European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2008; 12: 183-190

Protective effect of lycopene in cardiovascular disease

G. RICCIONI[1,2], B. MANCINI[2], E. DI ILIO[2], T. BUCCIARELLI[2], N. D'ORAZIO[2]

[1]Cardiology Unit, "San Camillo de Lellis" Hospital, Manfredonia, Foggia (Italy)
[2]Human Nutrition, Biomedical Sciences, University "G. D'Annunzio", Chieti (Italy)

Abstract. – Coronary artery disease (CAD) represents the primary cause of death in Western Countries with an high incidence on human health and community social costs. Oxidative stress induced by reactive oxygen species (ROS) plays an important role in the aetiology of this disease. In particular, the LDL-oxidization has a key role in the pathogenesis of atherosclerosis and cardiovascular heart diseases through the initiation of plaque formation process. Dietary phytochemical products such antioxidant vitamins (A,C,E) and bioactive food components (alpha- and beta-carotene) have shown an antioxidant effect in reducing both oxidative markers stress and LDL-oxidization process. Scientifical evidences support the beneficial roles of phytochemicals in the prevention of some chronic diseases. Lycopene, an oxygenated carotenoid with great antioxidant properties, has shown both in epidemiological studies and supplementation human trials a reduction of cardiovascular risk. However, controlled clinical trials and dietary intervention studies using well-defined subjects population haven't been provided a clear evidence of lycopene in the prevention of cardiovascular diseases. The present short review aims to evaluate the beneficial effect of lycopene in the prevention of cardiovascular disease.

Key Words:

Lycopene, Atherosclerosis, Cardiovascular risk, Vitamins, Oxidized LDL.

Abbreviatons

CVD: Cardiovascular diseases
CHD: Coronary heart disease
TIA: Transient ischemic attack
CHF: Congestive heart failure
LDL: Low density lipoprotein
HDL: High density lipoprotein
Lyc: Lycopene
NO: Nitric oxide
ROS: Reactive oxygen species
SOD: Superoxide dismutase
GSHpx: Glutathione peroxidase
MI: Myocardial infarction
LDL-C: Low density lipoprotein cholesterol
HDL-C: High cholesterol density cholesterol
PVD: Peripheral vascular disease
CRP: C-reactive protein

Introduction

Heart diseases and stroke represent an epidemic cause of death and disability in developed countries[1], responsible for about 30 percent of all deaths worldwide each year. The term cardiovascular diseases (CVD) include coronary heart disease (CHD) (myocardial infarction [MI], angina pectoris, coronary insufficiency, and coronary death), cerebrovascular diseases (stroke and transient ischemic attacks [TIA], peripheral vascular disease (PVD), congestive heart failure (CHF), hypertension, valvular and congenital heart disease[2].

Numerous epidemiological investigations have characterized the risk pattern for CHD. In particular age, male sex, elevated low density lipoprotein cholesterol (LDL-C) levels, low high density lipoprotein cholesterol (HDL-C) levels, diabetes mellitus, and cigarette smoking are key risk factors for CHD[3].

Along with genetic factors and age, lifestyle and diet are also considered important risk factors. In particular carotenoids, a group of phytochemicals substances responsible for different colors of the foods, play an important role in the prevention of human diseases and maintaining good health[4].

Corresponding Author: Graziano Riccioni, MD; e-mail: griccioni@hotmail.com

It is estimated that nearly one-third of all cancer deaths in the occidental developed countries could be prevented through an appropriate dietary modification. Various dietary antioxidants have shown considerable promise as effective agents for cancer prevention by reducing oxidative stress which has been implicated in the development of many diseases, including cancer. Therefore, for reducing the incidence of tumour formation, modifications in dietary habits, especially by increasing consumption of fruits and vegetables rich in antioxidants, are increasingly advocated. Accumulating research evidence suggests that many dietary factors may be used alone or in combination with traditional chemotherapeutic agents to prevent the occurrence of neoplasms, their metastatic spread, or even to treat cancer. The reduced cancer risk and lack of toxicity associated with high intake of fruits and vegetables suggest that specific concentrations of antioxidant agents from these dietary sources may produce cancer chemopreventive effects without causing significant levels of toxicity[5].

Epidemiological studies regarding intake of tomatoes and tomato-based products and blood lycopene reported an inverse associations between tomato intake or blood lycopene level and the risk of cancer. The evidence for a benefit was strongest for tumours of the prostate, lung, and stomach. Because the data are from observational studies, a cause-effect relationship cannot be established definitively. However, the consistency of the results across numerous studies in diverse populations, for case-control and prospective studies, and for dietary-based and blood-based investigations argues against bias or confounding as the explanation for these findings. Lycopene may account for or contribute to these benefits, but this possibility is not yet proven and requires further studies. Numerous other potentially beneficial compounds are present in tomatoes, and, conceivably, complex interactions among multiple components may contribute to the anticancer properties of tomatoes. The consistently lower risk of cancer for a variety of anatomic sites that is associated with higher consumption of tomatoes and tomato-based products adds further support for current dietary recommendations to increase fruit and vegetable consumption[6,7].

Antioxidant nutrients are believed to slow the progression of atherosclerosis because of their ability to inhibit the tissue damage deriving to oxidative process[8-10]. Lycopene (Lyc) is one of such dietary antioxidant naturally present in tomatoes and tomato products, which has received much attention recently[11].

Oxidative Stress

Advances in pathophysiological research suggested that CVD represent a *continuum pathophysiological process* that includes oxidative stress, endothelial dysfunction, inflammatory process, and vascular remodeling[12]. Normal endothelial function appears to depend greatly on the homeostatic balance between nitrix oxide (NO) and reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide[13].

ROS are highly reactive oxidant molecules generated endogenously through regular metabolic, lifestyle activity, and diet. They react with cellular components, causing oxidative damage to such critical cellular biomolecules as lipids, proteins and DNA. This damage may play a significant role in the pathogenesis of several chronic diseases[14-16].

Oxidative stress induced by ROS is characterized by production of oxidized-LDL (LDL_{ox}), that play a key role in the pathogenesis of atherosclerosis and represent a underlying disorder leading to heart attack and ischemic stroke[17].

In particular, the endothelial cells actively participate to the development of inflammatory reactions. The recruitment of leukocytes to sites of inflammation is initiated by endothelial secretion of chemotactic molecules and enhanced expression of adhesion molecules that interact with surface proteins and leukocytes[18]. Cytokynes and arachidonic acid metabolites of the leukocyte pathway derived from cells of vessels wall stimulates endothelial secretion of many of these molecules[19].

Inflammation characterizes all phases of atherothrombosis and provides a critical pathophysiological link between plaque formation and acute rupture, leading the occlusion and infarction. An inflammation complex novel risk factors including high sensitivity C-reactive protein (hsCRP), lipoprotein(a), homocysteine, and markers of fibrinolytic and hemostatic function such as fibrinogen, D-dimer, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor (PAI-1) antigens characterize the several phases of atherothrombosis. In particular, hsPCR, a circulating member of pentraxin family, plays a major rule in the human immune response. Although it is a primarily derived from the liver, recent data indicate that cells within human coronary arteries, particularly in the athero-

sclerotic intima, can elaborate CRP[20,21]. More than a simply marker of inflammation, CRP may influence directly vascular vulnerability through several mechanisms, including enhanced expression of local adhesion molecules, increased expression of endothelial PAI-1, reduced endothelial nitrix oxide bioactivity, altered LDL uptake by macrophages, and colocalization with complement within atherosclerotic lesions[22].

Large series of prospective epidemiological studies has demonstrated that CRP, when measured with high-sensitivity assays (hsCRP), strongly and independently predicts risk of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death even among apparently healthy individuals[23,24]. The hsCRP levels correlate only modestly with underlying atherosclerotic disease as measured by carotid intima-media thickness or by coronary calcification. This observation suggest that hsCRP does not simply reflect the presence od subclinical diseases but rather indicates an increased propendity for plaque disruption and/or thrombosis[25].

Antioxidants

Antioxidants are protective agents that inactivate ROS and therefore significantly delay or prevent oxidative damage. In particular, superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH_{Px}) are endogenous naturally antioxidants present within human cells. In addition, antioxidants such as vitamin E, vitamin C, polyphenols and carotenoids are available from foods. Current dietary guidelines to combat chronic diseases, including cancer and CHD, recommend increased intake of plant foods, including fruits and vegetables, which are rich sources of antioxidants[26,27]. The role of dietary antioxidants, including vitamin C, vitamin E, carotenoids and polyphenols, in disease prevention has received much attention recently and appears to have a wide range of antiatherogenic properties[28-31]. These observations may explain the epidemiological data indicating that diets rich in fruits and vegetables are associated with a reduced risk of numerous chronic diseases[32,33].

Lycopene (Lyc)

Chemical Structure

Lyc, the most predominant carotenoid in human plasma, a natural pigment synthesized by plants and microrganisms, is an acyclic isomer of beta-carotene[34,35] containing 11 conjugated and two non-conjugated double bonds arranged in a linear array[8,36]. Lyc from natural plant sources exists predominantly in an all-*trans* configuration, the most thermodynamically stable form, and in human plasma is present as an isomeric mixture, with 50% as *cis* isomers[37].

Because of its high number of conjugated dienes, lyc is one of the most potent antioxidants, with a singlet-oxygen-quenching ability twice as high as that of ß-carotene and 10 times higher than that of alpha-tocopherol[38-41]. Owing to their lipophilic nature, lyc and other carotenoids are found to concentrate in serum low-density and very-low-density lipoprotein fractions[35]. Lyc is also found to concentrate in the adrenal gland, testes, liver and prostate gland, where it is the most prominent carotenoid[42-45]. Tissue-specific lyc distribution may be important in the antioxidant role. However, unlike other carotenoids, lyc levels in serum or tissues do not correlate well with overall intake of fruits and vegetables[46,47].

Red fruits and vegetables, including tomatoes, watermelons, pink grapefruits, apricots and pink guavas, contain lyc[48]. Processed tomato products, such as juice, ketchup, paste, sauce and soup, all are good dietary sources of lyc[49].

Mechanisms of Action

Two major hypotheses have been proposed to explain the antiatherogenic activities of lyc: non-oxidative and oxidative mechanisms. The non-oxidative mechanisms has been suggested to be due to regulation of gap-junction communication in mouse embryo fibroblast cells[50,51].

The oxidative hypotesis include the oxidization of LDL as the primary initial step leading to its uptake by the macrophages inside the arterial wall and the formation of foam cells and atherosclerotic plaque[52]. The oxidative modifications reflect not only the polyunsaturated fatty acids (PUFA), but also oxidation of the cholesterol, phospholipids, and oxidative degradation of the apolipoprotein B itself[53]. In addition to the formation of foam cells and plaque in the arterial wall, components of LDL_{ox} can also influence other events that related to the increased risk of CHD[54]. These include their ability to increase cholesterol accumulation by macrophages, their ability to produce proteins with chemiotactic activity to monocytes and cytotoxic activity to a variety of cells causing endothelial injuries[55].

Lyc also has been shown to act as a hypocholesterolemic agent by inhibiting HMG-CoA (3-hydroxy-methylglutaryl-coenzyme A-reductase)[56], and has been hypothesized to prevent atherogenesis by protecting critical cellular biomolecules, including lipids, lipoproteins, proteins and DNA[57-59]. In healthy human subjects, lyc- or tomato-free diets resulted in loss of lyc and increased lipid oxidation[60], whereas dietary supplementation with lyc for 1 week increased serum lyc levels and reduced endogenous levels of oxidation of lipids, proteins, lipoproteins and DNA[57,58].

Lycopene and Cardiovascular Disease

Risk of Cardiovascular Disease

Several epidemiological and prospective studies have shown that the consumption of antioxidant vitamins such as vitamin E and beta-carotene may reduce the risk of CHD[61,62]. However, if some large-scale human trials have failed to confirm the protective effect of antioxidant supplementation (vitamin E and beta-carotene) observed in previously[9,10].

Few similar studies, *in vitro* and *in vivo*, have been performed with lyc. A number of studies *in vitro* have shown that lyc can protect native LDL from oxidation and can suppress cholesterol synthesis[54,55]. However, the predictability of *in vitro* LDL-oxidation as a marker of atherosclerosis has been questioned in recent years[63].

Epidemiological Studies

Several 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 lyc, the major responsible for the beneficial effects of these food products. In particular mediterranean diet is rich in tomatoes, tomato products, lyc, and other carotenoids, and is associated with lower risk of CHD[56,57,59].

In the Physicians' Health Study, Sesso et al[64] have founded in 499 patients with CVD (MI, stroke, CVD death, or revascularization procedures) an association between higher plasma lyc concentration and low risk of CVD.

In the CARDIA/YALTA study, Hozawa et al[65] have demonstrated that circulating serum carotenoids (alpha-carotene, beta-carotene, zeaxanthin/lutein, beta-cryptoxanthin, and lyc) were associated in apparently beneficial directions with markers of inflammations (leucocyte counts, fibrinogen, CRP), oxidative stress, and endothelial dysfunction (F2 isoprostane, serum ICAM-1, and P-selectin).

Lidebjer et al[66] in a case-control study on 139 subjects (39 with acute coronary syndrome, 50 with stable CAD, and 50 healthy controls) have demonstrated a significantly lower plasma levels of oxygenated carotenoids (lutein, zeaxanthin, lyc, beta-cryptoxanthin, alpha- and beta-carotene) in both patient groups.

The EURAMIC multicentre case-control study, has evaluated the relation between antioxidant status and acute MI. 1,389 subjects (662 cases and 717 controls) were recruited from 10 European countries to maximize the variability in exposure within the study. Adipose tissue antioxidant levels, which are better indicators of long-term exposure than blood antioxidant levels, were used as markers of antioxidant status. Biopsy specimens of adipose tissue, taken directly after the MI, were analysed for various carotenoids. After adjustment for a range of dietary variables (age, BMI, socioeconomic status, smoking, hypertension, and maternal-paternal hystory of the disease), only lyc levels, and not β-carotene levels, were found to be protective (odds ratio 0.52 for the contrast of the 10th and 90th percentile with a 0.005 p value). In particular this study have showed a various odds ratio in subjects never smokers (0.33), ex-smokers (0.41), and smokers (0.63) supporting the antioxidant hypothesis[66]. Similarly, Handelman et al[57] in a study from John Hopkins University showed that smokers with low levels of circulating carotenoids have an higher risk for subsequent MI.

Lower blood lyc levels were also found to be associated with increased risk for death in a population study comparing Lithuanian and Swedish cohorts with different rates of death from CAD[68]. Even in a Japanese population-based study (11.9 year follow-up) Ito et al[69] have found in 3,061 subjects (1,190 males and 1,871 females) an association between low hazard ratio for cardiovascular disease mortality and high serum values of carotenoids (alpha- and beta-carotene, lyc, total carotene values).

Only in the Atherosclerosis Risk in Communities (ARIC) case-control study, the fasting serum antioxidant levels of 231 cases and an equal number of control subjects were assessed in rela-

tionship to the intima-media thickness (IMT) as an indicator of asymptomatic early atherosclerosis. After controlling for other variables, an odds ratio of 0.81 was observed, but the *p* value for the association for lyc was not significant[70].

Supplementation Clinical Trials

Although epidemiological studies conducted so far provide convincing evidence for the role of lyc in CHD prevention, there is a good suggestion but not the proof of a causal relationship between lyc intake and risk of CHD. However, very such supplementation studies have been reported in literature.

Bose et al[71], recently, in a clinical case-control study on subjects with CHD have demonstrated a significant improvement in the level of serum enzymes involved in antioxidant activities (lipid peroxidation rate, SOD, GSH_{PX}) after 60 days of tomato supplementation in the CHD group respect to control group suggesting that the tomato lyc may have a considerable therapeutic potential as an antioxidant.

Visioli et al[72] in three-weeks supplementation study of tomato products (raw, sauce, and paste) on twelve females have found an increased significant lyc concentrations after tomato consumption and a reduced LDL-oxidizability suggesting an important role for tomato products in the prevention of lipid proxidation.

In a small dietary supplemention study[73], six healthy male subjects consumed 60 mg/day lyc for three months. At the end of treatment period, a significant 14% reduction in their plasma LDL-C level was observed.

In a randomized, crossover dietary intervention study[74], 19 non smokers healthy human subjects (10 male, 9 females) consumed lyc from traditional tomato products and nutritional supplement for one week (from 20 to 150 mg/day). Lyc was observed to be absorbed readily from all dietary sources, resulting in significantly increase in serum lyc levels and lower lipid lewels, protein, and DNA oxidation. In the same study[75] although there were no changes in serum total cholesterol and LDL-C and HDL-C, serum lipid peroxidation were significantly decreased and serum lyc levels increased.

Conversely, Peterson et al[76] have designed a single-blind, randomized, controlled, crossover dietary intervention study aimed to determine wheter an increase of five portions of fruits and vegetables in the form of soups and beverages have beneficial effects on markers of oxidative stress and CVD risk factors. After 4 weeks consumed carotenoid-rich or control vegetable soups and beverages they have found an increase of dietary carotenoids (alpha- and beta-carotene, lyc), vitamin C, alpha-tocopherol, potassium, and folate, but not alterations of oxidative stress markers or cardiovascular risk factors.

Conclusion

The current dietary recommendation to increase the consumption of fruits and vegetables rich in antioxidants has generated interest in the role of lyc in disease prevention. However, the evidence thus far is mainly suggestive, and the underlying mechanisms are not clearly understood[77,78].

Nutrition has a significant role in the prevention of many chronic disease as CVD[79,80]. A great intake of fruit and vegetables can help prevent heart disease and mortality by preventing the oxidation of cholesterol in arteries. Even epidemiological studies yielded conflicting results, carotenoids, in particular lyc, has an important role and play a key effect in the prevention of CVD.

Only few lyc dietary supplementation small studies have shown a mild significative both hypocholesterolemic effect than reduction of LDL oxidation. However, a clearly relationship between lyc intake supplementation and reduction risk of CHD is not fully established. For this reason long-term study are need for to evaluate a clear and significative link between lyc and risk of CVD.

Another important aspect regard the bioavailability of dietary lyc which is influenced in absorption by other antioxidants. Lyc has been shown to be better adsorbed from processed tomato products than from fresh tomatoes. Many factors (bioavailability, metabolism, isomerization, interaction with other carotenoids, and mechanism) are important in establishing long-term and well-designed future studies to evaluate the role of lyc in prevention of CHD.

References

1) Pearson TA. Cardiovascular disease in developing countries: myths, realities, and opportunities. Cardiovasc Drug Ther 1999; 13: 95-104.

2) Vasan RS, Benjamin EJ, Sullivan LM, D'Agostino RB. The burden of increasing worldwide cardiovascular disease. In: Hurst's, The Heart, Fuster C, Wayne AR, O'Rourke RA (eds), 11th edition, New York, McGraw-Hill, 2004.

3) Neaton J, Wentworth D. Serum cholesterol, blood pressure, cigarette smoking, and death from coronary heart disease. Overall findings and difference by age for 316.099 white man. Multiple Risk Factor Intervention Trial Research Group. Arch Intern Med 1992; 152: 56-64.

4) Rao AV, Rao GL. Carotenoid and human health. Pharmacol Res 2007; 55: 207-216.

5) Khan N, Afaq F, Mukhtar H. Cancer chemoprevention through dietary antioxidants: progress and promise. Antioxid Redox Signal 2008; 10: 475-510.

6) Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 1999; 91: 317-31.

7) Seren S, Lieberman R, Bayraktar UD, Heath E, Sahin K, Andic F, Kucuk O. Lycopene in cancer prevention and treatment. Am J Ther 2008; 15: 66-81.

8) Britton G. Structure and properties of carotenoids in relation to function. FASEB J 1995; 9: 1551-1558.

9) Riccioni G, Bucciarelli T, Mancini B, Di Ilio C, Capra V, D'Orazio N. The role of the antioxidant vitamin supplementation in the prevention of cardiovascular diseases. Expert Opin Investig Drugs 2007; 16: 25-32.

10) Riccioni G, Bucciarelli T, Mancini B, Corradi F, Di Ilio C, Mattei P, D'Orazio N. Antioxidant vitamin supplementation in cardiovascular diseases. Ann Clin Lab Sci 2007; 37: 89-95.

11) Rao AV, Ray MR, Rao LG. Lycopene. Adv Food Nutr Res 2006; 51: 99-164.

12) Dzau VJ, Antman EM, Black HR, Hayes DL, Manson JAE, Plutzy J. The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes. Part I: pathophysiology and clinical trial evidence (Risk factors through stable coronary artery disease). Circulation 2006; 114: 2850-2870.

13) Dzau J. Theodore Cooper lecture: Tissue angiotensin and pathobiology of vascular disease: a unifying hypothesis. Hypertension 2001; 37: 1047-1052.

14) Ames BN, Gold LS, Willett WC. Causes and prevention of cancer. Proc Natl Acad Sci USA 1995; 92: 5258-5265.

15) Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994; 344: 793-795.

16) Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet 1994; 344: 721-724.

17) Heller FR, Descamps O, Hondekijn JC. LDL oxidation: therapeutic perspectives. Atherosclerosis 1998;137 (Suppl): S25-S31.

18) Rosenfeld ME. Leukocyte recruitment into developing atherosclerotic lesion: the complex interaction between multiple molecules keeps getting more complex. Arterioscl Thromb Vasc Biol 2002; 22: 361-363.

19) Baggiolini M. Chemokines in pathology and medicine. J Intern Med 2001; 250: 91-104.

20) Calabro P, Wilerson JT, Yeh ET. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. Circulation 2003; 108: 1930-1932.

21) Jabs WJ, Theissing E, Nitschke M, Bechtel JF, Duchrow M, Mohamed S, Jahrbeck B, Sievers HH, Steinhoff J, Bartels C. Local generation of C-reactive protein in diseased coronary artery venous by pass grafts and normal vascular tissue. Circulation 2003; 108: 1428-1431.

22) Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. Circulation 2001; 103: 1194-1197.

23) Albert CM, Ma J, Rifai N, Stampfer MJ, Ridker PM. Prospective study of CRP, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. Circulation 2002; 105: 2595-2599.

24) Ridker PM, Cushman M, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral artery disease. JAMA 2001; 285: 2481-2485.

25) Burke AP, Tracy RP, Kolodgie F, Malcom GT, Zieske A, Kutys R, Pestaner J, Smialek J, Virmani R. Elevated C-reactive protein values and atherosclerosis in sudden coronary death: association with different pathologies. Circulation 2002; 105: 2019-2023.

26) Canada's food guide to healthy eating. Ottawa: Health Canada; 1992. Cat no H3953/1992E. Available:www.hcc.gc.ca/hppb/nutrition/pube/foodguid/foodguide.html.

27) Dietary guidelines for Americans. 5th ed. Home and Garden Bulletin no 232. Washington: US Department of Agriculture, US Department of Health and Human Services; 2000. Available: www.nal.usda.gov/fnic/dga.

28) 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.

29) Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 1995; 62: 1315S-1321S.

30) Feri B. Natural antioxidants in human health and disease. San Diego: Academic Press; 1994.

31) Food, Nutrition and the Prevention of Cancer: a global perspective. Washington: World Cancer Research Fund/American Institute for Cancer Research; 1997.

32) Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz G-A, Willett W-C. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993; 328: 1450-1456.

33) Gaziano JM, Manson JE, Branch LG, Colditz GA, Willett WC, Buring JE. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. Ann Epidemiol 1995; 5: 255-260.

34) Rao AV, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutr Res 1999; 19: 305-323.

35) Clinton SK. Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev 1998; 56: 35-51.

36) Zechmeister L, LeRosen AL, Went FW, Pauling L. Prolycopene, a naturally occurring stereoisomer of lycopene. Proc Natl Acad Sci USA 1991; 21: 468-474.

37) Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ. Cis-trans Lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev 1996; 5: 823-833.

38) Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. Antioxidant activities of carotenes and xanthophylls. FEBS Lett 1996; 384: 240-246.

39) 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.

40) Woodall AA, Lee SWM, Weesie RJ, Jackson MJ, Britton G. Oxidation of carotenoids by free radicals: relationship between structure and reactivity. Biochim Biophys Acta 1997; 1336: 33-42.

41) Di Mascio P, Kaiser S, Sies H. Lycopene as the most effective biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989; 274: 532-538.

42) Stahl W, Schwarz W, Sundquist AR, Sies H. Cis-trans isomers of lycopene and beta-carotene in human serum and tissues. Arch Biochem Biophys 1992; 294: 173-177.

43) Kaplan LA, Lau JM, Stein EA. Carotenoid composition, concentrations and relationships in various human organs. Clin Physiol Biochem 1990; 8: 1-10.

44) 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.

45) 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.

46) Michaud DS, Giovannucci EL, Ascherio A, Rimm EB, Forman MR, Sampson L. Associations of plasma carotenoid concentrations and dietary intake of specific carotenoids in samples of two prospective cohort studies using a new carotenoid database. Cancer Epidemiol Biomarkers Prev 1998; 7: 283-290.

47) Stahl W, Sies H. Lycopene: a biologically important carotenoid for humans? Arch Biochem Biophys 1996; 336: 1-9.

48) Nguyen ML, Schwartz SJ. Lycopene: chemical and biological properties. Food Technol 1999; 53: 38-45.

49) Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health disease. Mol Aspects Med 2005; 26: 459-516.

50) Zhang LX, Cooney RV, Bertram JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. Carcinogenesis 1991; 12: 2109-2114.

51) Zhang LX, Cooney RV, Bertram JS. Carotenoids upregulate connexin43 gene expression independent of their provitamin A or antioxidant properties. Cancer Res 1992; 52: 5707-5712.

52) Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994; 344: 793-795.

53) Witztum JL. Role of oxidized low densirty lipoprotein in atherogenesis. Br Heart J 1993; 69: S12-S18.

54) Frei B. Cardiovascular disease and nutrient antioxidants: role of low-density lipoprotein oxidation. Crit Rev Food Sci Nutr 1995; 351: 83-98.

55) Holvoet P, Collen D. Oxidized lipoproteins in atherosclerosis and thrombosis. FASEB J 1994; 8: 1279-1284.

56) 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 Commun 1997; 233: 658-662.

57) Agarwal S, Rao AV. Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. Lipids 1998; 33: 981-984.

58) 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.

59) 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.

60) Rao AV, Agarwal S. Effect of diet and smoking on serum lycopene and lipid peroxidation. Nutr Res 1998; 18: 713-721.

61) Kohlmeier L, Kark JD, Gomez-Garcia E, Martin BC, Steck SE, Kardinal AFM. Lycopene and miocardial infarction risk in the EURAMIC study. Am J Epidemiol 1997; 146: 618-626.

62) Agarwal S, Rao AV. Carotenoids and chronic disease. Drug Metabol Drug Interact 2000; 17: 189-209.

63) Zock P, Katan MB. Diet, LDL oxidation, and coronary artery disease. Am J Clin Nutr 1998; 68: 759-760.

64) Sesso HD, Buring JE, Norkus EP, Gaziano JM. Plasma lycopene, other carotenoids, retinol and the risk of cardiovascular disease in men. Am J Clin Nutr 2005; 81: 990-997.

65) Hozawa A, Jacobs DR, Steffes MW, Gross MD, Steffen LM, LEE DH. Relationships of circulating carotenoid concentrations with several markers of inflammation, oxidative stress, and endothelial dysfunction: the Coronary Artery Risk Development in Young Adults (CARDIA)/Young Adult Longitudinal Trends in Antioxidants (YALTA) study. Clin Chem 2007; 53: 447-455.

66) Lidebjer C, Leanderson P, Ernerudh J, Jonasson L. Low plasma levels of oxygenated carotenoids in patients with coronary artery disease. Nutr Metab Cardiovasc Dis 2007; 17: 448-456.

67) Handelman GJ, Parker L, Cross CE. Destruction of tocopherols, carotenoids and retinol in human plasma by cigarette smoke. Am J Clin Nutr 1996; 63: 559-565.

68) Kristenson M, Zieden B, Kucinskiene Z, Elinder LS, Bergdahl B, Elwing B. Antioxidant state and mortality from coronary heart disease in Lithuanian and Swedish men: concomitant cross sectional study of men aged 50. Br Med J 1997; 314: 629-633.

69) Ito Y, Kurata M, Suzuki K, Hamajima N, Hishida H, Aoki K. Cardiovascular disease mortality and serum carotenoid levels: a Japanese population-based follow-up study. J Epidemiol 2006; 16: 154-160.

70) Irribarren C, Folson AR, Jacobs DR, Cross MD, Belcher JD, Eckfeldt JH. Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis. Arterioscler Thromb Vasc Biol 1997; 17: 1171-1177.

71) Bose KS, Agrawal BK. Effect of lycopene from cooked tomatoes on serum antioxidant enzymes, lipid peroxidation rate and lipide profile in coronary heart disease. Singapore Med J 2007; 48: 415-420.

72) Visioli F, Riso P, Grande S, Galli C, Porrini M. Protective activity of tomato products on in vivo markers of lipid oxidation. Eur J Nutr 2003; 42: 201-206.

73) Fuhrman B, Elis A, Aviram M. Hypercholesterolemic effect of lycopene and beta-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophage. Biochem Biophys Res Commun 1997; 233: 658-662.

74) 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.

75) Agarwal S, Rao AV. Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. Lipids 1988; 33: 981-984.

76) Paterson E, Gordon MH, Niwat C, George TW, Parr L, Waroonphan S, Lovegrove JA. Supplementation with fruit and vegetable soups and beverages increas plasma carotenoid concentrations but does not alter marker of oxidative stress or cardiovascular risk factors. J Nutr 2006; 136: 2849-2855.

77) Voutilainen S, Nurmi T, Mursu U, Rissanen TH. Carotenoids and cardiovascular health. Am J Clin Nutr 2006; 83: 1265-1271.

78) Sesso HD. Carotenoids and cardiovascular disease: what research gaps remain? Curr Opin Lipidol 2006; 17: 11-16.

79) Das S, Otani H, Maulik N, Das DH. Lycopene, tomatoes, and coronary heart disease. Free Rad Res 2005; 39: 449-455.

80) Kaliora AC, Dedoussis GV, Schmidt H. Dietary antioxidants in preventing atherogenesis. Atherosclerosis 2006; 187: 1-17.