Oxidative stress as a possible pathogenic cofactor of post-menopausal osteoporosis: Existing evidence in support of the axis oestrogen deficiency-redox imbalance-bone loss

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Post-menopausal osteoporosis (PO) is one of the major health issues associated with menopause-related oestrogen withdrawal. Despite the intense research and the relevant progress achieved in the last two decades, the pathogenic mechanism underlying PO is still poorly understood. As a consequence of this gap in the knowledge, such disorder and the related complications are still difficult to be effectively prevented. A wealth of experimental and epidemiological/clinical evidence suggests that the endocrine change associated to menopausal transition might lead to a derangement of redox homeostasis, that is, the prelude to the health-threaten condition of oxidative stress (OxS). In turn, this (bio)chemical stress has been widely hypothesized to contribute, most likely in synergy with inflammation, to the development of menopause-related diseases, including PO. The main aim of this review is to discuss the current literature evidence on the association between post-menopausal oestrogen withdrawal, OxS and PO. It is also aimed to provide a critical overview of the most significant epidemiological studies on the effects of dietary antioxidants on bone health and to devise a strategy to overcome the limitations emerged and controversial results.

Key words Menopause - oestrogens - oxidative stress - post-menopausal osteoporosis - reactive oxygen species - redox homeostasis

Introduction

Post-menopausal osteoporosis (PO) is one of the major health problems associated with menopause-related oestrogen deprivation. The disease is characterized by a reduced bone mass and an impairment of bone architecture, with both leading to an increased risk in fragility fractures (FFs). FFs, in turn, are significantly associated to a high rate of disability and mortality.

According to an epidemiological study, 22 million women and 5.5 million men living in the European Union suffered from PO in 2010, with more than 3.5 million new FFs sustained. The incidence of osteoporosis in Asia is also steadily increasing mostly because this region is witnessing a rapid rise in elderly population. In particular, India is expected to have the highest total increase in people aged over 50 yr (from 120 million in 2013 to 620 million in 2050).
Moreover, the female population is estimated to go through menopause earlier than Caucasians (mean age 46 vs 51 yr). This fact, along with the expected increase in female life expectancy (from 68 to 73 yr in the period 2011-2021), will extend the time span of post-menopausal period and, hence, the likelihood of related disturbances.4

Since PO is a worldwide health issue, an effort should be made to better clarify the underlying pathogenic mechanism(s) so as to devise effective preventive strategies. One of the most intriguing hypotheses focuses on the pivotal role of oxidative stress (OxS) as a link between gonadal failure and clinical onset and progression of PO.5-7. By definition, OxS is the result of disruption of a finely tuned balance between antioxidants and oxidants, with the latter group including reactive oxygen species (ROS), reactive nitrogen species (RNS) and other less abundant substances.8,9 The main aim of this review is to illustrate and discuss the current evidence on the association between post-menopausal oestrogen withdrawal, uncontrolled ROS generation and PO. Furthermore, it is meant to provide a critical overview of the most significant epidemiological studies on the effects of dietary antioxidants on bone health and to devise a strategy to overcome the limitations emerged and controversial results.

Post-menopausal osteoporosis: From oestrogen decline to disease onset

To preserve structural integrity, the skeleton necessities to continuously remodel and repair the micro-cracks that take place in the two types of osseous tissue: the ‘spongy’ cancellous and the compact cortical bone. Bone remodelling cycle occurs through the balanced activities of its constituent cell types: bone-forming osteoblast, the bone-degrading osteoclast and the osteocyte, the primary trigger of the entire turnover process.10

Despite pivotal role of oestrogen on the bone, it is still difficult to identify the mechanism(s) by which these hormones modulate bone metabolism. The oestrogens are able to block bone resorption through two mechanisms: both by direct interaction with osteocytes and osteoclast and by regulation of T-cell and osteoblast formation and activity.11,12. As described in some reviews,13,14 the biological axis constituted by receptor activator of nuclear factor k-B (RANK), its natural ligand (RANKL) and decoy receptor for RANKL, osteoprotegerin (OPG), is the molecular link between oestrogen deficiency and bone loss.15. Noteworthy, RANK/RANKL/OPG axis also appears to play a key role in mediating the widely hypothesized connection between immune system and bone, central concept of osteoimmunology.13,14

Osteoclast precursors differentiate under the influence of macrophage colony-stimulating factor and RANKL.13. The latter cytokine, expressed in both membrane-bound (in osteoblasts) and soluble forms, promotes differentiation of osteoclast precursors and activates mature osteoclast to reabsorb bone, through the interaction with RANK.10. The osteoclastogenic effect can be prevented by OPG, which is produced by osteoblasts and stromal cells and competes with RANKL for RANK binding.14. In addition, oestrogens can decrease osteoclast precursor responsiveness to RANKL by binding to nuclear factor-kB (NF-kB), which prevents the production of RANKL and promotes the release of OPG. Finally, oestrogens interfere with osteoclast signalling pathways downstream to RANK, thereby inhibiting osteoclast differentiation.14. As a logical consequence, oestrogen withdrawal affects the ‘healthy’ balance between RANKL and OPG, with the result of prolonging osteoclast lifespan and accelerating bone resorption.14

Lymphocytes T and B may play an important role in the complicated network of biological interactions underlying oestrogen control of bone homeostasis.13,14. In particular, T-cells have been deemed to be the master regulators of both life cycle and metabolism of osteoclasts/osteoblasts. These immune cells are capable of inducing bone loss through the production of several bone-reabsorbing cytokines including RANKL, interleukin (IL)-1, IL-6 and tumour necrosis factor-α (TNF-α). Moreover, oestrogen decline has a direct effect on the synthesis of cytokines in T-cells, as shown in experiments on ovariectomized (OvX) rodents14 and in a few human studies.13,18. Our review does not aim at describing in detail the mechanisms implicated in the immune regulation of osteoporosis caused by oestrogen deficiency, which will not be dealt in-depth.

Pro-inflammatory cytokines are not the only factors that have been shown to be altered in a context of bone loss induced by oestrogen deficiency. Converging pre-clinical and epidemiological findings indicate that an excessive accumulation of oxidant substances, in particular, ROS, might contribute to the imbalance between bone resorption and formation that underlies PO development.
Reactive oxygen species (ROS) versus antioxidants: Perpetual struggle behind ageing processes and related diseases

ROS, the most abundant biological oxidants, can subtract electrons from all the biomolecules, including DNA, proteins, carbohydrates and lipids\(^9\). The modifications induced by these species are potentially toxic for cell biology because the induced damage easily spreads in the neighbouring cells if not adequately counteracted by the antioxidant system. A clear example of the contagious cytotoxicity of ROS is represented by the lipid peroxidation of cell membranes. Abstraction of one single electron from the polyunsaturated fatty acids incorporated in phospholipids triggers a chain reaction that diffuses throughout the lipid bilayer and finally leads to an irreversible structural and functional alteration of the plasmatic membranes\(^9\).

ROS are constantly produced in different cell sites: first, by mitochondria during the indispensable process of oxidative phosphorylation and, to a lesser extent, by cytosol, membrane, peroxisome and endoplasmatic reticulum, and hence, their impact on cell health is not always deleterious. Accordingly, it is now well established that the dichotomy ROS-oxidation causes biological damage and, if perpetrated, disease, only upon exceeding a critical threshold\(^9,19,20\). A number of endogens (e.g. inflammation and dysmetabolic syndrome) and environmental factors (e.g. pollution, smoking and nutrition) can accelerate the production of ROS, thereby increasing the risk of pathological conditions\(^9\). Moreover, the severity of the effects does not only depend on the quantity but also on the quality of the oxidant species involved. In fact, ROS family includes various members ranging from highly unstable/reactive-free radicals (i.e. molecules lacking one or more electrons) to poorly reactive non-radical molecules, such as hydrogen peroxide (H\(_2\)O\(_2\))\(^9,20\). The production of this compound depends on the enzyme superoxide dismutase (SOD). It catalyzes the dismutation of peroxide radical (O\(_2^-\)), which in turn is produced by different enzymes located in the mitochondria, cytosol or membrane\(^9\). In an hypothetical classification based on the antioxidant power, SOD and other enzymes are able to neutralize H\(_2\)O\(_2\), i.e. catalase (CAT) and glutathione peroxidase (Gpx) most likely occupy the first place\(^21\). These three enzymes have been indicated as the first line of defence, thanks to their unique ability to directly scavenge ROS \textit{in vivo}\(^20,21\). There are other endogen enzymatic and non-enzymatic molecules which effectively contrast such reactive species as glutathione (natural substrate of Gpx), thioredoxin, coenzyme Q and uric acid (UA, the most abundant antioxidant in plasma). The core of the antioxidant protective system belongs to a complex network of enzymes, signalling molecules and transcription factors that result from the evolutionary adaptation of cells to the risk of living in an oxygen-rich environment\(^9\).

Dietary antioxidants, such as vitamins E (also named α-tocopherol) and C, β-carotene and lycopene, are also important in this context. Even though almost all these molecules show a detectable antioxidant capacity in experimental conditions, only α-tocopherol seems to be really effective \textit{in vivo}\(^21\). This property depends on its relatively rapid kinetics of reaction, as well as on its high bodily concentration, especially in high ROS-vulnerable cell membranes\(^21\).

Growing pre-clinical evidence has highlighted the beneficial effects of a highly variegate category of food antioxidants: the polyphenols\(^21\). Several members of this family, in particular, curcumin (turmeric) and resveratrol (grapes), have the peculiar capacity to stimulate a general xenobiotic response in target cells. In fact, these activate multiple defensive genes, such as those encoding SOD and CAT\(^9,21\).

A central paradigm in the redox field is that ROS/RNS become pathological players only when they overwhelm the opposing forces of the innate (endogen) and acquired (dietary) antioxidants. The rupture of this redox homeostasis is the prelude to OxS, a condition that severely affects cell integrity and biology and predisposes to several diseases\(^9,19\).

Oestrogen deficiency: Starting point of oxidative stress-induced bone-degenerative processes heading to post-menopausal osteoporosis

OxS is considered the common pathogenic factor for the ‘menopausal syndrome’ that includes several disturbances (e.g. vasomotor complains, cognitive impairment and urogenital dystrophy) and diseases, such as PO. The oestrogen decline associated with menopausal transition is believed to be the ‘spark’ that triggers OxS.

Experimental and epidemiological evidence

Experiments on OvX animals provide the most convincing evidence in support of the potential antioxidant impact of 17 β-oestradiol (E2), \textit{i.e.} the predominant type of oestrogen. A vast body of studies shows that bilateral oophorectomy induces
a redox imbalance characterized by increased levels of lipoperoxidation markers and decreased activity of antioxidant enzymes. Notably, it has also been reported that oestrogen replacement suppresses OxS in OvX rats, thereby suggesting that the administration of exogenous hormones to the animals can reverse their pro-oxidative profile.

Despite the consistent experimental evidence, the concept of oestrogen as an antioxidant is not unanimously accepted mostly because of the contrasting results emerging from human epidemiological studies. Post-menopausal women showed higher serum levels of lipid peroxidation markers and lower levels of low molecular weight or enzymatic antioxidants compared to reproductive age women. In contrast, data from other reports failed to prove the occurrence of OxS after menopause and questioned the hypothesis of an inverse correlation between E2 levels and peripheral markers of oxidative damage.

The inconsistency of epidemiologic data on menopause-OxS relationship is due, at least in part, to the methodological limitations as well as to the differences in study design and in composition of population sample. Regardless of these drawbacks, there is still large consensus around the antioxidant potential of oestrogens. However, at present, the mechanisms by which these hormones provide tissue protection against oxidative challenge and the exact biochemical and cytological basis of the relation between OxS and osteoporosis are still unclear. Illustration of the most common hypotheses at this regard are briefed below.

**Oestrogens as antioxidants: Possible mechanisms of action**

**Hypothesis 1:** Oestrogens directly inhibit the peroxidative processes: The interest in the antioxidant proprieties of oestrogens was sparked by Sack et al., showing that intra-arterial infusion of E2 in the post-menopausal women induced a decrease in the peroxidative damage of low-density lipoprotein (LDL) and that these beneficial effects were reversed after treatment interruption. However, the physiological levels of E2, even in the reproductive age women, are much lower than the threshold at which oestrogens play direct antioxidant effect *in vitro*. Due to this evident constraint, along with subsequent negative results of the epidemiological studies, Sack’s original hypothesis has been almost definitely refused by the experts in the field.

**Hypothesis 2:** Oestrogens upregulate the expression of antioxidant enzymes: Viña and colleagues were the first to definitely prove that oestrogens modulated the molecular signalling pathways involved in cellular redox homeostasis. Along with other researchers, they have demonstrated that E2 stimulates the expression of SOD and Gpx. The interaction between E2 and mitochondrial receptors sharply decreases the rate of ROS formation and simultaneously increases the membrane integrity of these organelles. Accordingly, oestrogen deprivation in OvX animals has been repeatedly demonstrated to be associated with a downregulation of these defensive enzymes and with an increase of oxidative challenge.

**Hypothesis 3:** Oestrogens prevent the disruption of redox balance by suppressing pro-oxidant sources: E2 may prevent redox balance impairment indirectly, *i.e.*, by modulating and harmonizing the regional distribution of fat in female body. The level of oxidative damage is closely related with the amount, type and localization of body adiposity.

Menopause transition is associated with the preferential accumulation of fat in the abdominal region with a parallel increase in the visceral/subcutaneous fat ratio. In fact, oestrogen is able to regulate regional lipid accumulation mostly through the interaction with oestrogen receptor α (ERα) that is more expressed in subcutaneous than in visceral adipocytes. These receptors play a crucial role in the activity of adipocytes as well as in sexual dimorphism in fat distribution. The E2:ERα interaction appears to preserve and promote the typical gynoid (gluteofemoral) distribution, which is widely believed to be associated with a healthy metabolic profile. In contrast, the menopause-related accumulation of intra-abdominal fat leads to the onset of a chronic ‘low-grade inflammation’, which, in turn, contributes to the development of metabolic diseases, such as type 2 diabetes mellitus and cardiovascular disease.

The adverse effects of inflammation are further exacerbated by the concomitant increase in ROS production. One of the prominent effects of a persistent inflammatory state is the generation of a pro-oxidative environment due to the production of reactive species. For example, the exposure to inflammatory cytokines, such as IL-6, stimulates the generation of reactive species by phagocytic cells, such as macrophages and neutrophils. In turn, OxS enhances the release of pro-inflammatory mediators from immunocompetent cells, thereby triggering a toxic self-perpetuating vicious cycle recently named...
oxinflammation\cite{19,40}. This detrimental synergy is a latent feature of several diseases\cite{40} and may account for the close interplay between central adiposity and serum levels of oxidative damage markers in post-menopause, reported by various research groups including ours\cite{8,41-44}.

**Implication of oxidative stress in post-menopausal osteoporosis pathogenesis: Experimental and epidemiological evidence**

**In vitro and animal studies**

Evidence from animal studies supports the detrimental effect of OxS on bone health\cite{35,49}. Increase in the intracellular ROS has been shown to result from oophorectomy in animal models\cite{23,47}. It has been observed that ROS-enriched bone environment stimulates osteoclastogenesis in two ways: primarily, by potentiating the responsiveness of osteoclast precursors to RANKL, and secondarily, by inducing additional osteoclastogenic cytokines (\textit{i.e.} IL-1, IL-6 and IL-7)\cite{5,6}. Furthermore, the treatment with H$_2$O$_2$ did not enhance the expression of OPG, but rather RANKL in human stromal cells\cite{9}. Moreover, the induction of superoxide production was shown to increase both \textit{in vivo} and \textit{in vitro} the number and activity of osteoclasts\cite{44}.

Cumulating evidence suggests that OxS may also influence the cell cycle of osteoblasts. Two separate studies showed that ROS decreased the life span of these cells in OvX or aged mice\cite{45}. Overall, both endogenous and dietary antioxidants appeared to mitigate and delay bone loss in a number of animal studies. Consistently, low bone mass was detected in transgenic mice lacking cytoplasmic SOD, created by the deletion of the corresponding gene\cite{49}. Separate series of studies showed that various forms of vitamin E prevented the reduction in trabecular number and bone volume in OvX rats\cite{23,47}. Deng \textit{et al.}\cite{92} reported that mice supplemented with gamma-trienol were significantly protected from ovariectomy-induced bone loss. Growing pre-clinical evidence also suggests that commonly consumed antioxidant-rich fruits have a pronounced effect on bone through the promotion of bone formation and the prevention of bone resorption\cite{53}.

**Human studies**

The aforementioned compelling experimental evidence prompted a number of epidemiological studies on the topic. Multiple reports\cite{34,57} (including two from ourselves)\cite{30,51} highlighted an inverse and positive association between peripheral markers of oxidative damage and antioxidant status, respectively, and bone mass density (BMD) at femoral neck, total hip or lumbar spine. Such findings led us to further explore the involvement of redox processes on bone loss, by focussing our attention on peripheral markers of bone remodelling in a cohort of post-menopausal women. We showed that higher serum concentration of OxS marker was correlated with an increased bone resorption rate\cite{51}. In a more recent association study, our research group has provided further evidence in support of implication of OxS in the derangement of bone homeostasis\cite{48}. We measured the serum levels of RANKL, OPG and 8-hydroxy-2-deoxyguanosine, a widely used marker of DNA oxidation, in a sample of normal, osteopenic and osteoporotic post-menopausal women (n=124), and found a positive and independent correlation between the OxS marker and RANKL/OPG ratio within the osteopenic subsample\cite{38}.

Besides the evidence linking OxS to the pathogenesis of PO, some reports also addressed its relationship with the possible complications of such disease. In particular, a plasmatic indicator of OxS arose as a significant predictor for hip fracture in a prospective investigation on 996 women, followed up for up to 23 years\cite{54}.

Among the several antioxidants with a hypothesized association with osteoporosis, UA was the one showing the most consistent and convincing results. A retrospective study on 615 Japanese women reported a positive and independent correlation between the levels of UA and lumbar spine BMD\cite{55}. An Australian study involving 356 peri- and post-menopausal women sought to confirm the positive role of UA on bone turnover and assessed whether UA relates to changes in BMD longitudinally; multivariate analysis showed an association between UA levels and longitudinal change in BMD at several skeletal features\cite{56}. The putative protecting role of UA on bone metabolism has been confirmed in a large study involving 7502 women\cite{57}. Higher serum UA was associated with higher bone mass, lower bone turnover and lower prevalence of vertebral fracture. \textit{In vitro} experiments showed that UA suppressed osteoclastogenesis in a dose-dependent manner and reduced the production of ROS in osteoclast precursors\cite{57}.

Contrasting results are also worthy to be highlighted. Multiple studies failed to find a significant increase of OxS in osteoporotic as compared to...
healthy post-menopausal women. In our view, these negative findings do not rule out the role of reactive species in PO development but define the limits of their involvement. As this bone disease is multifactorial, ROS can be reasonably considered just one of the multiple components of its complex and multifaceted pathogenic process. Moreover, detectable changes in the blood markers are expected to occur in more severe pathologic conditions characterized by intense tissue damage and mitochondrial impairment or in chronic metabolic disorders, such as diabetes and vascular chronic inflammation. An additional point to consider is the intrinsic limitation of all the epidemiological studies dealing with OxS. The research has not yet validated a gold standard biomarker for the quantification of redox imbalance in biological fluids, and many of the indicators measured are scarcely specific and sensitive. Finally, most of the reports have a cross-sectional design, and hence, they take a ‘static’ snapshot of a population at a specific point of time. In the specific case of the studies in the framework of interest, the picture essentially consists of the comparison between women affected by PO and healthy controls. Such an approach raises two main issues. First, it hinders any firm conclusion about the causal role of OxS in the disease occurrence. Second, it intrinsically misevaluates the influence of covariates (body fat parameters, lifestyle and ethnicity, concomitant diseases, therapies, age differences, alcohol abuse, physical activity, diet and smoking) on the relationship between the two variables. Longitudinal design is thus the most proper approach to establish the causality of the observed statistical associations, but these types of investigation are problematic mainly because of the high inter-individual variability in the duration and age of onset of menopause.

Anti-osteoporotic therapies targeting oxidative stress: Current state of the art

The questions that inevitably arise from the reported evidence are: how can we translate the research findings and acquired knowledge into clinical practice? And, in line with the aim of this review, is there any evidence that targeting OxS could be beneficial in terms of prevention and treatment of PO?

At present, there are various effective drug treatments for the management of PO and for the prevention of subsequent FFs. Pharmacologic intervention is appropriate in case of previous fractures or osteoporosis at increased risk of fracture based on a fracture risk assessment tool, i.e. WHO FRAX. Whereas bisphosphonates, denosumab and teriparatide are used for the treatment of patients with a full-blown osteoporosis, selective oestrogen receptor modulators may be considered as the first-line treatment in preventing bone loss in women within 10 years of the menopause, side by side with the menopausal hormonal therapy (MHT) and the most recent combination of bazedoxifene and oestrogen.

Convergent data from research studies and clinical trials indicate that MHT normalizes turnover and preserves BMD at all skeletal sites, thereby decreasing vertebral and non-vertebral fractures significantly. Despite the initial concern with the safety of such treatment, recent studies reported encouraging results in this field. In particular, a 10 yr randomized trial completed in 2008 showed a significantly lower risk of mortality, heart failure and myocardial infarction without any apparent increase in risk of cancer in carefully selected women younger than 60 yr old receiving MHT early after menopause. Epidemiologic evidence is consistent with the data from animal studies on OvX rats receiving oestrogen replacement therapy (ERT). López-Grueso et al showed that ERT could prevent redox imbalance and counteract the typical post-surgical dysmetabolic profile only when administered early after oophorectomy. Based on these results, the authors postulated that ERT could upregulate the antioxidant enzymes and hence promote longevity. Unfortunately, to the best of our knowledge, there is no longitudinal study supporting this hypothesis in humans.

Along with pharmacological interventions, a reduction in the risk of osteoporosis should be achieved through lifestyle interventions, including exercise, adequate intake of calcium and proteins and avoidance of risk factors, such as sedentary lifestyle, smoking and alcohol abuse. According to wide-acknowledged guidelines, an adequate intake of calcium and vitamin D is mandatory to achieve peak bone mass and prevent post-menopausal bone loss. Nutrition can also be the source of several antioxidants, with potential beneficial effects on bone health. However, the reliability of the studies on the possible benefit of dietary antioxidants on bone homeostasis, fracture risk and markers of bone turnover is hampered by several drawbacks. First, the long-term effects of phytochemicals have not been assessed in randomized controlled clinical trials on post-menopausal women. Second, there are only a few
interventional studies, while observational research is highly heterogeneous in terms of study design and outcome measures. Finally, the studies published so far may produce biased results as these have mainly relied on dietary and food questionnaires and have not objectively assessed body stores through blood or urine analyses. Taking into account these multiple caveats, it is worth a brief overview of the reported findings.

The preventive effect of vitamin E on bone erosion observed in animal models prompted a number of large epidemiological surveys on the possible effect of its intake from diet and/or supplements. In a study on 951 current-smoking post-menopausal women, high intake of vitamin E was associated with a significantly lower risk of hip fracture. Accordingly, the supplementation with vitamin E was related to a reduced rate of fracture of any type in a large-scale epidemiological study (n=61,433 peri-menopausal women). In contrast with the aforementioned results, a longitudinal study conducted by MacDonald et al highlighted a direct association between increased vitamin E intake and greater femoral neck BMD loss. Finally, Wolf et al evaluated the effect of daily vitamin E intake (and serum level of the vitamin) in 11,068 elderly women and failed to show any significant association between vitamin E and BMD at any site in multivariate analysis. Besides vitamin E, many other studies evaluated the effect of other antioxidants on bone health. A large population-based case-control study showed that the intake of β-carotene and selenium, but not vitamin C, was related to a decreased rate of hip fracture. Promising results also arose from the handful of small epidemiological studies on lycopene, one of the isomers of β-carotene, that is, especially present in tomato fruit. In particular, two studies indicated that daily consumption of this phytochemical may suppress bone resorption as suggested by the apparent inverse relation between lycopene intake and serum levels of resorption markers. Unfortunately, the encouraging pre-clinical findings obtained with other bioactive compounds, especially present in fruits, such as flavonoids, resveratrol and pectin, have not been adequately replicated in humans yet. On the contrary, robust epidemiological evidence gathered in the last two decades yields a wide consensus around the positive impact of dietary intake of vegetables and, in particular, fruits on bone health.

**Summary and novel roads ahead**

The body of evidence supporting the pathogenic role of OxS in PO is mostly from *in vitro* or animal studies. Ageing and oestrogen deficiency represent the noxious combination that ‘breaches’ the first line of defence (i.e. antioxidant enzymes) against the potentially cytotoxic reactive species. The erosive activity of ROS on the bone has been well characterized. These chemically unstable molecules can affect bone health at various levels, influencing both indirectly (by stimulating the release of pro-absorptive cytokines) and directly the life cycle of osteoblasts/osteoclasts (Figure).

**Figure.** Cause-effect relationship between oestrogen decline, oxidative stress and post-menopausal osteoporosis. The menopause-associated decline in oestrogens (E2) results in a decrease of systemic and local (bone) protection against reactive oxygen species attack. This effect is due to the ability of 17β-oestradiol to act as a direct antioxidant and, most likely, to simultaneously upregulate the expression of the antioxidant enzymes and to contrast the accumulation of proinflammatory (and thus pro-oxidant) visceral fat. The uncontrolled increase of reactive oxygen species (ROS) leads to oxidative stress which, in turn, alters the balance between bone formation and resorption, thereby increasing the latter activity and contributing to the onset of post-menopausal osteoporosis.
This putative connection between OxS and the clinical manifestations of the menopausal syndrome has sparked a constellation of observational studies exploring the beneficial effects of dietary antioxidants on the post-menopausal women. However, it is impossible to reach a firm conclusion as to the usefulness of phytochemicals in this context, due to the numerous methodological and design limitations as well as to the lack of randomized clinical trials. Nonetheless, taking into account also the safety of these natural compounds, we firmly believe, along with many others, that this could be the right road ahead.

The future interventional studies with antioxidants should address some criteria that have not been adequately considered so far. First, the modification of the usual diet (with increased intake of fruits and vegetables) or the use of antioxidant supplements might be suggested to accompany MHT to preserve correct redox homeostasis. It is now well accepted that no antioxidant is able to contrast oxidative challenge by itself. On the contrary, this task can be achieved only in concert with other antioxidants. A typical example in this field is α-tocopherol; after reacting with ROS, it becomes itself a free radical, which can be neutralized by the finely orchestrated synergic action of a network of endogen antioxidants, including glutathione and reduced glutathione, and vitamin C. This consideration directly leads to the second benchmark to be addressed in the future trials: the interventions based on the administration of a single antioxidant are doomed to fail in the absence of an exhaustive picture of the individual redox profile.

Such a tailored approach can be designed by measuring the peripheral levels of oxidative damage both overall and of the single antioxidants. Third and last points to take into account are that all types of antioxidant act as preventive, but not repairing agents. Translating these assumptions into practice: if the aim of a therapy is to effectively reduce the ‘weight’ of OxS contribution to PO development, then it must be prescribed to patients with normal or moderately low BMD, but not to patients with full-blown osteoporosis. The interventions aiming to reduce OxS may be accompanied with classical prevention strategies such as lifestyle modifications, avoidance of recognized risk factors and adequate intake of vitamin D, calcium and proteins.

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