LYCOPENE

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Oxidative stress is now recognized as an important etiological factor in the causation of several chronic diseases including cancer, cardiovascular diseases, osteoporosis, and diabetes. Antioxidants play an important role in mitigating the damaging effects of oxidative stress on cells. Lycopene, a carotenoid antioxidant, has received considerable scientific interest in recent years. Epidemiological, tissue culture, and animal studies provide

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convincing evidence supporting the role of lycopene in the prevention of chronic diseases. Human intervention studies are now being conducted to validate epidemiological observations and to understand the mechanisms of action of lycopene in disease prevention. To obtain a better understanding of the role of lycopene in human health, this chapter reviews the most recent information pertaining to its chemistry, bioavailability, metabolism, role in the prevention of prostate cancer and cancer of other target organs, its role in cardiovascular diseases, osteoporosis, hypertension, and male infertility. A discussion of the most relevant molecular markers of cancer is also included as a guide to future researchers in this area. The chapter concludes by reviewing global intake levels of lycopene, suggested levels of intake, and future research directions.

I. INTRODUCTION

Lycopene, as a dietary source of a carotenoid antioxidant, has attracted considerable interest in recent years as an important phytochemical with a beneficial role in human health. Chronic diseases including cancer, cardiovascular disease, diabetes, and osteoporosis are the major causes of morbidity and mortality in the Western World. Along with genetic factors and age, lifestyle factors and diet are also considered important risk factors for these diseases (Agarwal and Rao, 2000a). The role of oxidative stress induced by reactive oxygen species (ROS) and the oxidative damage of important biomolecules is one of the main foci of research related to human diseases. Oxidative stress is thought to be involved in the cause and progression of several chronic diseases (Rao and Rao, 2004). Antioxidants are agents that inactivate ROS and provide protection from oxidative damage. Dietary guidelines for the prevention of chronic diseases, recognizing the importance of antioxidants, have recommended an increase in the consumption of fruits and vegetables that are good sources of dietary antioxidants. In addition to traditional antioxidant vitamins, such as vitamins A, E, and C, fruits and vegetables also contain several phytonutrient antioxidants. The two important classes of phytonutrient antioxidants include the carotenoids and polyphenols. *In vitro* cell culture studies, laboratory animal studies, case control and cohort studies, and dietary intervention studies have all provided evidence in support of the role of antioxidants in the prevention of cancer and other chronic diseases. The focus of this chapter will be on lycopene, a potent carotenoid antioxidant. Since animal and human experimental studies on the role of lycopene in cancer prevention are beginning to be undertaken, a brief overview of some important molecular markers of cancer, which could be used in these studies, is also reviewed.

The authors consider this to be important information in planning future animal and human clinical and intervention studies.

II. OXIDATIVE STRESS AND CHRONIC DISEASES

ROS are highly reactive oxidant molecules that are generated endogenously through regular metabolic activity, lifestyle activities, such as smoking and exercise, environmental factors, such as pollution and ultra violet radiation, and diet. Being highly reactive, they can cause oxidative damage to cellular components such as lipids, proteins, and DNA (Agarwal and Rao, 2000a). Under normal circumstances, presence of endogenous repair mechanisms, such as the antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GP_x) and catalase and antioxidant vitamins, minerals, and phytonutrients, help repair the damaged biomolecules. However, when these defense mechanisms are overwhelmed by ROS, it leads to permanent damage of the biomolecules resulting in increased risk of chronic diseases. Lipid peroxidation products are increased in a variety of oxidative stress conditions (Witztum, 1994). Premenopausal women with high mammographic tissue densities at high risk for breast cancer excrete higher levels of the lipid peroxidation product malondialdehyde (Boyd and McGuire, 1990; Boyd *et al.*, 1995). A 20% increase in the levels of lipid peroxides were found in prostate cancer patients compared to their matched controls with retinoic acid therapy (Rigas *et al.*, 1994). Oxidation of the low-density lipoprotein (LDL) has been associated with increased formation of atherosclerotic plaque, leading to coronary heart disease (CHD) (Arab and Steck, 2000; Parthasarathy *et al.*, 1992; Rao, 2002b). Similarly, DNA oxidation increases the risk of cancers and other pathological disorders. Cancer tissues contain increased levels of oxidized DNA adducts (Loft and Poulsen, 1996; Musarrat *et al.*, 1996; Wang *et al.*, 1996). Oxidation of intercellular proteins results in functional changes of enzymes that modulate cellular metabolism (Rao and Agarwal, 2000). Both DNA and protein oxidation are associated with the aging process (Ames *et al.*, 1993). ROS also induce the expression of a wide variety of transcription factors, such as NFkB, AP1, and oncogenes, such as *c-fos* and *c-jun*. Conformational changes are found in p53 protein, which mimic the mutant phenotype induced by ROS (Hainaut and Miller, 1993; Toledano and Leonard, 1991; Wasylyk and Wasylyk, 1993; Wei, 1992). By inducing these alterations, ROS can influence cell cycle mechanisms and ultimately lead to human health disorders. Increases in the tissue and body fluid levels of several biomarkers of oxidized lipids, proteins, and DNA have been shown during aging and also in patients with cancer and cardiovascular diseases (Ames *et al.*, 1996; Halliwell *et al.*, 1995; Pincemail, 1995; Stadtman, 1992;

Witztum, 1994). Dietary antioxidants, such as lycopene, can protect lipids, proteins, and DNA from oxidation and play a role in the prevention of such diseases.

III. CHEMISTRY AND DIETARY SOURCES OF LYCOPENE

Lycopene belongs to the family of carotenoid compounds found in fruits, vegetables and green plants. In plants these compounds are part of the photosynthetic machinery and are responsible for the yellow, orange, and red colors of fruits and vegetables. They are synthesized by plants and microorganisms but not by animals and humans. They are important dietary sources of vitamin A and are also excellent antioxidants (Paiva and Ressell, 1999). More than 600 carotenoids are found in nature, about 40 of which are present in a typical human diet, and about 20 have been identified in blood and tissues (Agarwal and Rao, 2000b). The principal carotenoids present in the diet and human body are β-carotene, α-carotene, lycopene, α-cryptoxanthine, lutein, and zeaxanthin, accounting for over 90% of all carotenoids (Gerster, 1997). All carotenoids posses certain common chemical features consisting of a polyisoprenoid structure, a long conjugated chain of double bonds in the central position of the molecules, and a near bilateral symmetry around the central double bond (Britton, 1995). However, modifications in the base structure by cyclization of the end groups and by introduction of oxygen functions yield different carotenoids giving them characteristic colors and antioxidant properties (Agarwal and Rao, 2000b). The antioxidant properties of carotenoids are due to their ability to quench singlet oxygen species. Since carotenoids contain several double bonds, they can undergo *cis–trans* isomerization. The *trans* form is considered to be more stable and is the most common form present in foods. The biological significance of the isomeric forms of carotenoids is not fully understood at the present time. Chemical structures of some common carotenoids are shown in Figure 1.

Lycopene, like other carotenoids, is a natural pigment synthesized by plants and microorganisms to absorb light during photosynthesis and to protect them against photosensitization. It is a noncyclic carotenoid having a molecular formula of $C_{40}H_{56}$ and a molecular weight of 536.85 daltons. It is a lipophylic compound that is insoluble in water. It is a red pigment absorbing light in the visible range and a petroleum ether solution of lycopene has λ_{max} of 472 nm and $\varepsilon^{1\%}$ 3450 (Rao and Agarwal, 1999). It is an open chain hydrocarbon containing 11 conjugated and 2 nonconjugated double bonds arranged in a linear array. As with other carotenoids, the double bonds in lycopene can undergo isomerization from *trans* to mono or ply-*cis* isomers by light, thermal energy, and chemical reactions. All-*trans*,

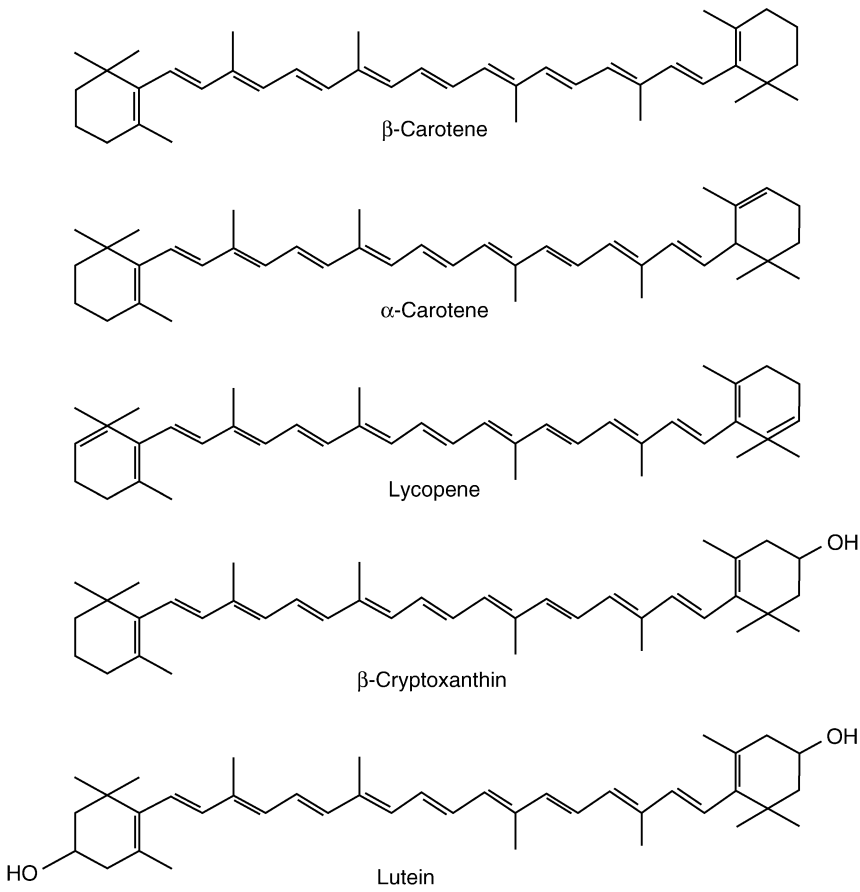

FIG. 1 Structures of major dietary carotenoids.

5-*cis*, 9-*cis*, 13-*cis*, and 15-*cis* are the most commonly identified isomeric forms of lycopene. Different isomeric forms of lycopene are shown in Figure 2.

Since lycopene lacks the β-ionic ring structure, unlike β-carotene, it lacks provitamin A activity. The biological activity of lycopene is thought to be primarily due to its antioxidant properties. However, other mechanisms, such as facilitating gap junction communication (GJC) (Aust *et al.*, 2003; Heber, 2002; Wertz *et al.*, 2004; Zhang *et al.*, 1991, 1992), stimulation of the immune system (Chew and Park, 2004; Heber, 2002; Heber and Lu, 2002; Kim *et al.*, 2004; Wertz *et al.*, 2004), endocrine-mediated pathways

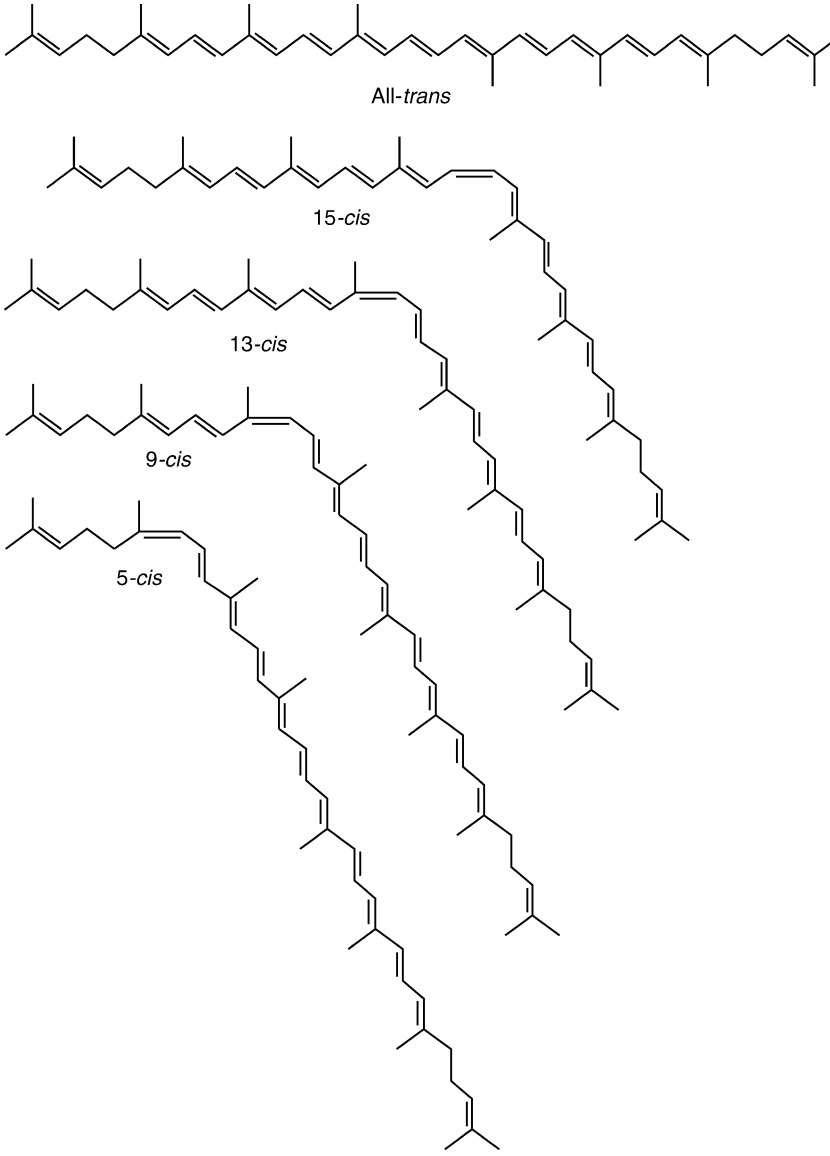

FIG. 2 Structures of *trans* and *cis* isomeric forms of lycopene.

TABLE I
LYCOPENE CONTENT OF COMMON FRUITS AND VEGETABLES

Fruits and vegetables	Lycopene (μg/g wet weight)
Tomatoes	8.8–42.0
Watermelon	23.0–72.0
Pink guava	54.0
Pink grapefruit	33.6
Papaya	20.0–53.0
Apricot	<0.1

Source: Lycopene content of tomato products and their contribution to dietary lycopene. Reprinted from Food Research International. 1999; **31**, pp. 737–741 by permission of Elsevier.

TABLE II
LYCOPENE CONTENT OF COMMON TOMATO BASED FOODS

Tomato products	Lycopene (μg/g weight)
Fresh tomatoes	8.8–42.0
Cooked tomatoes	37.0
Tomato sauce	62.0
Tomato paste	54.0–1500.0
Tomato soup (condensed)	79.9
Tomato powder	1126.3–1264.9
Tomato juice	50.0–116.0
Pizza sauce	127.1
Ketchup	99.0–134.4

Source: Lycopene content of tomato products and their contribution to dietary lycopene. Reprinted from Food Research International. 1999; **31**, pp. 737–741 by permission of Elsevier.

(Heber, 2002; Heber and Lu, 2002; Wertz *et al.*, 2004), and cell cycle regulations, have also been demonstrated.

Although red-colored fruits and vegetables are the most common sources of dietary lycopene, not all red-colored plants contain lycopene. Common food sources of lycopene are the tomatoes, processed tomato products, watermelons, pink guava, pink grapefruits, papaya, and apricots. The lycopene content of these foods are shown in Table I.

Tomatoes and tomato-based products account for more than 85% of the dietary lycopene in North America. Lycopene content of some common tomato-based foods is shown in Table II.

IV. ANALYTICAL METHODS OF MEASURING LYCOPENE IN FOOD AND OTHER BIOLOGICAL MATERIALS

Spectrophotometric methods and high-pressure liquid chromatography (HPLC) are used most commonly in the quantitative estimations of total lycopene in food and biological samples. It is first extracted from the samples using various organic solvents. Typically, lycopene from tomato products is extracted with hexane:methanol:acetone (2:1:1) mixture containing 2.5% butylated hydroxytoluene (BHT). The optical density of the hexane extract is then measure spectrophotometrically at 502 nm against a hexane blank. Concentrations of lycopene are then calculated using the extinction coefficient ($E^{\%}$) of 3150 (Rao and Agarwal, 1999). Results are reported as parts per million (ppm) of lycopene or as μg per unit weight of the food product. Alternatively, the hexane extract is analyzed by HPLC using reverse-phase C18 column and an absorbance detector (Agarwal *et al.*, 2001; Rao *et al.*, 1999). Lycopene is quantified from the HPLC profile by using purified lycopene standard available from several commercial sources. Rao *et al.* (1999) compared the spectrophotometric and HPLC methods and found the results to be in good agreement. The spectrophotometric method offers a convenient, fast, and less expensive procedure for the detection of total lycopene compared to the HPLC procedure. A large number of samples can be processed by this method in a relatively short period of time without compromising the accuracy. For the detection of the *cis* isomeric forms of lycopene, the HPLC system with an absorbance or electrochemical detectors is used (Agarwal *et al.*, 2001; Clinton *et al.*, 1996; Ferruzzi *et al.*, 2001). Typically, food samples are homogenized and then extracted with the hexane: methanol:acetone (2:1:1) mixture containing 2.5% BHT. The extracts are then analyzed by reverse-phase HPLC using a C30 polymeric HPLC column. Peaks are eluted with methanol:methyltert-butyl ether (62:38) and monitored at 460 nm using an absorbance detector (Agarwal *et al.*, 2001). Lycopene content in the serum and plasma samples is estimated by extracting the lycopene with hexane:methyl chloride (5:1) containing 0.015% BHT and analyzed using a Vydac 201HS54 reverse-phase analytical HPLC column and an absorbance detector set at 460 nm. The mobile phase used is a mixture of acetonitrile, methanol, methyl chloride, and water (7:7:2:0.16). Lycopene peaks are identified and quantified with the use of external standards. Analysis of lycopene in tissue samples requires that the samples first undergo saponification by incubation in sodium hydroxide. Samples are then extracted and analyzed as before for the serum and plasma samples (Rao *et al.*, 1999).

TABLE III
EFFECT OF PROCESSING ON LYCOPENE CONTENT OF TOMATO JUICE

Stage of processing	Processing temperature (°C)	Lycopene content (ppm) Mean ± SEM
Raw tomatoes	–	124.5 ± 1.6[a]
Scalded pulp	76	105.6 ± 0.8[a]
Salting tank	–	107.9 ± 0.3[a]
Sterilization tank	120	102.6 ± 0.4[a]
Processed juice	–	102.3 ± 0.8[a]

[a]Numbers with different letters are statistically significant ($p < 0.05$).
Source: Lycopene content of tomato products: Its stability, bioavailability and *in vivo* antioxidant properties. Reprinted from Journal of Medicinal Food. 2001; **4**, pp. 9–15 by permission of Mary Ann Liebert, Inc., Publishers.

TABLE IV
EFFECT OF STORAGE ON LYCOPENE CONTENT OF TOMATO JUICE

Storage condition	Lycopene content (ppm) Mean ± SEM
Fresh juice	91.1 ± 0.8[a]
4°C, 12 months	89.5 ± 0.6[a]
25°C, 12 months	92.0 ± 0.7[a]
37°C, 12 months	92.6 ± 0.6[a]

[a]Numbers with different letters are statistically significant ($p < 0.05$).
Source: Lycopene content of tomato products: Its stability, bioavailability and *in vivo* antioxidant properties. Reprinted from Journal of Medicinal Food. 2001; **4**, pp. 9–15 by permission of Mary Ann Liebert, Inc., Publishers.

V. STABILITY AND ANTIOXIDANT PROPERTIES OF LYCOPENE AND ITS ISOMERS

Although lycopene is a fairly stable molecule, it can undergo oxidative, thermal, and photodegradation. Studies evaluating the thermal stability of lycopene have shown it to be stable under the conditions of industrial processing. Agarwal *et al.* (2001) studied the effect of processing temperatures and storage on lycopene stability and its isomerization. They showed that the lycopene content of tomatoes remained unchanged during the multistep processing operations for the production of juice or paste and remained stable for up to 12 months of storage at ambient temperatures (Tables III and IV).

TABLE V
EFFECT OF HEATING TOMATO JUICE IN THE PRESENCE OF LIPIDS ON LYCOPENE ISOMERS

	Corn oil	Olive oil	Butter
Lycopene (n = 3)	[mean ± SEM]	[mean ± SEM]	[mean ± SEM]
% *Trans*	83.82 ± 1.81[a]	76.74 ± 0.3[b]	84.53 ± 1.67[a]
% *Cis*	16.18 ± 1.81[a]	23.26 ± 0.3[b]	15.47 ± 1.67[a]
	Corn oil	Olive oil	Butter
Lycopene (n = 3)	[mM/L]	[mM/L]	[mM/L]
All-*trans*	11666.98[a]	10681.51[b]	11765.81[a]
Cis	2266.65[a]	3258.48[b]	2167.19[a]

[a,b]Values are means ± SEM, n = 3. Values with different superscripts in a given row are significantly different, $P < 0.05$ (One-way ANOVA and Turkeys Test).

In the same study (Agarwal *et al.*, 2001), processed tomato products, such as tomato paste, ketchup, and juice, were shown to have a similar distribution of the *cis* and *trans* isomeric forms of lycopene. However, when the tomato juice was subjected to cooking temperatures in the presence of different oils, a noticeable increase in the formation of the *cis* isomers was observed in the presence of olive oil (Table V). Similar observations were also reported by Nguyen *et al.* (Nguyen and Schwartz, 1998). Conversion of the all-*trans* lycopene present in raw tomatoes to its *cis* isomeric form is of interest since the *cis* forms are generally considered to be more bioavailable. In another study (Chasse *et al.*, 2001), the stability of lycopene isomers were studied using an ab initio computational modeling. 5-*Cis* lycopene was found to be the most stable followed by all-*trans*. Lycopene is one of the most potent antioxidants, with a singlet-oxygen-quenching ability twice as high as that of β-carotene and ten times higher than that of α-tocopherol (Di Mascio *et al.*, 1989). In the computational model study (Chasse *et al.*, 2001), 5-*cis* lycopene had the highest antioxidant property as indicated by the ionization potential, followed by 9-*cis*. All-*trans* lycopene had the least antioxidant potential (Figure 3).

VI. BIOAVAILABILITY, TISSUE DISTRIBUTION, METABOLISM, AND SAFETY OF LYCOPENE

Lycopene levels in plasma and human tissues reflect dietary intake. In a study, when subjects consumed a tomato-free diet for 2 weeks, their lycopene levels dropped significantly (Rao and Agarwal, 1998a). Ingested lycopene is

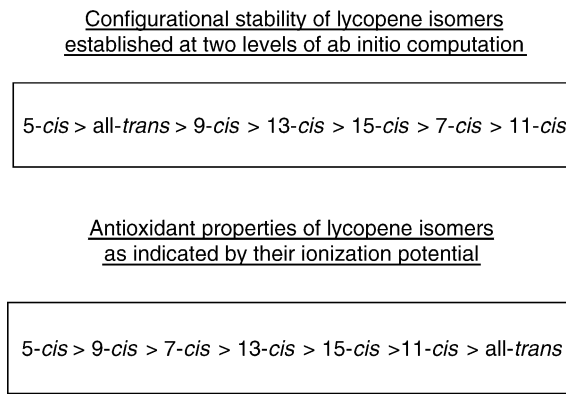

FIG. 3 Stability and antioxidant properties of lycopene isomers (Chasse *et al.*, 2001).

incorporated into dietary lipid micelles and absorbed into the intestinal mucosal lining via passive diffusion. They are then incorporated into chylomicrons and released into the lymphatic system for transport to the liver. Lycopene is transported by the lipoproteins into the plasma for distribution to the different organs (Parker, 1996). Owing to the lipophilic nature of lycopene, it was found to concentrate in the LDL and very low density lipoprotein (VLDL) fractions and not the high density lipoprotein (HDL) fraction of the serum (Rao and Agarwal, 1998a; Stahl and Sies, 1996). A schematic diagram of lycopene absorption and transportation is shown in Figure 4.

In humans, lycopene absorption is in the range of 10–30% with the remainder being excreted. Many biological and lifestyle factors influence the absorption of dietary lycopene including age, gender, hormonal status, body mass and composition, blood lipid levels, smoking, alcohol, and the presence of other carotenoids in the food (Rao and Agarwal, 1999; Stahl and Sies, 1992). Fasting serum lycopene levels were found to be higher and more reproducible than postprandial levels indicating that diet induced metabolic stress (Rao and Agarwal, 1998b). Although the lycopene levels in blood do not differ significantly between men and women (Brady *et al.*, 1997; Olmedilla *et al.*, 1994), in women, they were found to be influenced by the phases of menstrual cycle with a peak during midluteal phase (Forman *et al.*, 1996). Inconsistent results are reported in the literature regarding the effect of smoking on serum lycopene levels (Brady *et al.*, 1997; Peng *et al.*, 1995; Ross *et al.*, 1996). A study showed no significant differences in serum lycopene levels between smokers and non-smokers (Rao and Agarwal, 1998a). However, the serum lycopene levels in the smokers fell by 40% immediately after smoking three cigarettes. Exposing fresh plasma under *in vitro* conditions to cigarette smoke resulted in the depletion of the lycopene and other lipid-soluble antioxidants

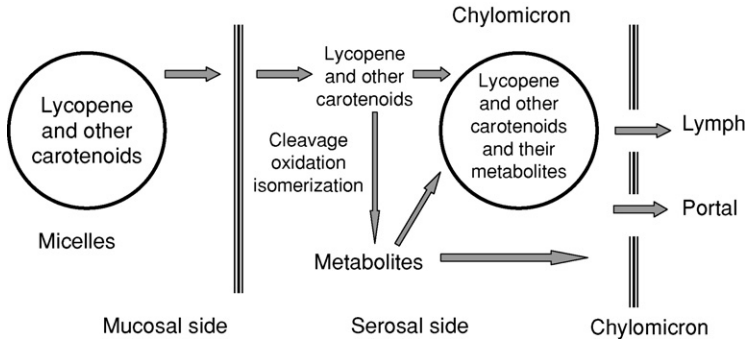

FIG. 4 Absorption and translocation of lycopene.

(Handelman *et al.*, 1996). Alcohol consumption was also shown to alter serum lycopene levels (Brady *et al.*, 1997). Other factors that influence the bioavailability of lycopene are its release from the food matrix due to processing, presence of dietary lipids, and heat-induced isomerization from the all-*trans* to *cis* conformation. They all enhance lycopene absorption into the body. Ingestion of cooked tomato juice in oil medium increased serum lycopene levels threefold whereas consumption of an equal amount of unprocessed juice did not have any effect (Stahl and Sies, 1992).

In another study, lycopene from tomato paste was shown to be 3.8 times more bioavailable than that from fresh tomato (Gärtner *et al.*, 1997). It is generally believed that conversion of all-*trans* lycopene to its *cis* isomeric form enhances its absorption (Stahl and Sies, 1992). Presence of β-carotene was shown to increase the absorption of lycopene in some studies, while the presence of canthaxanthin appears to decrease lycopene absorption (Blakely *et al.*, 1994; Gaziano *et al.*, 1995; Wahlqvist *et al.*, 1994). In a recent study (Rao and Shen, 2002), low levels of lycopene (5, 10, and 20 mg) were provided in the form of either ketchup or oleoresin capsules for 2 weeks to healthy human subjects. Serum lycopene levels at the beginning and end of the 2 weeks of treatment were compared. A significant increase in the serum lycopene levels was observed for both ketchup and capsules at all three levels of intake. Although the serum lycopene levels increased in a dose-dependent fashion with dietary intake, biomarkers of lipid and protein oxidation did not differ significantly between the treatments. The absorption efficiency of lycopene was observed to be greater at the lower levels of dietary intake.

Lycopene is the most predominant carotenoid in human plasma with a half-life of about 2–3 days (Stahl and Sies, 1996). Although the most prominent geometric isomers of lycopene in plants are the all-*trans*, in human plasma, lycopene is present as an isomeric mixture containing 50% of the

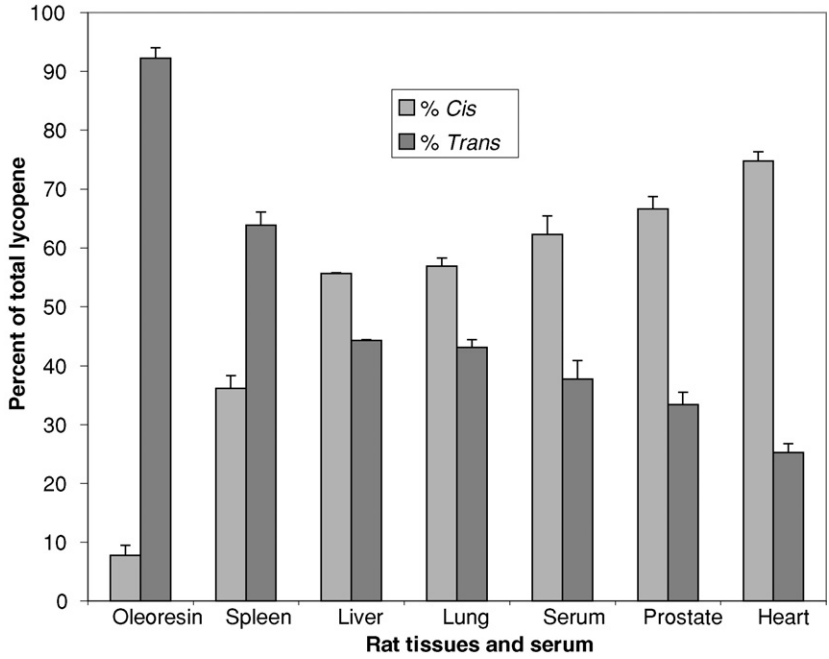

FIG. 5 *Cis* and *trans* lycopene isomers in rat serum and tissues. Values expressed are mean ± SEM, n = 3.

total lycopene as *cis* isomer (Rao and Agarwal, 2000). When animals were fed lycopene containing predominantly the all-*trans* isomeric form, serum and tissue lycopene showed the presence of *cis* lycopene (Jain *et al.*, 1999). Similar results were also observed in human serum (Figure 5).

There are also some indications of *in vivo trans* to *cis* isomerization reactions. Very little is known about the *in vivo* metabolism of lycopene. In a recent study, dos Anjos Ferreira *et al.* (2004) used the postmitochondrial fraction of rat intestinal mucosa to study lycopene metabolism. They identified two types of metabolic products of lycopene, cleavage products and oxidation products. Identified among the cleavage products were: 3-keto-apo-13-lycopenone and 3,4-dehydro-5,6-dihydro-15-apo-lycopenal. The oxidation products included: 2-ene-5,8-lycopenal-furanoxide, lycopene-5,6.5′,6′-diepoxide, lycopene-5,8-furanoxide isomer (I), lycopene-5,8-epoxide isomer (II), and 3-keto-lycopene-5′,8′-furanoxide. It is possible that similar metabolites of lycopene are also present *in vivo* in the presence of lipoxygenase enzymes. Nagao (2004) on the other hand showed *in vitro* cleavage of lycopene to acycloretinal, acycloretinoic acid, and apolycopenals in a

nonenzymatic manner. They also showed that these cleaved products of lycopene induced apoptosis of HL-60 human promyelocytic leukemia cells. Only a few metabolites, such as 5,6-dihydroxy-5,6-dihydro lycopene, have been detected in human plasma (Khachik *et al.*, 1995, 1997, 2002). It is suggested that lycopene may undergo *in vivo* oxidation to form epoxides, which then may be converted to the polar 5,6-dihydoxy-5,6 dihydro-lycopene through metabolic reduction. A controversy exists as to the role of primary lycopene or its polar metabolites that are the biologically active forms. Further research is needed to better understand this aspect of lycopene.

Following the absorption of lycopene, it is transported to various organs and accumulates in tissues. Tissue distribution of lycopene varies considerably suggesting unique biological effects on some tissues and not on others. The highest concentrations of lycopene are in the testes, adrenal glands, liver, and prostate. Table VI shows the lycopene levels in human tissues (Rao and Agarwal, 1999).

Lycopene and its oxidation products are present in human milk and other body fluids (Khachik *et al.*, 1997). Human seminal plasma also contains lycopene and its levels were lower in immunoinfertile men compared to normal individuals (Palan and Naz, 1996). Although the plasma or serum levels of lycopene are used commonly to assess its bioavailability, adipose tissue has been suggested as a better tissue for the assessment of body lycopene status (Kohlmeier *et al.*, 1997).

Historically, lycopene-containing fruits and vegetables have been consumed by humans without any safety problems. Several studies were undertaken to evaluate the safety of both natural and synthetic lycopene (Jonker *et al.*, 2003; Matulka *et al.*, 2004; Mellert *et al.*, 2002). In our studies we evaluated intake levels of lycopene, ingested in the form of tomato juice, tomato sauce, and nutritional supplement from 5 to 75 mg/day (Rao and Agarwal, 1998a) in healthy human subjects. No adverse effects of consuming lycopene were observed in these studies. In another study (Mellert *et al.*, 2002) two synthetic crystalline lycopene sources, BASF lycopene 10 CWD and Lyco Vit 10%, each containing approximately 10% lycopene were tested in rats. After ingesting the test products for 13 weeks at intake levels of up to 3000 mg/kg body weight/day, no adverse effects were observed.

Lycopene derived from a fungal biomass of *Blakeslea trispos*, suspended in sunflower oil at a concentration of 20% w/w, was tested for subchronic toxicity at concentrations of 0%, 0.25%, 0.50%, and 1.0% in rats for 90 days (Jonker *et al.*, 2003). No evidence of toxicity of lycopene at dietary intake levels up to 1.0% was observed in this study. The authors suggest the no-observed-effect level (NOEL) for this lycopene to be 1.0% in the diet, the highest dietary concentration tested. McClain and Bausch (2003) published

TABLE VI
LYCOPENE LEVELS IN HUMAN TISSUES

Tissue	Lycopene (nmol/g wet weight)
Adrenal	1.90–21.60
Breast	0.78
Colon	0.31
Kidney	0.15–0.62
Liver	1.28–5.72
Lung	0.22–0.57
Ovary	0.3
Pancreas	0.7
Prostate	0.8
Skin	0.42
Stomach	0.2
Testes	4.34–21.36

a summary of additional safety studies using synthetic lycopene. No teratogenic effects were observed in the two-generation rat study. Deposition of lycopene in plasma, liver, and other tissues also had no adverse effects. The red coloration of skin associated with the intake of high levels of lycopene disappeared after 13 weeks showing the reversibility of this effect. A condition identified as lycopenemia was reported when tomato juice was ingested in excess for prolonged periods of time resulting in increased serum lycopene levels and coloration of skin (Reich *et al.*, 1960). No other adverse effects were associated with the skin coloration. In consideration of the studies establishing the safety of lycopene for human consumption, the Food and Drug Administration in the United States of America granted generally recognized as safe (GRAS) status to lycopene as a nutritional supplement.

VII. MECHANISMS OF ACTION OF LYCOPENE

The biological activities of carotenoids, such as β-carotene, are related to their provitamin A activity within the body (Clinton, 1998). Since lycopene lacks the β-ionic ring structure, it does not have any provitamin A activity (Stahl and Sies, 1996). The biological effects of lycopene in humans have therefore been attributed to mechanisms other than vitamin A. Two major hypotheses have been proposed to explain the anticarcinogenic and antiatherogenic activities of lycopene: oxidative and nonoxidative mechanisms. The proposed mechanisms for the role of lycopene in the prevention of chronic diseases are summarized in Figure 6.

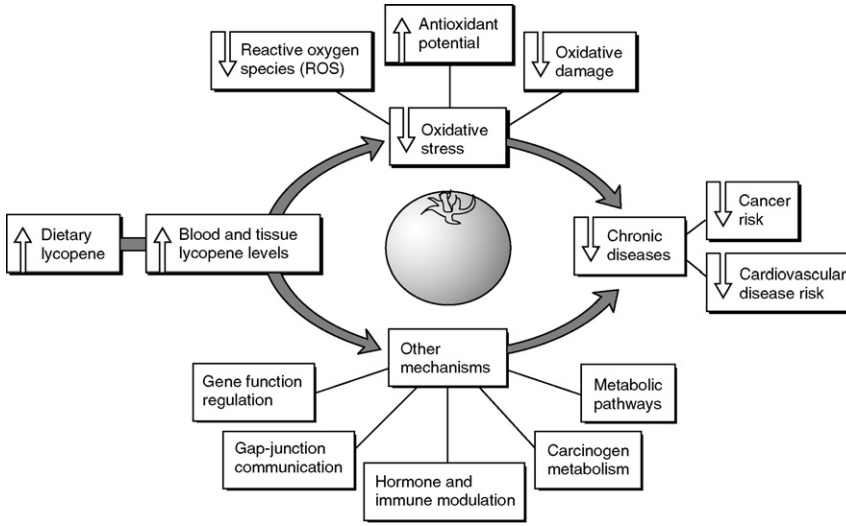

FIG. 6 Proposed mechanisms for the role of lycopene in chronic diseases. (Tomato lycopene and its role in human health and chronic diseases. Reprinted from CMAJ. 2000; **163**(6), pp. 739–744 by permission of 2000 CMA Media Inc.)

Heber (Heber and Lu, 2002) and Wertz *et al.* (2004) provided an overview of the mechanisms of action of lycopene. The antioxidant properties of lycopene constitute the major focus of research with regards to its biological effects. Dietary intake of lycopene has been shown to increase circulatory and tissue levels of lycopene. Acting as an antioxidant, it can trap ROS and reduce oxidative stress and damage to cellular components including lipids, proteins, and DNA (Agarwal and Rao, 2000a). Since oxidative damage of lipids, proteins, and DNA have all been implicated in the development of chronic diseases, such as cardiovascular diseases, cancer, and osteoporosis, lycopene acting as a potent antioxidant can reduce the risk of these diseases.

Included among the nonoxidative mechanisms are: inhibition of insulin-like growth factor-I (IGF-I) signaling, interleukin-6 (IL-6) expression, androgen signaling, improving GJC, induction of phase II drug-metabolizing enzymes and oxidative defense genes, and improving the immune response (Agarwal and Rao, 2000a; Wertz *et al.*, 2004). GJC between cells is thought to be one of the protective mechanisms related to cancer prevention. Many human tumors are deficient in GJC and its restoration or upregulation is associated with decreased proliferation of tumor cells. The anticarcinogenic effects of lycopene may be due to regulation of GJC as shown in mouse embryo fibroblast cells (Zhang *et al.*, 1991, 1992). Aust *et al.* (2003) reported

that the *in vitro* oxidation product of lycopene, 2,7,11-trimethyl-tetradeca-hexaene-1,14-dial, stimulated GJC in rat liver epithelial WB-F344 cells. Studies using human and animal cells have identified the expression of the connexin 43 geneas being upregulated by lycopene allowing for a direct intercellular GJC (Heber and Lu, 2002). Suppression of the carcinogen-induced phosphorylation of the regulatory proteins, such as p53 and R_b antioncogenes by lycopene, may also play an important role stopping cell division at the G-G_1 cell cycle phase (Matsushima-Nishiwaki *et al.*, 1995). Astrog *et al.* (1997) emphasize the lycopene-induced modulation of the liver-metabolizing enzymes as the underlying mechanism of protection against carcinogen-induced preneoplastic lesions in the rat liver. There is evidence to suggest that lycopene reduces cellular proliferation induced by insulin-like growth factors, which are potent mitogens, in various cancer cell lines (Pincemail, 1995). T cell differentiation (immunomodulation) was suggested to be the mechanism for the suppression of mammary tumor growth by lycopene treatments in SHN-retired mice (Kobayashi *et al.*, 1996; Nagasawa *et al.*, 1995).

In a study, Chew and Park (2004) showed that lycopene and other nonprovitamin A carotenoids induce immunoenhancement in animals. Bone marrow-derived dendritic cells are the most potent of the antigen-presenting cells. They initiate the immune response by presenting antigens to naïve T lymphocytes. Kim *et al.* (2004) using these dendritic cells showed that lycopene can significantly increase the phenotypic and functional maturation of these cells, especially in lipopolysaccharide-induced dendritic cell maturation. The authors suggest therapeutic application for lycopene via the manipulation of the dendritic cells. Lycopene also interacts synergistically with 1,25-dihydroxyvitamin D3 [1,25$(OH)_2D_3$] and leutin to regulate cell cycle progression, suggesting some interactions at a nuclear or subcellular level and specific positioning of different carotenoids in cell membranes (Amir *et al.*, 1999). Lycopene also acts as a hypocholesterolemic agent by inhibiting HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase that may be related to reducing the risk of cardiovascular diseases (Fuhramn *et al.*, 1997).

VIII. LYCOPENE AND HUMAN DISEASES

A. LYCOPENE AND THE PREVENTION OF CHRONIC DISEASES: THE HYPOTHESIS

The underlying hypothesis of oxidative stress, antioxidants, and chronic diseases is illustrated in Figure 7.

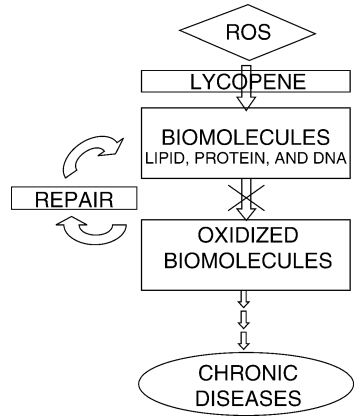

FIG. 7 Lycopene and the prevention of chronic diseases: The hypothesis. (Lycopene and the prevention of chronic diseases. Reprinted from Major findings from five international conferences. 2002. A.V. Rao, D. Heber, eds., p. 4. By permission of Caledonian Science Press.)

B. CANCER

1. Epidemiological evidence

Various epidemiological studies have suggested that a diet rich in a variety of fruits and vegetables results in a lower risk of cancer and other chronic diseases (Giovannucci, 1999). An early epidemiological study on elderly Americans indicated that high intake of tomatoes was associated with a significant reduction in mortality from cancers of all sites (Colditz *et al.*, 1985; Franceschi *et al.*, 1994). Since then several other articles have appeared in recent years reviewing the epidemiological data pertaining to the relationship between the intake of tomatoes, tomato products, and lycopene and the prevention of cancer. The Mediterranean diet, rich in tomatoes and tomato products, maybe responsible for the lower cancer incidence in that region (LaVecchia, 1997). In other epidemiological studies, serum and tissue levels of lycopene were inversely associated with the risk of prostate cancer, breast, cervical, and ovarian cancers, gastrointestinal tract cancers including stomach, colon, and the rectum, and lung cancer (Giovannucci, 1999; Giovannucci *et al.*, 1995, 2002). A review (Giovannucci, 1999) reported on the epidemiological studies including estimation of dietary intake of tomatoes and lycopene and the circulatory levels of lycopene in relation to the risk of cancers of various sites. The results were found to be consistent for a variety of cancers across numerous diverse populations and with the use of

several different study designs. None of the studies analyzed reported any adverse effects of consuming high levels of tomatoes or lycopene. Although a majority of the epidemiological studies reported inverse relationship between the consumption of tomatoes, lycopene and circulating levels of lycopene and the risk of cancers, other studies (Giovannucci, 1999) found no protective effect of serum and dietary lycopene on cancer risk.

2. *Molecular markers of cancer*

With increasing interest in undertaking animal experimental and human clinical and intervention studies to evaluate the role of lycopene in cancer prevention, it is important that well-established molecular and clinical markers of cancer be used in these studies. In general, the main clinical end points used in animal and human experiments are the tumor burden and volume and survival rates. Now that our understanding of cancer pathology has advanced, several molecular events are beginning to be recognized and used in research to evaluate the outcomes from intervention studies. A brief overview of some of the more important molecular markers of cancer that can and should be used in future studies is presented in this section.

Clinical evaluation of tumors by the conventional tumor lymph node metastases (TNM) staging system is used to determine prognosis and choice of treatment. This system takes into account size of the primary tumor (T), lymph node involvement (N), and occurrence of distant metastases (M). Tumor grading is a measure of cellular differentiation and is also used as a prognostic indicator. Significant effort to identify novel markers for clinical assessment or as a research tool has given rise to several putative molecular markers for diagnosis, prognosis, and response to therapy. In fact, over 85 markers for prostate cancer alone have been reported in the literature, although the clinical value of the majority of these markers has not yet been determined (Tricoli *et al.*, 2004).

Broad-spectrum molecular markers include those involved in proliferation, cell cycle, apoptosis, and vascularization. Several of these are well-established markers that have varying degrees of clinical value. One of the most reliable markers for cellular proliferation is Ki-67, and increased expression is associated with poor prognosis for prostate, breast, lung, and bladder cancer (Claudio *et al.*, 2002; Esteva *et al.*, 2004; Martin *et al.*, 2004; Santos *et al.*, 2003). In breast cancer, Ki-67 expression correlates well with the proliferating cell nuclear antigen (PCNA), which is also a marker for proliferation (Keshgegian and Cnaan, 1995). Both proteins are nuclear antigens that are expressed at high levels in rapidly dividing cells. However, the clinical value of Ki-67 and PCNA is inconsistent, and their correlation with prognosis varies with cancer type.

Proteins related to cell cycle and apoptosis, such as p53, survivin, cyclin D1, and cyclin E, have also been associated with malignancy and may serve as molecular markers of disease. The p53 tumor suppressor protein is involved in cell cycle checkpoint control and prevents cells from progressing through the cell cycle in the event of DNA damage. Although immunohistochemical analysis shows increased p53 expression in prostate cancer, inactivating mutations are associated with disease progression (Moul, 1999; Theodorescu *et al.*, 1997). Similarly, p53-inactivating mutations are associated with poor prognosis in breast and lung cancer (Powell *et al.*, 2000; Steels *et al.*, 2001). On the other hand, increased expression of survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, has been observed in multiple tumor types (reviewed in Altieri, 2003). Clinical studies show that survivin expression is associated with poor prognosis for breast cancer (Span *et al.*, 2004) and is associated with more aggressive forms of prostate and liver cancer (Morinaga *et al.*, 2004; Shariat *et al.*, 2004).

More recently, within this group of cell cycle regulatory proteins cyclin D1 and cyclin E, which promote the G1/S transition, have emerged as useful molecular markers for clinical cancer research. Overexpression of cyclin D1 is correlated with malignancy, metastasis, and progression for many types of cancer (reviewed in Fu *et al.*, 2004), while high levels of cyclin E are linked to poor prognosis of ovarian, breast, and lung cancer (Farley *et al.*, 2003; Lindahl *et al.*, 2004; Muller-Tidow *et al.*, 2001). Although the cyclins serve as molecular markers, therapeutic strategies that target cell cycle control have focused on inhibition of cyclin-dependent kinases (CDKs), which are the effector molecules associated with cyclins (Shapiro, 2004). However, strategies that decrease cyclin D1 or cyclin E expression or increase stability of CDK inhibitors, such as $p27^{kip1}$ and $p21^{cip1}$, are also of value (Swanton, 2004).

Tumor vascularization is an unfavorable predictor of metastasis and survival. Vascular endothelial growth factor (VEGF), which promotes neovascularization, is indicative of angiogenesis for several types of cancer (Callagy *et al.*, 2000; Gaffney *et al.*, 2003; Paley *et al.*, 1997; Strohmeyer *et al.*, 2000). A study demonstrated that increased VEGF expression correlated with increased microvesicle density in prostate tumor biopsies and that VEGF expression also correlated with disease progression (Strohmeyer *et al.*, 2004). Vascularization of tumors increases chances of metastasis by connecting tumors with the circulatory system. Consequently, brain metastases of breast cancer show increased VEGF expression in xenograft tumor models (Kim *et al.*, 2004).

Prostate-specific antigen (PSA) is one of the most reliable cancer-specific molecular markers. PSA expression is, for the most part, restricted to the prostate and elevated serum PSA indicates the possibility of malignant prostate cancer. Use of serum PSA as a diagnostic tool is still controversial since PSA levels are also elevated in benign prostatic hyperplasia (BPH),

which is a nonmalignant growth of the prostate gland (Gittes, 1991). Nevertheless, serum PSA levels are used effectively to monitor prostate cancer recurrence and progression following androgen withdrawal therapy (Sadar *et al.*, 1999). The prostate-specific membrane antigen (PSMA) has emerged as a reliable marker for prostate cancer diagnosis, while prostate stem cell antigen (PSCA) has provided a prostate-specific therapeutic target (reviewed in Tricoli *et al.*, 2004).

Molecular markers for breast cancer include osteopontin (OPN), estrogen and progesterone receptors (ER and PR, respectively), and Her2/neu. Although the role of OPN in breast cancer is not fully understood, convincing clinical evidence demonstrates that increased OPN expression in breast carcinoma cells is associated with malignancy and that plasma OPN is elevated in women with breast cancer metastasis (Singhal *et al.*, 1997; Tuck and Chambers, 2001; Tuck *et al.*, 1998). OPN is a secreted glycoprotein that is particularly abundant in bone and its binding to cell-surface integrins results in integrin-mediated signaling, which may be important for adhesion and migration of cancer cells to secondary sites (Allan *et al.*, 2005).

The ER, PR, and Her2/neu status of breast cancer are taken into consideration when choosing treatment strategies. Tumors that express ER and PR are more likely to respond to hormone therapy (Early Breast Cancer Trialists' Collaborative Group, 1992; Bardou *et al.*, 2003; McGuire, 1978), while overexpression of Her2/neu may indicate increased sensitivity to the therapeutic anti-Her2/neu antibody trastuzumab (Herceptin) (Baselga *et al.*, 1996; Pegram *et al.*, 1998). Isoforms of ER (ER-α and ER-β) and PR (PR-A and PR-B) have been identified, and expression of receptor isoforms must be taken into account when choosing therapeutic strategies (Fuqua and Cui, 2004). Her2/neu overexpression in breast cancer correlates with higher histological tumor grade, more aggressive disease, and poor prognosis (Menard *et al.*, 2001; Slamon *et al.*, 1987), possibly by increasing cell proliferation, invasiveness, and/or tumor angiogenesis (Ignatoski *et al.*, 2000; Petit *et al.*, 1997).

The growing number of putative molecular markers of cancer suggests that a single marker is not likely to be sufficient for predicting either disease outcome or response to treatment. Consequently, high-throughput genomic and proteomic approaches that have the capacity to assess expression of multiple biomarkers at the same time are becoming increasingly important in determining prognostic signatures of disease.

In a key study by van't Veer *et al.* (2002), DNA micro array analysis of 117 primary breast cancer tumors was used to identify a "poor prognosis" gene expression signature, consisting of 70 genes, that was highly prognostic for development of metastases and for overall survival. Minn *et al.* (2005) demonstrated that this poor prognosis genetic signature was unable to predict organ-specific metastatic potential. However, an experimental

metastasis xenograft model was used to identify a bone metastasis gene signature that had clinical relevance when applied retrospectively to genetic profiles of metastatic primary human tumors (Kang *et al.*, 2003; Minn *et al.*, 2005).

Although still in its infancy, clinical cancer proteomics also aims to identify molecular markers for detection, prognosis, and response to treatment, as well as novel therapeutic targets. Both matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Celis *et al.*, 2004; Le Naour *et al.*, 2001; Yanagisawa *et al.*, 2003) and surface-enhanced laser desorption/ionization-mass spectrometry (SELDI-MS)–based protein chip arrays (Carter *et al.*, 2002; Liu *et al.*, 2005; Shiwa *et al.*, 2003; Wong *et al.*, 2004) have been used to identify cancer biomarkers and to generate baseline protein expression profiles of malignant tumors.

The complexity of the signaling events in cancer progression and the multidimensional nature of the disease suggest that molecular portraits of tumors are likely to be more relevant than individual biomarkers as diagnostic and prognostic indicators. Ultimately, these molecular profiles will allow not only a more precise evaluation of dietary intervention strategies, such as lycopene, but also diagnostic and prognostic assessment. It will also allow clinicians to monitor patients' responses to therapy and to optimize treatment strategies.

3. *Prostate cancer*

Of all the cancers, the role of lycopene in the prevention of prostate cancer has been studied the most. The supporting evidence for lycopene comes from tissue culture, animal, epidemiological, and human experimental studies and was reviewed by Rao and Agarwal (1999). Lycopene, β-carotene, canthaxanthin, and retinoic acid were all shown to inhibit the growth of the DU145 prostate cancer cells (Hall, 1996). Kotake-Nasra *et al.* (2001) evaluated the effects of several carotenoids including lycopene on the viability of three human prostate cancer cell lines, PC-3, DU145, and LNCaP. The viability of all three cell lines was significantly reduced by lycopene and other carotenoids present in foods. Kim *et al.* (2002) measured the effect of lycopene on the proliferation of LNCaP human prostate cancer cells in culture. A new, water-dispersible lycopene was used in this study at concentrations of 10^{-6}, 10^{-5}, and 10^{-4} M. Lycopene at concentrations of 10^{-6} and 10^{-5} M significantly reduced the growth of LNCaP cells after 48, 72, and 96 hours of incubation by 24.5–42.8%. In a follow-up study, the authors expanded the concentration range of lycopene tested to 10^{-9} and 10^{-7} M. A dose-dependent decrease in cell growth was observed. The authors suggested that lycopene as an antioxidant may play an important role in

treating prostate cancer. In a review article (Heber and Lu, 2002) it was pointed out that lycopene, at physiological concentrations, can inhibit cancer cell growth by interfering with growth factor receptor signaling and cell cycle progression, specifically in prostate cancer cells, without any evidence of toxic effects or apoptosis of cells. Overall, *in vitro* tissue culture studies suggest that lycopene at physiological concentrations can reduce the growth of both androgen-dependent and androgen-independent prostate cancer cells. In an *in vivo* study, when men with localized prostate adenocarcinoma consumed tomato sauce-based pasta dishes providing 30 mg of lycopene per day for 3 weeks, the cells from prostate biopsies at the baseline and post-intervention resected tissues showed significant reduction in DNA damage (Bowen *et al.*, 2002).

Animal models have provided excellent systems to investigate *in vivo* biochemical consequences of administering lycopene in a well-defined, controlled environment, where the confounding variables could be kept to a minimum. Using laboratory mice, the radioprotective as well as antibacterial activities of lycopene were established almost 40 years ago (Forssberg *et al.*, 1959; Lingen *et al.*, 1959). Guttenplan *et al.* (2001) studied the effect of ingesting lycopene-rich tomato oleoresin at two doses on the *in vivo* mutagenesis in prostate cells of lacZ mice. Both short-term benzopyrene (BaP)-induced and long-term spontaneous mutagenesis was monitored. A nonsignificant inhibition of spontaneous mutagenesis in the prostate was observed only at the higher dose. However, lycopene was shown to inhibit mutagenesis slightly by the oleoresin. In another study (Boileau *et al.*, 2003), the effect of whole tomato powder (13 mg lycopene per kg diet), lycopene beadlets (161 mg lycopene per kg diet), and control beadlets (0 mg lycopene per kg diet) were evaluated for their effect on prostate cancer in a rat model. The results showed that the consumption of tomato powder, but not lycopene, inhibited prostate carcinogenesis. Based on these observations, the authors suggested that tomato products may contain compounds in addition to lycopene that modify prostate carcinogenesis. However, Limpens *et al.* (2004) pointed out that the levels of lycopene used in the form of beadlets may have been too high. In support of their argument, they tested the effect of two doses of synthetic lycopene (5 and 50 mg per kg body weight) in an orthotopic model of human prostate cancer cell line PC-346C in nude mice. Lycopene inhibited tumor growth and decreased PSA levels. This effect reached statistical significance only in the low-dose lycopene group suggesting that lycopene dosages might be relevant to their effects. Two other studies also reported on the effect of lycopene in prostate cancer. One study was conducted in mice using the 12T-10 Lady transgenic model, which spontaneously developed localized prostatic adenocarcinoma and neuroendocrine cancer followed by metastases that resembled the pathogenesis in humans (Venkateswaran

et al., 2004). Administration of lycopene, vitamin E, and selenium in combination was shown to dramatically inhibit prostate cancer development and increase the disease-free survival time. A strong correlation between the disease-free state and increased levels of the prognostic marker $p27^{Kip1}$ and a marked decrease in PCNA expression was observed giving some clues as to the mechanisms of action of the chemopreventive agents. In another study (Siler *et al.*, 2004), lycopene (200 ppm) alone, vitamin E (540 ppm) alone, or both of them in combination were tested in the MatLyLu Dunning prostate cancer model. They were used to supplement the diets for 4 weeks. Both lycopene and vitamin E accumulated in the tumor tissue and increased the necrotic area in the tumor. Lycopene interfered with local testosterone activation by downregulating 5-α-reductase and reducing the expression of steroid target genes. In addition, lycopene downregulated prostatic IGF-I and IL-6 expression. Based on these observations, the authors suggest that lycopene and vitamin E contribute to the reduction of prostate cancer by interfering with internal autocrine or paracrine loops of sex steroid hormones and growth factor activation and/or synthesis and signaling in the prostate. In contrast to these studies, lycopene had no effect on carcinogeneses with male F344 rats treated with 3,2′-dimethyl-4-aminobiphenol (DMAB) and 2-amino-1-methylimidazo[4,5-b]pyridine (PhIP) to induce prostate cancer (Imaida *et al.*, 2001).

Several epidemiological studies reported the inverse association between dietary lycopene intake and prostate cancer. In one of the earliest prospective trials (Mills *et al.*, 1989), a cohort of Seventh-Day Adventist men consuming high levels of tomato products, more than five times per week, had significantly decreased prostate cancer risk compared to the men consuming lower intakes of tomato products less than one time per week. The largest published Health Professionals Follow-up Study (Giovannucci *et al.*, 1995, 2002) reported an inverse relationship between the consumption of various tomato products and prostate cancer incidence. A 35% reduction in the risk of prostate cancer was observed for a consumption frequency of 10 or more servings of tomato products per week. The protective effect was stronger with more advanced or aggressive stages of prostate cancer. Of all the foods evaluated, tomato sauce was the strongest dietary predictor of reduced prostate cancer risk and serum lycopene levels. In another large nested case-control study, the Physicians Health Study (Gann *et al.*, 1999), prediagnostic plasma lycopene levels between confirmed cancer cases and controls were compared. A 41% reduction in the overall prostate cancer risk and a 60% reduction in patients with aggressive prostate cancer was observed in men with the highest plasma lycopene. All lycopene-supplemented groups together had an overall 37% reduction in risk. In this study, lycopene was the only carotenoid that significantly and consistently reduced the risk

of prostate cancer. Another nested case-control study (Hsing *et al.*, 1990) compared serum lycopene levels from men who developed prostate cancer with those of a matched group of control subjects. Results showed a modest 6.2% lower mean serum lycopene levels in subjects developing prostate cancer compared to the control group. Overall, of the several epidemiological studies, close to 60% of the studies observed a significant association between lycopene intake or plasma levels and the risk for prostate cancer. Comparing different studies is made difficult by the use of different methods for estimating the intake of lycopene, use of different databases, differentiating between the consumption of lycopene itself from the intake of lycopene-containing fruits and vegetables, and differences in the bioavailability of lycopene from different dietary sources.

There are only a few randomized, controlled clinical trials reported in the literature investigating the role of lycopene in prostate cancer. A recent case-control study assessed the status of oxidative stress and antioxidants in prostate cancer patients and compared them against age-matched control subjects (Rao *et al.*, 1999). Their dietary history was recorded in order to estimate the intake of lycopene. Fasting blood samples and biopsy tissue samples were obtained for analyses. Serum and tissue lycopene levels in prostate cancer patients were significantly lower by 44% than in the control subjects. None of the other carotenoids and vitamins A and E showed any differences (Figure 8). Analysis of the serum biomarkers of oxidation showed a significantly higher level of protein oxidation in the cancer patients compared to the control subjects. Significantly higher levels of serum PSA and lower levels of protein thiols were observed in the prostate cancer patients compared to their age-matched controls (Figure 9). Based on the observation that only lycopene levels were significantly lower in the prostate cancer patients, authors of the study suggested that lycopene is used preferentially as the dietary antioxidant. In a follow-up pharmacokinetic study (Rao, 2002a), use of lycopene by prostate cancer patients was investigated and compared with their age-matched controls. Following the collection of a fasting blood sample, 50 mg of lycopene in the form of tomato juice as the source of lycopene was given as a single dose. Hourly blood samples were then collected and analyzed for serum lycopene levels. Results are shown in Figure 10. It was concluded that the utilization rates of lycopene by both groups were similar. However, absorption of lycopene by prostate cancer patients seemed lower than the controls. The reasons for this observation are not fully understood. Two new studies addressed the role of lycopene in treating prostate cancer. In one trial (Kucuk *et al.*, 2001) newly diagnosed prostate cancer patients were randomly assigned to receive 15 mg lycopene or no supplement for a period of 3 weeks before radical prostatectomy. Seventy-three percent of the men in the intervention group receiving

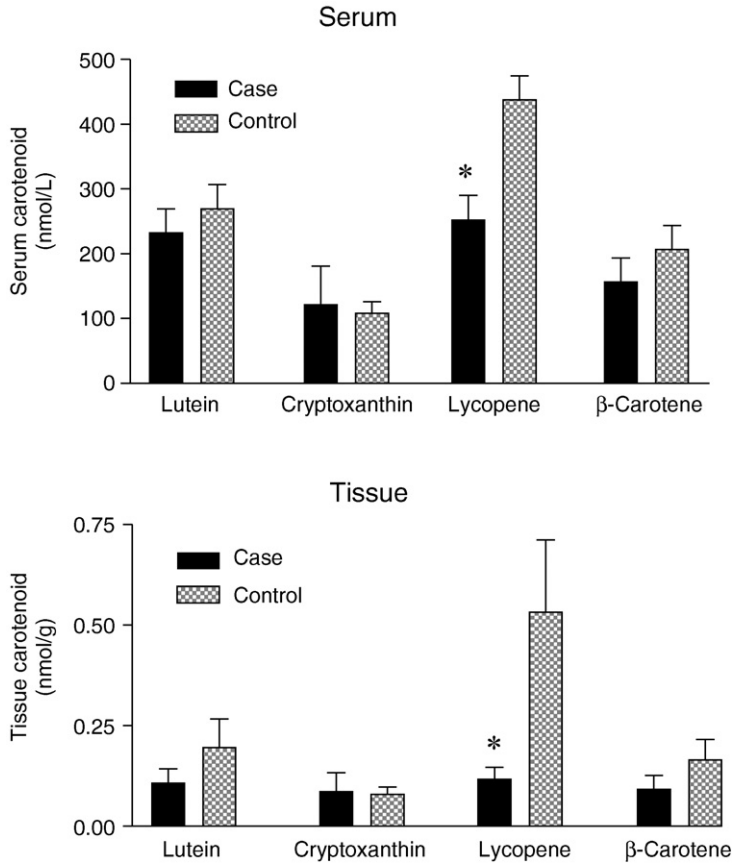

FIG. 8 Serum and tissue carotenoid levels in prostate cancer cases and controls (*$p < 0.05$). (Lycopene, tomatoes and health: New Perspectives 2000. Reprinted from Lycopene and the prevention of chronic diseases. Major findings from five international conferences. 2002. A.V. Rao, D. Heber, eds., p. 22. By permission of Caledonian Science Press.)

lycopene compared to 18% in the control group showed no involvement of surgical margins and/or extraprostate tissue with cancer. A statistically significant lower percentage of diffuse involvement of the prostate by high-grade prostatic intraepithelial neoplasms was also observed in the lycopene-treated group compared to the controls. Prostatic lycopene levels were significantly higher in the intervention group with a modest decrease in the serum PSA levels. A second study by the same authors (Kucuk *et al.*, 2002) also confirmed the previous observation that lycopene supplements reduced the growth of prostate cancer. These observations raise the possibility that lycopene may be used to treat patients with established prostate cancer.

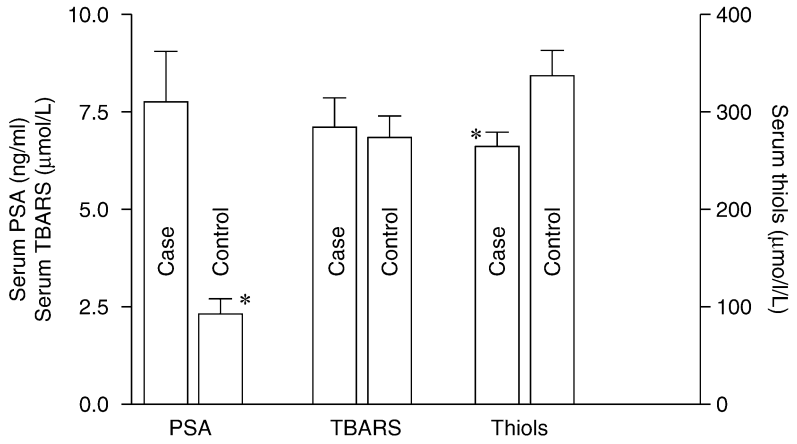

FIG. 9 Serum PSA, thiobarbituric acid substances (TBARS), and thiols in prostate cancer cases and controls (*$p < 0.05$). (Lycopene, tomatoes and health: New Perspectives 2000. Reprinted from Lycopene and the prevention of chronic diseases. Major findings from five international conferences. 2002. A.V. Rao, D. Heber, eds., p. 23. By permission of Caledonian Science Press.)

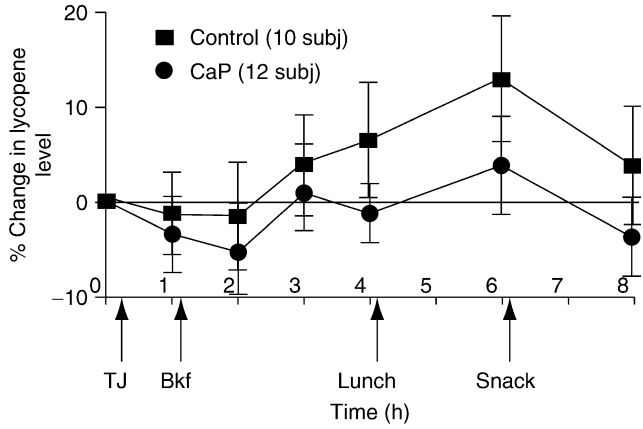

FIG. 10 Lycopene absorption kinetics in prostate cancer patients and controls. CaP, cancer patients; TJ, tomato juice; Bkf, breakfast. (Lycopene, tomatoes and health: New Perspectives 2000. Reprinted from Lycopene and the prevention of chronic diseases. Major findings from five international conferences. 2002. A.V. Rao, D. Heber, eds., p. 24. By permission of Caledonian Science Press.)

Other studies also provide supporting data for the role of lycopene in treating prostate cancer. When tomato sauce was used as a source of lycopene, providing 30 mg lycopene per day for 3 weeks preceding prostatectomy in

men diagnosed with prostate cancer, serum and prostate lycopene levels were elevated significantly (Bowen *et al.*, 2002). Oxidative damage to DNA was reduced and serum PSA levels declined significantly by 20% with lycopene treatment. In a study from India (Ansari and Gupta, 2003), where patients with metastasized prostate cancer were given either orchidectomy alone or in combination with 2 mg lycopene twice daily and followed for 2 years, serum PSA levels in the lycopene group were reduced more markedly than in the group with orchidectomy alone. Improvement was also observed in urine flow rates, bone scans, and survival. However, some questions regarding the relatively low doses of lycopene and the lack of dietary controls have been raised with regards to this study, requiring further studies to be undertaken in the future. Two new clinical trials sponsored by the National Cancer Institute are currently under way. The first of these two studies is a phase I investigation of lycopene for the chemoprevention of prostate cancer. In this study, healthy subjects with a baseline serum lycopene level of less than 600 nM will be given different doses of lycopene to evaluate dose-limiting toxicity and the maximum tolerated dose of lycopene given orally. The second study, a randomized control trial, will compare the effectiveness of lycopene and isoflavone administered in different doses with multivitamin supplements prior to surgery for the treatment of patients with stage I and stage II prostate cancer. A multivitamin supplementation group will be used as the control. Results from these studies will contribute significantly to our understanding of the role of lycopene in the treatment of established prostate cancer.

4. Other cancers

In addition to prostate cancer, there is growing evidence to indicate that lycopene may also play a role in the prevention of cancer of other sites including breast, lung, gastrointestinal, cervical, ovarian, and pancreatic cancers (Giovannucci, 1999). As with prostate cancer, the main evidence in support of the role of lycopene in the prevention of these cancers comes from cell culture, animal, and epidemiological studies. No clinical and dietary intervention studies have so far been reported. Lycopene was shown to interfere with cell cycle progression and IGF-I signaling in MCF-7 mammary cancer cells (Karas *et al.*, 2000). Inhibition of the proliferation of estrogen-dependent and estrogen-independent human breast cancer cells, MCF-7 and MDA-MB-231, treated with lycopene and other carotenoids was reported by Prakash *et al.* (2001). In another study, lycopene caused only a modest inhibition of the MCF-7 human mammary cancer cells compared to an open chain analogue of retinoic acid (Ben-Dor *et al.*, 2001). Inhibition of human breast and endometrial cancer cells by lycopene

is generally associated with the inhibition of cell cycle progression at the G (1) phase. When human breast cancer cells synchronized in the G (1) phase were treated with lycopene, the reduction in cell cycle progression was associated with reduction in the cyclin D levels and retention of p27, leading to the inhibition of G (1) CDK activities (Ben-Dor *et al.*, 2001). Multidrug resistance (MDR) of a majority of human tumor cells is responsible for the failure of therapeutic treatments. When mouse lymphoma and human breast cancer cells transfected with the MDR-1 gene were treated with lycopene, apoptosis of the cancer cells was induced, suggesting it as a possible drug resistance modifier in cancer therapy (Molnar *et al.*, 2004). In another study (Amir *et al.*, 1999), lycopene reduced cell cycle progression and differentiation in HL-60 promyelocytic leukemia cells. In the same study, lycopene had a synergistic effect with $1,25(OH)_2D_3$ on cell proliferation and differentiation and an additive effect on cell cycle progression. Oxidized lycopene, in a separate study, enhanced the inhibition of the growth of leukemia cells much more than the unoxidized lycopene (Nara *et al.*, 2001). Lycopene had similar inhibitory effects on the proliferation and differentiation of oral cancer cells (Livny *et al.*, 2002, 2003) and rat ascites hepatoma cells (Kozuki *et al.*, 2000).

Several animal studies have reported on the role of lycopene in cancers other than prostate. Lycopene inhibited the growth and development of C6 glioma cells (malignant brain cells) transplanted into rats (Wang *et al.*, 1959). The growth inhibition was more pronounced when it was given before the inoculation of glioma cells. Chronic intake of lycopene was shown to markedly delay the onset and reduced spontaneous mammary tumor growth and development in SHN virgin mice (Nagasawa *et al.*, 1995). This effect was associated with reduced mammary gland thymidylate synthetase activity and lowered levels of serum-free fatty acids and prolactin, a hormone known to be involved in breast cancer development by stimulating cell division. In another study (Sharoni *et al.*, 1997), a DMBA-induced rat mammary tumor model was used to compare the effect of lycopene-enriched tomato oleoresin with β-carotene on the initiation and progression of tumors. The lycopene-treated rats developed significantly fewer tumors, and the tumor area was smaller than the unsupplemented rats. β-Carotene showed no protection against the development of mammary cancer. However, when *N*-methylnitrosourea (MNU) was used to induce mammary tumorigenesis, lycopene had no effect on tumor incidence, latency, multiplicity, volume, or total tumor per group in rats (Cohen *et al.*, 1999). Colon cancer has also been studied using animal models. Several chemical carcinogens including azoxymethane (AOM), dimethylhydrazine (DMH), and MNU were used to induce carcinogenesis. The preneoplastic marker, aberrant crypt foci (ACF), was used in most of these experiments as the preneoplastic marker of carcinogenesis. In one study, lycopene and tomato juice

showed no effect on the incidence of colon cancer in B6C3F$_1$ mice (Kim *et al.*, 1998). Narisawa *et al.* (1998) reported that tomato juice but not lycopene significantly reduced the incidence of colon cancer in female F344/NSlc rats treated with MNU. In a study conducted in our laboratory (Jain *et al.*, 1999), the effect of lycopene on colon cancer was evaluated. Male Fischer 344 rats were treated with AOM to induce ACF in the colon. Lycopene in the form of 6% oleoresin was incorporated into an AIN93M diet at a concentration of 10 ppm lycopene. The ACF in lycopene-fed rats showed reduced number and size compared to the control group of rats. These effects were more pronounced when lycopene was fed during the promotion stage of carcinogenesis than during the initiation stage. In the same study, ingestion of lycopene by the rats reduced lipid and protein oxidation. Other animal studies showed similar protective effect of lycopene against lung (Kim *et al.*, 1997, 2000), liver (Gradelet *et al.*, 1998), urinary bladder (Okajima *et al.*, 1998), and hamster cheek pouch cancers (Bhuvaneswari *et al.*, 2002).

An extensive review of the epidemiological studies on the role of tomatoes, tomato-based products, and lycopene in cancer was published by Giovannucci (Giovannucci, 1999; Giovannucci *et al.*, 2002). Other studies showing the effect of lycopene on reducing the risk of stomach, other digestive tract, lung, breast, and cervix cancers were reviewed in the same articles (Giovannucci, 1999; Giovannucci *et al.*, 2002). Overall, consumption of lycopene-rich sources of foods, such as tomatoes and tomato-based products, show encouraging outcomes. However, the number of such studies at present is few, requiring further research in this important area of diet and cancer prevention.

C. CARDIOVASCULAR DISEASES

CHD is the major cause of death in North America and the rest of the Western World. It is also recognized as an important contributor of morbidity and mortality in the developing countries of the world. The emphasis so far has been on the relationship between serum cholesterol level as a biomarker for the risk of CHD. Oxidative stress induced by ROS is also considered to play an important part in the etiology of several chronic diseases including CHD (Ames *et al.*, 1993, 1996; Halliwell *et al.*, 1992, 1995; Pincemail, 1995; Stadtman, 1992; Witztum, 1994). Oxidation of the circulating LDL that carries cholesterol into the blood stream to oxidized LDL (LDL_{ox}) is thought to play a key role in the pathogenesis of arteriosclerosis, which is the underlying disorder leading to heart attack and ischemic strokes (Heller *et al.*, 1998; Parthasarathy *et al.*, 1992; Witztum, 1994) (Figure 11).

Antioxidant nutrients are believed to slow the progression of arteriosclerosis because of their ability to inhibit the damaging oxidative processes

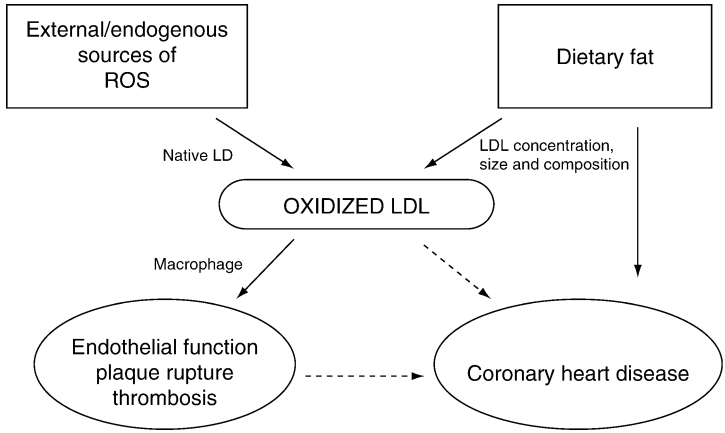

FIG. 11 LDL oxidation and coronary heart disease (Rao and Balachandran, 2004). (Role of antioxidant lycopene in heart disease. Reprinted from Antioxidants and cardiovascular disease. 2004. R. Nath, M. Khullar, Singal P.K., eds., pp. 62–83. By permission of Narosa Publishing House.)

(Heller *et al.*, 1998; Morris *et al.*, 1994; Parthasarathy *et al.*, 1992). Several retrospective and prospective epidemiological studies have shown that consumption of antioxidant vitamins, such as vitamin E, and β-carotene may reduce the risk of CHD (Agarwal and Rao, 2000a; Kohlmeier *et al.*, 1997). Randomized clinical trials also support the reduced risk for CHD with vitamin E supplementation (Stephens *et al.*, 1996; Virtamo *et al.*, 1998; Zock and Katan, 1998). The protective effect of vitamin E observed in these studies has been ascribed to its antioxidant properties. Support for the protective effect of antioxidants also comes from the observations that men and women with CHD exhibit lower levels of circulating antioxidants (Parthasarathy, 1998). However, some large-scale human trials have failed to confirm the protective effect of β-carotene and reported inconclusive results with vitamin E. In the recently completed Heart Outcomes Prevention Evaluation (HOPE) Study, supplementation with 400 IU/d of vitamin E for 4.5 years did not result in any beneficial effects on cardiovascular events in patients at high risk (Hodis *et al.*, 1995).

Lycopene as a dietary antioxidant has received much attention. Epidemiological studies have shown an inverse relationship between the incidence of CHD and the intake of tomatoes and lycopene and serum and adipose tissue lycopene levels (Arab and Steck, 2000; Kohlmeier *et al.*, 1997). These observations have generated scientific interest in lycopene as a preventative agent for CHD. Unlike the number of studies on vitamin E and β-carotene, only a few similar studies have been performed with lycopene (Agarwal and Rao, 2000a; Arab and Steck, 2000; Kohlmeier and Hastings, 1995; Rao and Agarwal, 2000)

in the prevention of CHD. A number of *in vitro* studies have shown that lycopene can protect native LDL from oxidation and suppress cholesterol synthesis (Dugas *et al.*, 1998; Fuhramn *et al.*, 1997). In the J-774 A.1 macrophage-like cell line, lycopene at a concentration of 10 μM induced 73% inhibition in cholesterol synthesis from acetate. A slightly lower inhibition was also observed with β-carotene (Fuhramn *et al.*, 1997). In this study, both the carotenoids augmented the activity of the macrophage LDL receptor. However, in another study, dietary enrichment of endothelial cells with β-carotene but not lycopene inhibited the oxidation of LDL (Dugas *et al.*, 1999). The predictability of *in vitro* LDL oxidation as a marker of arteriosclerosis or CHD has been questioned (Zock and Katan, 1998). Similarly in animal model studies, the increased resistance of extracted LDL *in vitro* to oxidation does not necessarily correlate to reduced risk of arteriosclerosis (Diaz *et al.*, 1997).

The evidence in support of the role of lycopene in the prevention of CHD stems primarily from the epidemiological observations on normal and at risk populations. Present knowledge largely relies on the data obtained from dietary estimates or plasma values in relation to the risk of CHD. Epidemiological studies have suggested that a diet rich in a variety of fruits and vegetables results in lower risk of CHD. Fruits and vegetables are in general good sources of dietary carotenoids including lycopene. The antioxidant properties of lycopene may be responsible for the beneficial effects of these food products. Mediterranean diets rich in tomatoes, tomato products, lycopene, and other carotenoids are associated with the lower incidence of arteriosclerosis and CHD. One of the earlier studies that investigated the relationship between serum antioxidant status including lycopene and myocardial infarctions (Street *et al.*, 1994) reported an odds ratio of 0.75. However, in this study there were no controls for other variables such as age, health status, and diet. The strongest population-based evidence comes from a recently reported multicenter case-control study (EURAMIC) that evaluated the relationship between adipose tissue antioxidant status and acute myocardial infarction (Kohlmeier *et al.*, 1997). Subjects (662 cases and 717 controls) from 10 European countries were recruited to maximize the variability in exposure within the study. Needle aspiration biopsy samples of the adipose tissue were taken shortly after the infarction and the levels of α- and β-carotenes, lycopene, and α-tocopherol measured. Adipose lycopene levels expressed as mg/g of fatty acids varied from the lowest 0.21 to the highest 0.36. After adjusting for age, body mass index, socioeconomic status, smoking, hypertension, and maternal and paternal history of the disease only lycopene, and not β-carotene levels, was found to be protective with an odds ratio of 0.52 for the contrast of the 10th and 90th percentiles with a p value of 0.005. The results also showed a dose–response relationship between each quintile of adipose tissue lycopene and the risk of myocardial infarction.

The protective potential of lycopene was maximal among individuals with highest polyunsaturated fat stores. The odds ratios for lycopene of subjects who never smoked, ex-smokers, and smokers were 0.33, 0.41, and 0.63, respectively. These findings seem to support the antioxidant hypothesis. A component of this larger EUREMIC study representing the Malaga region of Spain was analyzed further (Gomez-Aracena *et al.*, 1997). In this case-control study consisting of 100 cases and 102 controls, adipose tissue lycopene levels showed an odds ratio of 0.39 with a 95% confidence interval of 0.13 and 1.19. The *p* value for the trend was 0.04. In the Arteriosclerosis Risk in Communities (ARIC) case-control study, fasting serum antioxidant levels of 231 cases and an equal number of control subjects were assessed in relationship to the intima-media thickness as an indicator of asymptotic early arteriosclerosis (Iribarren *et al.*, 1997). After controlling for other variables, an odds ratio of 0.81 was observed but the *p* value for the association was not significant. Statistical significance was observed only for β-cryptoxanthin, lutein, and zeaxanthin. In a cross-sectional study comparing Lithuanian and Swedish populations showing diverging mortality rates from CHD, lower blood lycopene levels were found to be associated with increased risk and mortality from CHD (Kristenson *et al.*, 1997). In the Austrian stroke prevention study, lower levels of serum lycopene and α-tocopherol were reported in individuals from an elderly population at high risk for microangiopathy-related cerebral damage, which is considered as a risk factor for cerebrovascular disease (Schmidt *et al.*, 1997).

Although the epidemiological studies done so far provide evidence for the role of lycopene in CHD prevention, it is at best only suggestive and not proof of a causal relationship between lycopene intake and the risk of CHD. Such a proof can be obtained only by performing controlled clinical dietary intervention studies in which both the biomarkers of the status of oxidative stress and the disease are measured. To date, very few such intervention studies have been reported in the literature. In one study when healthy human subjects consumed a lycopene-free diet for a period of 2 weeks, their serum lycopene levels decreased by 50% by the end of the 2 weeks and at the same time an increase of 25% in the *in vivo* lipid oxidation was observed (Rao and Agarwal, 1998a). In a small dietary supplementation study, six healthy male subjects consumed 60 mg/day lycopene for 3 months. At the end of the treatment period, a significant 14% reduction in their plasma LDL cholesterol levels was observed (Fuhramn *et al.*, 1997). As part of the same study, the authors investigated the effect of lycopene, *in vitro*, on the activity of macrophage HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis *in vitro*. Based on these observations, they concluded that dietary supplements of carotenoids may act as moderate

hypocholesterolemic agents (Fuhramn *et al.*, 1997). In a randomized, crossover, dietary intervention study 19 healthy human subjects (10 males and 9 females), nonsmokers and not on any medication and vitamin supplements, consumed lycopene from traditional tomato products and nutritional supplements for 1 week. The levels of lycopene consumption ranged from 20 to 150 mg/day. Lycopene was absorbed readily from all dietary sources resulting in a significant increase in serum lycopene levels and lower levels of lipid, protein, and DNA oxidation (Rao and Agarwal, 1998b). In the same study, serum lipoproteins and LDL oxidation were also evaluated. LDL oxidation was estimated by measuring the levels of thiobarbituric acid-reactive substances (TBARS) and conjugated dienes (CD). Although there were no changes in serum total cholesterol and LDL and HDL cholesterols, serum lipid peroxidation and LDL cholesterol oxidation were significantly decreased as the serum lycopene levels increased (Agarwal and Rao, 1988) (Figure 12).

The rationale for the protective role of dietary antioxidants, such as lycopene, is scientifically valid. However, only a few studies have so far been performed with lycopene. Cardiovascular and cerebrovascular diseases are progressively degenerative diseases consisting of several stages that eventually lead to death (Rao, 2002b). Lycopene has been shown to protect LDL oxidation that characterizes the early events of the disease. Population-based epidemiological studies that use death due to cardiovascular disease (CVD)

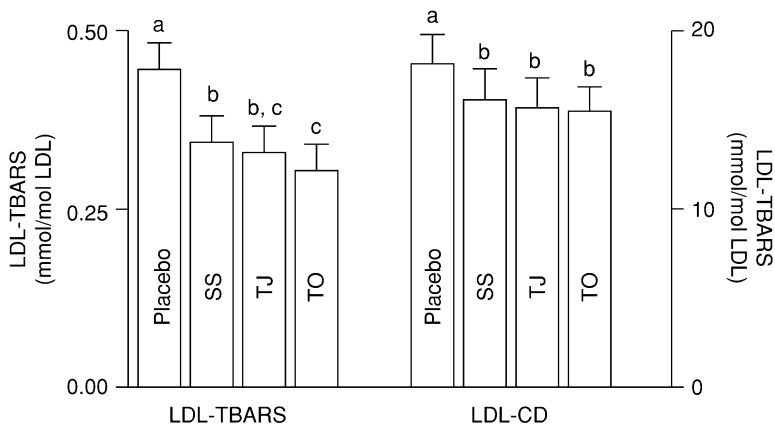

FIG. 12 Effect of dietary lycopene supplements on serum LDL oxidation (SS, spaghetti sauce; TJ, tomato juice; TO, tomato oleoresin). Bars with different letters are statistically different ($p < 0.05$). (Tomato lycopene and low density lipoprotein oxidation: A human dietary intervention study. Reprinted from Lipids. 1988; **33**, pp. 981–984 by permission of AOCS Press.)

as the end point have also provided evidence in support of the role of lycopene in the prevention of CVD. However, the link between early events in the disease, such as LDL oxidation, and the terminal outcome of death needs further studies. In the future, well-controlled clinical and dietary intervention studies evaluating the effectiveness of lycopene will provide useful information in the management of CVD. Important aspects of such studies will be to use well-defined subject populations, standardized outcome measures of oxidative stress and the disease, and lycopene ingestion that is representative of normal healthy dietary intakes.

D. OSTEOPOROSIS AND OTHER BONE DISORDERS

The role of lycopene in bone health to date is based on its potent antioxidant properties, the well-known role of oxidative stress in bone health, and the limited reported studies on the effects of lycopene in bone cells in culture. Therefore, in order to understand the role that lycopene can play in bone health, we have included a review of the reported studies on the role of oxidative stress in bone health and bone cells.

Bone is a dynamic tissue that is continuously being renewed throughout life by the process of bone remodeling involving the coupled events of removal of old bone by osteoclasts and formation of new bone by osteoblasts (for review see Chan and Duque, 2002; Kenny and Raisz, 2002; Mundy, 1999). The remodeling process is the result of the interactions of these cells with multiple molecular agents including hormones, growth factors, and cytokines. Disturbances in the remodeling process can lead to metabolic bone diseases (Lindsay and Cosman, 1990; Raisz, 1993). As will be reviewed later, oxidative stress, shown to control the functions of both osteoclasts and osteoblasts, may contribute to the pathogenesis of skeletal system including osteoporosis, the most prevalent metabolic bone disease.

1. Evidence associating oxidative stress and antioxidants with osteoporosis

ROS-induced oxidative stress is associated with the pathogenesis of osteoporosis. Epidemiological evidence suggests that certain antioxidants including vitamins C and E and β-carotene may reduce the risk of osteoporosis (Leveille *et al.*, 1997; Melhus *et al.*, 1999; Morton *et al.*, 2001; Singh, 1992) and counteract the adverse effects of oxidative stress on bone that is produced during strenuous exercise (Singh, 1992) and among smokers (Melhus *et al.*, 1999). Vitamins C, E, and A, uric acid, the antioxidant enzymes SOD in plasma and erythrocytes and GP_x in plasma were consistently lower in osteoporotic than in control subjects, while plasma levels of

malondialdehyde did not differ between groups. These results showed that antioxidant defenses are markedly decreased in osteoporotic women (Maggio *et al.*, 2003). Increased oxidative stress biomarker 8-*iso*-prostaglandin F alpha (8-iso-PGFα) is biochemically linked with reduced bone density (Basu *et al.*, 2001; Sontakke and Tare, 2002). The severity of osteoporosis was positively correlated with the level of the oxidative stress marker, lactic acid, in two men with mitochondrial DNA (mtDNA) deletions (Varanasi *et al.*, 1999), and a study of severe osteoporotic syndrome in relatively young males linked osteoporosis to an increase in oxidative stress (Polidori *et al.*, 2001). A correlation between serum glutathione reductases and bone densitometry values has been reported (Avitabile *et al.*, 1991). In spite of these reports, the cellular and molecular mechanisms involved in the role of oxidative stress in osteoporosis remain poorly defined.

Low bone density is also associated with oxidative stress in lower species. Thus, in ovariectomized rats melatonin has a bone-protective effect, which depends in part on its free radical-scavenging properties (Cardinali *et al.*, 2003). A mouse model that has been used to study the role of ROS in age-related disorders including osteoporosis is the accelerated mouse-senescence-prone P/2 (SAM-P/2) that generates increased oxygen radicals (Hosokawa, 2002; Udagawa, 2002). This model could be very useful in studying the role of lycopene in osteoporosis.

2. *Evidence associating oxidative stress and antioxidants with osteoblasts*

Very little work has been reported on the role of oxidative stress in osteoblasts. However, osteoblasts can be induced to produce intracellular ROS (Cortizo *et al.*, 2000; Liu *et al.*, 1999), which can cause a decrease in alkalinephosphatase (ALP) activity that is partially inhibited by vitamin E and cause cell death (Cortizo *et al.*, 2000; Liu *et al.*, 1999). Treatment of rat osteosarcoma ROS 17/2.8 cells with tumor necrosis factor-alpha (TNF-α) suppressed bone sialoprotein (BSP) gene transcription through a tyrosine kinase-dependent pathway that generates ROS (Samoto *et al.*, 2002). H_2O_2 modulated intracellular calcium (Ca^{2+}) activity in osteoblasts by increasing Ca^{2+} release from the intracellular Ca^{2+} stores (Nam *et al.*, 2002).

3. *Evidence associating oxidative stress and antioxidants with osteoclasts*

The mechanisms involved in the differentiation of osteoclasts and their ability to resorb bone are poorly understood. ROS may be involved in this process (Silverton, 1994). Both the H_2O_2 produced by endothelial cells (Zaidi

et al., 1993) intimately associated with osteoclasts and the H_2O_2 that is produced by osteoclasts (Bax *et al.*, 1992) increase osteoclastic activity and bone resorption. H_2O_2 may also be involved in the regulation of osteoclast formation (Suda *et al.*, 1993), differentiation of osteoclast precursors (Steinbeck *et al.*, 1998), and osteoclast motility (Bax *et al.*, 1992). The tartrate-resistant acid phosphatase (TRAP), found on the surface of osteoclasts, reacts with H_2O_2 to produce highly destructive ROS that target the degradation of collagen and other proteins (Halleen *et al.*, 1999). Superoxide was localized both intracellularly and at the osteoclast-bone interface using nitroblue tetrazolium (NBT), which is reduced to purple-colored formazan by ROS, suggesting the participation of superoxide in bone resorption (Key *et al.*, 1990) and in the formation and activation of osteoclasts (Garrett *et al.*, 1990). Osteoclastic superoxide is produced by NADPH oxidase (Darden *et al.*, 1996; Steinbeck *et al.*, 1994). However, Fraser *et al.* (1996) suggested that H_2O_2, not superoxide, stimulates bone resorption in mouse calvaria and that the earlier finding of stimulation by superoxide (Garrett *et al.*, 1990; Key *et al.*, 1994) may be due in part to conversion of this radical to H_2O_2. 1,25-Dihydroxyvitamin D_3 had a direct nongenomic effect on the generation of superoxide anion (O_2^-), which was inhibited by estrogen (Berger *et al.*, 1999). Estrogen has been reported to have an antioxidant property (Clarke *et al.*, 2001; Wagner *et al.*, 2001). Hormones known to stimulate bone resorption, such as parathyroid hormone (PTH) (Datta *et al.*, 1996) and $1,25(OH)_2D_3$, have stimulatory effects on ROS production in osteoclasts (Berger *et al.*, 1999) and hormones known to have inhibitory effects on bone resorption, such as calcitonin, inhibit ROS production (Berger *et al.*, 1999; Datta *et al.*, 1995).

Antioxidants also play a role in osteoclast activity. Osteoclasts produce the antioxidant enzyme SOD in the plasma membrane (Oursler *et al.*, 1991). ROS production in osteoclasts was inhibited after treating the cells with antioxidant enzymes such as SOD (Key *et al.*, 1990) and catalase (Suda *et al.*, 1993). ROS production in osteoclasts was also inhibited by estrogen (Berger *et al.*, 1999), the superoxide scavenger deferoxamine mesylate-manganese complex (Key *et al.*, 1994; Ries *et al.*, 1992), pyrrolidine dithiocarbamate (PDTC), and *N*-acetyl cysteine (NAC) (Hall *et al.*, 1995). The use of antioxidants from natural sources, such as fruits and vegetables, could be another way of inhibiting ROS. The use of lycopene in this regard is reviewed later.

4. In vitro *studies of lycopene in bone cells*

a. Effects of lycopene on osteoblasts. The studies on the effects of lycopene on osteoblasts are limited to two reports (Kim *et al.*, 2003; Park *et al.*, 1997). Kim *et al.* (2003) studied the effect of incubating osteoblast-like

SaOS-2 cells in 10^{-6} and 10^{-5} M lycopene or their respective vehicle controls on cell proliferation. They showed that lycopene stimulated cell proliferation as shown in Figure 13.

They also reported that lycopene had a stimulatory effect on ALP activity, a marker of osteoblastic differentiation in more mature cells, but depending on the time of addition, it had an inhibitory or no effect on younger SaOS-Dex cells (Figure 14). These findings constituted the first report on the effect of lycopene on human osteoblasts. In another study by Park *et al.* (1997), the effect of lycopene on MC3T3 cells (the osteoblastic cells of mice) was contrary to the findings of Kim *et al.* (2003). Park demonstrated that lycopene had an inhibitory effect on cell proliferation. Both studies, however, reported that ALP activity was stimulated. The discrepancy in the effect of lycopene on cell proliferation could be a result of species differences or experimental conditions. More studies are required to clarify the role of lycopene in osteoblasts.

b. Effects of lycopene on osteoclasts. To date, there are only two reported studies on the effects of lycopene in osteoclasts (Ishimi *et al.*, 1999; Rao *et al.*, 2003). Rao *et al.* (2003) cultured cells from bone marrow prepared from rat femur in 16-well calcium phosphate-coated Osteologic™ multitest slides (Millenium Biologix Inc.). Varying concentrations of lycopene in the

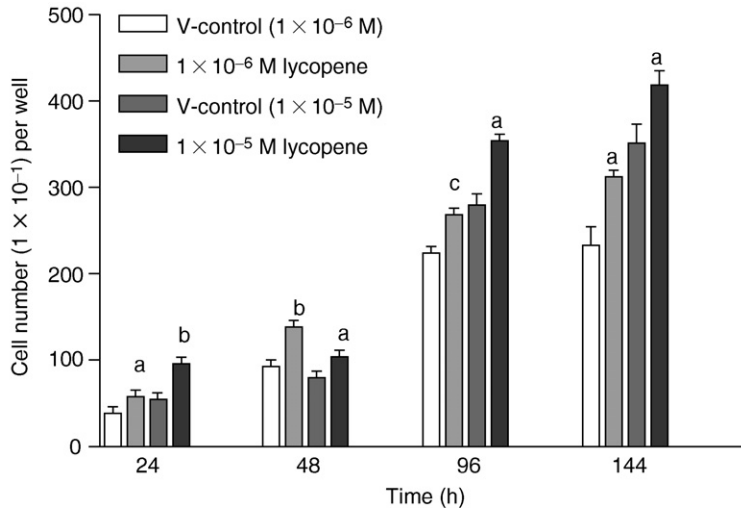

FIG. 13 Effect of lycopene on the proliferation of SaOS-2 cells. Compared with respective vehicle control of the same dilution: a = $p < 0.05$, b = $p < 0.001$, $c = p < 0.005$ (Kim *et al.*, 2003). (Lycopene II – Effect on osteoblasts: The carotenoid lycopene stimulates cell proliferations and alkaline phosphotase activity of SaOS-2 cells. Reprinted from Journal of Medicinal Food. 2003; **6**, pp. 79–86 by permission of Mary Ann Liebert, Inc., Publishers.)

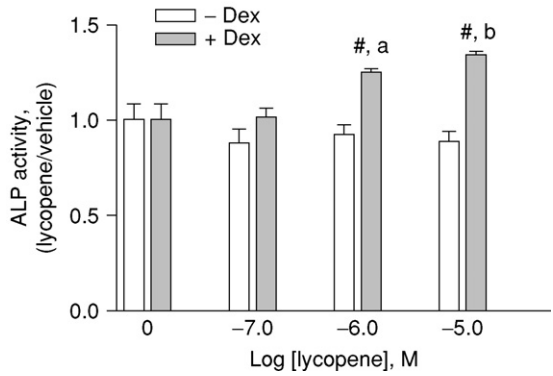

FIG. 14 Effect of lycopene on ALP activity # $p < 0.005$, comparison with zero control: a < 0.05, b < 0.01 (Kim *et al.*, 2003). (Lycopene II – Effect on osteoblasts: The carotenoid lycopene stimulates cell proliferation and alkaline phosphotase activity of SaOS-2 cells. Reprinted from Journal of Medicinal Food. 2003; **6**, pp. 79–86 by permission of Mary Ann Liebert, Inc., Publishers.)

absence or presence of the resorbing agent PTH-(1–34) were added at the start of culture and at each medium change every 48 hours. The effects of lycopene on mineral resorption are shown in Figure 15.

Lycopene inhibited the TRAP+ multinucleated cell formation in both vehicle- and PTH-treated cultures. The cells that were stained with the NBT reduction product formazan were decreased by treatment with 10^{-5} M lycopene, indicating that lycopene inhibited the formation of ROS-secreting osteoclasts (Figure 16).

Rao *et al.* (2003) 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 new and may be important in the pathogenesis, treatment, and prevention of osteoporosis.

The effects of lycopene on osteoclast formation and bone resorption were also reported by Ishimi *et al.* (1999) in murine osteoclasts formed in coculture with calvarial osteoblasts (Ishimi *et al.*, 1999). Their results differed from those of Rao *et al.* (2003) in that they found that lycopene inhibited the PTH-induced, but not the basal, TRAP+ multinucleated cell formation. Furthermore, they could not demonstrate any effect of lycopene on bone resorption. They also did not study the effect of lycopene on ROS production.

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

Postmenopause is associated with a global increase in bone turnover markers (Kushida *et al.*, 1995; Vernejoul, 1998). These markers predict bone loss

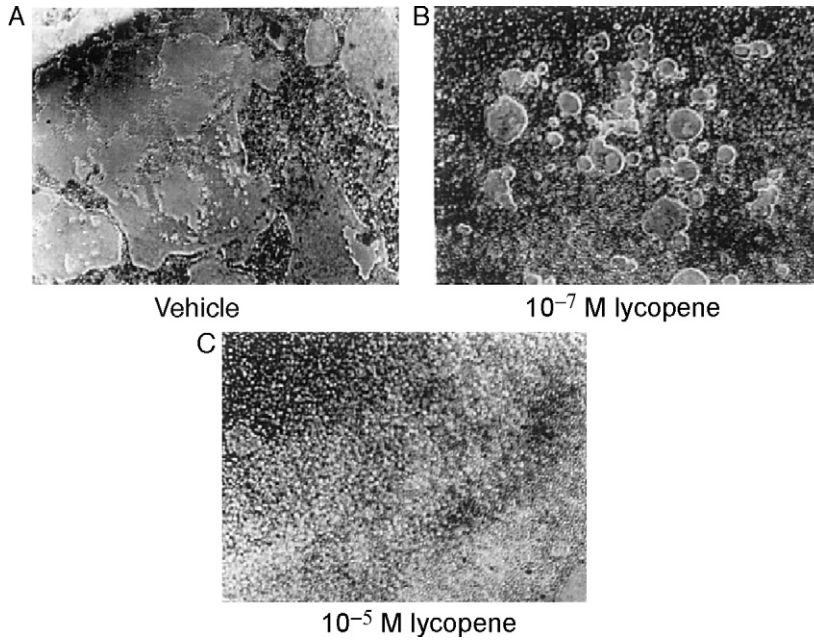

FIG. 15 Effect of lycopene on resorption of the calcium phosphate substrate coating of osteologic multitest slides in the presence of osteoclasts (Rao *et al.*, 2003). (Lycopene I – Effect on osteoclasts: Lycopene inhibits basal and parathyroid hormone-stimulated osteoclast formation and mineral resorption mediated by reactive oxygen species in ray bone marrow cultures. Reprint from Journal of Medicinal Food. 2003; **6**, pp. 69–78 by permission of Mary Ann Liebert, Inc., Publishers.)

and osteoporosis in postmenopausal women (Garnero *et al.*, 1996). One of the objectives of our current clinical study at St. Michael's Hospital is to test whether the serum lycopene correlates inversely with the oxidative stress parameters and bone turnover markers in postmenopausal women who are at risk for osteoporosis. Thirty-three women aged 50–60 were recruited and asked to complete a 7-day food intake record prior to giving fasting blood samples. Oxidative stress parameters, total antioxidant capacity, serum lycopene, and the bone turnover markers bone ALP (bone formation) and cross-linked N-telopeptides of type I collagen (NTx) (bone resorption) were measured from serum samples. The participants were grouped into quartiles according to their serum lycopene per kilogram body weight (nM/kg) and correlation analyses were carried out using the Newman-Keuls posttest. The most important and interesting findings to date are the correlation between lycopene intake and serum lycopene (Figure 17) indicating that lycopene is readily absorbed in the body, significant decreases in protein

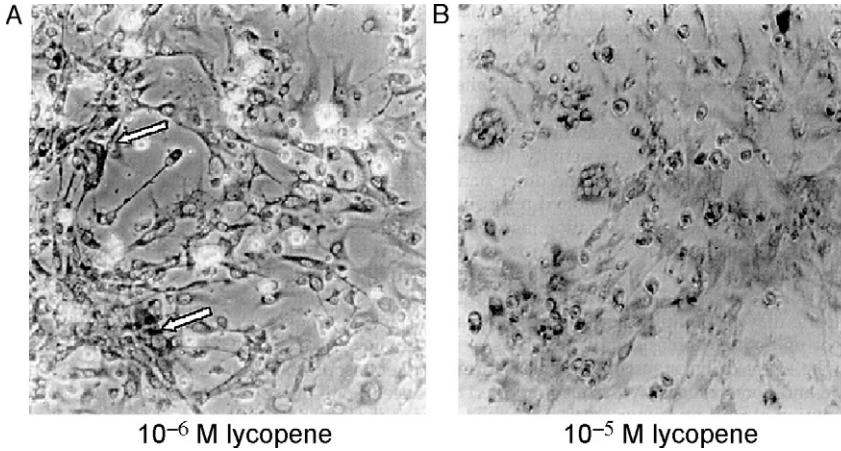

FIG. 16 Effect of lycopene on ROS production in osteoclasts (Rao *et al.*, 2003). (Lycopene I – Effect on osteoclasts: Lycopene inhibits basal and parathyroid hormone-stimulated osteoclast formation and mineral resorption mediated by reactive oxygen species in ray bone marrow cultures. Reprint from Journal of Medicinal Food. 2003; **6**, pp. 69–78 by permission of Mary Ann Liebert, Inc., Publishers.)

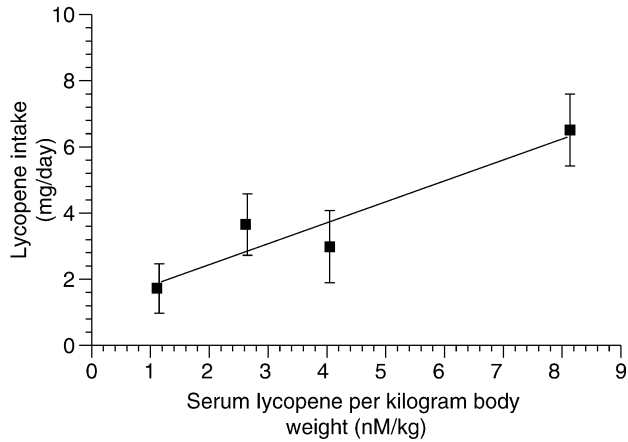

FIG. 17 Effect of lycopene intake on serum lycopene in 33 postmenopausal volunteers.

oxidation as indicated by increased thiols ($p < 0.05$) and decreased NTx values ($p < 0.005$) as levels of serum lycopene increase (Figure 18) (Rao *et al.*, 2005).

Since there was a significant positive correlation between serum lycopene levels and dietary lycopene intake as determined from the estimated food

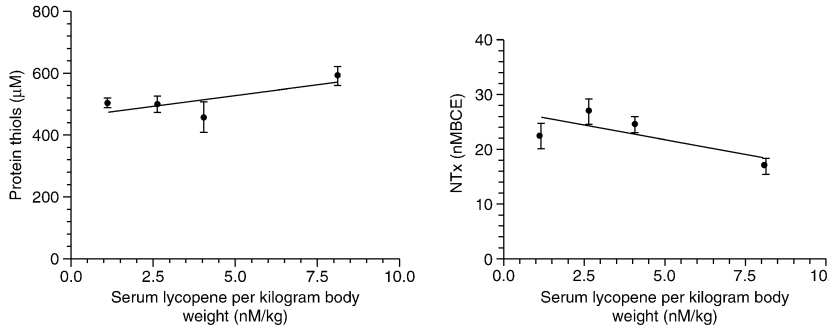

FIG. 18 Effect of serum lycopene on protein oxidation and bone turnover markers in 33 postmenopausal volunteers.

records ($p < 0.01$) (Figure 18), our results support the hypothesis that dietary lycopene acts as an effective antioxidant, reducing oxidative stress and bone turnover markers. Our observations suggest an important role for lycopene mediated via its antioxidant property in reducing the risk of osteoporosis. Dietary intervention studies with varying doses and sources of lycopene are currently being conducted to demonstrate the beneficial effects of lycopene in the prevention and management of osteoporosis.

E. HYPERTENSION

High blood pressure or hypertension is a major health problem effecting close to 25% of the adult population in North America. It is a condition commonly associated with narrowing of the arteries. It is known as the "silent killer" because there may be no symptoms until a person develops a fatal complication of the disease. Although the exact cause of hypertension is unknown, there are several factors and conditions that may contribute to its occurrence, including genetic factors, family history of hypertension, obesity, sedentary lifestyle, excess salt intake, alcohol, smoking, stress, age, hormone levels, abnormalities in the nervous and circulatory systems and kidneys, and the salt and water content in the body. Oxidative stress has also been implicated in the causation of hypertension (Friedman *et al.*, 2003; Lassegue and Griendling, 2004; Zini *et al.*, 1993). The ROS generated endogenously can effect multiple tissues, either directly or through nitric oxide depletion and includes contraction and endothelial dysfunction in the vasculature, hypertrophic remodeling in the blood vessels and myocardium, reabsorption of salt and decreased glomerular filtration in the kidney, and increased efferent sympathetic activity from the central nervous system (CNS). Although several pharmaceutical agents are used in the effective management of

hypertension, there has been considerable interest in the use of naturally occurring food components. Antioxidant polyphenols derived from green tea have been studied in this context and have been shown to be effective in controlling high blood pressure. Because of the potent antioxidant property of lycopene, it has also been studied for its role in hypertension. Paran and Engelhard (2001) using a single-blind, placebo-controlled trial studied the effect of tomato lycopene on blood pressure. Thirty, grade one hypertensive patients between the ages of 40–65 years, not requiring any blood pressure and lipid-lowering medications, were recruited into the study. After a 2-week run-in period for baseline evaluation, the patients were placed on 4-week placebo and 8-week treatment periods. The treatment consisted of ingesting tomato extract dietary supplement capsules that provided 15 mg lycopene every day. Results showed no significant changes in the diastolic blood pressure after the 8 weeks of treatment but did show a considerable reduction in the systolic blood pressure from the baseline value of 144 mm Hg to 134 mm Hg at the end of the lycopene treatment (Figure 19).

In another study (Moriel *et al.*, 2002), 11 patients with mild essential hypertension were compared with 11 healthy subjects for water- and lipid-soluble antioxidants and the concentrations of nitric oxide derivatives in the plasma. A significant reduction in plasma lycopene was observed in the hypertensive patients compared to the normal subjects. Similar reductions in ascorbate, urate, and β-carotene were also observed in this study. However, there were no differences in the nitrous oxide derivatives between the two groups. Hypertension and lymphatic circulation impairment are associated with liver cirrhosis. When patients with liver cirrhosis were compared to healthy matched controls, a significant reduction in serum lycopene, other carotenoid antioxidants, retinol, and α-tocopherol were observed in the cirrhotic patients. Based on these observations, the authors recommend thorough screening for the antioxidants and improved diet in the

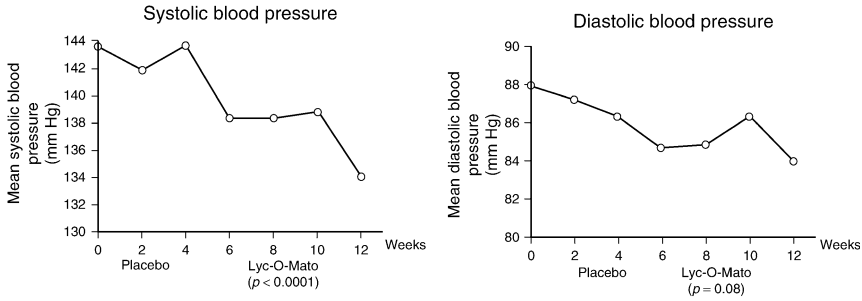

FIG. 19 Effect of tomato extract on systolic and diastolic blood pressure.

follow-up of liver cirrhosis patients and the link to hypertension (Rocchi *et al.*, 1991). A dietary approaches to stop hypertension (DASH) diet is recommended for lowering high blood pressure (Most, 2004). The DASH diet was designed to give beneficial levels of fibre, potassium, magnesium, and calcium. As such it contains more fruits, vegetables, and whole grains compared to control diets and is substantially higher in antioxidant phytochemicals. When the DASH diet was compared with the control diet, it was found to contain substantially higher levels of lycopene and other carotenoids, polyphenols, flavanols, flavanones, and flavan-3-ols. The beneficial effects of these phytochemicals in the management of blood pressure are now being recognized. Further clinical and intervention studies are required to better understand the role of lycopene in hypertension.

F. MALE INFERTILITY

Infertility affects 15% of all couples and at least 30–50% of these couples will have an abnormality detectable in the male partner, which contributes to their difficulty achieving a pregnancy. Therefore, it is estimated that about 7–10% of adult men in their reproductive years (20–50 years of age) are infertile and 25% of all men with infertility will have nonspecific or idiopathic infertility (Dubin and Amelar, 1971; Greenberg *et al.*, 1978; Johnson, 1975). For these men, medical therapy has generally been ineffective in improving sperm quality and fertility. Oxidative stress has been suggested as an important contributory factor in male infertility. Significant levels of ROS are detectable in the semen of up to 25% of infertile men, whereas fertile men do not produce detectable levels of ROS in their semen (Iwasaki and Gagnon, 1992; Zini *et al.*, 1993). The role of oxidative stress and antioxidants in male infertility is shown in Figure 20.

The identification of novel, less invasive treatments, such as vitamins and antioxidants, for male infertility could potentially have a great impact on the management of the infertile couple. To date, a small number of studies have evaluated the role of vitamins and antioxidants (mostly as single agents) in male infertility. In general, these studies suggest a beneficial role of antioxidant therapy in the treatment of male-factor infertility but additional studies are needed. In 1991, Fraga *et al.* (1991) demonstrated that dietary vitamin C has a beneficial effect on the integrity of sperm DNA (DNA oxidation) in male smokers. In a small placebo-controlled trial, Dawson *et al.* (1993) found that supplemental vitamin C improves sperm quality.

A number of investigators have evaluated the role of vitamin E and have reported improved sperm quality in controlled and uncontrolled trials (Geva *et al.*, 1996; Kessopoulou *et al.*, 1995; Suleiman *et al.*, 1996). In a small placebo-controlled trial, Lenzi *et al.* (1998) have reported that glutathione

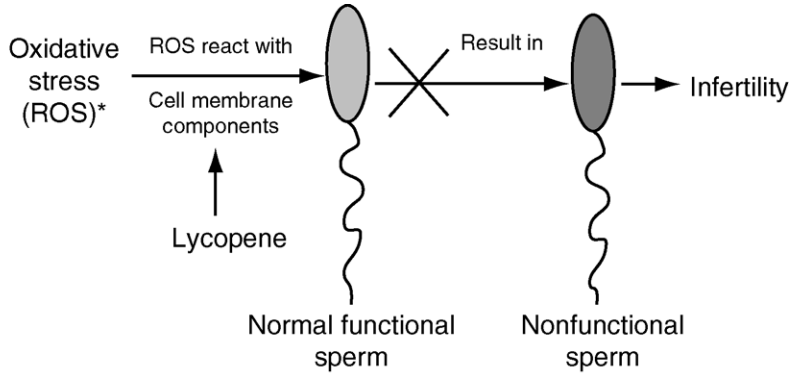

FIG. 20 Effect of oxidative stress and lycopene on sperm functionality.

(intramuscular) improves sperm quality significantly. The role of l-carnitine has been evaluated in uncontrolled trials with early promising results (Moncada *et al.*, 1992). Vitamins C and E and other antioxidants, including taurine (Alvarez and Storey, 1983), L-carnitine (Moncada *et al.*, 1992), and coenzyme Q10 (Alleva and Scaaraamucci, 1997; Lewin and Lavon, 1997), protect spermatozoa from oxidative stress *in vitro*. Since the recognition of lycopene as a potent antioxidant, and its preventive role in oxidative stress-mediated chronic diseases, researchers are beginning to investigate its role in protecting sperm from oxidative damage leading to infertility. Men with antibody-mediated infertility were found to have lower semen lycopene levels than fertile controls (Palan and Naz, 1996). In another study (Mohanty *et al.*, 2001), 50 infertile male volunteers between the ages of 21–50 years were recruited. The subjects had a normal hormonal profile of antisperm antibody titre and without any history of having taken any therapy for infertility or having obstructive azospermia. They consumed a daily dose of 8 mg lycopene in capsule form. The treatment was continued until sperm analysis showed optimal levels or until pregnancy of their partners was achieved. After a 12-month follow-up, it was reported that lycopene treatment resulted in significant increases in serum lycopene concentration. Significant improvements in sperm motility, sperm motility index, sperm morphology, and functional sperm concentration were also observed. The partners of 18 of the 50 subjects had successful pregnancies, accounting for a 36% success rate. Other studies are now in progress and their results will further advance our knowledge of the beneficial role of lycopene in male infertility (Figure 21).

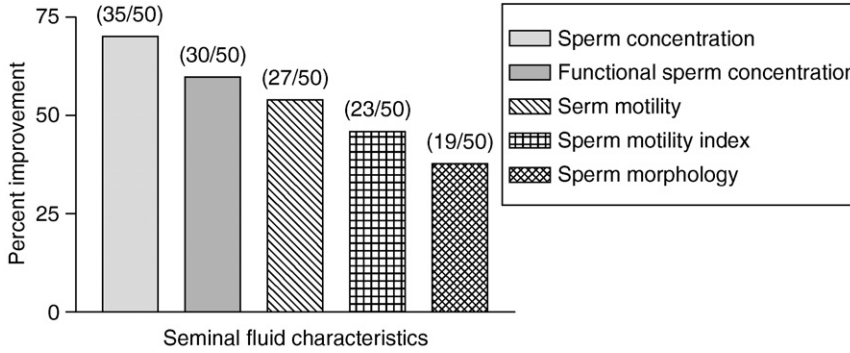

FIG. 21 Effect of lycopene on the sperm quality in infertile men (Mohanty *et al.*, 2001).

G. NEURODEGENERATIVE DISEASES

Neurodegenerative diseases (NDD) are a group of degenerative disorders of the nervous system, including the brain, spinal cord, and peripheral nerves. They include Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and epilepsy (Rao and Balachandran, 2003). They are a group of disorders with varied clinical importance and etiologies. Oxidative stress is now established as being an important causative factor as well as an ancillary factor in the pathogenesis of the NDD (Ebadi *et al.*, 1996; Singh *et al.*, 2004). The brain and nervous system are particularly vulnerable to free radical damage for a number of reasons as shown in Table VII.

The high lipid content of the nervous system, low antioxidant capacity, and the presence of iron, coupled with its high aerobic metabolic activity, make it particularly susceptible to oxidative damage (Halliwell, 1989; Rao and Balachandran, 2003; Rao and Rao, 2004). Several antioxidant systems have been shown to be effective in mitigating the neurotoxin effect of ROS. Important among them are the free radical-deactivating enzymes, SOD, glutathione peroxidase and catalase, free radical-scavenging agents that include vitamins A, C, and E, iron chelators, and selenium, and phytonutrients such as the carotenoids, flavonoids, and terpenoids (Rao and Balachandran, 2003; Singh *et al.*, 2004). Activity of the antioxidant enzymes was shown to be reduced in patients with Parkinson's disease (Ambani *et al.*, 1975; Fahn and Cohen, 1992; Kish *et al.*, 1985). As in Parkinson's, Alzheimer's, and Huntington's disease, and amyotrophic lateral sclerosis, increased levels of lipid peroxidation and oxidation of DNA were observed compared to controls, suggesting diets high in antioxidants might be effective in reducing the

TABLE VII
VULNERABILITY OF BRAIN TO FREE RADICAL DAMAGE

Brain consumes large quantities of oxygen for its relatively small weight contributing to the formation of ROS.
Membrane lipids in brain contain high levels of polyunsaturated fatty acid side chains that are prone to free radical attack.
Presence of iron and other transition metals in the brain can also contribute to the production of ROS.
Brain contains lower levels of antioxidant vitamins such as vitamins A, C, and E.
Brain contains low to moderate amounts of antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase, which play an important role in the inactivation of ROS.

risk of these diseases (Borlongan *et al.*, 1996; Ferrante *et al.*, 1997; Grant, 1997; Jenner, 1996; Retz *et al.*, 1998). Several *in vitro* studies have demonstrated the effectiveness of antioxidants in protecting nervous tissue from damage by the free radicals. Among the dietary antioxidants, most of the studies were directed at the role of vitamins A, C, and E and β-carotene. They were shown to prevent neuronal damage due to oxidative stress (Mitchell *et al.*, 1999). Similarly, in clinical trials, high doses of vitamins C and E were shown to reduce the rate of Parkinson's disease progression to the point of drug intervention by 2½ years (Fahn, 1991). Other epidemiological studies also provide supporting evidence for the role of vitamins A, C, and E and β-carotene in reducing the risk of NDD. Relatively small number of studies have been reported for the role of lycopene in NDD. In one study, significant reduction in lycopene levels was observed in Parkinson's disease and in vascular dementia (Foy *et al.*, 1999). The Austrian Stroke Prevention study showed lower serum lycopene and α-tocopherol levels to be associated with a high risk of microangiopathy (Schmidt *et al.*, 1997). In another case-control study of dietary risk factors for amyotrophic lateral sclerosis conducted in New England, a modest protective association was suggested for lycopene (Longnecker *et al.*, 2000). A correlation between high blood lycopene levels and functional capacity, such as the ability to perform self-care tasks, was reported in an elderly population in another study (Snowdon *et al.*, 1966). It is suggested that antioxidants, such as lycopene, may act directly on the neurons in an indirect manner affecting peripheral markers of oxidative stress (Sinclair *et al.*, 1998). The levels of lycopene in the CNS were present in much lower concentrations than in the other human tissues (Clinton, 1998). However, it is believed that lycopene can cross the blood–brain barrier and be effective in reducing the damage caused by ROS.

Although the present epidemiological, *in vitro*, and *in vivo* studies suggest an important role for lycopene in the prevention of NDD, further research needs to be done to gain a better understanding of its role in the managements of neuronal disorders that constitute an important human health problem globally.

H. OTHER HUMAN DISEASES

Since the recognition of the biological role of lycopene in the prevention of chronic diseases, the emphasis of the scientific community has been in the area of cancer, with special focus on prostate cancer. However, based on the hypothesis that oxidative stress may be an important etiological factor in the causation of most of the degenerative diseases and that lycopene is a potent antioxidant, the scientific community has started to study its role in diseases other than the ones reviewed in this chapter. These health disorders include skin and ocular diseases, rheumatoid arthritis, periodontal diseases, and inflammatory disorders. The scientific information pertaining to the role of lycopene in these diseases is still in its infancy. However, the rationale for undertaking these studies is scientifically valid and it is hoped that in the next 3–5 years several studies will be reported in the literature.

IX. DIETARY INTAKE LEVELS OF LYCOPENE

Since humans do not synthesize lycopene, it has to be provided through the diet. Estimating the dietary intakes of lycopene by various populations around the world is made difficult due to the variability in the reported levels of lycopene in food sources. However, several reports have appeared in the literature showing average daily intake levels of lycopene. Table VIII summarizes the reported levels of lycopene intake in different regions of the world (Block, 1994; Frorman *et al.*, 1993; Rao *et al.*, 1999; U.S. Department of Agriculture and Agricultural Research Service, 1998; U.S. Department of Agriculture and CSFII, 1994–1996). Other studies have indicated the average intake of lycopene in North America to be 5.3 mg/day. However, 50% of the population was shown to consume 1.86 mg/day or even less in some cases (Agarwal and Rao, 2000a; Rao and Agarwal, 1999).

Although there is a need to make more accurate estimations of lycopene intake by various populations, the general thinking among scientists is that the average intake levels of lycopene in North America are lower than the levels required for its beneficial biological effects. Hence, there may be a need to incorporate sources of lycopene, such as the tomato products, as part of a

TABLE VIII
LYCOPENE INTAKE LEVELS

Country	Average daily intake (mg)
United States of America	16.15
	5.93
	5.20
	3.70
Canada	25.20
Germany	1.30
United Kingdom	1.10
Finland	0.70

Source: Rao (2002a). Future Directions and intake recommendations. Reprinted from Lycopene and the prevention of chronic diseases. Major findings from five international conferences. 2002. A.V. Rao, D. Heber, eds., p. 43. By permission of Caledonian Science Press.

healthy diet and lifestyle. Lycopene supplements may also have a role in contributing to beneficial levels.

Until recently, nutritionists failed to recognize the importance of lycopene in human health due to its lack of provitamin A activity. As a result, at present, it is not considered as an ''essential'' nutrient and as such there are no established ''recommended daily intake'' (RDI) or ''recommended nutrient intake'' (RNI) levels for lycopene. However, with the recognition of the role of lycopene in human health, there is considerable interest now among the nutritionists and other health professionals to make ''suggestions'' based on scientific knowledge about daily intake levels. The main assumption regarding the role of lycopene in the prevention of cancer and other chronic diseases is that it has to be absorbed from the diet and be present at the site of its action (Rao and Agarwal, 1999). Information about the absorption and *in vivo* antioxidant properties of lycopene is therefore essential in formulating dietary guidelines. Serum and plasma levels of lycopene have been used extensively to assess the absorption of lycopene from dietary sources and to assess its biological significance. Adipose tissue levels of lycopene have also been used and are considered a more accurate reflection of lycopene status in the body. However, due to the invasive nature of collecting adipose tissue samples, circulatory levels are still considered as standard procedure to assess lycopene absorption. In addition to the levels of lycopene in serum/plasma, measuring the biomarkers of oxidative stress is also used to assess the biological activity of lycopene including its effect on the prevention of prostate cancer. The rationale being that in the presence of the antioxidant lycopene, the biomarkers of oxidative stress will be lower.

Since oxidative stress is related to the risk of chronic diseases, lower levels of oxidative stress are considered consistent with lower risk of these diseases. A few studies have also measured biochemical and pathobiological markers of cancer in patients after lycopene intervention (Kucuk *et al.*, 2001). However, the levels of lycopene used in these studies were based on preliminary studies investigating the absorption and oxidative stress status in healthy and at risk for cancer patients. Dose–response studies have not been undertaken.

In a recently reported study, when human subjects refrained from the consumption of lycopene-containing foods, their serum lycopene levels fell significantly within 2 weeks (Rao, 2002a; Rao and Agarwal, 1999). This observation was consistent with the fact that humans do not synthesize lycopene and have to be provided with dietary sources to maintain circulatory lycopene levels. Another study was undertaken to investigate the absorption of lycopene in healthy human subjects (Rao and Agarwal, 1998a). A total of 19 subjects (male and female) underwent a washout period during which they restrained from consuming foods that were known sources of lycopene. Following the washout period, subjects consumed tomato juice, tomato sauce, or lycopene supplements for a period of 1 week in a randomized crossover study design. Subjects also underwent a washout period between the treatments. Lycopene levels varied from 28 to 150 mg/day. Lycopene was found to be absorbed equally efficiently from all three sources of lycopene. Increased levels of serum lycopene paralleled significant reductions in lipid, protein, and DNA oxidation. Based on these studies, an intake level of 30–35 mg of lycopene per day was suggested. In a later study, lower levels of lycopene (5, 10, and 20 mg/day) from either tomato ketchup or lycopene supplement capsules were studied for their effect on serum lycopene levels and oxidative biomarkers (Rao and Shen, 2002). Once again, lycopene was absorbed equally well from both sources. Based on the results from this study, the previously "suggested" levels of lycopene intake of 30–35 mg/day were lowered to 7–8 mg/day. At these levels of intake, a maximum level of serum lycopene and reduction in lipid, protein, and DNA oxidation was observed.

Although the suggested level of 7–8 mg of lycopene per day is based on one recent study (Rao and Shen, 2002), a majority of other studies reported in the literature use a single level of intake of 15, 30, or 75 mg of lycopene to study its effect on prostate cancer and other diseases. Systematic dose-response studies have not yet been carried out using disease biomarkers as the end point. Under these conditions, the best estimate of lycopene intake is based on serum/plasma concentrations and biomarkers of oxidation.

X. CONCLUSIONS

Lycopene is a carotenoid antioxidant that has received considerable scientific interest in recent years. Although the chemistry and *in vitro* antioxidant properties of lycopene have been known for several years, its role in human health has just begun to be investigated. A publication in 1995 reported a significant inverse relationship between the intake and circulatory levels of lycopene and the risk of prostate cancer. We started our studies to investigate the bioavailability and *in vivo* antioxidant properties of lycopene and demonstrated that lycopene was absorbed readily from food sources and maintained its antioxidant properties *in vivo* as indicated by reduced levels of lipid, protein, and DNA oxidation. Till recently, most of the evidence in support of the role of lycopene in reducing the risk of cancers and other chronic diseases was based on epidemiological observations. Although these studies provide convincing evidence to suggest the beneficial role of lycopene in human health, they are at best indicative. More recently, several clinical and dietary intervention studies are beginning to be undertaken. It is to be expected that results from these and other studies that are yet to be undertaken will enhance our knowledge about lycopene and disease prevention and perhaps even the treatment of some chronic diseases. Equally important, these studies will help us understand the mechanisms of action of lycopene.

Oxidative stress is now being recognized as an important causative agent of many chronic diseases. Oxidation of lipids and in particular oxidation of LDL cholesterol is being associated with increasing the risk of CVD. Similarly, oxidation of DNA is associated with increased risk of cancers. Since most of the enzymes involved in metabolic and regulatory processes are proteins, its oxidation can lead to inactivation or improper activity of the enzymes, resulting in increased risk of diseases. The significance of these observations perhaps is to suggest that oxidative stress may be the main common feature of several diseases. Lycopene, being one of the most potent naturally occurring antioxidant, can be effective in reducing the risk of these diseases. Although the initial interest in lycopene was due to its role in the prevention of prostate cancer, it is now being investigated for its possible role in other diseases such as CVD, osteoporosis, hypertension, male infertility, macular degeneration, and diseases related to inflammation. Although the antioxidant property of lycopene is the major mechanism being investigated, evidence is beginning to appear which would suggest that the biological role of lycopene may also involve other mechanisms such as stimulating the GJC, immune stimulation, and metabolic regulations. These mechanisms need further research in the future.

Many questions still remain unclear regarding the biological role of lycopene. However, based on the current evidence, it would be prudent to include sources of lycopene as part of a healthy diet.

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