

# Lycopene: Its role in human health and disease

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## INTRODUCTION

Oxidative stress, induced by reactive oxygen species (ROS), is associated with the incidence of chronic diseases such as cancer, coronary heart disease (CHD) and osteoporosis (1). ROS are highly reactive oxidant molecules that are generated endogenously through normal metabolic processes, life style activity and the diet. Antioxidants provide an effective means to combat the deleterious effects of ROS and are increasingly being considered as strategic chemopreventive agents in the management of human diseases. Lycopene, a carotenoid phytonutrient, is the most potent antioxidant naturally present in many fruits and vegetables. However, tomatoes and processed tomato products constitute the major source of dietary lycopene accounting for up to 85% of the daily intake (2). There is considerable scientific interest in the role of lycopene in the prevention of several degenerative diseases.

## CHEMISTRY, BIOAVAILABILITY AND METABOLISM OF LYCOPENE

Lycopene is a highly unsaturated straight chain hydrocarbon with a total of 13 double bonds, 11 of which are conjugated. This unique nature of the lycopene molecule makes it a very potent antioxidant. *In vitro* studies have shown lycopene to be twice as potent as  $\beta$ -carotene and ten times that of  $\alpha$ -tocopherol in terms of its singlet oxygen quenching ability. Lycopene lacks the terminal  $\beta$ -ionic rings, and unlike  $\beta$ -carotene, lacks pro-vitamin A activity. Although in nature it exists predominantly in its all-trans isomeric form, it can undergo light, thermal energy and chemical reaction induced cis-isomerization. Several cis-isomers of lycopene have been identified in processed tomato products and biological fluids and tissues. In a recent study cis-isomers of lycopene were shown to be more stable, having higher antioxidant potential compared to the all-trans lycopene (3). The biological significance of lycopene isomerization is not fully understood. Lycopene absorption has been shown to be significantly higher in the thermally processed tomato products compared to raw tomatoes, and the processed products were shown to contain higher levels of cis-isomers of lycopene (4). It is therefore thought that cis-isomerization of lycopene enhance its absorption (5). Lycopene was shown to be readily absorbed from tomato juice, tomato sauce and supplements (6). Serum lycopene levels were shown to increase significantly upon the

consumption of tomato products and supplements with a concomitant decrease in the biomarkers of oxidation including the oxidation of serum lipids, LDL cholesterol, serum proteins and DNA. No adverse effects were observed in this study up to a daily intake of 150 mg lycopene for one week (6). Recent studies have shown that ingested lycopene is metabolized in the body. Several metabolites have now been identified and characterized (7). It is not known if these metabolites represent the biologically active forms of lycopene.

## LYCOPENE AND PROSTATE CANCER

Prostate cancer afflicts more than 10% of North American men and while this cancer is often curable by surgery or radiotherapy when confined to the prostate, in more than half of the patients the cancer recurs or spreads outside the gland at the time of diagnosis (8). In these patients with advanced disease there are very few therapeutic treatment options, highlighting a continuing need to explore new and emerging therapeutic options which harbor supportive pre-clinical and clinical efficacy data. Lycopene has emerged from the scientific literature over the past few years, to bear significant potential for consideration in both the treatment and prevention of prostate cancer

Numerous epidemiological studies and reviews have been carried out describing the role of lycopene in association with the prevention of prostate cancer (1,9). One of the earliest epidemiological studies showing an inverse relationship between the consumption of tomatoes and tomato products and the risk of prostate cancer was published in 1995 (10). In this study the beneficial properties of tomato products was attributed to lycopene. Since then, several other epidemiological, experimental and tissue culture studies have been reported providing further evidence for the role of lycopene in prostate cancer

One case control study in particular, conducted in 1982, examined the relationship between serum lycopene and other antioxidant levels on prostate cancer risk as well as aggressive prostate cancer incidence (11). Odds ratios were calculated for prostate cancer incidence using logistic regression models after a thirteen-year follow-up. Lycopene was the only antioxidant for which plasma lycopene was very strongly related to lower prostate cancer risk (upper quintile odds ratio = 0.40; P, trend = 0.006 for aggressive cancer). In tissue distribution studies carried out in rats, lycopene was found in liver, testes, stomach, intestine and prostates of rats fed a tomato oleoresin

diet (12). Physiological levels of lycopene were also detected in prostate, lung, mammary gland and serum of male and female rats fed a diet containing a carotenoid mixture extracted from tomatoes (13). Additional studies suggest that the *cis*-lycopene is the predominant isomer found in liver and that androgens modulate the metabolism of lycopene in the liver (14). Since prostate cancer is, at the onset, an androgen dependent disease, it seems, perhaps as a coincidence, that prostate cancer and lycopene metabolism share a dependency on this regulatory influence.

Rationalisation of the evidence for a reduction in prostate cancer risk has often been assigned to the antioxidant properties of lycopene (1,15) although recent evidence suggests that additional mechanisms beyond antioxidant property of lycopene may also be pertinent to prostate carcinogenesis. It is clear that many of the biological mechanism(s) of action of lycopene are not fully delineated, however numerous attempts have been made using cells in culture as a tool to unfold the mechanistic processes involved in its anticancer activity. Cytotoxic and antiproliferative effects of lycopene are indicated in prostate cancer cells (PC-3, DU 145, LNCaP). Similar effects of lycopene are also demonstrated for other cancer cell lines. Suppression of insulin-like growth factor (IGF-I)-mediated cell signaling reported in mammary carcinoma cells that is associated with its effects on proliferation may also hold true in case of prostate cancer (16-19). Elevated IGF-1 levels are thought to be correlated with an increased lifetime risk of developing prostate cancer. This finding, if confirmed clinically, provides a basis for the preventive properties of lycopene on prostate cancer development (20). Studies using KB-1 oral mucosa cells also show that cell signaling proteins are significantly impacted by lycopene treatment procuring an enhancement in gap junction communication (21). Furthermore, while not directly studied in a prostate cancer cell line, 1,25-dihydroxy-vitamin D<sub>3</sub>, an active product of vitamin D<sub>3</sub> metabolism with known anti-proliferative potency against prostate cancer, has been shown to sensitize synergistically the inhibition of cell proliferation and differentiation upon co-exposure with lycopene in a promyelocytic leukemia cell line (22). This study suggests that lycopene, not only acts in an antiproliferative sense on its own, but also works in concert when combined with other dietary factors.

More recently, clinical reports have also been presented suggesting a therapeutic application for lycopene in prostate cancer treatment (23,24). Pre-operative lycopene administration to men two weeks prior to radical prostatectomy was shown to decrease the number and size of cancerous foci in the prostate as well as associated high grade prostatic intra-epithelial neoplasia (PIN) in treated men as compared with control treated men (23). The treated group also had less involvement of surgical margins and the prostate tissue sectioned and used for immunohistochemical staining displayed a significant increase in connexin 43, a cellular gap junction protein, and bax, a pro-apoptotic protein in treated as compared with control tissues. However, while the results of this study are positive, the treatment modality used cannot be definitively assigned as lycopene since the authors describe the use of analytical HPLC to demonstrate that there was significant carotenoid and retinoid content in the supplement administered (23). A second clinical study describes a single case response of hormone refractory prostate cancer to lycopene (24). Extensive nodal disease was reported in a man whose serum PSA was 365 ng/ml in March 1999. After 2 years of daily lycopene (10 mg per day) and saw palmetto treatment (300 mg orally 3 times per day), the man was reported to be asymptomatic (24). This study was uncontrolled with respect to both placebo group, compliance and product quality and so there remains some questionability regarding definitive interpretation of the results. The patient in this study used saw palmetto in conjunction with lycopene and

so, again, the compound of interest was not the sole product ultimately being tested. However, the outcome of these studies suggests a potential therapeutic role for lycopene as well as a use in the prevention of prostate cancer.

As more evidence emerges in support of the anticancer and therapeutic benefits of lycopene, and as we begin to unravel more mechanistic detail of its activities, men living with prostate cancer are increasingly encouraged that an additional portion of tomato based foods can significantly benefit their fight against a disease which is the number one cancer affecting male population

## LYCOPENE AND OTHER CANCERS

The Mediterranean diet, which is rich in vegetables and fruits, including tomatoes, has been suggested to be responsible for the lower cancer rates in that region (25). Dietary intake of tomatoes and tomato products has been found to be associated with a lower risk of a variety of cancers in several epidemiological studies (26). A high intake of tomatoes was linked to protective effects against digestive tract cancers in a case-control study (27) and a 50% reduction in rates of death from cancers at all sites in an elderly US population (28). Results of a recently published met-analysis of epidemiological studies evaluating the relationship between dietary estimations of tomato intake and the circulatory levels of lycopene with the risk of cancers was reported (26). Of the 72 studies reviewed, 57 showed an inverse association, and 35 of these inverse associations were statistically significant. Although the strongest association was observed for prostate, lung and stomach cancers, risk of other cancers including breast, cervical and uterine cancers were also shown to be inversely associated. Several tissue culture studies have also shown the protective effect of lycopene against the growth of different cancer cell lines (1). A recent study demonstrated the inhibitory effect of tomato lycopene on the growth of breast cancer cells. Animal studies have also shown the protective effect of dietary lycopene against colon, lung and breast cancers - reviewed in Ref. 2.

Although, the role of lycopene in the prevention of prostate cancer has been studied more extensively, human intervention studies with tomatoes and lycopene on cancers of other sites are now beginning to be undertaken. Results of such studies will undoubtedly be reported in the scientific literature in the future which will contribute to our knowledge in this important area of human health.

## LYCOPENE AND CORONARY HEART DISEASE (CHD)

So far, serum cholesterol level has been the main focus as the risk factor for CHD. However, ROS-induced oxidative stress is now recognized as an important etiological factor of CHD (29). Oxidation of the circulating low-density lipoprotein (LDL) to oxidized LDL (LDL<sub>ox</sub>) is thought to play a key role in the pathogenesis of the disease (30,31). Lycopene, as a potent antioxidant, is therefore thought to play an important protective role against CHD. *In vitro* studies have shown that lycopene can protect native LDL from oxidation and also inhibit cholesterol synthesis (32,33). Animal intervention studies have also shown lycopene to increase the resistance of the extracted LDL *in vitro* to oxidation. However, epidemiological studies provide the main evidence in support of the role of lycopene in the prevention of CHD (29,34,35). In a cross sectional study (36), Lithuanian population, who are at a high risk of mortality from CHD was compared to a lower risk Swedish population and were shown to have lower blood lycopene levels. These observations suggested low blood lycopene levels to be associated with increased risk and mortality from CHD. In another case control population

study (37), cases that exceeded 90th percentile of intima-media thickness for all arterial segments, had lower levels of lycopene. Similarly, Rissanen et al (38) using a randomized, double blind, placebo-controlled population study showed a direct association between low plasma lycopene concentrations and the onset of early arteriosclerosis, manifested as increased intima-media thickness of the common carotid artery, in middle-aged men living in Eastern Finland. The same authors, in a follow-up study, showed low level of serum lycopene to be associated with an increased risk of arteriosclerosis vascular events in middle-aged men who were previously free of CHD and stroke (35). Based on the observed inverse relationship between plasma lycopene and intima-media thickness, Gianetti et al suggested a protective role for lycopene against arteriosclerosis (39). The EUEAMIC study (40) which is a multicenter case-control study evaluating the relationship between adipose tissue antioxidant status and acute myocardial infarction is perhaps the strongest population based evidence in support of the role of lycopene in the prevention of CHD. In this study, subjects recruited from 10 different European countries with acute myocardial infarctions were compared with controls. Adipose tissue samples were taken by needle aspiration biopsy procedures shortly after the infarction and used to measure  $\alpha$ - and  $\beta$ -carotenes, lycopene, and  $\alpha$ -tocopherol levels. Only lycopene, and not the other antioxidants, was found to have a significant inverse relationship with the risk of myocardial infarction (40,41). In addition to the epidemiological studies, a few dietary intervention studies have also been reported in the literature. In one study, upon consuming a lycopene-free diet by healthy human subjects for a period of two weeks, serum lycopene levels were reduced by 50% (42). An increase of 25% in serum lipid oxidation was also observed in this study. In one small study, six healthy human subjects consumed 60 mg/day of lycopene for a period of 3 months. A significant 14% drop in their plasma LDL cholesterol level was observed (33) at the end of the treatment period suggesting a hypocholesterolemic property of lycopene. However, other studies, using different levels of lycopene intake for shorter periods of time did not report reductions in serum total or LDL cholesterol levels upon ingesting lycopene (6). A significant reduction in LDL oxidation was observed in another randomized, cross-over dietary intervention study (43) when 19 healthy human subjects consumed 20-150 mg lycopene daily from tomato juice, tomato sauce and a nutritional supplement for a period of one week.

In summary, although there is convincing epidemiological and *in vitro* evidence in support of the role of lycopene in the prevention of CHD, only a few clinical trials have so far been undertaken. More dietary intervention studies are needed to fully understand the mechanisms of action of lycopene in CHD

## LYCOPENE AND BONE HEALTH

The potent antioxidant properties of lycopene, the strong evidence for the role of oxidative stress in bone health, and the limited reported studies on the effects of lycopene in bone cells in culture may have important implications for the beneficial role of lycopene in bone health.

Bone is a dynamic organ that undergoes continuous remodeling, the tightly regulated coupling between the resorption of old bone by osteoclasts and the formation of new bone by osteoblasts that is fundamental to normal bone physiology. Disturbances in bone remodeling lead to bone diseases (44-47). Oxidative stress, shown to control the function of both osteoclasts and osteoblasts, may contribute to the pathogenesis of skeletal system including the most prevalent metabolic disease, osteoporosis.

Very little work has been reported on the role of oxidative stress in osteoblasts. However, previous reports demonstrated that ROS is involved in osteoblast function (48-50). The reports on the

effect of lycopene in osteoblasts came from two laboratories (51,52). Kim et al (52) showed that lycopene stimulated the proliferation of the osteoblast-like SaOS-2 cells. Lycopene also stimulated alkaline phosphatase activity in the more mature cells grown in the presence of dexamethasone (SaOS+Dex cells), but inhibited or had no effect in younger cells grown in the absence of dexamethasone (SaOS-Dex cells), depending on the time of addition. This findings are the first report on the effect of lycopene on human osteoblasts. Kim et al (52) suggested that there are three possible mechanisms by which lycopene could have exerted the effects in osteoblasts: it could act as a potent antioxidant, inhibiting oxidative damage caused by the highly reactive oxygen species (ROS) produced intracellularly in SaOS-2 cells or it could involve cell cycling genes (17) or gap junction communication (53) as were demonstrated in other cell systems. On the other hand, Park et al (51) reported that lycopene had an inhibitory effect on the cell proliferation of MC3T3 cells that are osteoblastic cells of lower species. Both studies however reported a stimulation of alkaline phosphatase activity. The difference in the effect of lycopene on cell proliferation between the two studies could be due to species differences, experimental conditions or the stage of differentiation at which lycopene was added. More studies are required to clarify the role of lycopene in osteoblasts

Although the mechanisms involved in the differentiation of osteoclasts and its ability to resorb bone are poorly understood, one theory suggests that ROS are involved in these processes. A number of studies revealed that ROS increase bone resorption (54-59). Others suggested that ROS may be involved in the regulation of osteoclast formation (60) and osteoclast motility (61).

The effect of lycopene on osteoclasts has been reported by Ishimi et al (62) and Rao et al (63). Rao et al (63) cultured cells from bone marrow prepared from rat femur into 16-well calcium phosphate-coated Osteologic™ slides and added varying concentrations of lycopene in the absence or presence of the resorbing agent parathyroid hormone-1-34 [PTH-(1-34)] at the start of culture and at each medium change every 48 hours. Their results showed that lycopene inhibited the TRAP+ multinucleated cell formation in both vehicle- and PTH-treated cultures. The cells that had purple-colored formazan staining, a result of the nitroblue tetrazolium (NBT) reduction, were decreased by treatment with  $10^{-5}$  M lycopene, indicating that lycopene inhibited the formation of ROS-secreting osteoclasts. Based on these observations, it was concluded that lycopene inhibited basal and PTH-stimulated osteoclastic mineral resorption and formation of TRAP+ multinucleated osteoclasts, as well as the ROS produced by osteoclasts. These findings are novel and may be important in the role of lycopene in the prevention of osteoporosis. Ishimi et al (62) also reported the effects of lycopene on murine osteoclasts which formed when co-cultured with calvarial osteoblasts. Their results showed that lycopene inhibited only the PTH-induced, but not the basal TRAP+ multinucleated osteoclasts formation. Furthermore, they could not show any effect of lycopene on bone resorption nor did they study its effect on ROS production

There is evidence to suggest that ROS-induced oxidative stress is associated with the pathogenesis of osteoporosis. Epidemiological studies presented evidence suggesting that certain antioxidants including vitamin C, E and beta-carotene may reduce the risk of osteoporosis (64-67) and counteract the adverse effects on bone of the oxidative stress produced during strenuous exercise (66) and in heavy smokers (64). In a recent study, Maggio et al (68) demonstrated that women with osteoporosis had markedly decreased plasma antioxidants. A biochemical link between reduced bone density and increased oxidative stress biomarker 8-iso-prostaglandin F alpha (8-iso-PGF $\alpha$ ) has been reported (69,70). Positive correlation was found between the severity of osteoporosis and the level of

oxidative stress marker lactic acid in 2 men with mitochondrial deletion (mtDNA) (71). Another study of severe osteoporotic syndrome in relatively young males showed evidence linking osteoporosis to an increase in oxidative stress (72). In spite of these reports, the cellular and molecular mechanisms involved in the role of oxidative stress in osteoporosis remain poorly defined. To our knowledge, the role of lycopene in osteoporosis has not yet been reported. A study is being carried out in our laboratory to study the role of lycopene in postmenopausal women who are at risk of osteoporosis. Postmenopause is associated with a global increase in bone turnover markers (73,74). High bone turnover markers have been shown to predict the risk of bone loss and osteoporosis in postmenopausal women (75). Our current clinical study is designed to test whether oxidative stress, antioxidant status and antioxidant enzyme polymorphism correlate with high bone turnover markers in postmenopausal women who are at risk of osteoporosis. An intervention study is also in progress in which postmenopausal volunteers will be given regular tomato juice, tomato juice rich in lycopene, lycopene capsule or placebo capsule after a wash-out period without lycopene consumption. It is expected that there will be a decrease in bone turnover and oxidative stress markers and an increase in antioxidant status in postmenopausal women taking tomato juice or lycopene capsules. If this expectation is fulfilled, then lycopene may provide a dietary alternative to drug therapy for women who are risk of osteoporosis.

In summary, although there is epidemiological evidence in support of the beneficial effects of tomatoes and tomato products, the predominant source of lycopene, in the prevention of osteoporosis in the Mediterranean population, the direct role of lycopene has not yet been explored. The effect of lycopene on osteoblasts (51,52) and osteoclasts (62,63) that have been reported may provide evidence that lycopene may indeed be important for the prevention of osteoporosis.

## LYCOPENE AND OTHER HUMAN DISEASES

The main emphasis on the role of lycopene in human health thus far has been in the areas of cancer and CHD. Recently, its role in the prevention of osteoporosis is also being investigated. A study was recently reported that investigated the effect of lycopene-rich tomato extract on blood pressure in hypertensive patients (76). In this study, 30 grade one-hypertensive patients between the ages of 40-65 years consumed a placebo capsule containing no lycopene for 4 weeks followed by 15 mg lycopene per day for 8 weeks. Following a base line assessment, subjects were followed every two weeks for clinical, dietary and blood pressure measurements. A drop of 10 mm Hg in the systolic blood pressure was observed at the end of the treatment period compared to the base line measurement. No differences were observed in the diastolic blood pressure. Since the patients were only marginally hypertensive, the observed reduction in the systolic blood pressure is considered significant. Lycopene may offer an alternative dietary management opportunity for hypertension to the currently used pharmaceutical products.

Another disorder that is associated with human health is male infertility. It is estimated that 7-10% of adult men in their reproductive years are infertile (77-79). One of the important contributory factors for male infertility is oxidative stress. Significant levels of ROS are detectable in the semen of up to 25% infertile men (80,81). Human spermatozoa are particularly vulnerable to oxidative damage due to the abundance of unsaturated fatty acids in the sperm plasma membrane. Rapid depletion of ATP in the sperm and oxidative DNA damage in the presence of ROS are other factors that contribute to the loss of functionality of the sperm (82-84). A few studies have suggested the beneficial role of antioxidant therapy in the treatment of male-factor infertility (83). Very few studies have so far been

reported for the use of lycopene in the management of male-factor infertility. Lower semen lycopene levels in men with antibody-mediated infertility compared to fertile controls were reported in one study (85). In a more recent study (86), 50 infertile men consumed 8 mg lycopene daily in the form of lycopene-rich tomato extract capsules. Sperm samples were analyzed at regular time periods. In this study, lycopene treatment resulted in significant increases in serum lycopene levels in all subjects. Lycopene treatment also increased sperm concentration, sperm motility and morphology, and the sperm functional concentration. Follow up studies showed a successful pregnancy rate of 36% in their partners. Other similar studies are now in progress.

Neurodegenerative diseases are a group of disorders of the central and lateral nervous system that cause much suffering and deaths around the world. Oxidative damage of the neurons is now recognized as a causative factor in the etiology of these disorders. Similar to the human sperm, brain and the nerves system are also vulnerable to the free radical damage since the membrane lipids in brain contain high levels of polyunsaturated fatty acid side chains. For the relatively small weight of the brain, it consumes large quantities of oxygen, contributing further to the formation of ROS (87,88). Other factors which also play an important part include the presence of iron and other transition metals responsible for the production of ROS (89) and low levels of antioxidant enzymes in the brain (90). Antioxidants, as protective agents against oxidative damage, have therefore been studied in relation to neurodegenerative diseases. Parkinson's disease and vascular dementia patients were shown to have lower levels of blood lycopene. Similarly, lower levels of serum lycopene and  $\alpha$ -tocopherol were observed to be related to a high risk of microangiopathy in the Austrian Stroke prevention study (91). A modest protective association was suggested for lycopene in the case of amyotrophic lateral sclerosis (92). In an elderly population, high blood levels of lycopene were positively related to their functional capacity, measured as the ability to perform self-care tasks (93). In view of the importance of the neurodegenerative diseases, further studies will undoubtedly be carried out in the future to evaluate the effectiveness of lycopene in the management of these diseases

## RECOMMENDED INTAKE LEVELS OF LYCOPENE

Estimating the daily intake of lycopene has been difficult due to the variability of reported values in the food sources. Values varying from 3.70 to 16.15 mg have been reported for the United States of America (35). Reported values for Finland, United Kingdom and Germany were 0.7, 1.1 and 1.3 mg respectively (35). A survey in Canada showed daily intake of lycopene to be 25.2 mg (94). On an average, the daily intake of lycopene is estimated to be 3.7 mg. Based on previously reported studies a daily intake of 25-30 mg lycopene was initially suggested (35). However, based on the results of a recent study where absorption of lycopene from tomato ketchup and supplement at the intake levels of 5, 10 and 20 mg daily for one week were evaluated, the suggested daily intake of lycopene was modified to 5-10 mg (95,96). This level of intake can easily be achieved by ingesting several dietary sources of lycopene.

## SUMMARY

A brief review of the literature relating to lycopene and its role in human health is presented in this article. ROS-induced oxidative stress is suggested as being basic to several human diseases. Antioxidants, having the ability to mitigate the damaging effects of ROS, provide an effective means of preventing chronic diseases. A naturally occurring and a potent antioxidant of recent

interest is lycopene present in tomatoes and tomato products. The major focus of studies relating to the role of lycopene in human health to date has been in the area of prostate cancer. However, more recently, several studies are being reported in the literature on the effect of lycopene in the prevention of other cancers and CHD. Few studies have also evaluated its role in hypertension, male infertility and neurodegenerative diseases. The role of oxidative stress and antioxidants in osteoporosis has been reported, but the role of lycopene is yet to be established. With its broad spectrum of beneficial applications to human health, lycopene is being considered as a functional ingredient having nutraceutical properties. The emerging area of 'Complimentary Health Care' offers many opportunities for compounds such as lycopene to be of great interest to the food and related industries as well as to the public health organizations.

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