Chapter 10

Oxidative Stress and Antioxidants in the Risk of Osteoporosis — Role of Phytochemical Antioxidants Lycopene and Polyphenol-containing Nutritional Supplements

L.G. Rao and A.V. Rao

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http://dx.doi.org/10.5772/60446

1. Introduction

Osteoporosis is a systemic disease that is characterized by low bone mass and deterioration of the microarchitecture of bone, resulting in an increased risk of fracture in postmenopausal women and men over 50 years old. Several factors have been identified that contribute to the risk of osteoporosis, and oxidative stress has now emerged as one of the most important life style risk factor associated with loss of bone mass. Phytochemicals as antioxidants have been shown to counteract the deleterious effects of oxidative stress in the risk of osteoporosis. This review will include an overview on osteoporosis, the deleterious effects of oxidative stress and the beneficial effects of phytochemical antioxidants, with emphasis on the results of our clinical studies on the phytochemical lycopene and polyphenols present in nutritional supplements.

2. Osteoporosis – An overview

Osteoporosis is a metabolic bone disease known as "the silent thief" because the gradual loss of bone associated with this disease usually occurs over the years, and there are usually no noticeable symptoms until the bones are so fragile that a fracture occurs [1]. Osteoporosis is "a major public health threat" that is projected to results to 8.1 million fractures (78 % women, 22 % men) during the period between 2010 and 2050 [2]. Approximately 1 in 2 women and 1 in 5 men older than 50 years will eventually experience osteoporotic fractures [3]. The condition costs our healthcare system $18 billion per year [4]. Newer findings on all aspects of osteoporosis have increased exponentially. The more importantly ones are discovering an ever

increasing number of risk factors including oxidative stress, opening up new knowledge on the involvement of the bone forming cells osteoblasts and the bone resorbing cells osteoclasts in the development of osteoporosis, the introduction and improvement of more sensitive diagnostic instruments, and finding new drugs and the nutritional alternatives for the prevention and treatment of osteoporosis. Advances in the knowledge on osteoporosis is not without pitfalls. Hormone Replacement Therapy (HRT), once a first line of treatment for osteoporosis has been discontinued due to side effects [5]. It is becoming more evident that the drugs known as bisphosphonates, although effective in stopping the resoption of bone and preventing osteoporosis in women, are associated with a number of side effects [6, 7]. Because of this, a number of women are now resorting to other modes of treatment, including that from natural food components. Our laboratory has carried out studies on the use of phytochemical antioxidants such as lycopene and polyphenols present in nutritional supplements as possible alternatives and/or complementary to drugs in the treatment and prevention of osteoporosis. This chapter will include an overview on osteoporosis, oxidative stress as a risk factor in the development of osteoporosis and a review of studies on the use of antioxidants in counteracting oxidative stress in the prevention of osteoporosis. These topics should put our research in perspective and offer a rationale to our study approaches. Finally we will highlight our pioneering clinical studies on the lipid-soluble phytochemical antioxidant lycopene and the water-soluble antioxidant polyphenols present in a nutritional supplement in the prevention of risk for osteoporosis in postmenopausal women.

2.1. Risk factors for osteoporosis

Some of the risk factors for osteoporosis [8, 9] are presented in Table 1 [10]. The risk factors that are of interest in our studies are the oxidative stress-generating factors, including nutrition deficiency, low antioxidant status, smoking, alcohol intake, excessive sports activity and caffeine intake.

Unmodifiable	Modifiable
Race	Chronic inactivity
Sex	Low body weight
Age	Low lifetime calcium intake
Genetics	Medication used
Body size	Oxidative stress-related
Family History	Factors:
Previous Fractures	Smoking
	High Alcohol intake
	Low antioxidant status
	Nutrition deficiency
	Excessive sports activity
	Excessive caffeine intake

Table 1. Risk Factors for Osteoporosis

http://dx.doi.org/10.5772/60446

2.2. Prevention and treatment of osteoporosis

Until 10 years ago, the first line of treatment for women who have gone through menopause and were diagnosed with osteoporosis was hormone replacement therapy (HRT). However, results of the Women's Health Initiative (WHI) revealed that women taking HRT had higher risks for breast cancer, cardiovascular events, blood clots, cognitive decline, and more [5]. This treatment for osteoporosis has since been discontinued and is prescribed only for a short period of time to alleviate hot flashes in menopausal women [11]. The current treatments which inhibit bone resorption that are approved by the Food and Drug Administration (FDA) include a number of bisphosphonates under specific trademarks [12]. Some are taken daily while others are formulated for weekly, monthly or intermittent oral use [13, 14]. The newer bisphophonates are injectables such as Zoledronate and Ibandronate [14]. Other drugs available include calcitonin; Raloxifene (Evista), the Selective Estrogen Receptor Modulator (SERM) and strontium renalate [15]. Parathyroid hormone, PTH1-34 or teriparatide (Forteo), is the only anabolic agent currently approved for use by the FDA [16, 17]. The new class of osteoporosis drug now approved for use is a human monoclonal antibody (Denosumab) which bind to RANKL, imitating the effects of OPG and acting as an inhibitor of RANKL [18]. Other drugs are still being tested clinically for osteoporotic treatment and prevention [16].

None of the drugs are without side effects. Side effects that emerged in clinical trials include acute phase response with iv treatment or high-dose oral therapy and esophageal irritation with oral administration. Osteonecrosis of the jaw, musculoskeletal complaints, and atypical fractures are some uncommon side effects that have been noted with wide clinical use of bisphosphonate. The number of these events are small, and a clear cause-and-effect relationship between events and bisphosphonate treatment has not been established. Accumulation of Bisphosphonates in the bone create a reservoir leading to continued release from bone for months or years and provide some residual anti-fracture reduction long after treatment is stopped [19]. As a result, there is a recommendation for a drug holiday after 5 –10 yr of treatment with bisphosphonate [7, 19]. The length of the holiday is based on previous duration of treatment, BMD status and fracture risk. Studies with alendronate and risedronate showed that if treatment is stopped after 3–5 yr, there is at least 1–2 yr persisting anti-fracture efficacy. The consensus from expert panels [7] for those who are not on holiday is not to stop the use of drug since the side effects are often rare, and that the benefits outweigh the side effects. In the balance, most individuals who have osteoporosis are much better taking an osteoporosis medication [6].

2.3. Alternative approach to prevention and treatment of osteoporosis

Diet is now recognized as an important life-style factor in the management of bone health [20]. Given that many nutrients have been identified as being beneficial to bone health [21, 22], there is strong scientific support for the potential benefits of incorporating therapeutic nutritional interventions with contemporary pharmaceutical treatments [23]. As a result of the possible adverse side effects of HRT [5] and the ever increasing reports on the side effects of bisphosphonates that are prescribed for the management of postmenopausal osteoporosis [19], complementary and alternative medicine (CAM) is in demand as an alternative for the prevention

and treatment of osteoporosis [24]. CAM is the term for medical practices, services and products that are not a part of standard care. Some of the approaches include exercise, acupuncture, diet, herbs rich in polyphenols and nutritional supplements including calcium, zinc, magnesium boron and other vitamins and minerals [24]. Recent dietary guidelines for the prevention of chronic diseases have recommended an increase in the consumption of fruits and vegetables worldwide [25] that are good sources of dietary antioxidants [26]. The beneficial effects of antioxidants in bone health and osteoporosis are demonstrated epidemiologically and through clinical intervention. As will be reviewed in this chapter, our clinical studies on lycopene treatment and nutritional supplements containing polyphenols and other nutritional components showed positive results on bone health.

3. Oxidative stress and antioxidants – An overview

Oxidative stress is caused by reactive oxygen species (ROS) which are the main by-products formed in the cells of aerobic organisms that can initiate autocatalytic reactions in such a way that the target molecules get converted into free radicals causing a chain of damage [27]. There is ample evidence to show that oxidative stress induces an increase in the rate of bone loss and is therefore a risk factor for osteoporosis. Epidemiological evidence in humans and studies in animals indicate that aging and the associated increase in reactive oxygen species (ROS) are responsible for bone loss [28]. Oxidative stress is associated with the activity and function of both the osteoblasts and osteoclasts cells, the two major bone cells involved in the pathogenesis of osteoporosis [29, 30].

Under normal physiological conditions, the cells can fight free radical attack or oxidative stress by promoting antioxidant defenses. A number of endogenous defense mechanisms are present in the body, including the metal chelating proteins and the endogenous antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [31]. Exogenous antioxidants come from dietary sources present in fruits and vegetables containing several phytonutrient antioxidants two of which are the potent antioxidant lipid-soluble lycopene and the water-soluble antioxidant polyphenols [32]. In cases where the exogenous antioxidants or antioxidants from diet fail to prevent oxidative damage, the repair antioxidants come into play which include DNA repair enzymes, protease, lipase, and transferase [33]. When antioxidants loses its fight with oxidative stress, diseases associated with oxidative stress develop, which include cardiovascular disease, cancer, diabetes, neurological diseases and osteoporosis [26].

3.1. Lycopene, a lipid-soluble phytochemical antioxidant

The role of lycopene in the prevention of human diseases is supported by a number of evidence and previously reviewed [34, 35]. Since then, there have been several epidemiological as well as clinical intervention studies showing the relationship between lycopene intake and the prevention of cancers at other sites, as well as coronary heart disease, diabetes, hypertension, macular degenerative disease, neurodegenerative disease and male infertility [26]. The role of

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lycopene in bone health has so far been based on its potent antioxidant properties, the well-known role of oxidative stress in bone health, the limited studies on the effects of lycopene in bone cells in culture [34, 35] and the results of epidemiological studies [36, 37]. To date our clinical intervention studies at St. Michael's Hospital on the role of lycopene and elucidation of its mechanism in lowering the risk for osteoporosis in postmenopausal women (aged 50 to 60 years) are so far the only clinical studies reported in the literature.

3.2. Polyphenols, the water-soluble antioxidant

Polyphenols are a class of water-soluble molecules naturally found in plants [38]. It is estimated that there are 10,000 different phytonutrients (phyto, meaning from plants). The health benefits associated with fruits, vegetables, tea, red wine, and Mediterranean diets are probably linked to the polyphenol antioxidants [21, 39, 40]. The polyphenols of interest in our study are a mixture of flavonoids such as quercetin, apigenin, kaempferol and luteolin present in the supplement greens+™ [41]. greens+™ in combination with another supplement, bone builder™, were used in our study on osteoblasts cells and in clinical intervention studies on the prevention of risk of osteoporosis in postmenopausal women as will be reviewed below.

4. Studies on the antioxidants polyphenols and lycopene

4.1. Studies on lycopene

The direct role of lycopene in osteoblasts and osteoclasts, the cells involved in the pathogenesis of osteoporosis, is now being unraveled. This involvement is further supported by both epidemiological and clinical intervention with lycopene in postmenopausal women who are at risk of osteoporosis [29, 30].

An epidemiological study to determine the beneficial role of lycopene in the prevention of risk for osteoporosis was carried out by Rao et al [36]. In a cross-sectional study, 33 postmenopausal women aged 50–60 years participants were recruited and asked to provide seven-day dietary records and blood samples for analyses of total antioxidant capacity; oxidative stress parameters including lipid peroxidation and protein oxidation; and bone turnover markers including bone resorption marker NTX and bone formation marker. Their results showed that the estimated dietary lycopene had a significant and direct correlation with serum lycopene, suggesting that lycopene from the diet is bioavailable. Their conclusion that the higher serum lycopene was associated with a low NTx ($p<0.005$) and lower protein oxidation ($p<0.05$) supports the antioxidative properties of lycopene involvement in its mechanisms of action in bone [36].

The overall conclusions that can be derived from the cross-sectional study is that lycopene has a role in the prevention of risk for osteoporosis. Further clinical studies described below support this conclusion.

Mackinnon et al [42] studied whether the elevated dose obtained through lycopene supplementation compared to intakes typically obtained from the usual daily diet was more beneficial in reducing bone turnover markers. Serum lycopene, bone turnover markers and oxidative stress parameter data were compared between postmenopausal women who were supplemented with lycopene and those who obtained both a low and high intake lycopene from daily food diet. Results showed that women who consumed lycopene supplement had significantly lower TBARS values than participants who obtained a low intake or high intake lycopene through their usual daily diets. These differences in TBARS value may be attributed to a significantly higher concentration of serum 5-*cis* in lycopene-supplemented participants compared to participants who obtained their lycopene from low or high usual daily diet. This suggests that it is the 5-*cis* isomer, with the most potent antioxidant capacity which, at higher concentrations, decreases bone turnover markers due to its ability to provide the greatest protection against oxidative stress. It also appears to show that supplementation with lycopene may be necessary in spite of the daily intake of lycopene [42].

Another study was carried out to determine the effects of a lycopene-restricted diet on oxidative stress parameters and bone turnover markers in postmenopausal women [43]. Results showed that restricting the participants from consuming lycopene-containing products resulted in significant decreases in serum lycopene, α-/β-carotene and lutein/zeaxanthin, with the overall change in the serum carotenoids being lower than that seen for lycopene. All configurations of lycopene (all trans, 5-cis- and other cis lycopene) were found to be decreased and the antioxidant enzymes SOD and CAT were also significantly depressed after lycopene restriction. These changes were accompanied by a significant increase in the bone resorption marker NTx. The important conclusion from this study is the possibility that the significant increase in the bone resorption marker NTx could lead to a long-term decrease in BMD and increased fracture risk as was observed by Brown et al. [44]; longer restriction period may be detrimental to a group of postmenopausal women who were already at high risk for osteoporosis. Therefore, shorter wash-out periods of no lycopene consumption is all that is needed in clinical trials examining the effects of lycopene on bone health [43].

A clinical fully randomized controlled intervention study was next carried out by Mackinnon et al [45] to investigate directly the effects of lycopene supplementation on decreasing the risk for osteoporosis. Lycopene supplements include lycopene capsules, tomato juice with normal amount of lycopene, tomato juice with high amount of lycopene. They have shown that after the 4-month duration, the LYCOPENE-supplemented group had a significant increase in total antioxidant capacity, decrease in oxidative stress parameters protein oxidation as shown by increase in thiol values and lipid peroxidation as shown by TBARS which correlated to a decrease in NTx; all changes were significantly different from the PLACEBO group. These findings suggest that lycopene obtained in the form of tomato juice or capsule exerted equivalent antioxidant potency in reducing the risk of osteoporosis in postmenopausal women [45].

Mackinnon et al studied whether polymorphism plays a role in the development of osteoporosis [46]. To do this, Mackinnon et al. studied the role of 172T→A or 584A→G polymorphisms

http://dx.doi.org/10.5772/60446

of the paraoxonase 1 (PON 1) in modulating the effects of serum lycopene on antioxidant capacity, oxidative stress parameters and bone turnover markers, and in women between the ages of 25-70 years). Their results showed that the PON1 polymorphism modified the association between lycopene and NTx and BAP, an interaction that may also moderate the risk of osteoporosis [46].

In another study, they showed that there was a significant interaction between PON1 genotype and change in TBARS ($p<0.05$) suggesting that supplementation with lycopene resulted in decreased lipid peroxidation, which interacted with the PON1 genotype to decrease bone resorption markers in postmenopausal women. These results provided a mechanistic evidence of how intervention with lycopene may act to decrease lipid peroxidation and thus the risk of osteoporosis in postmenopausal women [45, 47].

In conclusion, the demand for the use of other natural food components in the management of postmenopausal osteoporosis has increased due to reports on the adverse side effects of the conventional therapy (eg, HRT and bisphosphonates). The studies reviewed above revealed evidence that antioxidants such as lycopene can counteract the damaging effects of oxidative stress brought about by ROS that lead to the development of osteoporosis. The results of studies reviewed here indicate that lycopene maybe useful either as a dietary alternative to drug therapy or as a complement to the drugs presently approved for used by women at risk of osteoporosis.

4.2. Studies on polyphenols

It is well known that polyphenols have a role in the prevention of chronic diseases such as cancers, diabetes, cardiovascular diseases, neurodegenerative diseases, and osteoporosis. Interest on polyphenols and bone health has increased in the last 10 years [48-51]. The anabolic role of phytonutrients and especially polyphenols in bone was reviewed by Horcajada [49], the mechanisms of action of polyphenol in osteoblast function and its interaction with osteoclasts was reviewed by Trzeciakiewicz [50] and the beneficial effects of green tea polyphenols has been reviewed [52, 53].

Currently, most of the research on polyphenols and their effects have emerged from *in vitro* and *in vivo* animal studies with only a few clinical studies available. In our recent review, we have included tables listing all the studies on polyphenols *in vitro* bone cell culture and the epidemiologic studies on the protective effects of polyphenol consumption against osteoporosis [54].

Combinations of polyphenols have also been studied. One such source is the nutritional supplement greens+™, a blend of several herbal and botanical products containing a substantial amount of polyphenols including quercetin, apigenin and luteolin [41] which act as antioxidants and therefore should be able to counteract oxidative stress. Thus, Rao et al [55] have shown that greens+™, is more effective in stimulating osteoblasts to form more bone nodules in a dose-dependant manner than epicatechin, the main polyphenol found in green

tea. We have further shown that this stimulatory effect is accompanied by decreases in the reactive oxygen species H_2O_2 [56].

Two additional nutritional supplements have since been formulated which may prove to be good for bone health. These are the bone builder™ and the greens+bone builder™ ; the latter is the original greens+™ product that has been supplemented with the bone builder™ formula which contains several compounds including vitamins, minerals, and antioxidants. These various components have been separately shown to have some beneficial effect on bone [57]. Using the human osteoblast SaOS-2 cells, Rao et al showed that similarly to the greens+™, the water-soluble bone-builderTM extract had a significant dose-dependent stimulatory effect on bone nodules formation [58]. It was additionally shown that similarly to the greens+™, the watersoluble bone-builder™ extract had a significant dose-dependent stimulatory effect on bone nodules formation [58]. In a later study, they have shown that when the two supplements, greens+*TM* and bone builder*TM*, were tested as combination, the effects were six times more effective than either one alone [59]. This led Rao et al to believe that synergistic effects of greens +*TM* and bone builder*TM* may have a beneficial effect on osteoporosis. A product called greens +bone builder™ is available commercially [57].

A clinical study was then carried out to evaluate the effect of the nutritional supplement greens + bone builder*TM* for 8 weeks on the risk for osteoporosis in postmenopausal women compared to placebo control [60, 61]. Results have shown that there was an increase in total antioxidant capacity, as well as a decrease in both lipid and protein oxidation over a 4 and 8-weeks of intervention with greens+ bone builder*TM* compared to placebo [60] suggesting that the nutritional supplement may have a beneficial effect on bone health by mitigating the effects of oxidative stress. In order to test whether the antioxidant properties of greens+bone builder™ can prevent the risk of osteoporosis in postmenopausal women. Kang et al [60] also measured the serum bone turnover markers, C-terminal telopeptide of type I collagen (CTX) as indicator of bone resorption, and procollagen type I N-terminal propeptide (PINP) as indicator of bone formation and determined their correlations with the serum antioxidant capacity, and the oxidative stress parameters lipid peroxidation, protein oxidation. Statistical analysis showed that at 8 weeks, the greens +bone builder™ supplement group significantly decreased the bone resorption marker CTX, while the Placebo group showed no significant changes. The supplement group was also significantly different from that of the Placebo group in all parameters measured. This decrease CTX correlated to the increase in their serum total antioxidant capacity and decreases in oxidative parameters protein oxidation lipid peroxidation [61]. These results suggest that a daily supplementation with polyphenols and micronutrients may be important in reducing oxidative damage by reducing bone resorption, thereby reducing the risk of osteoporosis in postmenopausal women [60, 61].

In summary, studies reported in the literature on the role of polyphenols in bone health have exploded in the last 10 years, but most of the reports involved in vitro studies in osteoclasts and osteoblasts, animal studies and epidemiologicai studies. There is little doubt from the excellent studies reported that oxidative stress is one of the primary culprits responsible for

http://dx.doi.org/10.5772/60446

the pathogenesis of osteoporosis via its role in osteoclastic resoption and the detrimental effects on the bone-forming osteoblasts. To date, only four clinical intervention studies have been reported, including ours. It is easy to see why it is very difficult to evaluate the role of polyphenols since, as we learned from this review, there are at least 8,000 different polyphenols identified to date, and each one probably having different effects on humans. Additionally, polyphenols are present in food with other constituents that may also be beneficial to bone health. In our clinical study, we combined the effects of a combination of polyphenols present in the nutritional supplement from greens+™ with the nutritional components present in bone builder™ such as minerals, vitamins and other nutrients. It is possible that the effects of greens +bone builder™ in increasing total antioxidant capacity, decreasing the oxidative stress markers protein oxidation and lipid peroxidation which correlated to the decrease in bone turnover marker for bone resorption is a result of the combined effects of the different polyphenols it contained with those of the other nutritional components present in the bone builder™. It remained for future studies to zero in on specific component that is responsible for its beneficial effect.

In conclusion, we showed that oxidative stress due to ROS that are shown to cause the development of osteoporosis may be prevented by supplementation with the antioxidants lycopene and polyphenols. Results of in vitro studies in osteoblasts and osteoclasts, animal intervention studies, epidemiological studies and clinical intervention studies on lycopene and polyphenols are evidence for their potential use as alternative or complementary agent with other established drugs approved for the prevention or treatment of osteoporosis in postmenopausal women.

Acknowledgements

Funding for this research into Oxidative Stress, Antioxidants and Bone Health is shared by H.J. Heinz Co (Canada), Kagome Co. (Japan), LycoRed Natural Product Industries, Ltd. (Israel), Genuine Health Ltd (Canada), Millenium Biologix Inc. (Canada), and matched by the Canadian Institutes of Health Research (CIHR). We sincerely thanked the valuable contributions to this research by the following students/graduate students and staff at the Calcium Research Laboratory, Department of Medicine at St Michael's Hospital and the University of Toronto and Department of Nutritional Sciences, University of Toronto: Dr. Bala Balachandran, Jaclyn Beca, Dawn Snyder, Loren Chan, Honglei Shen, Salva Sadeghi, Ayesha Quireshi, Dr. Erin Mackinnon and Nancy Kang. Their contributions were based on their experimental data, written reports published/in press manuscripts/theses. We would also like to thank to Dr. R.G. Josse for providing us with his medical expertise as well as allowing us access to his list of patients we were able to recruit. Special thanks to Dr. H. Vandenberghe for carrying out the CTX assay and for her valuable suggestions.

Author details

L.G. Rao[1*] and A.V. Rao[2]

*Address all correspondence to: leticia.rao@utoronto.ca

1 Department of Medicine, St Michael's Hospital and University of Toronto, Canada

2 Department of Nutritional Sciences, University of Toronto, Canada

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