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Will Tomatoes Prevent Osteoporosis?

BY LETICIA G. RAO, PH.D.

Osteoporosis is a major metabolic bone disease that occurs primarily in women over the age of 50 because of the loss of estrogen during menopause. Oxidative stress as a risk factor for osteoporosis has garnered a lot of interest. Caused by reactive oxygen species (ROS), oxidative stress is involved in the activity and function of osteoblasts and osteoclasts, the two major bone cells implicated in the pathogenesis of osteoporosis. However, the cellular and molecular mechanisms involved in this association, the action of ROS, and the role played by dietary antioxidants (eg, lycopene) are not clear and the subject of continued study. Lycopene is a potent antioxidant that is present mainly in tomatoes and tomato products, as well as in small amounts in some fruits and vegetables. Based on epidemiological data, clinical studies, and in vitro cell culture studies, lycopene has been associated with the prevention of major human chronic diseases, including certain types of cancer, cardiovascular disease, hypertension, macular degenerative disease, and male infertility. The ongoing clinical study at the Calcium Research Laboratory at St. Michael's Hospital is the first to evaluate lycopene in nutritional supplement and tomato juice in the prevention of osteoporosis in postmenopausal women. This issue of *Endocrinology Rounds* focuses on this ongoing research and the role of oxidative stress as a risk factor for osteoporosis.

Osteoporosis, a major metabolic bone disease

Bone is a dynamic tissue that is continuously renewed throughout life by the process of bone remodelling, which involves the coupled events of removal of old bone by osteoclasts and formation of new bone by osteoblasts.^{1,2} The remodelling process is the result of interactions between these cells and multiple molecular agents, including hormones, growth factors, and cytokines. Disturbances in the bone remodelling process can lead to metabolic bone diseases.^{3,4} Osteoporosis is a major metabolic bone disease characterized by low bone mass and microarchitectural deterioration of bone tissue, causing enhanced bone fragility and increased risk of fracture.⁵ It is known as the "silent disease" and affects 1 in 4 women and 1 in 8 men. Osteoporosis predominantly affects menopausal women aged >50 years due to the loss of estrogen; more than 1.4 million Canadian women are affected. In postmenopausal bone loss, the remodelling process becomes significantly more active with a primary increase in bone resorption and a counterbalancing, but insufficient increase in bone formation.⁶ Some of the risk factors for osteoporosis are listed in Table 1.^{7,8} The risk factor that will be reviewed in this issue is oxidative stress.

Oxidative stress and antioxidants

Oxidative stress results from the weakening of antioxidant defense or an excess production of reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^\cdot), and other free radicals.⁹ Oxidative stress may also result from normal metabolic activity or environmental factors such as diet. Uncontrolled, ROS will damage different biological targets, including lipids, DNA, and proteins. ROS production increases with age¹⁰ and is associated with several chronic diseases (Figure 1). The major antioxidant defense systems present in the body are superoxide dismutase (SOD),



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Table 1: Risk factors for osteoporosis**Risk factors that cannot be changed**

Race
Sex and age
Genetics
Previous fractures

Risk factors that can be changed

Chronic inactivity
Microgravity
Excessive sports activity
Low body weight
Low lifetime calcium intake
Hormones
Medication
Oxidative stress-related factors
Smoking
Low antioxidant status
Excessive lipid intake
Nutrition deficiency

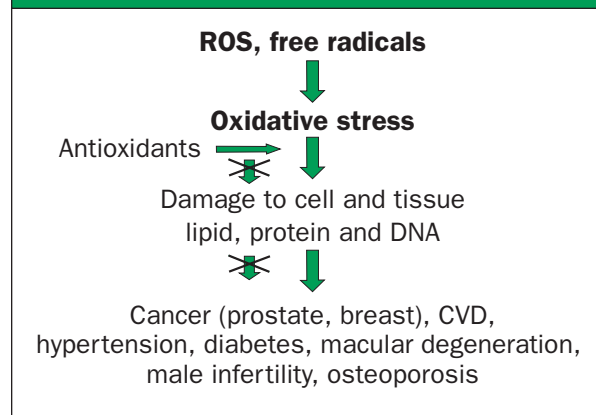
glutathione-S-transferases (GSTs), glutathione-S peroxidase (GPX), and catalase.¹¹ Antioxidants in the diet, notably vitamin C, vitamin E, β -carotene, selenium, lycopene, and polyphenols provide additional defense against oxidative stress.¹²

Clinical studies on oxidative stress and antioxidants in osteoporosis

ROS-induced oxidative stress has been associated with the pathogenesis of osteoporosis. Epidemiological studies suggest that certain antioxidants (eg, vitamin C, E, and β -carotene) may reduce the risk of osteoporosis^{13,14} and counteract the adverse effects of oxidative stress on bone that are produced during strenuous exercise¹⁵ and heavy smoking.¹³ Women with osteoporosis have markedly decreased plasma antioxidants.¹⁶ Increased oxidative stress biomarker 8-iso-prostaglandin F alpha (8-iso-PGF α) is biochemically linked with reduced bone density.^{17,18} The severity of osteoporosis was positively correlated with the level of the oxidative stress marker, lactic acid, in 2 men with mitochondrial deletion (mtDNA)¹⁹ and a study of severe osteoporotic syndrome in relatively young males linked osteoporosis to an increase in oxidative stress.²⁰ In spite of these reports, the cellular and molecular mechanisms involved in the role of oxidative stress in osteoporosis remain poorly defined.

Studies on oxidative stress and antioxidants in osteoclasts

The mechanisms involved in the differentiation of osteoclasts and their ability to resorb bone are poorly understood; however, one theory suggests that ROS are involved in this process.²¹ Both the hydrogen peroxide (H_2O_2) that is produced by endothelial cells²² intimately

Figure 1: Role of oxidative stress and antioxidants in cell and tissue damage leading to chronic disease

associated with osteoclasts and the H_2O_2 that is produced by osteoclasts²³ increase osteoclastic activity and bone resorption. H_2O_2 may also be involved in the regulation of osteoclast formation,²⁴ differentiation of osteoclast precursor clonal cell line HD-11EM cells,²⁵ and osteoclast motility.²³ The tartrate-resistant acid phosphatase (TRAP), found on the surfaces of osteoclasts, have the capacity to react with H_2O_2 to produce highly destructive ROS that target the degradation of collagen and other proteins.²⁶

Superoxide has been localized both intracellularly and at the osteoclast-bone interface using nitroblue tetrazolium (NBT), which is reduced to purple-coloured formazan by ROS, suggesting the participation of superoxides in bone resorption.²⁷ Osteoclastic superoxide is produced by NADPH oxidase.^{28,29} 1,25-dihydroxy-vitamin D₃ (1,25(OH)₂D₃) has a direct non-genomic effect that is inhibited by estrogen during the generation of superoxide anion (O_2^-).³⁰ Estrogen has also been reported to have an antioxidant property.^{31,32} Hormones known to stimulate bone resorption (eg, parathyroid hormone [PTH]³³ and 1,25(OH)₂D₃) have been reported to have stimulatory effects on ROS production in osteoclasts,³⁰ whereas hormones known to have inhibitory effects on bone resorption (eg, calcitonin) inhibit ROS production.^{30,34}

Antioxidants also play a role in osteoclast activity. Osteoclasts produce the antioxidant enzyme, SOD, in the plasma membrane.³⁵ ROS production in osteoclasts is inhibited after treating the cells with antioxidant enzymes such as SOD²⁷ and catalase.²⁴ ROS production in osteoclasts is also inhibited by estrogen,³⁰ the superoxide scavenger deferoxamine mesylate-manganese complex,^{36,37} pyrrolidine dithiocarbamate (PDTC), and N-acetyl cysteine (NAC).³⁸ The use of antioxidants from natural sources (eg, fruits and vegetables) may be another way of inhibiting ROS. The use of lycopene in this regard is reviewed below.

Studies on oxidative stress and antioxidants in osteoblasts

Very little work has been reported on the role of oxidative stress in osteoblasts. However, previous reports demonstrate that osteoblasts can be induced to produce intracellular ROS,^{39,40} which can cause cell death and decrease ALP activity;⁴⁰ this can be partially inhibited by vitamin E.³⁹ Treatment of rat osteosarcoma ROS 17/2.8 cells with tumour necrosis factor alpha (TNF- α) (10 ng/ml) suppressed bone sialoprotein (BSP) gene transcription through a tyrosine kinase-dependent pathway that generates ROS.⁴¹ H₂O₂ modulated intracellular calcium (Ca²⁺) activity in osteoblasts by increasing Ca²⁺ release from the intracellular Ca²⁺ stores.⁴² In a recent study, we showed that polyphenols, a group of important water-soluble antioxidants from tea and herbal preparations (eg, Greens+), inhibited ROS production and stimulated the mineralized bone nodule formation in the osteoblast-like SaOS-2 cells (unpublished observations).

The potent antioxidant lycopene

Lycopene is a potent antioxidant that is not synthesized in the body. Although 85% of dietary lycopene in North America is obtained from tomatoes and processed tomato products,¹² it can also be obtained from watermelon, pink guavas, and pink grapefruit.⁴³ Lycopene is absorbed more efficiently from processed tomato products than from raw tomatoes because it is converted from the all-trans to the cis-isomeric configuration with heat processing. Since lycopene is a lipid-soluble compound that is absorbed via a chylomicron-mediated mechanism, the presence of small amounts of lipids further enhance its absorption. The reported average daily intake levels of lycopene vary considerably from country to country, from 0.7 mg per day in Finland to 25 mg per day in Canada. However, a generally accepted universal level of daily intake is 2.5 mg.⁴⁴ There is no official recommended daily intake of lycopene, but based on published research, a daily intake of 7 mg is suggested.

Lycopene is a carotenoid acyclic isomer of β -carotene, with no vitamin A activity.⁴⁵ It is a highly unsaturated, straight-chained hydrocarbon containing a total of 13 double bonds, of which 11 are conjugated,⁴⁶ making it one of the most potent antioxidants.⁴⁷ The singlet oxygen-quenching ability of lycopene is twice that of β -carotene and 10 times that of α -tocopherol.⁴⁸ The chemistry and antioxidant properties of lycopene have been comprehensively reviewed.⁴⁹ The health benefits of lycopene may be due to its potent antioxidant property, although other mechanisms (eg, its effects on gap junction communication⁵⁰ and cell cycling⁵¹) are possibilities. There is now strong evidence to support the role of lycopene in the prevention of human diseases. Since the initial epidemiological observations suggesting an inverse relation-

ship between the intake of tomatoes and lycopene and the incidence of prostate cancer,¹² there have been several epidemiological and intervention studies showing the relationship between lycopene intake and the prevention of cancers at other sites, as well as coronary heart disease, hypertension, diabetes, macular degenerative disease, male infertility, and neurodegenerative disease.⁵² Human clinical trials are now being undertaken and reported in the literature and the American Food and Drug Administration (FDA) is reviewing the literature on lycopene and considering approval of the health claims by February, 2005. Go to www.fda.gov and search for "lycopene."

The role of lycopene in bone health has so far been based on its potent antioxidant properties, the well-known role of oxidative stress in bone health, and the limited studies on the effects of lycopene in bone cells in culture (see below). Data from our clinical studies at St. Michael's Hospital on the role of lycopene in postmenopausal women (aged 50 to 60 years), who are at risk for osteoporosis, are included in this review.⁵³

In vitro studies on lycopene in bone cells

Effect of lycopene on osteoclasts

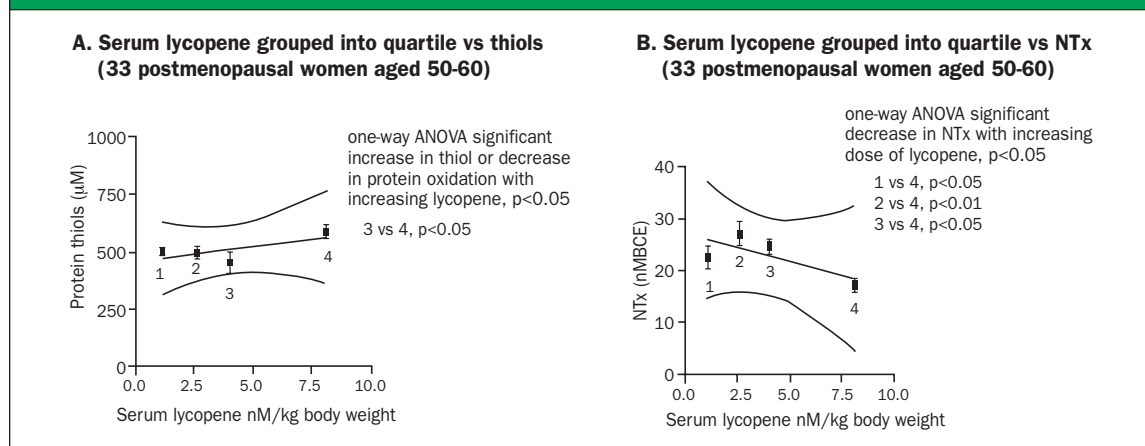
To date, there have been only 2 studies on the effects of lycopene on osteoclasts.^{54,55} Rao et al⁵⁵ cultured cells from bone marrow prepared from rat femur in 16 well, calcium phosphate-coated OsteologicTM multi-test slides. Varying concentrations of lycopene – in the absence or presence of the resorbing agent parathyroid hormone (PTH) (1-34) – were added at the start of culture and at each medium change every 48 hours. The effects of lycopene on mineral resorption, TRAP+ multinucleated osteoclast formation, and NBT-staining were studied. Lycopene inhibited TRAP+ multinucleated cell formation in both vehicle- and PTH-treated cultures. The cells that were stained with the NBT reduction product formazan were decreased in number after treatment with 10⁻⁵ M lycopene, indicating that lycopene inhibited the formation of ROS-secreting osteoclasts. These findings are new and may be important in the pathogenesis, treatment, and prevention of osteoporosis.

The effects of lycopene on osteoclast formation and bone resorption were also reported by Ishimi et al in murine osteoclasts formed in co-culture with calvarial osteoblasts.⁵⁴ Their results differed from those of Rao⁵⁵ in that they found that lycopene inhibited PTH-induced, but not basal, TRAP+ multinucleated cell formation. Furthermore, they could not demonstrate any effect of lycopene on bone resorption. They also did not study the effect of lycopene on ROS production.

Effects of lycopene on osteoblasts

Studies on the effects of lycopene on osteoblasts are also limited to 2 reports.^{56,57} Kim et al⁵⁷ showed that lycopene stimulated the proliferation of osteoblast-like

Figure 2: Correlation between lycopene consumption and oxidative stress parameter and bone resorption marker in postmenopausal women at risk of osteoporosis



SaOS-2 cells. They also reported that lycopene had a stimulatory effect on alkaline phosphatase activity, a marker of osteoblastic differentiation in more mature cells but, depending on the time of addition, had an inhibitory or no effect on younger SaOS-Dex cells. These findings comprised the first report on the effect of lycopene on human osteoblasts. In another study by Park et al,⁵⁶ the effect of lycopene on MC3T3 cells (the osteoblastic cells of mice) was contrary to the findings of Kim et al.⁵⁷ Park demonstrated that lycopene had an inhibitory effect on cell proliferation. Both studies, however, reported that alkaline phosphatase activity was stimulated. The discrepancy in the effects of lycopene on cell proliferation could be a result of species differences or experimental conditions. More studies are required to clarify the role of lycopene on osteoblasts.

Clinical studies on the role of lycopene in postmenopausal women at risk of osteoporosis

Postmenopause is associated with a global increase in bone turnover markers,^{58,59} which predict bone loss and osteoporosis in postmenopausal women.⁶ One of the objectives of our current clinical study at St. Michael's Hospital is to test whether serum lycopene correlates inversely with oxidative stress parameters and bone turnover markers in postmenopausal women who are at risk of osteoporosis. Thirty-three women, aged 50-60 years, were recruited and asked to complete a 7-day food intake record prior to giving fasting blood samples. Oxidative stress parameters, total antioxidant capacity, serum lycopene, and bone turnover markers (bone alkaline phosphatase [bone formation] and cross-linked N-telopeptides of type I collagen (NTx) [bone resorption]) were measured from serum

samples. The participants were grouped into quartiles according to their serum lycopene per kilogram body weight (nM/kg) and correlation analyses were carried out using the Newman-Keuls post-test. The most important and interesting findings to date are the significant decreases in protein oxidation (as indicated by increased thiols [$p < 0.05$]) and NTx values ($p < 0.005$) as levels of serum lycopene increase (Figure 2).⁵³ Since there was a significant positive correlation between serum lycopene levels and dietary lycopene intake, as determined from the estimated food records ($p < 0.01$) (data not shown), our results support the hypothesis that dietary lycopene is absorbed in the body and acts as an effective antioxidant, reducing oxidative stress and bone turnover markers. Our observations suggest an important role for lycopene in reducing the risk of osteoporosis that is mediated via its antioxidant property. Dietary intervention studies with varying doses and sources of lycopene are currently being conducted to demonstrate the beneficial effects of lycopene in the prevention and management of osteoporosis.

Conclusion

Although there is epidemiological evidence to support the beneficial effects of tomatoes and tomato products in the prevention of osteoporosis in the Mediterranean population, the direct role of lycopene, the potent antioxidant component in tomatoes, has not yet been explored. The effects of lycopene on osteoblasts^{56,57} and osteoclasts^{54,55} that have so far been reported and the involvement of oxidative stress in the pathogenesis of osteoporosis (as reviewed above) provide evidence for the importance of lycopene in the prevention of osteoporosis. Our ongoing clinical study at St. Michael's Hospital is the first to evaluate lycopene in the prevention of

osteoporosis in postmenopausal women. Although it is too early to suggest that eating tomatoes and tomato products will prevent osteoporosis, it would be a healthy practice to include tomatoes and tomato products in the diet as a source of lycopene for the prevention of oxidative stress-related chronic diseases, including osteoporosis. The final results of our study may indicate that lycopene can be used either as a dietary alternative to drug therapy or as a complement to drugs used in women at risk of osteoporosis.

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Abstract of interest

Lycopene and human health (A review)

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There is evidence to suggest an association between oxidative stress induced by reactive oxygen species (ROS) and chronic diseases. Antioxidants, by virtue of their ability to mitigate the damaging effects of ROS, have generated interest in their use as chemopreventive agents for a number of chronic diseases. Lycopene is the most potent natural antioxidant carotenoid occurring naturally in many fruits and vegetables and predominantly in tomatoes and tomato products. There is considerable interest in the role of lycopene in the prevention of human diseases. Initial epidemiological observations suggested an inverse relationship between the intake of tomatoes and lycopene and the incidence of prostate cancer. There is now evidence

to suggest a similar relationship between lycopene intake and the prevention of cancers of other sites, and coronary heart disease. Several human intervention studies are now reported in the literature in support of the role of lycopene in chronic diseases. Recent studies are evaluating the effect of lycopene in the management of osteoporosis, hypertension and male infertility among other human diseases. Although the evidence in support of lycopene in disease prevention is mainly based on epidemiological studies, human clinical trials are now being undertaken and reported in the literature.

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Upcoming Meetings

25-29 June 2005

Second Joint Meeting of the European Calcified Tissue Society (ECTS) and International Bone and Mineral Society (IBMS)

Geneva, Switzerland

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27th Annual Meeting of the American Society for Bone and Mineral Research

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