Oxidative stress as a possible therapeutic target for osteoporosis associated with aging

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Summary
Senile or involutional osteoporosis is a major problem in the developed world. Recent studies point to increased oxidative stress associated with aging, whether biological or chronological, as an important factor in its development. In this review paper, we focus on bone tissue disorders related to aging, the source of oxidative stress and negative influence on bone tissue. Finally, we consider the potential oxidative stress therapies currently being developed for this disease.

Key words: oxidative stress, osteoporosis, aging, fragility.
Introduction
The aging population in developed nations has led to an increase in the prevalence and incidence of osteoporosis. An estimated 200 million people suffer with this condition worldwide1.

Defined as a decrease in bone mass and quality that increases the risk of fracture2, osteoporosis is closely related to aging. Although the factors involved have not been fully identified, those associated with involutional osteoporosis include estrogen after menopause3, glucocorticoid deficit involved have not been fully identified, those associated with involutional osteoporosis include estrogen after menopause3, glucocorticoid deficit involved have not been fully identified, those associated with involutional osteoporosis include estrogen after menopause3, glucocorticoid deficit involved have not been fully identified, those associated with involutional osteoporosis include estrogen after menopause3. Also, in bred mice in which bone mass is regulated primarily by genetic factors, bone loss associated with age may assume up to 10% of the total bone mass, which is attributed to decreased bone remodeling3,4. As observed in rodents, humans initially tend to lose trabecular bone with age, especially in women3, related in part to a decrease in physical activity and, therefore, the mechanical stimuli in the tissue4. From 70 years, decreased cortical thickness is more pronounced with a concomitant increase in the intra-cortical porosity of the femur. The medullar area increases both in men and women4. These changes are associated with increased risk of osteoporotic fractures. However, in both mice and humans, the mechanical properties of bone are relatively conserved through a sustained increase in sub-periosteal mineral, which increases inertia time4.

Bone disorders associated with aging
Bone tissue undergoes a continuous remodeling process, with considerable regenerative capacity and adaptation to physiological changes. This process takes place in so-called bone remodeling units, consisting of different cell types: osteoclasts, osteoblasts and osteocytes (fully differentiated osteoblasts embedded in the mineralized matrix and actual orchestrator of remodeling process)5. Bone remodeling is highly regulated by genetic, mechanical, hormonal and local factors which determine the outcome of bone balance.

Peak bone mass is reached during puberty in women and somewhat later in males. The latter group develop a higher bone mass and present larger, wider bones, while the female bone structure tends to be smaller in diameter and cortical thickness. From about 30 years of age, a negative bone balance is observed in both sexes (with a predominance of bone resorption) which leads to a gradual loss of bone mass similar in both sexes, initially in the trabecular bone and later in the cortical6. This decline is accelerated after menopause in women due to loss of estrogens, agents with proven antioxidant properties, which maintain lower bone mass than in the case of men during aging. With age, metabolic disorders that affect the bones occur: neuromuscular changes related to lack of mobility; increased endogenous glucocorticoid production and renal failure with decreased synthesis of calcitriol. Moreover, with aging, bone collagen fibers undergo structural changes and the bone loses the ability to repair microfractures5. All this contributes to the increased incidence of fractures.

Most current concepts on the development of senile osteoporosis have been obtained from studies in experimental models, mainly in rodents. However, when interpreting these results, some bone peculiarities in rodents compared to humans must be taken into account, such as continuous modeling bone from the growth plate, the absence of menopause, as well as a lack of Haversian cortical bone system. However, as in humans, rodents have shown bone mass loss and a deterioration of structure and of long bone regenerative capacity associated with aging7,8. The bone loss in aged rats is related to a decrease in osteoblast maturation and the increased number of osteoclasts compared to osteoblasts in the trabecular bone8. Also, in bred mice in which bone mass is regulated primarily by genetic factors, bone loss associated with age may assume up to 10% of the total bone mass, which is attributed to decreased bone remodeling3-5. As observed in rodents, humans initially tend to lose trabecular bone with age, especially in women9, related in part to a decrease in physical activity and, therefore, the mechanical stimuli in the tissue10. From 70 years, decreased cortical thickness is more pronounced with a concomitant increase in the intra-cortical porosity of the femur. The medullar area increases both in men and women10. These changes are associated with increased risk of osteoporotic fractures. However, in both mice and humans, the mechanical properties of bone are relatively conserved through a sustained increase in sub-periosteal mineral, which increases inertia time10.

Mechanisms associated with bone aging
The underlying molecular mechanisms of involutional osteoporosis have begun to be elucidated in recent years. Associated with age, there has been a decrease in the osteoprotegerin (OPG) ratio/ligand receptor activator of nuclear factor (NF). This ratio is an important modulator of the remodeled bone21. Both OPG and RANKL are produced and secreted into the extracellular medium by osteoblastic cells and osteocytes. In fact, studies in mice models indicate that osteocytes produce most RANKL, thus directly influencing bone remodeling22-23. OPG is a soluble decoy receptor that captures RANKL in the extracellular medium (or on the surface of osteoblasts) and prevents it from binding to its receptor (RANK) in cells of osteoclastic lineage, thereby preventing the maturation and activation of osteoclasts. Thus, the OPG/RANKL relationship is an important anabolic/catabolic balance factor during bone remodeling11. Thus, the decreased OPG/RANKL relationship with age is consistent with increased osteoclast precursors in the bone marrow of old mice12. Osteocyte apoptosis plays an important role in bone loss associated with age and to immobilization or lack of stimuli16-20 and also associated with an increased RANKL expression11. Moreover, in old mice of the C57BL/6 strain, an increase in the production of endogenous glucocorticoids has been observed through the activation of the enzyme 11 beta-hydroxysteroid dehydrogenase type 1. This is related to reduced viability of bone cells (osteoblasts and osteoclasts) and angiogenesis, a key process in bone formation1. Several factors may affect the rate of fracture repair with age11. With aging, there is a decrease in bone marrow osteoprogenitor, which occurs in parallel with increased adipogenesis11. Both osteoblasts and adipocytes share a mesenchymal precursor cell differentiated either lineage depending on the microenvironment which are exposed these cells. Furthermore, osteoblasts from old...
mice RANKL production increase parallel to the decrease in expression of OPG. This alteration results in increased osteoclastogenesis and osteoclast activity. It is noteworthy that there are a decreased number of endothelial cells and angiogenesis, which may contribute negatively to the process of bone repair in older people.

Recently an increase in bone mass and reduced risk of fractures have been observed in elderly subjects who undergo angiotensin II receptor antagonist treatment. The drug's apparent beneficial effect on the bone is attributed to the inhibitory action of angiotensin II on various osteoblast differentiation markers, such as runt-related transcription factor 2 (Runx2), essential for osteoblast differentiation, osteocalcin and the increase of RANKL, which favors osteoclast differentiation. These data suggest that high blood pressure which is prevalent in the elderly could also contribute to involutional osteoporosis.

Sclerostin, the osteocyte-derived product of the Sost gene, is a potent inhibitor of bone formation through the binding to receptors associated with low density lipoprotein 5 and 6, inhibiting the canonical Wnt. Recent studies have shown that circulating sclerostin increases in post-menopausal women and with age in both sexes, which could be considered as second messengers of inflammatory response. These changes could lead to activation of the pathway Wnt39. Inhibiting its action, hence the absence of Klotho protein appears aforementioned mediated activation of FoxO phosphorylation engagement with oxidative stress inhibiting action osteogenic factors60.

Aging can be seen as a consequence of the imbalance between oxidizing agents produced naturally in cell metabolism and antioxidant defenses, with a predominance of the first. This is known as oxidative stress, which involves the oxidation of biomolecules and functional loss of cells48. Increased oxidative stress, carried out primarily in the mitochondria, is based on the overproduction of reactive oxygen species (ROS) such as superoxide anion (O2-), hydroxyl radicals (OH) and hydrogen peroxide (H2O2).

This increase cannot be properly balanced by antioxidants systems such as superoxide dismutase (SOD), catalase (CAT) enzymes glutathione cycle (glutathione reductase and glutathione peroxidase) and thioredoxin, among others. Excess ROS with chronological (and/or biological) age oxidizes DNA, proteins and lipids and induces the phosphorylation of mitochondria p66sh protein, leading to cell death. Recently, oxidative stress has been found to have important functions in cell signaling. In this context, ROS can be considered second messengers of inflammatory response. In fact, oxidation and inflammation are two closely related processes that increase with age.

Although some researchers have raised questions about whether oxidative stress is a cause or consequence of aging, in recent years it has been implicated in the bone deterioration. Using various animal models: premature aging, osteoporosis due to estrogen deficit (after ovariectomy) or diabetes, increased oxidative stress markers was found to decrease bone formation mechanisms55-59. The effects of oxidative stress to induce deleterious effects on bone tissue are not yet well known. Increased ROS may stabilize forkhead box O (FoxO) transcription, an important family of transcription regulators of many genes. Its functions include control of glucose metabolism, tumorigenesis and cell defense against oxidative stress. FoxO 1 and 3 are expressed in the bone 56, where they seem to play a key role in maintaining bone formation. It has been shown that genetic deletion of FoxOs in mice increases oxidative stress in bone and induces bone loss trabecular and cortical, associated with increased osteoblast/osteocytic apoptosis and a decrease bone formation. The activation involves FoxO phosphorylation engagement with the beta-catenin causing gene induction of oxidative stress response, as GADD45 and CAT. In fact, the protective action of oxidative stress of Klotho protein appears aforementioned mediated activation FoxOs. Furthermore, activation of the FoxO prevents beta-catenin to act as transcription factor in stimulating the proliferation and differentiation of osteoblasts.

Increased ROS in bone cells causes damage and apoptosis genomic DNA of osteoblasts and osteocytes. In addition, lipid peroxidation dependent lipoxygenase activated by oxidative stress plays an important role in bone loss associated with aging. This is evidenced by analyzing the expression of the lipoxygenase and ALOX12 and formation Alox15 4-hydroxynonenal, a product of lipid peroxidation, increased bone in older mice. It has also been shown that products of lipid oxidation inhibiting action osteogenic factors68.
Furthermore, the increase of ROS has been linked to an increase of osteoclastogenesis and osteoclast activity. It has recently been shown that the enzyme nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX 4) plays a key role in osteoclastogenesis. Mice deficient of this enzyme, which produces constitutively ROS have a high bone mass and osteoclast markers deficit; also in human bone samples high osteoclast activity is correlated with increased activity of NOX 4. Furthermore, it is noted that in situations of increased ROS associated with experimental DM, are mixed results. While some authors have observed an increase in osteoclast activity, it has been suggested that could be related to the greater severity of DM, however, other DM models, osteoclastic activity is reduced. In fact, studies using murine osteoclasts pre-incubated in the presence of high glucose appear to confirm their inhibitory effect on osteoclasts. Thus, differences in the degree of DM, strain and age of the animal, could contribute to the varying levels of bone resorption observed in different models.

Possible oxidative stress therapies in senile osteoporosis
The development of new anabolic therapies for osteoporosis that combine increased bone mass with its ability to neutralize the harmful effects of oxidative stress is of great interest. An intuitive approach to prevent bone loss with age would be based on the antioxidant administration. However, it pointed out that classic antioxidants, such as the CAT or N-acetylcysteine, exert undesirable effects on bone tissue as authentic anti-osteoclastogenic act as agents interfering with bone remodeling. In addition, such agents inhibit the canonical Wnt/beta-catenin whose activation is vitally important for maintaining bone formation, partly by inducing the seizure of activating the protein disheveled by the regulatory protein redox balance, nucleoredoxin. Recently, the bone anabolic effect has been associated with intermittent administration of parathyroid hormone (PTH) with its stress oxidative properties, such as the decrease in the amount of ROS, inhibition of phosphorylation of p66shc adaptor protein and increasing the amount of total glutathione. The advantage of this treatment with PTH versus the clas-
Figure 2. Osteogenic actions of PTH through the Wnt/beta-catenin pathway. PTH is able to directly activate the Wnt pathway by binding of the type 1 receptor (PTH1R) with coreceptor Wnt proteins, the related receptor low-density lipoprotein 6 (LRP6). Furthermore, phosphorylation of Akt activation produced by PTH1R results in FoxO degradation, which favors beta-catenin stabilization.

In vitro studies and animal models suggest that resveratrol, a compound bifenilic group of polyphenolic antioxidants present in the skin of grapes and other fruits, could be a potential anti-osteoporotic agent. This compound increases the proliferation and differentiation of osteoblast in the pre-MC3T3-E1 mouse in vitro. Furthermore, administering resveratrol to mesenchymal cells derived from human embryonic stem cells has been shown to induce the expression of mature Runx2 and osteoblasts. This mechanism of action of resveratrol appears to be mediated by SIRT1 deacetylation activation which increases FoxO3a expression and complex formation with resveratrol, increasing Runx2 expression (Figure 3). SIRT1 could also increase the activity of Runx2 directly by deacetylation of this transcription factor in pre-osteoblast cells. In recent research into older rats, administering resveratrol (10 mg/kg daily for 10 weeks) has been shown to improve bone quality and bone biomechanical properties of the osteoporotic bone. Although these pre-clinical results are promising, there are still no hard data to confirm the efficacy of resveratrol in senile osteoporosis in humans. However, of note is a recent study conducted in obese and osteopenic patients, in which oral administration of resveratrol (1 g daily for 16 weeks) significantly increased bone mass, and the amount of bone alkaline phosphatase, compared to the placebo group. Recent reports indicate that mice deficient in SIRT6, another deacetylase related to the response to oxidative stress, present an osteoporotic phenotype at an early age. The absence of SIRT6 is associated with overexpression of Runx2, osterix and OPG as well as the increased Wnt pathway inhibitor, Dickkopf 1, which leads to a deficit of osteoblast and osteoclast maturation. These data suggest that SIRT6 could be a therapeutic target in involutional osteoporosis. Furthermore, glucocorticoid excess also induces oxidative stress. In this situation, the oxidative stress observed in plasma reticulum can be reversed by translation initiation factor 2α phosphorylation, which disrupts protein translation. A dephosphorylation inhibitor compound, salubrin, has recently been shown to prevent deficit mineralization of osteoblasts treated with glucocorticoids in vitro as well as osteoblast and osteocyte apoptosis in an osteoporotic mouse model by prednisolone administration.

Conclusions

The progressive aging of the population in the developed world leads to increased musculoskeletal disorders, including osteoporosis. Osteoporosis and increased fragility of the elderly population are a socioeconomic challenge of the first magnitude. Different factors contribute to bone loss in the elderly, among which stands out as a common element increased oxidative stress (Figure 4). Thus, reducing oxidative stress could be a useful tool to combat involutional osteoporosis. However, the fact that oxidative stress compounds could interfere with the bone remodeling or key anabolic pathways for bone formation, such as the Wnt signaling pathway, requires certain considerations prior to therapeutic use. We must also take into account the physiological role of ROS, which act as secondary messengers of many metabolic pathways; therefore its uncontrolled inhibition could lead to unwanted side effects in bone cells. Further research is needed to determine the true effect of antioxidant therapies and appropriate dosing schedules to avoid deleterious action on bone remodeling. Taking into account these considerations, therapies aimed at neutralizing oxidative stress to prevent or alter the course of involutional osteoporosis would represent an obvious medical breakthrough.

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Bibliography


Figure 3. Osteogenic action of the resveratrol by interaction with SirT1. Resveratrol induces increased osteogenic factor activity by Runx2 deacetylation transcription mediated SirT1 deacetylase. It also promotes the formation of a transcription complex between FoxO3a and SIRT1 that promotes increased Runx2 expression.

Figure 4. Role of oxidative stress in bone damage associated with aging. Alteration of mitochondrial homeostasis with age causes generation of excessive amounts of ROS that exceed the capacity of cellular detoxification systems. Bone-forming cells in, excess ROS results: an increase in receptor expression activated gamma peroxisome proliferator (PPAR-gamma3); FoxO coupling with PPAR beta-catenin, which inhibits the Wnt pathway; and p66 protein phosphorylation inducing apoptosis. This excess ROS favors increased osteoclast activity and osteolastogenesis. Together these facts altered bone remodeling. The oxidative stress activity of agents such as PTH (and PTHrP), resveratrol and salubrinal are associated with osteogenic actions.