The effect of Lycopene as Immunotherapy and Immuno-modulatory in Lung Cancer, Tumor and Autoimmunity Treatment and Prevention

Dr. ZelalemKirosBitsue, EmnetAschalewFekede, Dr. ZelalemKirosBitsue,
United States of Africa Health Organization “AHO”,
Department of Immunology, International Campus, Tehran University of Medical SciencesTeklehaimanot General Hospital

Abstract: Experimental studies demonstrated that lycopene may inhibit the growth of several cultured lung cancer cells and prevent lung tumorigenesis in animal models through various mechanisms, including a modulation of redox status, cell cycle arrest and/or apoptosis induction, a regulation of growth factor signaling, changes in cell growth-related enzymes, an enhancement of gap junction communication and a prevention of smoke-induced inflammation. In addition, lycopene also inhibited cell invasion, angiogenesis, and metastasis.

The in vitro and in vivo experiments showing that lycopene not only enhances the antioxidant response of prostate cells, but that it is even able to inhibit proliferation, induce apoptosis and decrease the metastatic capacity of prostate cancer cells.

Evidence is accumulating to suggest other mechanisms such as modulation of intercellular gap junction communication, hormonal and immune system and metabolic pathways may also be involved.

In this article, I discuss the interaction of Lycopene with other Antioxidants, Mechanisms action of lycopene, Antitumor Effects of Lycopene in Lung Cancer Cells, Anti-carcinogenic effects of lycopene Anti-atherogenic effects of lycopene, In Vivo Models and Clinical Studies.

Key Word: Lycopene, Immunotherapy and Immunomodulatory, Animal model, Cancer, Tumor and autoimmunity

The Table of Content

1. Introduction

Cancer and cardiovascular disease (CVD) are two of the leading causes of death in Globe. Many epidemiological studies have concluded that a diet rich in fruits and vegetables reduces the incidence of heart disease and cancer in humans (1),(2),(3). Lycopene belongs to a group of naturally-occurring pigments known as carotenoids. Carotenoids are colored compounds found in the photosynthetic pigments of fruits and vegetables which provide them their bright colors and benefit human health by playing an important role in cell function.Lycopene has unique structural and chemical features that may contribute to specific biological properties. Unlike many other natural compounds; lycopene is generally stable to processing when present in the plant tissue matrix (4).

It is red, lipophilic and naturally occurring in many fruits and vegetables, with tomatoes and tomato-based products containing the highest concentrations of bioavailable lycopene. Several epidemiological studies have linked increased lycopene consumption with decreased prostate cancer risk (3),(5).Recently, lycopene has also been studied in relation to its potential health effects. The antioxidant properties of lycopene are thought to be primarily responsible for its beneficial properties.

There are relatively few reports on lung cancer chemopreventive effects of lycopene or tomato carotenoids in animal models. A good number, but not all, of these studies indicates a protective effect on lung tumorigenesis(6),(7),(8).These include the following: the optimal dose and form of lycopene, interactions among lycopene and other carotenoids and fat soluble vitamins, the role of dietary fat in regulating lycopene uptake and disposition, organ and tissue specificity, and the problem of extrapolation from animal models to human populations (6),(9),(10),(11).

Considerable evidence from several epidemiological studies suggests that lycopene has anti-carcinogenic and antiatherogenic potential, the effects of which have been attributed primarily to its antioxidant properties (lycopene quenches singlet oxygen almost twice as well as b-carotene does). These epidemiological leads have stimulated a number of animal model and cell culture studies designed to test this hypothesis and to establish its beneficial effects (5),(12),(13).

2. Interaction of Lycopen with other Antioxidants

In lipid bilayer of cellular membrane, lycopene is expected to be a poor antioxidant due to its lesser interaction with aqueous phase radicals. However, the role of lycopene as a lipid phase

antioxidant should not be neglected. The combinations of lycopene and other antioxidants such as vitamin C, vitamin E and β-carotene has exhibited higher scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical than their individual antioxidant activity (14). Besides, lycopene combined with other antioxidants also gave a better inhibiting effect towards dienehydroperoxides produced from linoleic methyl ester with 2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN) induced oxidation.(15). Lycopene was also reported to help in repairing the vitamin E radical (reaction 10) and the products from this reaction radical cation will be repaired by vitamin C (reactions 11 and 12).(16).

(10) Lycopene + TOH+˙ → TOH + Lycopene+˙

(11) Lycopene+˙ + ASCH2 → Lycopene + ASCH˙ + H+

(12) Lycopene+˙ +ASCH- → Lycopene + ASCH˙- + H+

Previously, lycopene was reported to react effectively with vitamin E radical in the lipophilic compartment (17). Inversely, their reaction with the hydrophilic vitamin C was expected to be less effective (18). In one study, the author had suggested a model for the synergistic interactions among the antioxidants located in the hydrophilic and lipophilic compartments of plasma. Besides, there might be lycopene-carotenoid interaction in biological system (reaction 13). A study done using multilamellar liposomes showed that lycopene and lutein was the best combination toward AMVN-induced oxidation (19). Lycopene is the strongest reducing agent and able to reduce the radical cations of lutein and zeaxanthin, but not β-carotene (20),(21).

(13) Carotenoid+˙+Lycopene → Carotenoid + Lycopene+˙ Different interpretations of reactions between lycopene with vitamin E and vitamin C is also reported (22),(23),(24). Lycopene is suggested to protect tocopherol through the electron transfer to form α-tocopheroxyl radical (α-TO˙) (reaction 14) (25).

(14) α-TO˙ + Lycopene → α-TOH + Lycopene+˙ On the other hand, some researchers suggested that α- tocopherol (α-TOH) could reduce lycopene+˙to regenerate the intact lycopene (reaction 15).(26).

(15) α -TOH + Lycopene+˙ → α-TO˙ + Lycopene However, a different reaction of lycopene radical cation (lycopene+˙) and α-tocopherol (α-TOH) or δ-tocopheroxyl radical (δ-TO˙) was also reported116 as the following reactions (reactions 16 and 17).

(16) α-TOH + Lycopene+˙ → α-TO˙ + Lycopene
(17) δ-TO˙ + Lycopene → δ-TOH + Lycopene+˙
In non-polar solvents, carotenoids will probably react with α-tocopherol radical cation (α-TOH+˙) rather than
with α-tocopherol anion (α-TOH-) as given in the reaction 18.(26).

(18) α -TOH+˙ + Lycopene → α-TOH- + Lycopene+˙
However, the reaction between lycopene and ascorbic acid increase the decay rate of Lycopene+˙due to the following reaction (reaction 19).(26),(22),(27).

(19) Lycopene+˙+ AscH- → Lycopene + Asc-˙+ H+ Lycopene in combination with other antioxidants such as vitamins E and C, polyphenols and other carotenoids have wide potential for human health.(28). Recent formulations of antioxidant mixtures in the development of nutritional products have been in favour for their health benefits.(29).

3. Mechanisms action of lycopene

A cellular and molecular study have shown lycopene to be one of the most potent antioxidants and has been suggested to prevent atherogenesis by protecting critical bimolecules such as DNA, proteins, lipids and low density lipoproteins (30). Lycopene, because of its high number of conjugated double bonds, exhibits higher singlet oxygen quenching ability compared to β-carotene or α-tocopherol(31). Cis lycopene has been shown to predominate in both benign and malignant prostate tissues, suggesting a possible beneficial effect of high cis-isomer concentrations, and also the involvement of tissue isomerases in vivo isomerization from all trans to cis form (32),(33), have shown that 9- cis- β-carotene is a better antioxidant than its all-trans counterpart, no such mechanistic data have been reported in case of individual lycopene isomers (34), reported a significant increase in 5-cis lycopene concentrations following a 1- week lycopene-restricted diet, and a subsequent reduction in 5-cis, and a concomitant increase in cis-β, cis-D and cis-E lycopene isomers during the 15-day dietary intervention with tomato products in healthy individuals. Although this study reported a decrease in LDL oxidizability due to the intervention withtomato lycopene, the individual antioxidant role of lycopene isomers and their inter conversions remain unclear. At a physiological concentration of 0.3 µmol/1, lycopene has been shown to inhibit growth of non-neoplastic human prostate epithelial cells in vitro, through cell cycle arrest which may be of significant implications in preventing benign prostate hyperplasia, a risk factor for prostate cancer (35). Lycopene has also been shown to significantly reduce LNCaP human prostate cancer cell survival in a dose-dependent manner, and this anti-neoplastic action may be explained by increased DNA damage at high lycopene concentrations (> 5µm), whereas lower levels oflycopene reduced malondialdehyde formation, with no effects on DNA (36). Physiologically attainable concentrations of lycopene have been shown to induce mitochondrial apoptosis in LNCaP human prostate cancer cells, although no effects were observed on cellular proliferation or necrosis (37). Lycopene has also been shown to interfere in lipid metabolism, lipid oxidation and corresponding development of atherosclerosis. Lycopene treatment has been shown to cause a 37% suppression of cellular cholesterol synthesis in J-774A.1 macrophage cell line, and augment the activity of macrophage LDL receptors (38). Oxidized LDLs are highly atherogenic as they stimulate cholesterol accumulation and foam cell formation, initiating the fatty streaks of atherosclerosis (39). LDL susceptibility to oxidative modifications is decrease by an acyl analog of platelet-activating (PAF), acyl-PAF, which experts its beneficial role during the initiation and progression of atherosclerosis. Purified lycopene in association with α- tocopherol or tomato lipophillic extracts has been shown to enhance acyl-PAF biosynthesis in endothelial cells during oxidative stress,further reported comparative data in which tomato oleoresin exhibited superior capacity to inhibit in vitro LDL oxidation in comparison with pure lycopene by up to fivefold. A combination of purified lycopene (5µmol/I) with α-toopherol in the concentration range of 1-10µmol/I resulted in a significant greater inhibition of in vitro LDL oxidation, than theexpected additive individual inhibitions. In this study, purified lycopene was also shown toact synergistically with other natural antioxidants like the flavonoid glabridin, the phenolicsrosmarinic acid and carnosic acid, and garlic acid in inhibiting LDL oxidation in vitro.

These observations suggested a superior antiatherogeneic characteristic of tomato oleoresin over pure lycopene. The combination of lycopene with other natural antioxidants, as in tomatoes, may be more potent in inhibiting lipid peroxidation, than lycopene per se. The antiatherogenic effects of lycopene are generally believed to be due to its antioxidant properties. Dietary lycopene increases blood and tissue lycopene levels and acting as an antioxidant, lycopene traps reactive oxygen species and reduce the oxidative damage tolipids (lipoproteins and membrane lipids), proteins including important enzymes, and DNA, therapy lowering oxidative stress. This reduced oxidative stress then leads to a reduced risk for chronic diseases associated with oxidative stress such as cardiovascular disease (40). Alternatively, some non-oxidative mechanisms may be responsible for the beneficial effects of lycopene. The increased lycopene status in the body may regulate gene functions, improve intercellular communication, modulate hormone and immune response, or regulate metabolism, thus lowering the risk for chronic disease (41). A possible mechanism speculated for the protective role of lycopene in heart disease is via the inhibition of cellular HMGCoAreducate, the rate-limiting enzyme in cholesterol synthesis (38).

4. Antitumoral Effects of Lycopene in Lung Cancer Cells

Reactive oxygen species (ROS) and the related oxidative damage have been implicated in the pathogenesis of various human chronic diseases(42),(43),(44). Lycopene is one of the most potent antioxidants(45), and has been suggested to prevent carcinogenesis by protecting critical biomolecules including lipids, proteins and DNA(45),(30). Several studies have indicated that lycopene is an effective antioxidant and free radical scavenger. Lycopene, because of its high number of conjugated double bonds, exhibits higher singlet oxygen quenching ability compared to β□carotene or α□tocopherol(31). In cell culture, lycopene was shown to inhibit nitration of proteins and DNA strand breakage caused by peroxynitrite treatment of Chinese hamster lung fibroblasts(46). Oxidative DNA damage caused by the redox-cycling of catechol-estrogens in plasmid DNA and Chinese hamster lung fibroblasts was also reduced by lycopene (47). In Hep3B cells treated with H2O2, lycopene was found to reduce DNA damage in a dose-dependent manner as indicated by the comet assay (48). A number of studies showed that lycopene inhibited the growth of human cancer cells grown in cultures. The growth-inhibitory effects of lycopene were observed not only in lung cancer cells, but also in other cell types, including prostate, breast, hepatoma, stomach, colon and oral cancer cells (49),(48),(50),(51),(52),(53),(54),(55),(56),(57),(58),(59). In some studies, lycopene has been reported to be more effective as an anticancer agent than □ β or α□carotene (49). Most studies on cell proliferation with lycopene treatment show that the carotenoid induced a cell cycle arrest at the G1 phase and that such an effect increased with the dose of lycopene (50),(51),(52),(53),(54),(55),(56),(57),(58),(59). Very few have reported no effect on inhibition of cell proliferation, but effect on apoptosis induction from lycopene treatment (58),(59),(37),(60). Moreover, lycopene has been shown to inhibit tumor metastasis in vitro(61),(62),(63). A limitation of cell culture studies is the extremely hydrophobicity of lycopene, which is an obstacle for conducting cell culture studies. Since lycopene is insoluble in water, steps must be taken to enhance its solubility in cell culture media or buffers before in vitro studies may be

carried out. Approaches that have been implemented to deliver lycopene to cells in culture have included the use of organic solvents such as tetrahydrofuran, liposomes, emulsifiers, lipoproteins in fetal bovine serum, and water-dispersible beadlets. The addition of lycopene in such different modalities may induce considerable variations between laboratories in terms of efficacy of lycopene concentrations and incorporation into the cells.

5. Anti-carcinogenic effects of lycopene

A number of epidemiologic data on the relationship between risk of cancer and dietary intake of lycopene from tomatoes and tomato products are available.(7), in an epidemiological study found thatlycopene intake is correlated with a diminished risk for prostate cancer. This study monitored dietary habits and the incidence rate of prostate cancer in approximately 48,000 men for 4 years and assessed over 46 different fruits, vegetables and related products on the basis of their consumption frequency. Of the 46 fruits and vegetables or related products that were analyzed, only four were significantly associated with lower prostate cancer risk. Of the four, tomato sauce, tomatoes, and pizza sauce, but not strawberries, were primary sources of lycopene. They found that men who ate 10 or more servings per week of tomato products, including tomatoes, tomato sauce, and pizza sauce, were up to 34% less likely to develop prostate cancer, and those who ate 4–7 servings per week were 20% less likely to develop the cancer. Tomato sauce was the strongest dietary predictor of reduced prostate cancer risk (66%), and the major predictor of serum lycopene levels. This relationship between lycopene intake and lower prostate cancer risk was independent of other factors and stronger for advanced cases of the cancer. Intakes of other carotenoids such as b-carotene, a-carotene, lutein, and b-cryptoxanthin were not associated with reduced risk of prostate cancer; only lycopene was related to lower risk. These findings suggest that tomato-based foods may be especially beneficial in reducing prostate cancer risk.

Stronger evidence exists for an association between lycopene intake and lower prostate cancer risk from the Physicians Health Study where pre-diagnostic plasma lycopene levels among 578 cases were compared to that of 1294 controls. Men with higher plasma lycopene levels had an approximately 25% reduction in overall prostate cancer risk, and a statistically significant 44% reduction in risk of aggressive prostate cancer(64). The relationship between lycopene and the prevention of prostate cancer is also supported by studies that have examined the plasma levels of lycopene in humans. In a case-control study by (5), to investigate the serum and prostate tissue lycopene and other major carotenoid concentrations in prostate cancer patients, significantly lower serum and tissue lycopene levels (44 and 78%, respectively) were observed in the prostate cancer patients than in their agematched controls. Concentrations of serum and tissue b-carotene and other carotenoids did not differ in the two groups. Similarly,(65). found that lycopene was the only antioxidant that occurred at a significantly lower level in men who developed prostate cancer compared to status-matched controls. However, other studies found no protective effect of serum and dietary lycopene on prostate cancer risk(66),(67). and thus more research is needed to elucidate the possible protective effects of lycopene against prostate cancer. Results from a case-control study in Italy (2706 cases of cancer of the oral cavity, pharynx, esophagus, stomach, colon and rectum vs. 2879 controls) indicated that a high intake of tomatoes and tomato-based food was associated with reduced risk of digestive tract (especially stomach, colon and rectal) cancers(68). Aside from this epidemiological evidence of a

possible protective effect of lycopene against cancer, several animal and tissue/cell culture studies have demonstrated the anticarcinogenic potential of lycopene.(49), in a cell culture study investigated lycopene's antiproliferative properties in comparison with those of a- and b-carotene using endometrial, mammary and lung human cancer cells.
Their results show that lycopene inhibited the growth of the endometrial, breast and lung cancer cells, and that a- and bcarotene were far less effective growth inhibitors than lycopene. In addition to its inhibitory effect on endometrial cancer cell growth, lycopene also suppressed insulin-like growth factor-I-stimulated growth. Insulin-like growth factors are major autocrine/paracrine regulators of mammary and endometrial cancer cell growth; therefore, investigating the role of lycopene interference in this major autocrine/paracrine system may contribute to understanding of the mechanism whereby lycopene inhibits endometrial cancer and other tumors. In another study by(53), to measure the effect of lycopene on the proliferation of LNCaP human prostate cancer cells in culture, lycopene was added to cells at concentrations of 10K6, 10K5 and 10K4 M and were allowed to grow for 24, 48, 72 and 96 h. Lycopene concentrations of 10K6 and 10K5 M significantly reduced the growth of LNCaP cells after 48, 72 and 96 h of incubation by 24.4–42.8%. To further determine whether there was a dose–response effect, a lower range of lycopene concentrations (10K9–10K7 M) was used in a follow-up experiment. Lycopene significantly reduced the growth of cells incubated for 24, 48, 72 or 96 h in a dose-dependent manner.(69),studied the effect of chronic intake of lycopene on mammary tumor growth and development in SHN virgin mice (mice genetically susceptible to developing breast tumors). They found out that lycopene-fed mice had delayed onset and reduced spontaneous mammary tumor growth and development. This suppression was associated with a reduced activity of mammary gland thymidylatesynthetase, an essential enzyme required for DNA synthesis, lower levels of serum free fatty acids and prolactin, a hormone known to be involved in breast cancer development by stimulating cell division. Another study by(70), investigated the cancer chemopreventive potential of lycopene in a multi-organ carcinogenesis mice model (B6C3F1 mice) during the post-initiation stage. The incidences and multiplicities of lung adenomas plus carcinomas combined in male mice receiving 50 ppm lycopene in their drinking water were significantly decreased when compared to the group receiving 25 ppm lycopene and the controls. The results suggest that lycopene exerts a chemopreventive effect limited to male lung carcinogenesis when given in the post-initiation stage to B6C3F1 mice. No effect was observed in females. The effect of lycopene on iron-induced oxidative stress in cells (exposed to ferric nitrilotriacetate) has also been studied by(71). Cells supplemented with lycopene showed 86% reduction in thiobarbituric acid-reactive substances (TBARS) and 77% reduction in 8-oxo-7,8-dihydro-20-deoxyguanosine (8- oxodGuo) levels, indicating a reduction in lipid peroxidation and DNA oxidation, respectively. These results indicate that lycopene has a protective effect on lipid peroxidation and oxidative DNA damage in cell culture, demonstrating a protective role against tumor production associated with oxidative damage. Lycopene may also be useful as a therapeutic agent in prostate cancer. This was demonstrated by(72), in a randomized clinical trial evaluating the effects of lycopene supplementation in patients with prostate cancer. Patients received 15 mg of lycopene twice daily for 3 weeks before radical prostatectomy. After 3 weeks of supplementation, 80% of subjects in the lycopene group had smaller tumors compared with those in the control group (45% of subjects) who received no supplementation. Their results also show that plasma prostate-specific antigen levels decreased by 18% in the lycopene group, whereas it increased by 14% in the control group. These results suggest that lycopene supplementation of 30 mg daily may be sufficient to modulate clinical markers of prostate cancer and be useful in the treatment of the disease. However, larger

clinical trials are needed to further investigate the potential of lycopene as a therapeutic agent in prostate cancer.

6. Anti-atherogenic effects of lycopene

Scientific evidence indicates that oxidation of lowdensity lipoproteins (LDL), which carry cholesterol in the blood stream plays an important role in the development of atherosclerosis, the underlying disorder leading to heart attacks and ischemic strokes (73). Several studies indicate that consuming the antioxidant lycopene that is contained in tomatoes and tomato products can reduce the risk of CVD. Available evidence from the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) study suggests that the thickness of the innermost wall of blood vessels and the risk of myocardial infarction were reduced in persons with higher serum and adipose tissue concentrations of lycopene (74). This finding suggests that the serum lycopene concentration may play a role in the early stages of atherosclerosis. A thick artery wall is a sign of early atherosclerosis, and increased thickness of the intima media has been shown to predict coronary events. Similarly, the relationship between plasma lycopene concentration and intima-media thickness of the common carotid artery wall (CCA-IMT) was investigated in 520 middle-aged men and women aged 45–69 years as part of the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study(75). Low levels of plasma lycopene were associated with a 17.8% increment in CCA-IMT in men, while there was no significant difference among women. These findings also suggest that low plasma lycopene concentrations are associated with early atherosclerosis, evidenced by increased CCA-IMT in middle-aged men. Findings from the Rotterdam study (76), showed modest inverse associations between levels of serum lycopene and atherosclerosis, assessed by the presence of calcified plaques in the abdominal aorta. Study population comprised of 108 cases of aortic atherosclerosis and 109 controls aged 55 years and over. The association between serum lycopene levels and atherosclerosis was most pronounced among subjects who were current and former smokers. No association with risk of aortic calcification for the serum carotenoids a-carotene, b-carotene, lutein, and zeaxanthin was observed.

These results suggest that lycopene may play a protective role inthe development of atherosclerosis. Results from the European Study of Antioxidants, Myocardial Infarction, and Cancer of the breast (The EURAMIC study) also show that men with the highest concentrations of lycopene in their adipose tissue biopsy had a 48% reduction in risk of myocardial infarction compared with men with the lowest adipose lycopene concentrations (77). An increase in LDL oxidation is known to be associated with an increased risk of atherosclerosis and coronary heart disease (78),(23), investigated the effect of dietary supplementation of lycopene on LDL oxidation in 19 healthy human subjects. Dietary lycopene was provided using tomato juice, spaghetti sauce and tomato oleoresin for a period of 1 week each. Blood samples were collected at the end of each treatment, and TBARS and conjugated dienes (CD) were measured to estimate LDL oxidation. In addition to significantly increasing serum lycopene levels by at least twofold, lycopene supplementation significantly reduced serum lipid peroxidation and LDL oxidation. The average decrease of LDL– TBARSand LDL–CD for the tomato products treatment over placebo was 25 and 13%, respectively. These results suggest significance for lycopene in decreasing risk for coronary heart disease. Results from the ongoing Women's Health Study (WHS) showed that women with the highest intake of tomato-based foods rich in lycopene had a reduced risk for CVD compared to women with a low intake of those foods (79).

Results showed that women who consumed seven servings or more of tomatobased foods like tomato sauce and pizza each week had a nearly 30% risk reduction in total CVD compared to the group with intakes of less than one serving per week. The researchers also found out that women who ate more than 10 servings per week had an even more pronounced reduction in risk (65%) for specific CVD outcomes such as heart attack or stroke. Though not statistically significant, the strongest association of dietary lycopene with CVD protection was seen among women with a median dietary lycopene intake of 20.2 mg/day, who had a 33% reduction in risk of the disease when compared with women with the lowest dietary lycopene intake (3.3 mg/day). Lycopene has also been shown to have anhypocholesterolemic effect both in vivo and in vitro. In a small dietary supplementation study, six healthy male subjects were fed 60 mg/day lycopene for 3 months. At the end of the treatment period, a significant 14% reduction in plasma LDL cholesterol levels was observed in vivo with no effect on HDL cholesterol concentrations (38). In the same study, the authors investigated the hypocholesterolemic effect of lycopene in macrophages in vitro. Incubation of macrophage cells with lycopene in vitro resulted in a 73% decrease in cholesterol synthesis. Their results suggest that lycopene inhibits macrophage cholesterol synthesis by inhibiting cellular HMGCoAreductase, the rate limiting enzyme in cholesterol synthesis in vitro. Oxidative stress and the expression of adhesion molecules on vascular endothelial cells are regarded as important features in the pathogenesis of atherosclerosis, and certain nutrients that have antioxidant properties may protect endothelial cells by interfering with cytokineinduced endothelial cell dysfunction(80),(81);examined in vitro the effect of the five most prevalent plasma carotenoids—a-carotene, b-carotene, lutein, zeaxanthin and lycopene—on the expression of key adhesion molecules involved in the atherosclerosis process, and determined the subsequent binding of U937 monocytic cells when carotenoids were incubated with human aortic endothelial cells (HAEC). While other carotenoids were ineffective, lycopene attenuated interleukin-1b-stimulated and spontaneous HAEC adhesion to U937 monocytic cells by 20 and 25%, respectively. Thus, among all the carotenoids tested, lycopene appears to be the most effective in reducing both HAEC adhesion to monocytes and expression of adhesion molecules on the cell surface. These results suggest an important role for lycopene in attenuating atherogenesis, and are consistent with findings from studies linking lycopene with reduced risk for CVD.

7. In Vivo Models

The first *in vivo* effects of lycopene were reported by Lindberg and co-workers in 1959. The group showed that the ingestion of lycopene led to an increased survival of previously irradiated mice and to a lower incidence of radiation-induced peritoneal tumours(82),(83). Nevertheless, the first animal studies concerning the effects of lycopene on prostate cancer were not performed until the first studies had been published linking lycopene or tomato products with an antitumorigenic effect in prostate cancer patients. Though plenty of clinical studies have been performed so far, only a few preclinical *in vivo* studies have been published. In August 2011, Ilic*et al*. identified 64 clinical studies(84), whereas at the same time, only 12 animal models had been published investigating the effects of lycopene on prostatecancer(85),(86),(87),(88),(89),(90),(91),(92),(93),(94).In the early 2000s, mainly syngeneic and chemically-induced models of prostate cancer have been used. Imaida*et al*. have been the first to test the chemopreventive efficacy of lycopene using a chemically-induced rat prostate cancer model (six-week-old male F344 rats) (89). After carcinogen exposure with 3,2'-dimethyl-

4-aminobiphenol for 20 weeks, lycopene purified from tomato extracts (99.9%, LycoRed™, Beer-Sheva, Israel) was incorporated in the basal diet (Oriental MF; Oriental Yeast co. Ltd, Tokyo, Japan) at a dosage of 15–45 ppm for 40 weeks. The authors found a significantly decreased incidence of prostatic intraepithelial neoplasia and carcinoma of the ventral prostate in animals treated with lycopene. However, these results have not been reproducible in subsequent experiments (89). Guttenplan and co-workers examined the effects of dietary supplementation of a lycopene-rich tomato oleoresin on benzo[a]pyrene induced mutagenesis in six-week-old male *lac*Z mice (88). The supplement consisted mainly of a 3.7% lycopene suspension (69 mM) in medium chain triglycerides (Cognis Corporation, LaGrange, IL, USA). The suspension contained different other natural carotenoids extracted from tomatoes (0.3% phytofluene, 0.44% *Z*-carotene, 0.47% 2,6-cyclolycopene-1,5-diol and 1.2% β-carotene) and was incorporated into an AIN-76A diet at a concentration of 7 and 14 g/kg diet for eight weeks in parallel to carcinogen exposure. Carcinogen-induced tumorigenesis in prostates from the lycopene treated groups was decreased as compared to a control group without lycopene supplementation. However, the differences have not been significant (88). In 2003, Boileau*et al*. showed that supplementation with water-dispersible beadlets of synthetic lycopene (Hoffman-La Roche, Basel, Switzerland) at a concentration of 2.5 g of beadlets/kg of AIN-93G diet had no effects on the prostate cancer-specific survival of male Wistar rats. To induce carcinogenesis, they were previously treated with cyproterone, testosterone and *N*-methyl-*N*-nitrosourea(86). However, the authors found that powder derived from heat-processed tomato paste (Armour/Del Monte Foods, San Francisco, CA, USA) incorporated into the diet at the same concentration as the synthetic lycopene was able to increase survival(86). Siler and co-workers used the MatLyLu-cell line to establish prostate tumours in 8–10-week-old male Copenhagen rats (91). Supplementation of synthetic lycopene at a dosage of 200 ppm (lycopene 5% TG, DSM Nutritional Products, Basel, Switzerland) incorporated into a standard basal diet (Kliba #2019, ProvimiKliba AG, Kaiseraugst, Switzerland) for four weeks led to a significant increase of necrotic area within the tumours when compared to animals without lycopene supplementation. Hence, the authors suggested that lycopene can lead to a reduction of the prostatic tumour mass (91). The same group found that synthetic lycopene induces anti-androgen and anti-inflammatory effects, both in cancerous and in healthy prostate tissue(92). Venkateswaran*et al*. have been the first to use a transgenic mouse model to investigate the effects of lycopene on prostate cancer (93). They incorporated a mixture of antioxidants containing 800 IU vitamin E, 200 µg selenium and 50 mg lycopene into a standard diet (Purina Mills Test Diet, Richmond, Indiana, USA). After 25 weeks, they euthanized the animals and observed a four-fold reduction in the incidence of prostate cancer compared to untreated mice. Unfortunately, the authors did not provide any information about the formulations of the antioxidants used. The contribution of the different micronutrients was also not evaluated (93). In 2005 Tang *et al*.(58). analysed the effect of natural lycopene on the growth of androgen-independent human DU145 prostate cancer cells after subcutaneous injection (1 × 107 cells/100 µLMatrigel™) into the flanks of 4–6 week old male BALB/c nude mice. The lycopene-formulation used consisted of >95% pure lycopene with 6% lycopene oleoresin extracted from tomatoes. After tumour cell injection, the mice were gavaged five days per week with different dosages of lycopene (0, 10, 100 and 300 mg/kg body weight) for eight weeks. The authors showed a decrease in tumour growth by 55.6% and 75.8% in mice treated with 100 mg and 300 mg lycopene. Mice injected with DU145 cells pre-treated with 20 µmol/L lycopene did not show any tumour formation after one month(58). Canene-Adams *et al*. implanted Dunning R3327-H tumour tissue with Matrigel™ (100 mg of tumour/mL Matrigel™) into the flanks of four-week-old male Copenhagen rats (87). Synthetic lycopene beadlets

(lycopene 5% TG, DSM Nutritional Products, Basel, Switzerland) or powder derived from tomatoes (Gilroy Foods, Gilroy, CA, USA) was incorporated into an AIN 93G-based diet, and supplementation started one month prior to tumour tissue transplantation. Tumour analysis at 18 weeks after tumour transplantation showed that 23 nmol or 224 nmol of synthetic lycopene beadlets per gram of diet insignificantly reduced tumour weights, whereas tomato powder at 13 nmol per gram of diet was able to significantly reduce the weight of the tumours as compared to the control groups (87). In 2010, Konijeti*et al*.(95),used a TRAMP model to compare the effects of lycopene beadlets (lycopene 10%, DSM Nutritional Products, Parsippany, NJ, USA) and tomato paste (Campbell's Soup Company, Camden, NJ, USA) on the incidence of prostate cancer. Both supplements were incorporated into the basal AIN 93 diet with 100 mg lycopene/kg diet since the age of weaning. In contrast to previously published studies (86),(96), the authors found a significantly decreased incidence of prostate cancer in those animals treated with synthetic lycopene, but not in the animals treated with the tomato paste (95).
In 2011, Tang and co-workers (85), investigated the effect of synthetic lycopene and docetaxel on the survival and growth rate of xenograftedtumours. DU145 prostate cancer cells were injected at a concentration of 1 × 106 cells/100 µL PBS into the right flank of NCR-*nu/nu* nude mice. After the tumours reached a size of approximately 200 mm3, the mice were treated either with 15 mg/kg body weight of synthetic microencapsulated lycopene (LycoVit™ 10% CWD, BASF Corporation, Shreveport, LA, USA) via gavage or with an intraperitoneal injection of docetaxel or with a combination of both. Docetaxel plus lycopene led to a significantly higher tumour regression and increase in survival when compared to docetaxel alone. The synthetic lycopene was able to enhance the antitumor capacity of docetaxel, even when it was given at a suboptimal dosing. However, lycopene alone had no effects on tumour regression or survival (85). Yang and colleagues implanted human PC3 prostate cancer cells (1 × 107 cells/100 µL PBS) into the flanks of 6–8-week-old athymic nude mice (94). Subsequently, the mice were gavaged with 4 or 16 mg/kg body weight of lycopene (97%, Wako Pure Chemical Industries, Japan; purified from tomatoes) or 16 mg/kg β-carotene suspended in corn oil twice a day for seven weeks. The authors showed that both lycopene and β-carotene were able to significantly decrease tumour volume and weight when compared to a control group without supplementation. The lycopene effect has been shown to be dosage-dependent (94).
The results of previously published animal studies are in large parts inconsistent. This is mainly due to the heterogeneity of the lycopene formulations and animal models used., lycopene formulations vary from synthetic lycopene to purified naturally derived lycopene and non-purified tomato powder or mixtures of different substances. Several other critical issues need to be considered, such as the dosage, form and bioavailability of the active component and the timing of the treatment. Nevertheless, the preclinical data strongly suggests an antitumorigenic activity of lycopene and its different formulations, either alone or in combination with other substances.

8. Clinical Studies

The interest in lycopene and its potential protective role in prevention of chronic diseases stems largely from epidemiological observations on normal and at-risk populations. The present knowledge largely relies on the data obtained from dietary estimates or plasma values in relation to chronic diseases. Epidemiological investigations to study the role of lycopene in relation to chronic diseases has focused primarily on cancers. The Mediterranean diet, which is

rich in fruits and vegetables, including tomatoes, has been suggested to be responsible for the lower cancer incidences in that region (97). Dietary intake of tomatoes and tomato products has been found to be associated with a lower risk of a variety of cancers in a number of epidemiological studies (98). In a prospective cohort study, Colditz*et al.* (99),investigated the frequency of intake of different types of vegetables and cancer deaths in 1271 elderly persons from Massachusetts. High intake of tomatoes was linked to a 50% reduction in mortality from cancers at all sites. Carrots and other carotenoid-rich vegetables had no effect(99).

The most impressive results came from U S Health Professionals Follow-up Study evaluating the intake of various carotenoids and retinol, from a food frequency questionnaire, in relation to risk of prostate cancer(7). The estimated intake of lycopene from various tomato products, and not any othercarotenoid, was inversely related to the risk of prostate cancer. A risk reduction of almost 35% was observed for a consumption frequency of 10 or more servings of tomato products per week, and the protective effects were even stronger for more advanced or aggressive prostate cancer(7). Similarly serum and tissue levels of lycopene were inversely associated with prostate cancer risk in recent case-control and cohort studies (5),(65). It is noteworthy that no significant association with other major carotenoids including b-carotene was observed in these studies(5),(65). High intake of tomatoes was consistently associated with reduced risk of digestive tract (especially stomach, colon and rectal) cancers in a case control study from Italy, where cases were patients with histologically confirmed cancers of oral cavity, pharynx, esophagus, stomach, colon and rectum and controls were patients with unrelated conditions(68). Similarly, an inverse association between lycopene (estimated intakes or serum levels) and breast cancer risk was reported by some investigators in epidemiological investigations. However, these observations were not confirmed by other investigators (100),(101),(102). In a recent case-control study from the Breast Cancer Serum Bank in Columbia, Missouri, only serum lycopene and none of the other antioxidants showed a significant inverse relationship with breast cancer risk (103). Dietary intake of lycopene as well as serum lycopene levels showed an inverse association with the risk of cervical intraepithelial neoplasia in another case-control study (104). In a cohort study, serum lycopene levels were found to be inversely related to the risk of bladder cancer (105). It appears that cancer risk is inversely associated with lycopene status, which can be improved by dietary sources rich in this carotenoid as well as through supplements. Giovannucci(98), recently reviewed 72 epidemiological studies including ecological, case-control, dietary studies and blood specime-based investigations on tomatoes, tomato-based products, lycopene and cancer. A significant number of studies analyzed demonstrated an inverse relationship between intakes of tomatoes or plasma lycopene levels and cancer. The strongest associations were observed for cancers of the prostate, lung and stomach. However, for cancers of pancreas, colon and rectum, esophagus, oral mucosa, breast and cervix the associations were only suggestive. These results were consistent across numerous diverse populations and with the use of several different study designs. None of the studies analyzed indicated increased risk of cancer (98). Oxidation of LDL which carries cholesterol into the blood stream has been hypothesized to play an important role in the causation of atherosclerosis, the underlying disorder leading to heart attack and ischemic strokes (106),(107). Antioxidant nutrients are believed to slow the progression of atherosclerosis because of their ability to inhibit damaging oxidative processes (78),(107),(108). Several epidemiological studies have provided evidence for the protective effect of vitamin E, which has been ascribed to its antioxidant properties (108),(109),(110),(111). However many dietary intervention trials involving a-tocopherol or b-carotene have yielded inconclusive results.

Similar studies have not been performed with lycopene. A recent multicenter case-control study (EURAMIC) evaluated the relationship between adipose tissue antioxidant status (a- and b-carotene and lycopene) and acute myocardial infarction (77). Subjects were recruited from 10 European countries to maximize the variability in exposure within the study. After adjusting for a range of dietary variables, only lycopene, and not b-carotene, levels were found to be protective (77). The protective potential of lycopene was maximal among individuals with highest polyunsaturated fat stores, supporting the antioxidant theory (77). Similarly lower blood lycopene levels were also found to be associated with increased risk and mortality from coronary heart disease in a concomitant cross-sectional study comparing Lithuanian and Swedish populations showing diverging mortality rates from coronary heart disease (112). Limitations of the epidemiological studies undertaken to date include heterogeneous population, levels of serum carotenoids, duration of the study and biomarkers of the disease. Further studies should address these study variables to provide a more precise role of lycopene in disease prevention. Although there is compelling epidemiological evidence in support of the role of lycopene in cancer and heart disease prevention, it only provides suggestive evidence rather than experimental proof. To date a very limited number of human intervention trials have been performed investigating the effectiveness of lycopene intake on lowering cancer and heart disease risk. Oxidative damage to lipids, proteins and DNA has been suggested to be involved in the causation and progression of cancer and heart disease (42),(113). A 50% loss of serum lycopene with an 25% increase in lipid oxidation (TBARS) was observed in human subjects ingesting a lycopene-free diet for two weeks(114). Consumption of vegetable juices and tomato juice containing lycopene has been shown to reduce DNA breaks in healthy subjects(30). Studies involving healthy human subjects in our laboratory indicated that lycopene from traditional tomato products is absorbed readily, increases serum levels and lowers oxidative damage to lipids, lipoproteins, proteins and DNA(23),(45). The level of consumption of tomato products used in this study was one to two servings/day (126 g spaghetti sauce or 500 mL tomato juice per day); that was easily achievable and in keeping with the current dietary recommendations pertaining to healthy eating. There are suggestions that tomato extract supplementation in the form of capsules lowered the PSA levels in prostate cancer patients (115)In a small clinical trial involving six male subjects, dietary supplementation of lycopene (60 mg/d for three months) resulted in 14% reduction of plasma LDL levels and thus acted as a moderate hypocholesterolemic agent (38).

9. Conclusion

There is a great need for well-designed human intervention studies that take into consideration study designs including subject selection, specific markers of analysis, the levels of carotenoids being tested, metabolism and isomerization of lycopene and their biological significance.

It is only through such studies that our understanding of the anticancer role played by tomato lycopene will be enhanced and help us to develop complementary strategies for the prevention, treatment and management of lung cancer.

Attempts to recapitulate the antitumorigenic activity of lycopene in animal models have been in some parts highly significant and showed interesting data, suggesting the potential of lycopene to reduce tumour growth rate and to increase the survival of the animals. Recent systematic

reviews could show that lycopene is able to decrease the serum PSA-levels in patients with prostate hyperplasia or cancer, demonstrating its effect on proliferating prostate cells [81,99].

The recommended daily intake of lycopene has been set at 35 mg that can be obtained by consuming two glasses of tomato juice or through a combination of tomato products (Rao and Agarwal, 2000). These foods may have both chemopreventive as well as chemotherapeutic values. Lycopene has shown distinct antioxidant and anticarcinogenic effects at cellular levels, and definitely contributes to the health benefits of consumption of tomato products.

It is readily absorbed from different food sources, distributes to different tissues and maintains its antioxidant properties in the body. It is suggested to have anti-cell-proliferative, anticarcinogenic and antiatherogenic activities.

Recent metaanalysis of the epidemiological literature indicated that higher intake or serum levels of lycopene are related to reduced risk for several human cancers. Although antioxidant properties of lycopene are thought to be responsible primarily for its biological effects, other mechanisms are also being identified.

More mechanistic studies, as well as controlled clinical intervention trials involving healthy subjects, subjects at high risk for cancer and heart disease and patients with chronic diseases, are needed to understand fully its health promoting effects and establish clear dietary guidelines.

10. Reference

1. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of agricultural and food chemistry. 2002;50(10):3010-4.
2. Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao D-Y, Katz NB. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. Experimental Biology and Medicine. 2002;227(10):845-51.
3. Sharoni Y, Danilenko M, Levy J. Molecular mechanisms for the anticancer activity of the carotenoid lycopene. Drug development research. 2000;50(3-4):448-56.
4. Khan N, Adhami VM, Mukhtar H. Apoptosis by dietary agents for prevention and treatment of cancer. Biochemical pharmacology. 2008;76(11):1333-9.
5. Rao AV, Fleshner N, Agarwal S. Serum and tissue lycopene and biomarkers of oxidation in prostate cancer patients: a case-control study. Nutrition and cancer. 1999;33(2):159-64.
6. Baysal T, Ersus S, Starmans D. Supercritical CO2 extraction of β-carotene and lycopene from tomato paste waste. Journal of Agricultural and Food Chemistry. 2000;48(11):5507-11.

7. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retino in relation to risk of prostate cancer. Journal of the national cancer institute. 1995;87(23):1767-76.
8. Stahl W, Sies H. Lycopene: a biologically important carotenoid for humans? Archives of Biochemistry and biophysics. 1996;336(1):1-9.
9. Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman JW. Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. The Journal of nutrition. 1999;129(6):1176-81.
10. Riso P, Pinder A, Santangelo A, Porrini M. Does tomato consumption effectively increase the resistance of lymphocyte DNA to oxidative damage? The American journal of clinical nutrition. 1999;69(4):712-8.
11. Shi J, Maguer ML. Lycopene in tomatoes: chemical and physical properties affected by food processing. Critical reviews in food science and nutrition. 2000;40(1):1-42.
12. Weisburger JH. Lycopene and tomato products in health promotion. Experimental Biology and Medicine. 2002;227(10):924-7.
13. Wu K, Schwartz SJ, Platz EA, Clinton SK, Erdman JW, Ferruzzi MG, et al. Variations in plasma lycopene and specific isomers over time in a cohort of US men. The Journal of nutrition. 2003;133(6):1930-6.
14. Liu D, Shi J, Ibarra AC, Kakuda Y, Xue SJ. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β-carotene mixtures on the DPPH free radical. LWT-Food Science and Technology. 2008;41(7):1344-9.
15. Shi J, Qu Q, Kakuda Y, Xue SJ, Jiang Y, Koide S, et al. Investigation of the antioxidant and synergistic activity of lycopene and other natural antioxidants using LAME and AMVN model systems. Journal of food composition and analysis. 2007;20(7):603-8.
16. Truscott T. β-Carotene and disease: a suggested pro-oxidant and anti-oxidant mechanism and speculations concerning its role in cigarette smoking. Journal of Photochemistry and Photobiology B: Biology. 1996;35(3):233-5.
17. Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. Archives of Biochemistry and biophysics. 2001;385(1):20-7.
18. Yeum K-J, Russell RM, Krinsky NI, Aldini G. Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma. Archives of Biochemistry and Biophysics. 2004;430(1):97-103.
19. Stahl W, Junghans A, de Boer B, Driomina ES, Briviba K, Sies H. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. FEBS letters. 1998;427(2):305-8.
20. Edge R, Land EJ, McGarvey D, Mulroy L, Truscott TG. Relative one-electron reduction potentials of carotenoid radical cations and the interactions of carotenoids with the vitamin E radical cation. Journal of the American Chemical Society. 1998;120(17):4087-90.
21. Scheer H. The pigments. Light-harvesting antennas in photosynthesis: Springer; 2003. p. 29-81.
22. Krinsky NI, Yeum K-J. Carotenoid–radical interactions. Biochemical and biophysical research communications. 2003;305(3):754-60.
23. Agarwal S, Rao AV. Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. Lipids. 1998;33(10):981-4.
24. Kiokias S, Gordon MH. Antioxidant properties of carotenoids in vitro and in vivo. Food Reviews International. 2004;20(2):99-121.

25. Böhm F, Edge R, Land EJ, McGarvey DJ, Truscott TG. Carotenoids enhance vitamin E antioxidant efficiency. Journal of the american chemical society. 1997;119(3):621-2.
26. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Molecular aspects of medicine. 2005;26(6):459-516.
27. Walter P, Hornig D, Moser U. Functions of vitamins beyond recommended dietary allowances: Karger Medical and Scientific Publishers; 2001.
28. Shixian Q, Dai Y, Kakuda Y, Shi J, Mittal G, Yeung D, et al. Synergistic anti-oxidative effects of lycopene with other bioactive compounds. Food Reviews International. 2005;21(3):295-311.
29. Castro I, Barros SM, Marquez UL, Motizuki M, Sawada TH. Optimization of the antioxidant capacity of a mixture of carotenoids and α-tocopherol in the development of a nutritional supplement. Food research international. 2005;38(8):861-6.
30. Pool-Zobel B, Bub A, Müller H, Wollowski I, Rechkemmer G. Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. Carcinogenesis. 1997;18(9):1847-50.
31. Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Archives of biochemistry and biophysics. 1989;274(2):532-8.
32. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, et al. cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiology Biomarkers & Prevention. 1996;5(10):823-33.
33. Levin G, Yeshurun M, Mokady S. In vivo antiperoxidative effect of 9-cis β-carotene compared with that of the all-trans isomer. 1997.
34. Hadley CW, Clinton SK, Schwartz SJ. The consumption of processed tomato products enhances plasma lycopene concentrations in association with a reduced lipoprotein sensitivity to oxidative damage. The Journal of nutrition. 2003;133(3):727-32.
35. Obermüller-Jevic UC, Olano-Martin E, Corbacho AM, Eiserich JP, Van der Vliet A, Valacchi G, et al. Lycopene inhibits the growth of normal human prostate epithelial cells in vitro. The Journal of nutrition. 2003;133(11):3356-60.
36. Hwang ES, Bowen PE. Effects of lycopene and tomato paste extracts on DNA and lipid oxidation in LNCaP human prostate cancer cells. Biofactors. 2005;23(2):97-105.
37. Hantz HL, Young LF, Martin KR. Physiologically attainable concentrations of lycopene induce mitochondrial apoptosis in LNCaP human prostate cancer cells. Experimental Biology and Medicine. 2005;230(3):171-9.
38. Fuhrman B, Elis A, Aviram M. Hypocholesterolemic effect of lycopene and β-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages. Biochemical and biophysical research communications. 1997;233(3):658-62.
39. Libby P. Inflammation and cardiovascular disease mechanisms. The American journal of clinical nutrition. 2006;83(2):456S-60S.
40. Omoni AO, Aluko RE. The anti-carcinogenic and anti-atherogenic effects of lycopene: a review. Trends in Food Science & Technology. 2005;16(8):344-50.
41. Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. Canadian Medical Association Journal. 2000;163(6):739-44.
42. Pincemail J. Free radicals and antioxidants in human diseases. Analysis of free radicals in biological systems: Springer; 1995. p. 83-98.
43. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proceedings of the National Academy of Sciences. 1995;92(12):5258-65.

44. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. Antioxidant activities of carotenes and xanthophylls. FEBS letters. 1996;384(3):240-2.
45. Rao A, Agarwal S. Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. 1998.
46. Muzandu K, Ishizuka M, Sakamoto KQ, Shaban Z, El Bohi K, Kazusaka A, et al. Effect of lycopene and β-carotene on peroxynitrite-mediated cellular modifications. Toxicology and applied pharmacology. 2006;215(3):330-40.
47. Muzandu K, Shaban Z, Ishizuka M, Kazusaka A, Fujita S. Lycopene and beta-carotene ameliorate catechol estrogen-mediated DNA damage. Japanese Journal of Veterinary Research. 2005;52(4):173-84.
48. Park YO, Hwang ES, Moon TW. The effect of lycopene on cell growth and oxidative DNA damage of Hep3B human hepatoma cells. Biofactors. 2005;23(3):129-39.
49. Levy J, Bosin E, Feldman B, Giat Y, Miinster A, Danilenko M, et al. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. 1995.
50. Chalabi N, Le Corre L, Maurizis JC, Bignon Y, Bernard-Gallon D. The effects of lycopene on the proliferation of human breast cells and BRCA1 and BRCA2 gene expression. European Journal of Cancer. 2004;40(11):1768-75.
51. Fornelli F, Leone A, Verdesca I, Minervini F, Zacheo G. The influence of lycopene on the proliferation of human breast cell line (MCF-7). Toxicology in vitro. 2007;21(2):217-23.
52. Hwang E-S, Bowen PE. Cell cycle arrest and induction of apoptosis by lycopene in LNCaP human prostate cancer cells. Journal of medicinal food. 2004;7(3):284-9.
53. Kim L, Rao AV, Rao LG. Effect of lycopene on prostate LNCaP cancer cells in culture. Journal of medicinal food. 2002;5(4):181-7.
54. Livny O, Kaplan I, Reifen R, Polak-Charcon S, Madar Z, Schwartz B. Lycopene inhibits proliferation and enhances gap-junction communication of KB-1 human oral tumor cells. The Journal of nutrition. 2002;132(12):3754-9.
55. Pastori M, Pfander H, Boscoboinik D, Azzi A. Lycopene in association with α-tocopherol inhibits at physiological concentrations proliferation of prostate carcinoma cells. Biochemical and biophysical research communications. 1998;250(3):582-5.
56. Reddy MK, Alexander-Lindo RL, Nair MG. Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors. Journal of Agricultural and Food Chemistry. 2005;53(23):9268-73.
57. Salman H, Bergman M, Djaldetti M, Bessler H. Lycopene affects proliferation and apoptosis of four malignant cell lines. Biomedicine & pharmacotherapy. 2007;61(6):366-9.
58. Tang L, Jin T, Zeng X, Wang J-S. Lycopene inhibits the growth of human androgen-independent prostate cancer cells in vitro and in BALB/c nude mice. The Journal of nutrition. 2005;135(2):287-90.
59. Palozza P, Colangelo M, Simone R, Catalano A, Boninsegna A, Lanza P, et al. Lycopene induces cell growth inhibition by altering mevalonate pathway and Ras signaling in cancer cell lines. Carcinogenesis. 2010;31(10):1813-21.
60. Burgess LC, Rice E, Fischer T, Seekins JR, Burgess TP, Sticka SJ, et al. Lycopene has limited effect on cell proliferation in only two of seven human cell lines (both cancerous and noncancerous) in an in vitro system with doses across the physiological range. Toxicology in vitro. 2008;22(5):1297-300.
61. Huang C-S, Shih M-K, Chuang C-H, Hu M-L. Lycopene inhibits cell migration and invasion and upregulates Nm23-H1 in a highly invasive hepatocarcinoma, SK-Hep-1 cells. The Journal of nutrition. 2005;135(9):2119-23.

62. Hwang E-S, Lee HJ. Inhibitory effects of lycopene on the adhesion, invasion, and migration of SK-Hep1 human hepatoma cells. Experimental Biology and Medicine. 2006;231(3):322-7.
63. Huang C-S, Fan Y-E, Lin C-Y, Hu M-L. Lycopene inhibits matrix metalloproteinase-9 expression and down-regulates the binding activity of nuclear factor-kappa B and stimulatory protein-1. The Journal of nutritional biochemistry. 2007;18(7):449-56.
64. Chan JM, Stampfer MJ, Giovannucci EL, editors. What causes prostate cancer? A brief summary of the epidemiology. Seminars in cancer biology; 1998: Elsevier.
65. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, et al. Lower prostate cancer risk in men with elevated plasma lycopene levels results of a prospective analysis. Cancer research. 1999;59(6):1225-30.
66. Key T, Silcocks P, Davey G, Appleby P, Bishop D. A case-control study of diet and prostate cancer. British journal of cancer. 1997;76(5):678.
67. Nomura A, Stemmermann GN, Lee J, Craft NE. Serum micronutrients and prostate cancer in Japanese Americans in Hawaii. Cancer Epidemiology Biomarkers & Prevention. 1997;6(7):487-91.
68. Franceschi S, Bidoli E, Vecchia CL, Talamini R, D'Avanzo B, Negri E. Tomatoes and risk of digestive-tract cancers. International Journal of Cancer. 1994;59(2):181-4.
69. Nagasawa H, Mitamura T, Sakamoto S, Yamamoto K. Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. Anticancer research. 1994;15(4):1173-8.
70. Kim DJ, Takasuka N, Kim JM, Sekine K, Ota T, Asamoto M, et al. Chemoprevention by lycopene of mouse lung neoplasia after combined initiation treatment with DEN, MNU and DMH. Cancer letters. 1997;120(1):15-22.
71. Matos HR, Di Mascio P, Medeiros MH. Protective effect of lycopene on lipid peroxidation and oxidative DNA damage in cell culture. Archives of Biochemistry and Biophysics. 2000;383(1):56-9.
72. Kucuk O, Sarkar FH, Sakr W, Djuric Z, Pollak MN, Khachik F, et al. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. Cancer Epidemiology Biomarkers & Prevention. 2001;10(8):861-8.
73. Rao A. Lycopene, tomatoes, and the prevention of coronary heart disease. Experimental Biology and Medicine. 2002;227(10):908-13.
74. Rissanen TH, Voutilainen S, Nyyssönen K, Salonen R, Kaplan GA, Salonen JT. Serum lycopene concentrations and carotid atherosclerosis: the Kuopio ischaemic heart disease risk factor study. The American journal of clinical nutrition. 2003;77(1):133-8.
75. Rissanen T, Voutilainen S, Nyyssönen K, Salonen R, Salonen JT. Low plasma lycopene concentration is associated with increased intima-media thickness of the carotid artery wall. Arteriosclerosis, thrombosis, and vascular biology. 2000;20(12):2677-81.
76. Klipstein-Grobusch K, Launer L, Geleijnse J, Boeing H, Hofman A, Witteman J. Serum carotenoids and atherosclerosis: the Rotterdam Study. Atherosclerosis. 2000;148(1):49-56.
77. Kohlmeier L, Kark JD, Gomez-Gracia E, Martin BC, Steck SE, Kardinaal AF, et al. Lycopene and myocardial infarction risk in the EURAMIC Study. American Journal of Epidemiology. 1997;146(8):618-26.
78. PARTHASARATHY S. Mechanisms by which dietary antioxidants may prevent cardiovascular diseases. Journal of Medicinal Food. 1998;1(1):45-51.
79. Sesso HD, Liu S, Gaziano JM, Buring JE. Dietary lycopene, tomato-based food products and cardiovascular disease in women. The Journal of nutrition. 2003;133(7):2336-41.

80. Hennig B, Toborek M, McClain CJ, Diana JN. Nutritional implications in vascular endothelial cell metabolism. Journal of the American College of Nutrition. 1996;15(4):345-58.
81. Martin K, Wu D, Meydani M. The effect of carotenoids on the expression of cell surface adhesion molecules and binding of monocytes to human aortic endothelial cells. Atherosclerosis. 2000;150(2):265-74.
82. Forssberg A, Lingen C, Ernster L, Lindberg O. Modification of the X-irradiation syndrome by lycopene. Experimental Cell Research. 1959;16(1):7-14.
83. Lingen C, Ernster L, Lindberg O. The promoting effect of lycopene on the non-specific resistance of animals. Experimental cell research. 1959;16(2):384-93.
84. Hadiani MR, Farhangi R, Soleimani H, Rastegar H, Cheraghali AM. Evaluation of heavy metals contamination in Iranian foodstuffs: canned tomato paste and tomato sauce (ketchup). Food Additives & Contaminants: Part B. 2014;7(1):74-8.
85. Tang Y, Parmakhtiar B, Simoneau AR, Xie J, Fruehauf J, Lilly M, et al. Lycopene enhances docetaxel's effect in castration-resistant prostate cancer associated with insulin-like growth factor I receptor levels. Neoplasia. 2011;13(2):108-19.
86. Boileau TW-M, Liao Z, Kim S, Lemeshow S, Erdman Jr JW, Clinton SK. Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. Journal of the National Cancer Institute. 2003;95(21):1578-86.
87. Canene-Adams K, Lindshield BL, Wang S, Jeffery EH, Clinton SK, Erdman JW. Combinations of tomato and broccoli enhance antitumor activity in dunning r3327-h prostate adenocarcinomas. Cancer research. 2007;67(2):836-43.
88. Guttenplan JB, Chen M, Kosinska W, Thompson S, Zhao Z, Cohen LA. Effects of a lycopene-rich diet on spontaneous and benzo [a] pyrene-induced mutagenesis in prostate, colon and lungs of the lacZ mouse. Cancer letters. 2001;164(1):1-6.
89. Imaida K, Tamano S, Kato K, Ikeda Y, Asamoto M, Takahashi S, et al. Lack of chemopreventive effects of lycopene and curcumin on experimental rat prostate carcinogenesis. Carcinogenesis. 2001;22(3):467-72.
90. Kavanaugh CJ, Trumbo PR, Ellwood KC. The US Food and Drug Administration's evidence-based review for qualified health claims: tomatoes, lycopene, and cancer. Journal of the National Cancer Institute. 2007;99(14):1074-85.
91. Siler U, Barella L, Spitzer V, Schnorr J, Lein M, Goralczyk R, et al. Lycopene and vitamin E interfere with autocrine/paracrine loops in the Dunning prostate cancer model. The FASEB journal. 2004;18(9):1019-21.
92. Siler U, Herzog A, Spitzer V, Seifert N, Denelavas A, Hunziker PB, et al. Lycopene effects on rat normal prostate and prostate tumor tissue. The Journal of nutrition. 2005;135(8):2050S-2S.
93. Venkateswaran V, Fleshner NE, Sugar LM, Klotz LH. Antioxidants block prostate cancer in lady transgenic mice. Cancer research. 2004;64(16):5891-6.
94. Yang CM, Yen YT, Huang CS, Hu ML. Growth inhibitory efficacy of lycopene and β-carotene against androgen-independent prostate tumor cells xenografted in nude mice. Molecular nutrition & food research. 2011;55(4):606-12.
95. Konijeti R, Henning S, Moro A, Sheikh A, Elashoff D, Shapiro A, et al. Chemoprevention of prostate cancer with lycopene in the TRAMP model. The Prostate. 2010;70(14):1547-54.
96. Canene-Adams K. Review: The tomato as a functional food. J Nutr. 2005;134:1226-30.
97. La Vecchia C. Mediterranean epidemiological evidence on tomatoes and the prevention of digestive-tract cancers. Experimental Biology and Medicine. 1998;218(2):125-8.

98. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. Journal of the national cancer institute. 1999;91(4):317-31.
99. Colditz GA, Branch LG, Lipnick RJ, Willett W, Rosner B, Posner B, et al. Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. The American journal of clinical nutrition. 1985;41(1):32-6.
100. Potischman N, McCulloch CE, Byers T, Nemoto T, Stubbe N, Milch R, et al. Breast cancer and dietary and plasma concentrations of carotenoids and vitamin A. The American journal of clinical nutrition. 1990;52(5):909-15.
101. Järvinen R, Knekt P, Seppänen R, Teppo L. Diet and breast cancer risk in a cohort of Finnish women. Cancer letters. 1997;114(1):251-3.
102. Zhang S, Tang G, Russell RM, Mayzel KA, Stampfer MJ, Willett WC, et al. Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. The American journal of clinical nutrition. 1997;66(3):626-32.
103. Dorgan JF, Sowell A, Swanson CA, Potischman N, Miller R, Schussler N, et al. Relationships of serum carotenoids, retinol, α-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States). Cancer Causes & Control. 1998;9(1):89-97.
104. Van Eenwyk J, Davis FG, Bowen PE. Dietary and serum carotenoids and cervical intraepithelial neoplasia. International Journal of Cancer. 1991;48(1):34-8.
105. Helzlsouer KJ, Comstock GW, Morris JS. Selenium, lycopene, α-tocopherol, β-carotene, retinol, and subsequent bladder cancer. Cancer Research. 1989;49(21):6144-8.
106. Witztum JL. The oxidation hypothesis of atherosclerosis. The Lancet. 1994;344(8925):793-5.
107. Parthasarathy S, Steinberg D, Witztum J. The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. Annual review of medicine. 1992;43(1):219-25.
108. Hodis HN, Mack WJ, LaBree L, Cashin-Hemphill L, Sevanian A, Johnson R, et al. Serial coronary angiographic evidence that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis. Jama. 1995;273(23):1849-54.
109. Morris DL, Kritchevsky SB, Davis C. Serum carotenoids and coronary heart disease: The lipid research clinics coronary primary prevention trial and follow-up study. Jama. 1994;272(18):1439-41.
110. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. New England Journal of Medicine. 1993;328(20):1450-6.
111. Handelman GJ, Packer L, Cross CE. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. The American journal of clinical nutrition. 1996;63(4):559-65.
112. Kristenson M, Zieden B, Kucinskiene Z, Abaravicius A, RazinkovienË L, Elinder LS, et al. Antioxidant state and mortality from coronary heart disease in Lithuanian and Swedish men: concomitant cross sectional study of men aged 50. Bmj. 1997;314(7081):629.
113. Haliwell B. Free radicals antioxidants and human disease: curiosity, cause or consequence. Lancet. 1994;344(7):21-724.
114. Rao A, Agarwal S. Effect of diet and smoking on serum lycopene and lipid peroxidation. Nutrition research. 1998;18(4):713-21.
115. Kucuk O, Sakr W, Sarkar F, Djuric Z, Li Y, Velazquez F, et al. Lycopene supplementation in men with prostate cancer (PCa) reduces grade and volume of preneoplacia (PIN) and tumor,

decreases serum PSA and modulates biomarkers of growth and differentiation. Karmano Cancer Institute, Wayne State University, Detroit, MI. 1999.