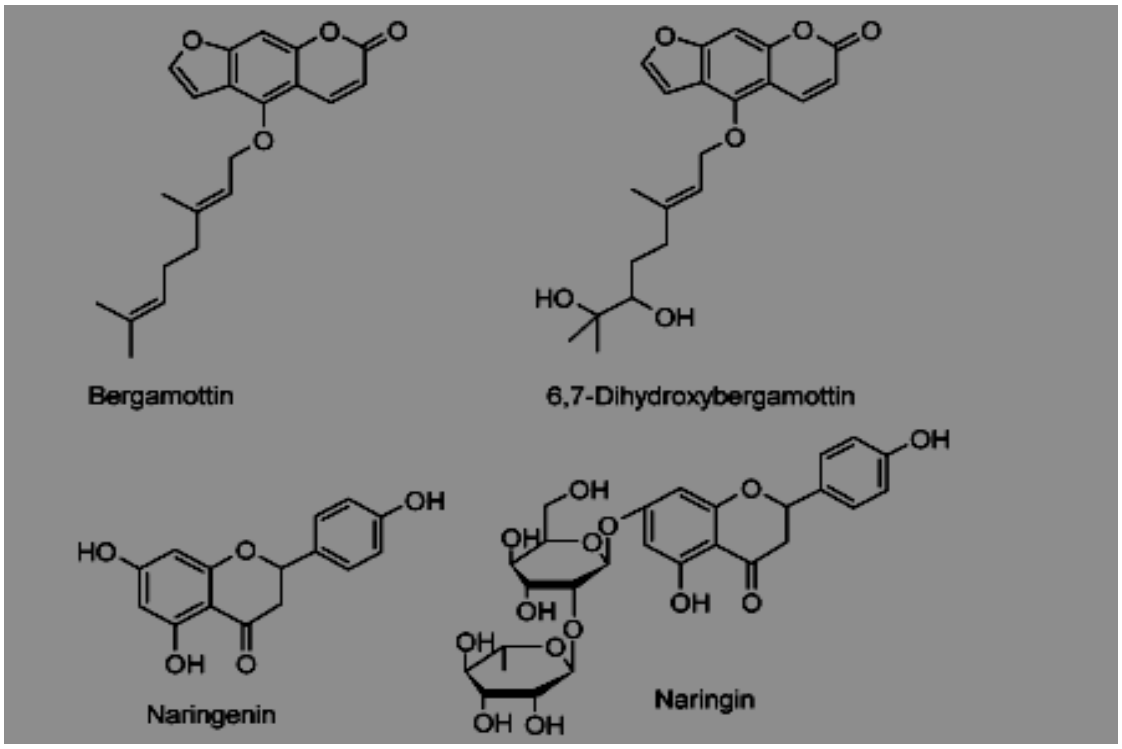

Fig. 13: Structures of flavonoids and furanocoumarins present in grapefruit juice (Zhang and Jennifer, 2004).

5.7.3. Osteoporotic treatment and bone remodelling:
Bone remodelling is the key to understanding the pathophysiology of systemic bone loss: postmenopausal and senile osteoporosis, glucocorticoid-induced osteoporosis, osteoporosis associated with chronic inflammation and osteoporosis following organ transplantation are to be distinguished. Other causes of osteoporosis are tumour burden, low mechanical loading due to bed rest or space flight and malnutrition. Each type of osteoporosis has its particular causes that influence the parameters of bone remodelling. Moreover, therapeutic options to decrease the fracture risk in osteoporotic patients such as bisphosphonates, selective oestrogen-receptor modulators (SERMs), PTH, strontium renelate, hormone replacement therapy, calcitonin, and vitamin D and calcium supplements all affect bone remodelling through different means.
In bone, the active vitamin D metabolite stimulates the activity of the osteoclasts and also has effects on the osteoblasts, where it increases the production of extracellular matrix proteins such as osteocalcin and osteopontin. The effects of 1, $25(OH)_2D_3$ on intestinal calcium absorption involve stimulation of the synthesis of calbindins. This group of proteins is known to bind calcium and transports it against a concentration gradient. Calbindins have also been found in the kidney and may be involved in 1, $25(OH)_2D_3$-dependent reabsorption of calcium. In addition, 1, $25(OH)_2D_3$ inhibits the synthesis and secretion of PTH in the parathyroid gland. Therapeutic application of $1,25(OH)_2D_3$ and calcium can lower the probability of hip and all non-vertebral fractures in elderly women who lived in care homes by 29% and 24%, respectively [80, 81, 82].

5.7.4. Osteoporotic treatment and bone regeneration:
Biophysical stimulations such as pulsed electromagnetic fields and low-intensity pulsed ultrasounds are considered to have positive effects on bone regeneration [83, 84]. Other therapeutic concepts are based on the application of growth factors, cells, and matrices that play a role during bone regeneration. Growth and differentiation factors for mesenchymal cells can be of either natural sources such as autologous preparations of platelet rich plasma [85] or from biotechnology such as recombinant BMP-2 or BMP-7, which are approved for the treatment of non-healing fractures and for spinal fusion [86].

In addition, metabolites that activate prostaglandin receptors 2 and 4 can stimulate bone regeneration [87, 88]. Angiogenic factors are investigated based on the understanding that blood capillaries are essential for bone regeneration [89]. Another strategy to enhance bone regeneration is supplementation of the defect site with additional osteogenic cells that can be isolated from autologous sources, e.g. bone marrow aspirates and the periosteum [90, 91]. Gene therapeutic approaches follow the concept that the transfected cells serve as a bioreactor for therapeutic growth factors over a prolonged time, but this method has not reached the level of routine clinical use [92, 93, 94].

5.7.5. Osteoporotic treatment and osteoclast regulation:

Bisphosphonates are incorporated into the bone matrix, and once released and endocytosed by the osteoclast, they inhibit farnesyl diphosphate synthase, which is required for the synthesis of GTB-binding proteins such as Rho, Rab and Cdc42. As these proteins are essential for osteoclast activity and survival, the overall resorption rate of osteoclasts is diminished and bone remodelling slows down.

Other targets for inhibition of osteoclast function involve the RANKL–RANK–OPG axis, H+-ATPase, carboanhydrase II, or cathepsin K, the $\alpha_V\beta_3$ integrin receptor, the c-src kinase, and p38 [(95)]. One should take into account that the osteoclasts provide a paracrine microenvironment with osteoblasts and their progenitor cells are potential target cells.

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