CAROTENOIDS AND CHRONIC DISEASES

Sanjiv Agarwal and A.V. Rao*

Department of Nutritional Sciences, University of Toronto, 150 College Street, Toronto, ON, Canada M5S 3E2

CONTENTS

---

* Author for correspondence; e-mail: v.rao@utoronto.ca

SUMMARY

Chronic diseases such as cancer and cardiovascular diseases are the major causes of deaths in North America. Dietary intake of fruits and vegetables has been suggested to have protective effects against such chronic diseases. Carotenoids are important plant pigments which are thought to contribute towards the beneficial effects of fruit and vegetable consumption. This review focuses on the role of carotenoids and particularly lycopene in chronic diseases.

KEY WORDS

carotenoids, cancer, cardiovascular disease, prevention, lycopene, oxidation, diet, tomato

1. INTRODUCTION

Cancer and cardiovascular diseases are the major causes of deaths in North America. Although genetic factors and age are important in determining the risk, dietary component is also a major risk factor associated with the diseases /1/. The role of reactive oxygen species (ROS) and oxidative damage to biomolecules is one of the main foci of recent research related to cancer and cardiovascular diseases. Oxidative stress has been widely postulated to be involved in the causation and progression of several chronic diseases /2-8/. Dietary antioxidants, which inactivate ROS and provide protection from oxidative damage /2-8/, are being considered as important preventive strategic molecules.

Recent dietary guidelines to combat chronic diseases recommend an increased consumption of plant foods including fruits, vegetables, cereals and legumes. Plant foods are rich sources of antioxidant phytochemicals, such as the carotenoids. In laboratory animal, cell culture, case control or cohort studies they appear to protect against the development of cancer /9-11/. Carotenoids are plant pigments commonly found in fruits and vegetables and are considered to be the important micronutrients responsible for the protective effects. Their ability to act as precursors of vitamin A and their antioxidant properties are thought to be responsible for their anticarcinogenic and antiatherogenic activity. This review focuses on carotenoids and particularly lycopene and human health and disease.

2. OXIDATIVE STRESS AND CHRONIC DISEASES

There is convincing evidence to indicate that ROS generated both endogenously and also in response to external factors such as diet and life-style may be a significant factor in the etiology of several degenerative diseases including cancer and cardiovascular disease (CVD) /2-8/. ROS are generated endogenously as byproducts of normal metabolic processes and cause oxidative damage to important biomolecules such as lipids, proteins and DNA, which if unrepaired accumulates and leads to physiological attrition and an increased risk of several chronic diseases (Fig. 1).

Oxidation of low density lipoproteins (LDL) is an important factor in atherosclerotic plaque formation leading to coronary heart disease /5/. Intracellular protein oxidation results in functional changes modulating cellular metabolism /6/. Oxidative modification of DNA bases leads to mutation and altered gene function resulting in cancer /12,13/. Oxidative damage and mutations in mitochondrial DNA lead to mitochondrial dysfunctions resulting in a variety of pathological disorders /14/. ROS induce the expression of a wide variety of transcription factors, such as NFkB, AP1, and oncogenes, such as c-*fos* and c-*jun*. Furthermore, ROS also induce conformational changes in p53 protein which mimics the mutant phenotype. ROS, therefore, by inducing these alterations, influence cell cycle mechanisms and ultimately lead to the development of cancer /15-18/.

Several biomarkers of oxidized lipids, protein and DNA have been found to be increased in the tissues and body fluids of patients with cancer and cardiovascular disease, and also during aging /2-8/. Lipid peroxidation products have been found to increase in a variety of oxidative stress conditions /5/. Premenopausal women with high mammographic tissue densities, a strong risk factor for breast cancer, were found to excrete more malondialdehyde, a lipid peroxidation product /19,20/. Increased oxidation of proteins and DNA was also shown to be involved in the aging process /21,22/. About 20% higher levels of lipid peroxides were found in prostate cancer patients compared to their matched controls with retinoic acid therapy /23/. Cancer tissues also show an increased amount of DNA adducts of oxidative damage /13,24,25/.

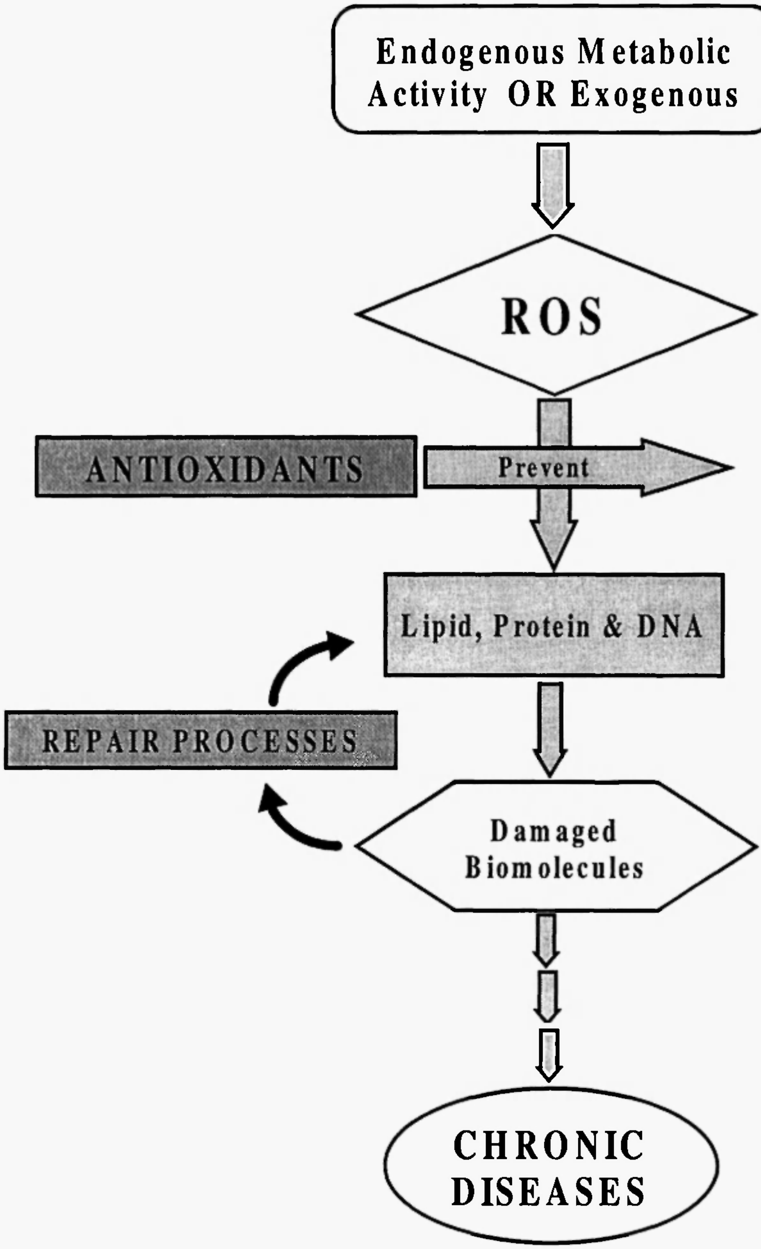

Fig. 1: Role of ROS and antioxidants in the chronic disease process.

3. CAROTENOIDS

Carotenoids are important pigments synthesized by plants and microorganisms but not by animals. They are present in the human diet as micro-components of fruits and vegetables and are thought to contribute to the inverse relationship between fruit and vegetable consumption and the risk of CVD, cancer and other chronic diseases /26,27/. They are important dietary sources of vitamin A and are also excellent antioxidants /26/. In plants, these pigments are part of the photosynthetic machinery and are responsible for the yellow, orange and red colors of fruits and vegetables. The importance of these carotenoids in plants lies mainly in protection against photo-damage.

There are more than 600 carotenoids found in nature, about 40 of which are present in a typical human diet, and about 20 have been identified in blood and tissues. β-Carotene, α-carotene, lycopene, α-cryptoxanthin and lutein account for over 90% of the carotenoids in the diet and the human body /28/. All carotenoids posses certain common chemical features: 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 /29/. Modifications in the base structure by cyclization of the end groups and by introduction of oxygen functions yield different carotenoids (Fig. 2). Carotenoids are rich in conjugated double bonds and therefore can undergo *cis-trans* isomerization. The *trans* form is more stable and is the most common form in foods. Very little is known about the specific roles and relative importance of *cis-trans* isomers of carotenoids in humans.

4. DIETARY SOURCES, ABSORPTION AND METABOLISM

Carotenoids are present in several types of foods, but most carotenoids in the diet are provided by deeply pigmented vegetables, fruits and juices /30,31/. The carotenoid pattern of common fruits and vegetables is shown in Table 1. In general, yellow-orange vegetables and fruits provide most of the dietary β-carotene and α-carotene, orange fruits provide α-cryptoxanthin, dark green vegetables provide lutein and tomatoes and tomato products are a major source of lycopene in the diet. Cooking and processing of foods affect carotenoid contents. In general the most common household cooking

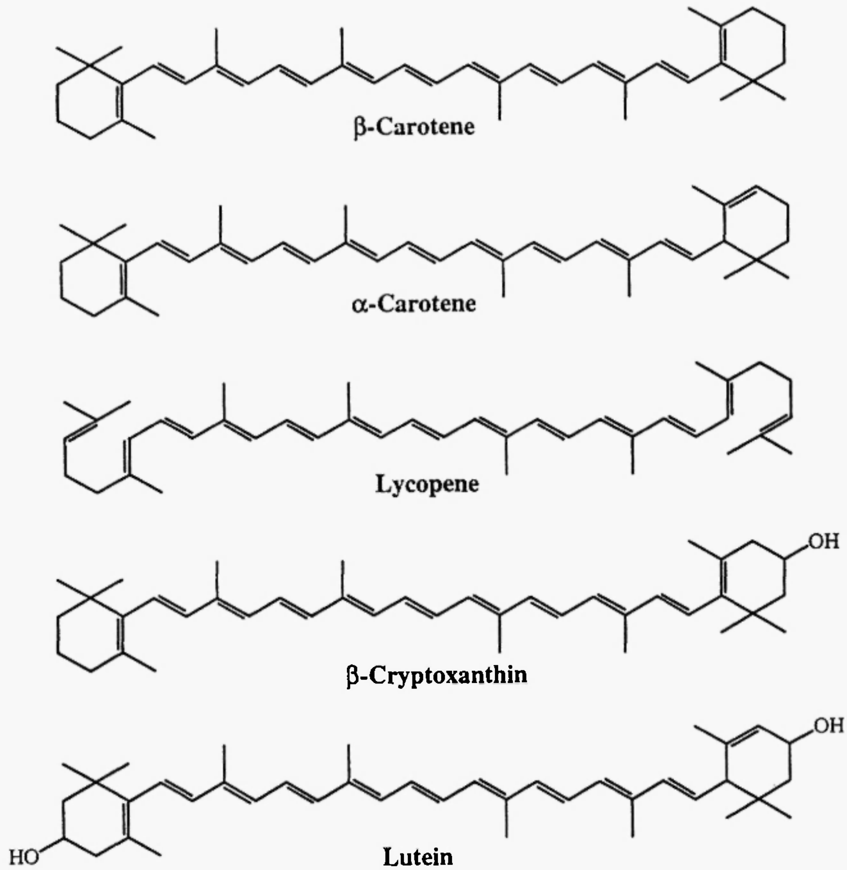

Fig. 2: Structures of major dietary carotenoids.

methods, such as microwave cooking, steaming and boiling in a small amount of water, do not drastically alter the carotenoid contents of vegetables. However, drying, extreme heat or excessive cooking time results in oxidative destruction of carotenoids /32/. At present there is no dietary reference intake (DRI) for carotenoids. Quantities of carotenoids in diet are difficult to estimate, partially because the methods used for the estimation are not sensitive and specific. In addition, the given values do not always take into account variations due to season.

TABLE 1

Examples of major contributors of carotenoids in North American diet /30,31/

Carotenoid	**Food Source**	**Amount** (μg/100 g)
β-Carotene	Apricot, dried	17,600
	Carrots, cooked	9,771
	Spinach, cooked	5,300
	Green Collard	5,400
	Canteloupe	3,000
	Beet Green	2,560
	Broccoli, cooked	1,300
	Tomato, raw	520
α-Carotene	Carrots, cooked	3,723
Lycopene	Tomatoes, raw	3,100
	Tomato Juice	10,000
	Tomato Paste	36,500
	Tomato Ketchup	12,390
	Tomato Sauce	13,060
β-Cryptoxanthin	Tangerine	1,060
	Papaya	470
Lutein	Spinach, cooked	12,475
	Green collard	16,300
	Beet, green	7,700
	Broccoli, cooked	1,839
	Green peas, cooked	1,690

Very little is known about the absorption and transport of major carotenoids except for β-carotene. In humans, appreciable amounts of carotenoids are absorbed intact by the mucosal cells and subsequently appear unchanged in the circulation and peripheral tissues /33,34/. Food processing and cooking operations such as mechanical homogenization or heating, which release carotenoids from the food matrix, enhance their bioavailability /35,36/. In the intestine, they are incorporated into the micelles formed from dietary fat and bile acids, which facilitates absorption via passive diffusion. The micellar carotenoids are then incorporated into the chylomicrones, released into the lymphatic system, and follow chylomicron metabolism. They are then taken up by the liver, incorporated into lipoproteins and released into the blood stream for transport to different tissues. Carotenoids are taken up differentially by different tissues, but little is known of the factors relating to this process. Adipose tissue is the primary storage depot for these carotenoids /33,34/.

5. ROLE IN DISEASE PREVENTION

Although the chemistry of carotenoids has been studied extensively their biological role remains unclear. Carotenoids play important physiological roles in human health. Not all carotenoids clearly serve as a dietary source of vitamin A. They also exhibit biological activity as antioxidants, in gap junction communication, in cell growth regulation, in modulating gene expression, in immune response and as modulators of biotransformation reactions involving drug metabolizing enzymes /26,27,37,38/. Carotenoids have been implicated as protective agents against cancer, CVD, age related macular degeneration (AMD) and cataract. Figure 3 summarizes the biological role of carotenoids in disease prevention. Epidemiological evidence has suggested a positive link between higher dietary intake and tissue concentrations and reduced risk of cancer and CVD /26,27/. A recent analysis of several epidemiological studies reported that people consuming large amounts of tomatoes and tomato products, rich sources of the carotenoid lycopene, or with high levels of circulating lycopene in their body, are less likely to develop several types of cancers than those consuming lesser amounts or having lower levels /39/. However, recent intervention trials using high doses of β-

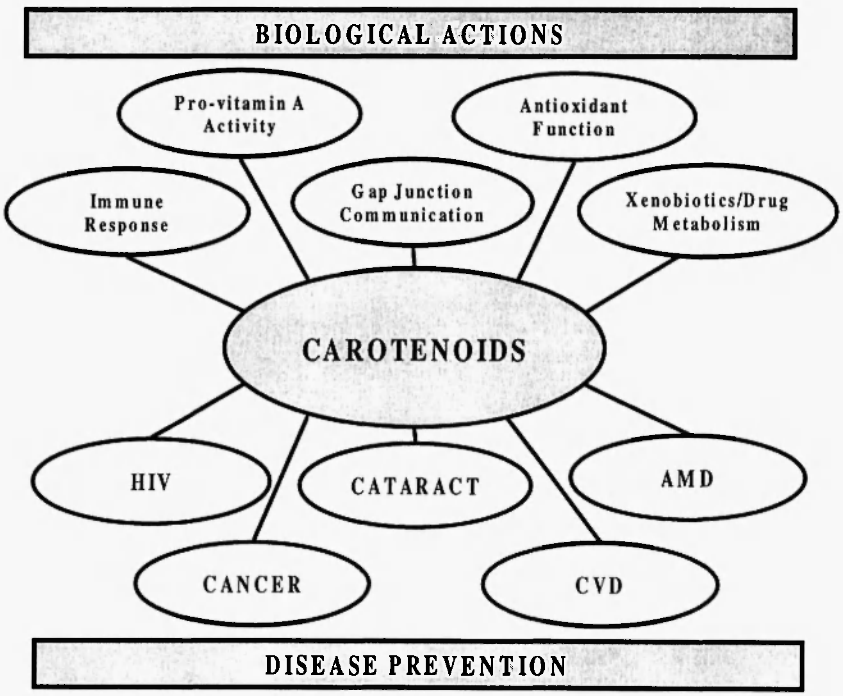

Fig. 3: Role of carotenoids in the prevention of chronic diseases.

carotene supplements did not show protective effects /40-42/. On the other hand, pharmacological supplementation with β-carotene increased lung cancer incidences in smokers in the Alpha-Tocopherol Beta-Carotene (ATBC) trial /43/, and increased mortality from CVD in a group of smokers, former smokers and asbestos exposed individuals in the β-Cartotene and Retinol Efficiency Trial (CARET) /44/. It appears that β-carotene induces a biphasic response by promoting health when taken at dietary levels, but may have adverse effects when taken in high amounts. Of all the carotenoids, lycopene has received most attention in recent years.

6. LYCOPENE

Lycopene is a natural pigment synthesized by plants and micro-organisms but not by animals. Some red fruits and vegetables, including tomatoes, watermelons, pink grapefruit, apricots and pink guavas, are rich sources of lycopene /45/. It is a carotenoid, an acyclic isomer of β-carotene, with no vitamin A activity /46/. Lycopene is one of the most potent antioxidants /47-50/ and its singlet oxygen quenching ability is twice that of β-carotene and 10 times that of α-tocopherol /50/. It is a highly unsaturated hydrocarbon containing 11 conjugated and two non-conjugated double bonds. As a polyene, it undergoes light-, thermal energy- or chemical reaction-induced *cis-trans* isomerization (Fig. 4) /45,51/.

Processed tomato products, such as juice, ketchup, paste, sauce and soup, are all rich sources of lycopene. In a recent study /52/, the average daily dietary lycopene intake levels were assessed by administering a food frequency questionnaire and were estimated to be 25 mg/day with processed tomato products accounting for 50% of the total intake. Lycopene from processed tomato products appears to be more bioavailable than from raw tomatoes /36,53/. The release of lycopene from the food matrix due to processing, presence of dietary lipids and heat induces a *trans* to *cis* transformation which enhances lycopene bioavailability /54/. The bioavailability of lycopene is also affected by the dose and presence of other carotenoids such as β-carotene /55/. However, the comparative bioavailabilities of lycopene from different tomato products, such as paste, juice, ketchup, sauce and soup, are not known.

Lycopene is the most predominant carotenoid in human plasma and its level is affected by several biological and lifestyle factors /54/. Owing to their lipophilic nature, lycopene and other carotenoids are found to concentrate in LDL and VLDL fractions of the serum /56/. Lycopene is known to accumulate in human tissues but its distribution is non-uniform. Lycopene is also found to concentrate and be the most prominant carotenoid in adrenal gland, testes, liver and prostate /57-61/. The tissue-specific lycopene distribution may exert unique biological effects in some tissues but not in others.

7. ISOMERIZATION OF LYCOPENE AND ITS POSSIBLE BIOLOGICAL SIGNIFICANCE

Lycopene from natural plant sources exist predominantly in *trans* configuration, the most thermodynamically stable form /45,51/. In

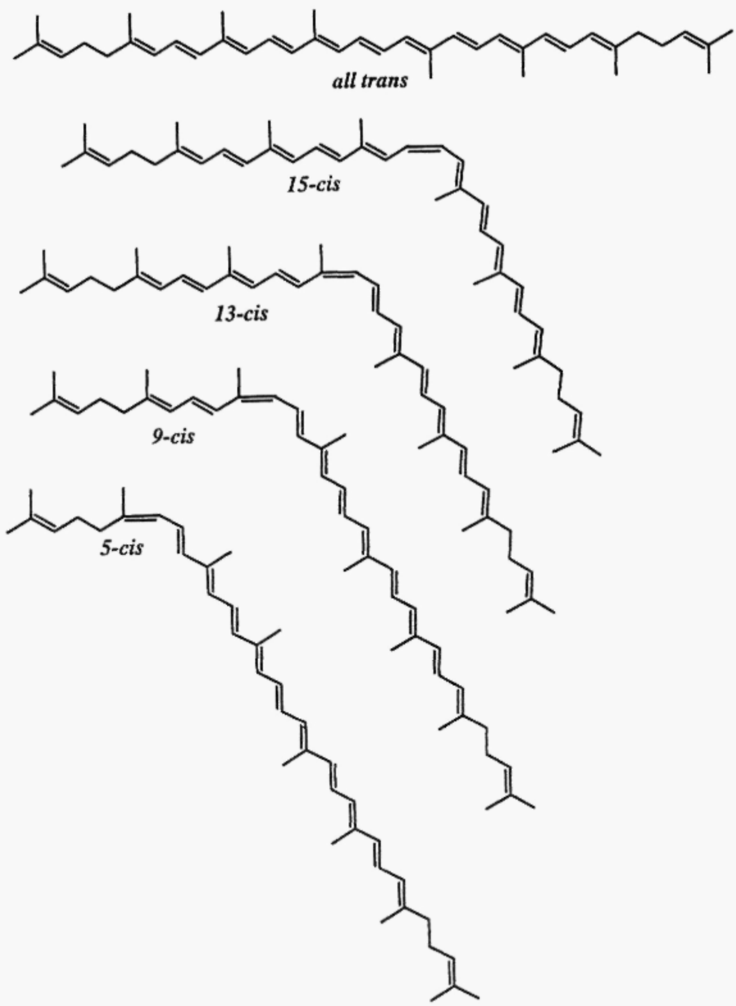

Fig. 4: Structures of *cis* and *trans* isomers of lycopene.

human plasma, lycopene is an isomeric mixture containing 50% of the total lycopene as *cis* isomers. All-*trans*, 5-*cis*, 9-*cis*, 13-*cis* and 15-*cis* are the most commonly identified isomeric forms of lycopene (Fig. 4) /62/. The relative biological significance of the various geometric isomers of lycopene is unclear.

Lycopene, ingested in its natural *trans* form found in tomatoes, is poorly absorbed. Recent studies have shown that heat processing of tomatoes and tomato products induces isomerization of lycopene to predominantly the *cis* form which in turn increases its bioavailability /53/. Epidemiological studies have supported the hypothesis that consumption of tomatoes and heat processed tomato products, such as in the Mediterranean diet, may reduce the risk of coronary heart disease by preventing the oxidation of LDL. Giovannucci *et al.* /63/ also suggested that only the intake of processed tomato products was related to a reduced risk of prostate cancer, probably because of their high *cis* isomer content of lycopene. However, Sakamoto *et al.* /64/ observed that ingestion of canned tomato juice, containing mainly *trans* lycopene, increased the *cis* isomers of lycopene in serum. They concluded that isomerization reactions may be taking place in the body. High concentrations of *cis* isomers were also observed in human serum and prostate tissue /62/, suggesting that tissue isomerases might be involved in *in vivo* isomerization of lycopene from all-*trans* to *cis* form.

A recently completed study /65/ demonstrated that serum and tissue total lycopene levels of prostate cancer patients were significantly lower than in their age-matched controls. It is hypothesized that cancer patients perhaps lack the ability to isomerize dietary lycopene and therefore do not absorb lycopene efficiently. A possibility also exists that due to increased oxidative stress in cancer patients, lycopene is utilized rapidly, resulting in lower levels. A combination of both decreased absorption and increased utilization may also be responsible for the low levels of lycopene observed in prostate cancer patients. A similar situation may also result in impaired lycopene absorption in other population groups.

8. TOMATO LYCOPENE AND CANCER RISK

Lycopene may play an important protective role in several human cancers. As early as 1959, lycopene was demonstrated to provide radioprotection and stimulate nonspecific resistance towards bacterial infections in animals /66,67/. Levy *et al.* /68/ showed that lycopene inhibited the growth of human endometrial, mammary and lung cancer cells grown in cultures, and was more effective than α- and β-carotene. Similar results have also been reported in rats and mice developing either carcinogen-induced or spontaneous tumors in mammary gland, liver, colon and bladder /69-72/.

The Mediterranean diet, which is rich in fruits and vegetables including tomatoes, has been suggested to be responsible for the lower cancer incidences in that region /73/. Dietary intake of tomatoes and tomato products have been found to be associated with a lower risk of a variety of cancers in a number of epidemiological studies /39/. High intake of tomatoes was linked to protective effects against digestive tract cancers in a case control study /74/ and 50% reduction in mortality from cancers at all sites in an elderly American population /75/. The most impressive results came from the US Health Professionals Follow-up Study which evaluated the intake of various carotenoids and retinol, from a food frequency questionnaire, in relation to prostate cancer risk /63/. The estimated intake of lycopene from various tomato products and not any other carotenoid, was inversely related to the risk of prostate cancer. A risk reduction of almost 35% was observed for a consumption frequency of 10 or more servings of tomato products per week and the protective effects were even stronger for more advanced or aggressive prostate cancer /63/. Serum and tissue levels of lycopene were inversely associated with breast cancer /76/ and prostate cancer risk in recent studies /65,77/. No significant association with other major carotenoids including β-carotene was observed in these studies /65,77/.

Giovannucci /39/ recently reviewed epidemiological studies, including ecological, case-control and dietary studies, and blood specimen based investigations on tomatoes, tomato-based products, lycopene and cancer. Among 72 studies, 57 reported an inverse association between tomato intake or circulating lycopene levels and risk of several types of cancers, and 35 of these inverse associations were statistically significant /69/. About half the studies analyzed reported a relative risk of 0.6 or less when comparing high and low intakes of tomatoes or levels of lycopene. These results were 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 high tomato intake or high lycopene levels /69/.

Although there is convincing epidemiological evidence on the role of lycopene in cancer prevention, this evidence is only suggestive rather than proof. To date a very limited number of human intervention trials have been performed investigating the bioavailability, *in vivo* antioxidant properties and the efficacy of lycopene intake in lowering the risk of cancer. Loss of serum lycopene with an increase in lipid oxidation was observed in human subjects ingesting a lycopene-free diet for two weeks /33/. In a small clinical intervention trial, one week dietary supplementation with lycopene from tomato sauce and juice decreased oxidative damage to lipids, lipoproteins, proteins and DNA /78-80/. Oxidative damage to critical biomolecules has been suggested to be involved in the causation and progression of cancer /2-8/. Consumption of vegetable juices and tomato juice containing lycopene has been shown to reduce DNA strand breaks /79,80/. There are indications that tomato extract supplementation in the form of capsules lowered the PSA levels in prostate cancer patients /81/.

9. TOMATO LYCOPENE AND RISK OF CARDIOVASCULAR DISEASE

Cardiovascular diseases are one of the leading causes of mortality in North America. A large body of scientific evidence indicates that oxidation of LDL which carry cholesterol into the blood stream may play an important role in the causation of atherosclerosis, the underlying disorder leading to heart attack and ischemic strokes /5,82,83/. Antioxidant nutrients are believed to slow the progression of atherosclerosis because of their ability to inhibit damaging oxidative processes /83-85/. Several controlled clinical trials and epidemiological studies have provided evidence for the protective effect of vitamin E which has been ascribed to its antioxidant properties /86-88/. Recent studies indicate that consuming tomatoes and tomato products containing lycopene reduces the risk of cardiovascular diseases. Lycopene also appears to have powerful antioxidant properties and the reduction in cardiovascular disease risk was attributed to the antioxidant effect of lycopene /78,89/.

Lower blood lycopene levels were found to be associated with increased risk and mortality from coronary heart disease in a population study comparing Lithuanian and Swedish populations showing divergent mortality rates from coronary heart disease /90/. A recent multicenter case-control study (EURAMIC) evaluated the relationship between antioxidant status and acute myocardial infarction /89/. Patients were recruited from ten European countries to maximize the variability in exposure within the study. Adipose tissue antioxidant levels, which are good indicators of long-term exposure, were used as markers of antioxidant status. Adipose tissue biopsies were taken directly following the infarction and were analyzed for carotenoids and vitamin E. After adjusting for a range of dietary variables, only lycopene, and not β-carotene levels, was found to be protective /89/.

Since lycopene, a lipid soluble antioxidant, is transported in the blood mainly in the lipoproteins, a single oral dose of lycopene along with β-carotene, lutein, cathaxanthin and vitamin E prevented the *ex vivo* oxidation of isolated LDL /91/. In tissue culture studies, lycopene was found to suppress cholesterol synthesis with a simultaneous increase in LDL receptor activity in macrophages leading to a faster clearance of LDL from plasma /92/. In the same study, dietary supplementation with tomato lycopene in healthy male subjects also lowered the plasma LDL levels by 14%, thus confirming the *in vitro* results /92/. In a recent study on healthy human subjects /78/, supplementation of the diet with lycopene from tomato products for one week increased serum lycopene levels and significantly lowered the levels of oxidized LDL. The results of this study suggest that consumption of tomato products inhibits oxidative damage to serum LDL and thus may be helpful in reducing the risk of heart disease. In this study, the tomato products were consumed as one or two servings per day for one week. This consumption level is easily achievable and is consistent with current dietary guidelines for healthy eating.

10. LYCOPENE AND OTHER HEALTH BENEFITS

Tomato lycopene has been implicated in several other diseases, including HIV infections, cataract and diseases of the central nervous system. HIV positive women as well as HIV infected children were found to have lower serum lycopene levels compared to controls /93,94/. Age related macular degeneration (AMD) is believed to be, at least partly, promoted by oxidative stress due to exposure of retinal pigment to light and oxygen. Although the human retina contains very little or no lycopene, low serum lycopene levels were found to be associated with a high risk of AMD /95/. In a recent Austrian Stroke Prevention Study /96/, lower serum lycopene and α-tocopherol levels were also found to be related to a high risk of microangiopathy-related cerebral damage, a risk factor for cerebrovascular disease, in the elderly population. Children with cystic fibrosis were also found to have significantly lower levels of serum lycopene and other carotenoids than healthy children /97/.

11. CONCLUDING REMARKS

Dietary guidelines for the prevention of cancer and coronary heart disease recommend increased consumption of plant foods including fruits, vegetables, cereals and legumes. The presence of micronutrient phytochemical compounds is now being recognized as playing an important role in the disease prevention properties of plant foods. Epidemiological evidence is compatible with a possible protective role of carotenoids and particularly lycopene against the risk of cancer and cardiovascular disease. However, epidemiological evidence is suggestive and not conclusive. Moreover, the underlying mechanisms are also not clear. To date the emphasis has been on β-carotene and lycopene. The roles of other carotenoids such as lutein in macular degeneration, canthaxanthin in cancer and CVD are beginning to be addressed. Since a balanced diet provides a variety of carotenoids, further research is critical in understanding the role of these carotenoids for formulation of dietary guidelines for healthy eating and prevention of disease.

REFERENCES

1. Trichopoulos D, Willett WC. Introduction: nutrition and cancer. Cancer Causes Control 1996; 7: 3-4.
2. Ames BN, Gold LS, Willet, WC. Causes and prevention of cancer. Proc Natl Acad Sci USA 1995; 92: 5258-5265.
3. Pincemail J. Free radicals and antioxidants in human disease. In: Favier AE, Cadet J, Kalyanaraman B, Fontecave M, Pierre J-L. eds. Analysis of Free Radicals in Biological Systems. Basel: Birkhäuser Verlag, 1995; 83-98.
4. Stadtman ER. Protein oxidation and aging. Science 1992; 257: 1220-1224.
5. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994; 344: 793-795.
6. Ames BN, Shigenaga MK, Hagan TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993; 90: 7915-7922.
7. Halliwell B, Murcia MA, Chirico S, Aruoma OI. Free radicals and antioxidants in food and in vivo: what they do and how they work. Crit Rev Food Sci Nutr 1995; 35: 7-20.
8. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet 1994; 344: 721-724.

9. Wynder EL. Cancer Prevention: Optimizing Life-Styles with Special Reference to Nutritional Carcinogenesis. Monographs of the National Cancer Institute 1992; 12: 87-91.
10. Hirayama J. A large scale cohort study on cancer risk by diet with special reference to the risk reducing effects of green-yellow vegetable consumption. In: Hayashi Y, ed. Diet, Nutrition and Cancer. Tokyo: Japan Scientific Society Press, 1986; 41-53.
11. Messina MJ, Persky V, Setchell KDR, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr Cancer 1994; 21: 113-131.
12. Ames BN, Shigenaga MK, Gold LS. DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. Environ Health Persp 1993; 101 (Suppl 5): 35-44.
13. Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med 1996; 74: 297-312.
14. Wallace DC, Shoffner JM, Trounce I, Brown MD, Ballinger SW, Corral-Debrinski M, Horton T, Jun AS, Lott MT. Mitochondrial DNA mutations in human degenerative diseases and aging. Biochim Biophys Acta 1995; 1271: 141-151.
15. Wei H. Activation of oncogenes and/or inactivation of anti-oncogene by reactive oxygen species. Med Hypotheses 1992; 39: 267-270.
16. Toledano MB, Leonard WJ. Modulation of transcription factor NF6B binding activity by oxidation-reduction in vitro. Proc Natl Acad Sci USA 1991; 88: 4328-4332.
17. Wasylyk C, Wasylyk B. Oncogene conversion of Ets affects redox regulation in vivo and in vitro. Nucl Acid Res 1993; 21: 523-529.
18. Hainaut P, Miller J. Redox modulation of p53 conformation and sequence-specific DNA binding. Cancer Res 1993; 53: 4469-4473.
19. Boyd NF, McGuire V. Evidence of lipid peroxidation in premenopausal women with mammographic dysplasia. Cancer Lett 1990; 50: 31-37.
20. Boyd NF, Connelly P, Byng J, Yaffe M, Draper H, Little L, Jones D, Martin L, Lockwood G, Tritchler D. Plasma lipids, lipoproteins, and mammographic densities. Cancer Epidem Biomarker Prev 1995; 4: 727-733.
21. Agarwal S. Sohal RS. DNA oxidative damage and life expectancy in houseflies. Proc Natl Acad Sci USA 1994; 91: 12332-12335.
22. Sohal RS, Agarwal S, Dubey A, Orr WC. Protein oxidative damage is associated with life expectancy of houseflies. Proc Natl Acad Sci USA 1993; 90: 7255-7259.
23. Rigas JR, Warrell RP Jr, Young CW. Elevated plasma lipid peroxide content correlates with rapid plasma clearance of all-trans-retinoic acid in patients with advanced cancer. Cancer Res 1994; 54: 2125-2128.
24. Musarrat J, Arezinawilson J, Wani AA. Prognostic and etiologic relevance of 8-hydroxyguanosine in human breast carcinogenesis. Eur J Cancer 1996; 32: 1209-1214.
25. Wang M, Dhingra K, Hittelman WN, Liehr JG, de Andrade M, Donghui L. Lipid peroxidation-induced putative malondialdehyde-DNA adducts in human breast tissues. Cancer Epidem Biomarker Prev 1996; 5: 705-710.

26. Paiva SAR, Ressell RM. β-Carotene and other carotenoids as antioxidants. J Am Coll Nutr 1999; 18: 426-433.
27. Astrog P. Food carotenoids and cancer prevention: an overview of current research. Trends Food Sci Technol 1997; 8: 406-413.
28. Gerster H. The potential role of lycopene for human health. J Am Coll Nutr 1997; 16: 109-126.
29. Britton G. Structure and properties of carotenoids in relation to function. FASEB J 1995; 9: 1551-1558.
30. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoids in fruits and vegetables: an evaluation of analytical data. J Am Diet Assoc 1993; 93: 284-296.
31. Scott KJ, Hart DJ. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chem 1995; 54: 101-111.
32. Thane C, Reddy S. Processing of fruits and vegetables: effect on carotenoids. Nutr Food Sci 1997; 2: 58-65.
33. Erdman JW Jr, Bierer TL, Gugger ET. Absorption and transport of carotenoids. Ann NY Acad Sci 1993; 691: 76-85.
34. Parker RS. Absorption, metabolism and transport of carotenoids. FASEB J 1996; 10: 542-551.
35. Parker RS. Bioavailability of carotenoids. Eur J Clin Nutr 1997; 51: S86-S90.
36. Gartner C, Stahl W, Sies H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. Am J Clin Nutr 1997; 66: 116-122.
37. Jewell C, O'Brien NM. Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat. Br J Nutr 1999; 81: 235-242.
38. Bertram JS. Carotenoids and gene regulation. Nutr Rev 1999; 57: 182-191.
39 Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 1999; 91: 317-331.
40. Carotenoid Research Initiative Group. Beta-carotene and the carotenoids: beyond the intervention trials. Nutr Rev 1996; 54: 185-188.
41. Cooper DA, Eldridge AL, Peters JC. Dietary carotenoids and lung cancer: a review of recent research. Nutr Rev 1999; 57: 133-145.
42. Gaziano M. Antioxidants in cardiovascular disease: randomized trials. Nutr Rev 1996; 54: 175-177.
43. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effects of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994; 330: 1029-1035.
44. Omenn GS, Goodman GE, Thornquist MD Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996; 334: 1150-1155.
45 Nguyen ML, Schwartz SJ. Lycopene: chemical and biological properties. Food Technol 1999; 53: 38-45.

46 Stahl W, Sies H. Lycopene: a biologically important carotenoid for humans? Arch Biochem Biophys 1996; 336: 1-9.
47. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. Antioxidant activities of carotenes and xanthophylls. FEBS Lett 1996; 384: 240-246.
48. Mortensen A, Skibsted LH. Relative stability of carotenoid radical cations and homologue tocopheroxyl radicals. A real time kinetic study of antioxidant hierarchy. FEBS Lett 1997; 417: 261-266.
49. Woodall AA, Lee SW-M, Weesie RJ, Jackson MJ, Britton G. Oxidation of carotenoids by free radicals: relationship between structure and reactivity. Biochim Biophys Acta 1997; 1336: 33-42.
50. DiMascio P, Kaiser S, Sies H. Lycopene as the most effective biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989; 274: 532-538.
51. Zechmeister L, LeRosen AL, Went FW, Pauling L. Prolycopene, a naturally occurring stereoisomer of lycopene. Proc Natl Acad Sci USA 1941; 21: 468-474.
52. Rao AV, Waseem Z, Agarwal S. Lycopene contents of tomatoes and tomato products and their contribution to dietary lycopene. Food Res Intl 1998; 31: 737-741.
53. Stahl W, Sies H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J Nutr 1992; 122: 2161-2166.
54. Rao AV, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutr Res 1999; 19: 305-323.
55. Johnson EJ, Qin J, Krinsky NI, Russell RM. Ingestion by men of a combined dose of β-carotene and lycopene does not affect the absorption of β-carotene but improves that of lycopene. J Nutr 1997; 127: 1833-1837.
56 Rao AV, Agarwal S. Effect of diet and smoking on serum lycopene and lipid peroxidation. Nutr Res 1998; 18: 713-721.
57. Stahl W, Schwarz W, Sundquist AR, Sies H. *Cis-trans* isomers of lycopene and β-carotene in human serum and tissues. Arch Biochem Biophys 1992; 294: 173-177.
58. Kaplan LA, Lau JM, Stein EA. Carotenoid composition, concentrations and relationships in various human organs. Clin Physiol Biochem 1990; 8: 1-10.
59. Schmitz HH, Poor CL, Wellman RB, Erdman JW Jr. Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. J Nutr 1991; 121: 1613-1621.
60. Nierenberg DW, Nann SL. A method for determining concentrations of retinol, tocopherol, and five carotenoids in human plasma and tissue samples. Am J Clin Nutr 1992; 56: 417-426.
61. Jain CK, Agarwal S, Rao AV. The effect of dietary lycopene on bioavailability, tissue distribution, in-vivo antioxidant properties and colonic preneoplasia in rats. Nutr Res 1999; 19:1383-1391.

62. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, Erdman JW. *Cis-trans* lycopene isomers, carotenoids and retinol in the human prostate. Cancer Epidem Biomarker Prev 1996; 5: 823-833.
63. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 1995; 87: 1767-1776.
64. Sakamoto H, Mori H, Ojima F, Ishiguro Y, Arimoto S, Imae Y, Nanba T, Ogawa M, Fukuba H. Elevation of serum carotenoids after continual ingestion of tomato juice. J Jpn Soc Nutr Food Sci 1994; 47: 93-99.
65. Rao AV, Fleshner N, Agarwal S. Serum and tissue lycopene and biomarkers of oxidation in prostate cancer patients: a case control study. Nutr Cancer 1999; 33: 159-164.
66. Forssberg A, Lingen C, Ernster L, Lindenberg O. Modification of X-irradiation syndrome by lycopene. Exp Cell Res 1959; 16: 7-14.
67. Lingen C, Ernster L, Lindenberg O. The promoting effects of lycopene on the non-specific resistance of animals. Exp Cell Res 1959; 16: 384-393.
68. Levy J, Bosin E, Feldmen B, Giat Y, Miinster A, Danilenko M, Sharoni Y. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. Nutr Cancer 1995; 24: 257-266.
69. Nagasawa H, Mitamura T, Sakamoto S, Yamamoto K. Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. Anticancer Res 1995; 15: 1173-1178.
70. Sharoni Y, Giron E, Rise M, Levy J. Effects of lycopene-enriched tomato oleoresin on 7,12-dimethyl-benz[a]anthracene-induced rat mammary tumors. Cancer Detection Prev 1997; 21: 118-123.
71. Okajima E, Tsutsumi M, Ozono S, Akai H, Denda A, Nishino H, Oshima S, Sakamoto H, Konishi Y. Inhibitory effect of tomato juice on rat urinary bladder carcinogenesis after N-butyl-N-(4-hydroybutyl)nitrosamine initiation. Jpn J Cancer Res 1998; 89: 22-26.
72. Narisawa T, Fukaura Y, Hasebe M, Nomura S, Oshima S, Sakamoto H, Inakuma T, Ishiguro Y, Takayasu J, Nishino H. Prevention of N-methylnitrosourea-induced colon carcinogenesis in F344 rats by lycopene. Jpn J Cancer Res 1998; 89: 1003-1008.
73. LaVecchia C. Mediterranean epidemiological evidence on tomatoes and the prevention of digestive tract cancers. Proc Soc Exp Biol Med 1997; 218: 125-128.
74. Franceschi S, Bidoli E, LaVeccia C, Talamini R, D'Avanzo B, Negri E. Tomatoes and risk of digestive-tract cancers. Int J Cancer 1994; 59: 181-184.
75. Colditz GA, Branch LG, Lipnic RJ. Increased green and yellow vegetables intake and lowered cancer death in an elderly population. Am J Clin Nutr 1985; 41: 32-36.
76. Dorgan JF, Sowell A, Swanson CA, Potischman N, Miller R, Schussler N, Stephenson HE Jr. Relationship of serum carotenoids, retinol, α-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States). Cancer Cause Control 1998; 9: 89-97.

77. Gann P, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, Stampfer MJ. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. Cancer Res 1999; 59: 1225-1230.
78. Agarwal S, Rao AV. Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. Lipids 1998; 33: 981-984.
79. Rao AV, Agarwal S. Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. Nutr Cancer 1998; 31: 199-203.
80. Pool-Zobel BL, Bub A, Muller H, Wollowski I, Rechkemmer G. Consumption of vegetables reduces genetic damage in humans: first result of a human intervention trial with carotenoid-rich foods. Carcinogenesis 1997; 18: 1847-1850.
81. Kucuk O, Sakr FH, Djuric Z, Li YW, Velazquez F, Banerjee M, Bertram JS, Crissman JD, Wood DP. Lycopene supplementation in men with prostate cancer (Pca) reduces grade and of preneoplasia (PIN) and tumor, decreases serum prostate specific antigen and modulates biomarkers of growth and differentiation. Intl Conf Diet Prev Cancer, Tampere, Finland, 1999; P1.13 (abst).
82. Parthasarathy S, Steinberg D, Witztum JL. The role of oxidized low-density lipoproteins in pathogenesis of atherosclerosis. Ann Rev Med 1992; 43: 219-225.
83. Heller FR, Descamps O, Hondekijn JC. LDL oxidation: therapeutic perspectives. Atherosclerosis 1998; 137: S25-S31.
84. Parthasarathy S. Mechanism by which dietary antioxidants may prevent cardiovascular diseases. J Med Food 1998; 1: 45-51.
85. Morris DL, Kritchevsky SB, Davis CE. Serum carotenoids and coronary heart disease: the Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study. JAMA 1994; 272: 1439-1441.
86. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993; 328: 1450-1456.
87. Hodis HN, Mack WJ, LaBree L, Cashin-Hemphill L, Sevanian A, Johnson R, Azen SP. Serial coronary angiographic evidence that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis. JAMA 1995; 273: 1849-1854.
88. Paolisso G, Gambardella A, Giugliano D, Galzerano D, Amato L, Volpe C, Balbi V, Varricchio M, D'Onofrio F. Chronic intake of pharmacological doses of vitamin E might be useful in the therapy of elderly patients with coronary heart disease. Am J Clin Nutr 1995; 61: 848-852.
89. Kohlmeir L, Kark JD, Gomez-Gracia E, Martin BC, Steck SE, Kardinaal AFM, Ringstad J, Thamm M, Masaev V, Riemersma R, Martin-Moreno JM, Huttunen JK, Kok FJ. Lycopene and myocardial infarction risk in the EURAMIC study. Am J Epidemiol 1997; 146: 618-626.
90. Kristenson M, Zieden B, Kucinskiene Z, Elinder LS, Bergdahl B, Elwing B, Abaravicius A, Razinkoviene L, Calkauskas H, Olsson A. Antioxidant state

and mortality from coronary heart disease in Lithuanian and Swedish men: concomitant cross sectional study of men aged 50. BMJ 1997; 314: 629-633.
91. Linseisen J, Hoffman J, Riedl J, Wolfram G. Effect of a single dose of anti-oxidant mixture (vitamin E, carotenoids) on the formation of cholesterol oxidation products after ex-vivo LDL oxidation in humans. Eur J Med Res 1998; 3: 5-12.
92. Fuhramn B, Elis A, Aviram M. Hypocholesterolemic effect of lycopene and β-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophage. Biochem Biophys Res Comm 1997; 233: 658-662.
93. Coodley GO, Coodley MK, Nelson HD. Micronutrients in HIV-infected women. J Women Health 1995; 4: 303-311.
94. Periquet BA, Jammes NM, Lambert WE, Tricoire J, Moussa MM, Garcia J, Ghisolfi J, Thouvenot JP. Micronutrient levels in HIV-1-infected children. AIDS 1995; 9: 887-893.
95. Mares-Perlman JA, Brady WE, Klein R, Klein BEK, Bowen P, Sapuntzakis SM, Palta M. Serum antioxidants and age-related macular degeneration in a population-based case-control study. Arch Ophthalmol 1995; 113: 1518-1523.
96. Schmidt R, Fazekas F, Hayn M, Schmidt H, Kapeller P, Roob G, Offenbacher H, Schumacher M, Eber B, Weinrauch V, Kostner GM, Esterbauer H. Risk factors for microangiopathy-related cerebral damage in Austrian stroke prevention study. J Neurol Sci 1997; 152: 15-21.
97. Homnick DN, Cox JH, DeLoof MJ, Ringer TV. Carotenoid levels in normal children and in children with cystic fibrosis. J Pediatr 1993; 122: 703-707.