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Review Article

Are antioxidants helpful for disease prevention?

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Abstract

Free radicals are produced continuously in the cells as part of normal cellular function, however excess production might play a role in pathophysiology of many disease conditions, including cancer, Alzheimer's disease, atherosclerosis and some of the drug-induced toxicity. Many basic research studies and observational epidemiologic studies in human suggest that antioxidants can prevent oxidative damage. However, this is still a controversial issue because the results of clinical trials have been inconsistent. This article provides a brief overview of some of the diseases which are associated with free radicals, then discusses the roles of some of dietary antioxidant supplements in disease prevention, with particular reference to the findings of latest clinical trials.

Keywords: Free radicals; Antioxidants; Disease prevention; Clinical trials

INTRODUCTION

Oxygen is an indispensable element for life. However under certain conditions it can have serious deleterious effects on the body. Oxygen can produce highly reactive compounds called reactive oxygen species (ROS). Many such reactive species are free radicals containing an unpaired electron. They have a tendency to either accept or donate an electron and are, therefore, unstable and highly reactive. Hydroxyl radical (OH), the superoxide radical (O_2), the nitric oxide radical (NO) and the lipid peroxyl radical (LOO) are amongst the most important oxygen-containing radicals formed in the body. Free radicals can attack a number of macromolecules including lipids, proteins and DNA resulting in the cellular damage (1-3).

Main sources of free radicals

Free radicals and other ROS are derived either from the enogenous metabolic processes in the human body or from external sources. The former includes mitochondrial respiration, peroxisomal metabolism, phagocyte activity, arachidonate pathways, inflammation, ischaemia, exercise and reactions involving iron and other transition metals. External sources include exposure to radiation, ozone, cigarette smoke, air pollutant and industrial chemicals (2).

Defense systems

Antioxidants are molecules that can safely react with free radicals to neutralize or terminate the chain reaction before vital molecules are damaged. They exert their defense mechanisms in a number of ways:
1) Enzymatic systems which catalyze removal of free radicals and ROS, e.g. catalase and superoxide dismutase. 2) Sacrificial antioxidants which donate oxygen to free radicals, such as vitamin C and vitamin E. 3) Proteins that minimize the availability of pro-oxidants, such as transferrin, heptoglobins, haemopexin and metallothionein. 4) Proteins that protect molecules by other mechanisms, e.g. heat shock protein (4,5).

Oxidative stress and diseases

Oxidative stress is an imbalance between the

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production of ROS and the defense systems to readily detoxify them (1). In human, oxidative damage and free radicals are associated with a number of diseases including atherosclerosis (3), Alzheimer's disease (6), cancer (7), ocular disease (8), diabetes (9), rheumatoid arthritis (10) and motor neuron disease (11). Many studies have shown the benefit of antioxidants in the prevention or delaying the course of these diseases. Amongst these antioxidant dietary supplements such as vitamin E, vitamin C and beta-carotene are widely used. However, the results of clinical trials have been inconsistent. In addition, the role of oxidative stress in the xenobiotics-induced toxicity has been a subject of intense interest. It is likely that antioxidants can prevent oxidative stress-mediated toxicity since it may be caused by oxygen free radicals which are produced by drugs. The protective effect of N-acetylcysteine from acetaminophen-induced hepatotoxicity is an example. It has been demonstrated that vitamin E has promising effects in protecting against toxicity induced by vancomycin (12), gentamycin (13) and cisplatin (14). Tempol and 2,3-dihydroxy-benzoic acid can prevent vancomycin-induced nephrotoxicity in rat (15) and antioxidants could ameliorate some of phenytoin adverse effects (16).

Many basic research studies and obser-vational epidemiologic studies in human suggest that antioxidants can prevent oxidative damage in that individuals who consume large amounts of fruits and vegetables seem to experience lower rates of diseases caused by oxidative damage. However, results of clinical trials have been inconsistent or in some cases there has been a lack of large scale randomized clinical trials. This article provides an overview of the findings of the latest clinical trials.

Antioxidants and prevention of athero-sclerosis

Lipoprotein oxidation is a key early stage in the development of atherosclerosis. Oxidized LDL is known to promote atherogenesis through foam cell formation and inflammatory responses. Free radicals have been implicated in the oxidative modification of LDL and several basic research studies strongly suggest that progression of the atherosclerotic lesions can be delayed by intervention with anti-oxidants (3,17,18). It has been advised that general population consume a balanced diet with more emphasize on antioxidants (19). However there are discrepancies in epidemio-logical evidence and clinical trails, and few long-term trials have evaluated the effect of antioxidants in men at initially low risk of cardiovascular disease.

In a recent randomized, double-blind, placebo-controlled factorial trial of vitamin E and vitamin C, designed by Physicians Health Study II and carried out between 1997 and 2007, neither vitamin C (500 mg daily) nor vitamin E (400 IU daily) could reduce the risk of major cardiovascular events in 14,641 US male physicians with initially age of 50 years or older. During the follow-up (mean of 8 years), there were 1245 confirmed major cardiovascular events. Vitamin E had no effect on the incidence of major cardiovascular events, compared to placebo (both vitamin E and placebo groups, 10.9 events per 1000 person-years; hazard ratio (HR), 1.01 [95% confidence interval (CI), 0.90-1.13]; P=0.86), as well as total myocardial infarction (HR, 0.90 [95% CI, 0.75-1.07]; P=0.22), total stroke (HR, 1.07 [95% CI, 0.89-1.29]; P=0.45), and cardiovascular mortality (HR, 1.07 [95% CI, 0.90-1.28]; P=0.43). Similarly, compared with placebo, vitamin C had no significant effect on major cardiovascular events (in vitamin C and placebo groups, there were 10.8 and 10.9 events per 1000 person-years, respectively; HR, 0.99 [95% CI, 0.89-1.11]; P=0.91), as well as total myocardial infarction (HR, 1.04 [95% CI, 0.87-1.24]; P=0.65), total stroke (HR, 0.89 [95% CI, 0.74-1.07]; P=0.21), and cardiovascular mortality (HR, 1.02 [95% CI, 0.85-1.21]; P=0.86). Neither vitamin E (HR, 1.07 [95% CI, 0.97-1.18]; P=0.15) nor vitamin C (HR, 1.07 [95% CI, 0.97-1.18]; P=0.16) had a significant effect on total mortality, however, vitamin E was associated with an increased risk of hemorrhagic stroke (HR, 1.74 [95% CI, 1.04-2.91]). These data provide no support for the use of vitamin E and vitamin C alone or in combination for the prevention of cardiovas-cular disease in middle-aged and old men (20).

In another randomized, placebo-controlled factorial trial of vitamin E, part of the women's health study (WHS), conducted between 1992 and 2004, 39,876 apparently healthy US female health care professionals aged at least 45 years were randomly assigned to receive vitamin E or placebo and aspirin or placebo (21). Women were administrated 600 IU of natural-source vitamin E on alternate days and were followed up for an average of 10.1 years. During follow-up, there were 482 major cardiovascular events in the vitamin E group and 517 in the placebo group, a non significant 7% risk reduction (relative risk [RR], 0.93, 95% [CI], 0.82-1.05; *P*=0.26). There were no significant effects on the incidences of myocardial infarction (RR, 1.01; 95% CI, 0.82-1.23; *P*=0.96) or stroke (RR, 0.98; 95% CI, 0.82-1.17; *P*=0.82), as well as ischemic or hemorrhagic stroke. For cardiovascular death, there was a significant 24% reduction (RR, 0.76; 95% CI, 0.59-0.98; *P*=0.03). However, there was no significant effect of vitamin E on total mortality (636 in the vitamin E group and 615 in the placebo group; RR, 1.04; 95% CI, 0.93-1.16; *P*=0.53). The data from this large trial do not support recommending vitamin E supplementation for cardiovascular disease prevention among healthy women (21).

Earlier in another randomized, double-blind trial conducted between 1997 and 2002 in the United States and Canada 423 postmenopausal women with coronary disease were studied to determine whether hormone replacement therapy (HRT) or antioxidant supplements, alone or in combination, influence the progression of coronary artery disease, as measured by serial quantitative coronary angiography. The study found that, compared with the placebo, neither vitamin E (400 IU) twice daily plus vitamin C (500 mg) twice daily alone nor in combination with HRT provide cardio-vascular benefit. Instead, a potential for harm was suggested with each treatment (22).

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Antioxidants and prevention of cancer

The underlying cause of cancer is thought to be damage to DNA, much of which is oxidative in nature. These oxidative processes, the mechanisms of which not fully understood, occur during the promotional stage of carcinogenesis. Therefore, it is plausible that antioxidants may be able to interfere with the metabolic activation of chemical carcinogens, cause regression of pre-malignant lesions or inhibit their development into cancer (3).

Evidence from basic research and observational epidemiologic studies suggest that individuals with high intakes of fruits and vegetables experience lower risks of developing cancer. Although there are many compounds in fruits and vegetables that may potentially influence cancer risk, it is generally assumed that certain antioxidants such as vitamin E, Vitamin C and beta-carotene may be responsible for the lower cancer rates. However, the few randomized trials of vitamin E, vitamin C or beta-carotene supplementation show no overall benefits; some even suggest harm. The findings of recent trials are summarized below:

The Physicians' Health Study II Randomized Controlled Trial also evaluated whether long-term vitamin E (400 IU every other day) or vitamin C (500 mg daily) supplementation decreases the risk of prostate and total cancer events among men (23). During a mean follow-up of 8.0 years, there were 1943 confirmed incident cases of total cancers and 1008 cases of prostate cancer. Compared with placebo, vitamin E had no effect on the incidence of total cancer (vitamin E and placebo groups, 17.8 and 17.3 cases per 1000 person-years; HR, 1.04; 95% CI, 0.95-1.13; *P*=0.41) or prostate cancer (vitamin E and placebo groups, 9.1 and 9.5 events per 1000 person-years; HR, 0.97; 95% CI, 0.85-1.09; *P*=0.58). There was also no significant effect of vitamin C on total cancer (vitamin C and placebo groups, 17.6 and 17.5 events per 1000 person-years; HR, 1.01; 95%CI, 0.92-1.10; *P*=0.86) or prostate cancer (vitamin C and placebo groups, 9.4 and 9.2 cases per 1000 person-years; HR, 1.02; 95% CI, 0.90-1.15; *P*=0.80). Neither vitamin E nor vitamin C had a significant effect on colorectal, lung, or other site-specific cancers. Stratification by various cancer risk factors demonstrated no significant modification of the effect of vitamin E on prostate cancer risk or either supplement on total cancer risk. In this large, long-term trial neither vitamin E nor vitamin C supplemen-

tation reduced the risk of prostate or total cancer in middle-aged and older men (23).

The women's health study cited above also evaluated the effect of vitamin E supplementation on the prevention of cancer (21). The study found that during the follow-up, there was no significant effect on the incidences of total cancer (1437 cases in the vitamin E group and 1428 in the placebo group; RR, 1.01; 95% CI, 0.94-1.08; *P*=0.87), breast (RR, 1.00; 95% CI, 0.90-1.12; *P*=0.95), lung (RR, 1.09; 95% CI, 0.83-1.44; *P*=0.52), or colon cancers (RR, 1.00; 95% CI, 0.77-1.31; *P*=0.99). Cancer deaths also did not differ significantly between groups. There was no significant effect of vitamin E on total mortality (636 in the vitamin E group and 615 in the placebo group; RR, 1.04; 95% CI, 0.93-1.16; *P*=0.53). The data from this large trial indicated that 600 IU of natural-source vitamin E taken every other day provided no overall benefit for prevention of cancer among healthy women (21). A third component of this study also evaluated the effects of beta-carotene supplementation in the prevention of cancer and cardiovascular disease. The study concluded that among 39,876 healthy women aged 45 and over randomly assigned to receive beta-carotene (50 mg on alternate days) or placebo, there were no statistically significant differences in incidence of cancer, cardiovascular disease, or total mortality after a median of 4.1 years (2.1 years' treatment plus 2.0 years' follow-up) (24).

A Systematic Review and Meta-analysis evaluated randomized clinical trails of antioxidants on the prevention of cancer (25). The review examined literature up to August 2005 and identified 12 high quality trails (including the women's health study) with a combined population of 104,196. Eligible antioxidants included beta-carotene, selenium, zinc, vitamin C and vitamin E alone or in combination with other antioxidant supplements. The review concludes that antioxidant supplementation, particularly with beta-carotene and vitamin E, does not reduce primary cancer incidence or cancer mortality. Beta-carotene supplementation might increase the risk of smoking-related cancers, as well as cancer mortality, and should be avoided by tobacco users. Selenium supplementation might reduce cancer incidence and cancer mortality in men, but not in women. Further research is needed to confirm the chemopreventive effect of selenium (25).

Antioxidants and prevention of ocular disease

Oxidative processes are thought to be an important contributing factor in the development of both cataracts and the age-related disorder of the retina, maculopathy. Oxidation, induced mainly by exposure to UV light, is believed to be a major cause of damage to the proteins of the lens. The oxidized protein precipitates and causes cloudiness of the lens. Antioxidants and antioxidant enzymes inactivate harmful free radicals and proteases degradation and remove the damaged portion from the lens, but the oxidative damage occurs at a faster rate. The oxidized protein may therefore accumulate, and with time, the damage becomes irreversible.

There is substantial interest in determining whether antioxidant lower risks of cataract development and progression. Basic laboratory studies and observational epidemiologic studies in humans support this possibility. Supplementation with antioxidant vitamins and minerals prevents or delays cataract development *in vitro* and in animal models. Individuals with higher intakes of fruits and vegetables, or higher plasma levels of various antioxidant nutrients, tend to have lower risks of cataract (26). However, the data for individual nutrients, including vitamins C and E and the carotenoids, are inconsistent. Rrecent randomized trials have found vitamin E and C ineffective against cataract.

A large randomized, double-masked trial examined the effect of beta-carotene supplementation on the development of age-related cataract in men. Healthy US physicians 40 to 84 years old (n = 22,071) were randomly assigned to receive either beta-carotene (50 mg on alternate days) or placebo for 12 years. The study found no difference between the beta-carotene and placebo groups in the overall incidence of cataract (998 cases vs. 1017 cases; RR, 1.00; 95% CI, 0.91-1.09) or cataract extraction (584 vs. 593, RR, 1.00;

95% CI, 0.89-1.12). However, among current smokers at baseline, beta-carotene appeared to ease their excess risk of cataract by about one fourth (27).

The women's health study also investigated whether vitamin E supplementation decreases the risk of age-related cataract in women (28). Participants (n = 39,876) were assigned randomly to receive either 600 IU natural-source vitamin E on alternate days or placebo and were followed up for the presence of cataract for an average of 9.7 years. Study found no significant difference between the vitamin E and placebo groups in the incidence of cataract (1159 vs. 1217 cases; RR, 0.96; 95% CI, 0.88–1.04). In subgroup analyses of subtypes, there were no significant effects of vitamin E on the incidence of nuclear, cortical, or posterior subcapsular cataract. The study concludes that 600 IU vitamin E taken every other day provides no benefit for age-related cataract or subtypes (28).

Another randomized, double-blind, placebo-controlled clinical trial evaluated the effect of a multivitamin/mineral supplement on the development or progression of age-related lens opacities. Participants (n = 1,020) aged 55 to 75 years and with early or no cataract, were randomly assigned to a daily tablet of multivitamin/mineral formulation (including vitamin A 5000 IU, vitamin C 60 mg, and vitamin E 30 IU) or a placebo and were observed for an average of 9.0 ± 2.4 years. Compared with placebo, total lens events were less common in participants who took the multivitamin/mineral formulation, but treatment had opposite effects on the development or progression of nuclear and posterior subcapsular cataract opacities, the two most visually important opacity subtypes. No statistically significant treatment effects were seen for cortical opacities, moderate visual acuity loss, or cataract surgery (29).

Antioxidants and prevention of skin aging

The reactions which add hydroxyl groups to the amino acids proline and lysine in the collagen molecule, via prolylhydroxylase and lysyl hydroxylase, both require vitamin C as a cofactor. Hydroxylation allows the collagen molecule to assume its triple helix structure, making vitamin C essential to the development and maintenance of scar tissue, blood vessels, and cartilage (30,31). In addition, topically applied vitamin C seems to enhance the mRNA level of collagens I and III, their processing enzymes, and the tissue inhibitor of matrix metalloproteinase 1 in the human dermis (32).

A randomized, double-blind, placebo-controlled trail conducted in France examined whether supplementation with a combination of antioxidant vitamins and minerals could reduce the risk of skin cancers (33). Adults (7876 women and 5141 men) were randomized to take an oral daily capsule of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg beta-carotene, 100 µg selenium, and 20 mg zinc) or an antioxidant placebo. The participants were followed-up for a median time of 7.5 years. A total of 157 cases of all types of skin cancer were reported, from which 25 were melanomas. Subgroup analysis of data showed that in women, the incidence of skin cancer was higher in the antioxidant group compared to placebo (adjusted HR, 1.68; *P*=0.03). In men, however, the incidence did not differ between the 2 treatment groups (adjusted HR, 0.69; *P*=0.11). The incidence of melanoma also appeared to be higher in the antioxidant group for women (adjusted HR, 4.31; *P*=0.02). The incidence of non-melanoma skin cancer did not differ between the antioxidant and placebo groups for women (adjusted HR, 1.37; *P*=0.22) or men (adjusted HR, 0.72; *P*=0.19) (33).

A double-blind randomized trial was performed to evaluate the clinical effects of a cream containing vitamin C vs. excipient on photoaged skin (34). Twenty healthy French female volunteers aged 51–59 years presenting with photoaged skin were given a topically applied cream containing 5% vitamin C and its excipient on their low-neck and arms over a 6-month period in view to evaluate efficacy and safety of such treatment. Clinical assessments included evaluation at the beginning and after 3 and 6 months of daily treatment. Cutaneous biopsies were obtained at the end of the trial and examined using immunohistochemistry and electron microscopy. Clinical examination by a dermatologist and self-assessment by the

volunteers demonstrated a significant improvement, in terms of the ‘global score’, on the vitamin C-treated side compared with the placebo. The study reported a highly significant increase in the density of skin microrelief and a decrease of the deep furrows. Ultrastructural evidence of the elastic tissue repair was also obtained and confirmed the favorable results of the clinical and skin surface examinations (34).

CONCLUSION

At moderate concentration free radicals and radical-derived ROS play an important role as regulatory mediators in signaling processes. Many of the ROS-mediated responses in fact protect the cells against oxidative stress and reestablish "redox homeostasis". At high concentrations, however, free radicals and radical-derived, non-radical reactive species are hazardous for living organisms and harm all major cellular constituents. ROS are generated as adverse side products of the oxidative energy metabolism in mitochondria (35-37). It is generally accepted that an excessive and/or sustained increase in ROS production plays an important role in the pathogenesis of many diseases including cancer, atherosclerosis, diabetes mellitus and neurodegenerative diseases. In addition, the process of aging may result, at least in part, from radical-mediated oxidative damage (1). Accordingly, there is a considerable interest to develop procedures to ameliorate undesirable ROS production in order to delay aging and oxidative stress-related diseases. Dietary antioxidants are widely used to ameliorate excessive oxidative stress, however, scientific proof of their efficacy is inconsistent. Epidemiological studies have established a positive correlation between the intake of fruits and vegetables and prevention of diseases thought to be caused by oxidative damage (38,39). However, there are many compounds in fruits and vegetables that potentially may beneficial. Antioxidant Supplements which are considered as medicinal products should undergo sufficient evaluation before marketing since at high doses they can be potentially harmful (40).

The findings of the latest large scale randomized clinical trials indicate that neither vitamin E nor vitamin C supplementation reduces the risk of major cardiovascular events in middle-aged and older men, healthy women or postmenopausal women. There may be even an increase in total and cardiovascular mortality in the latter group (20-22). Postmenopausal women with coronary disease should therefore be discouraged from using high doses of vitamins C and E. Similarly antioxidant supplementation, particularly with vitamin E, vitamin C and beta-carotene does not reduce primary cancer incidence or cancer mortality. Beta-carotene supplementation might even increase the risk of smoking-related cancers, as well as cancer mortality, and should be avoided by smokers (23-25). The data also indicate that neither Vitamin E nor beta-carotene supplementation affects the overall incidence of cataract or cataract extraction (27-29).

Selenium supplementation on the other hand might reduce cancer incidence, especially in men. Further research is needed to confirm the chemopreventive effect of selenium (25).

It should be noted however that the effects of supplements were evaluated in populations mostly from countries without overt deficiencies of specific supplements. Accordingly, it is difficult to assess how antioxidant supplements may affect disease prevention or mortality in populations with specific needs or insufficiency in micronutrients.

REFERENCES

1. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44-84.
2. Langseth L. Oxidants, antioxidants and disease prevention. ILSI. 1996;16: 840-841.
3. Bagchi K, Puri S. Free radicals and antioxidants in health and disease . EMHJ. 1998;4:350-360.
4. Vertuani S, Angusti A, Manfredini S. The antioxidants and pro-antioxidants network: an overview. Curr Pharm. 2004;14:1677-1694.
5. Christe Valko M, Leibfritz D, Moncol J, Cronin M, Mazur M, Telser J. Free radicals and oxidative stress: oxidants and antioxidants. Exp Physiol. 2000;82:291-295.
6. Nunomura A, Castellani R, Zhu X, Moreira P, Perry G, Smith M. Involvement of oxidative stress in

Alzheimer disease. J Neuropathol Exp Neurol. 2006;65:631-641.
7. Tamimi RM, Hankinson SE, Campos H, Spiegelman D, Zhang S, Colditz GA, et al. Plasma carotenoids, retinol, and tocopherols and risk of breast cancer. Am J Epidemiol. 2005;161:153-157.
8. Hoffman F. Antioxidant vitamins newsletter. Nutr Rev. 1997;14:234-236.
9. Davì G, Falco A, Patrono C. Lipid peroxidation in diabetes mellitus. Antioxid Redox Signal. 2005; 7:256-268.
10. Hitchon C, El-Gabalawy H. Oxidation in rheumatoid arthritis. Arthritis Res Ther. 2004;6:265-278.
11. Cookson M, Shaw P. Oxidative stress and motor neuron disease. Brain Pathol. 2000; 9:165-186.
12. Naghibi B, Hajhashemi V, Ghafghazi T, Talebi A, Taheri D. The effect of Vitamin E in vancomycin-induced toxicity in rats. Res Pharm Sci. 2006;1:104-111.
13. Abdel-Nahafim AB, Abdel-Wahab MH, Attia F. Protective effects of vitamin E against gentamaycin–induced nephrotoxicity in rats. Pharmacol Res. 1999;40:183-187.
14. Appenroth D, Frob S, Kersten L. Protective effects of vitamin E on cisplatin nephrotoxicity in developing rats. Arch toxicol. 1999;71:677-683.
15. Naghibi B, Hajhashemi V, Ghafghazi T, Talebi A, Taheri D. 2,3-dihydroxybenzoic acid and tempol can prevent from of vancomycin-induced nephrotoxicity in rats. Toxicol. 2007;3:192-199.
16. Aghaye Ghazvini MR, Ghafghazi T, Hajhashemi V, Ani M. Effects of long-term phenytoin or phenytoin plus ascorbic acid administration on urethane anesthetic effect in rats: a controlled study. Curr Ther Res. 2002;10:649-653.
17. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet. 1994;17:793-795.
18. Jialal L, Devaraj S. Low-density lipoprotein oxidation, antioxidants, and atherosclerosis: a clinical biochemistry perspective. Clin Chem. 1996;42:498-506.
19. Penny M, Etherton K, Lichtenstein AH, Howard BV, Steinberg D, Witztum J. Antioxidant vitamin supplements and cardiovascular disease. Circulation. 2004;110:637-664.
20. Sesso HD, Buring J.E, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. JAMA. 2008;300:2121-2133.
21. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer. The women's health study: a randomized controlled trial. JAMA. 2005;294:56-65.
22. Waters DD, Alderman EL, Hsia J, Howard BV, Cobb FR, Rogers WJ, et al. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in post-menopausal women: a randomized controlled trial. JAMA. 2002;288:2432-2440.
23. Gaziano, JM, Glynn, RJ, Christen, WG, Kurth T,Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. JAMA. 2009;301:52-62.
24. Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease. The women's health study. J Natl Cancer Inst. 1999;91:2102-2106.
25. Bardia A, Tleyjeh IM, Cerhan JR, Sood AK, Limburg PJ, Erwin PJ, et al. Efficacy of antioxidant supplementation in reducing primary cancer incidence and mortality: systematic review and meta-analysis. Mayo Clin Proc. 2008;83:23-34.
26. Taylor A. Role of nutrients in delaying cataracts. Ann NY Acad Sci. 1992;669:111-123.
27. Christen, WG, Manson, JE, Glynn, RJ, Gaziano M, Sperduto SD, Buring JE, et al. A randomized trial of beta-carotene and age-related cataract in US physicians. Arch Ophthalmol. 2003;121:372-378.
28. Christen, WG, Glynn, RJ, Chew, EY, Buring, JE. Vitamin E and age-related cataract in a randomized trial of women. Ophthalmol. 2008;115:822-829.
29. Maraini, G, Sperduto, RD, Ferris, F, Clemons TE, Rosmini F, Ferrigno L. A randomized, double-masked, placebo-controlled clinical trial of multivitamin supplementation for age-related lens opacities. Clinical trial of nutritional supplements and age-related cataract report no. 3. Ophthalmol. 2008;115:599-607.
30. Prockop DJ, Kivirikko K. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem. 1995; 64: 403-434.
31. Peterkofsky B. Ascorbate requirement for hydroxylation and secretion of procollagen: relationship to inhibition of collagen synthesis in scurvy. Am J Clin Nutr. 1991;54:1135-1140.
32. Nusgens BV, Humbert P, Rougier A, Colige AC, Haftek M, Lambert CA, et al. Topically applied vitamin C enhances the mRNA level of collagens I and III, their processing enzymes and tissue inhibitor of matrix metalloproteinase in the human dermis. Dermatol. 2001;116:853-859.
33. Hercberg S, Ezzedine KH, Guinot CH, Preziosi P, Galan P, Bertrais S, et al. Antioxidant supplementation increases the risk of skin cancers in women but not in men. J Nutr. 2007;137:2098-2105.
34. Humbert PG, Haftek M, Creidi P, Lapière C, Nusgens B, Richard A, et al. Topical ascorbic acid on photoaged skin. Clinical, topographical and ultrastructural evaluation: double-blind study vs. placebo. Exp Dermatol. 2003;12:237-244.
35. Droge W. Free radicals in the physiological control of cell function. Phys Rev. 2002;82:47-95
36. Roth S, Dröge W. Regulation of T cell activation and T cell growth factor (TCGF) production by hydrogen peroxide. Cell Immunol. 1987;108:417-424.
37. Mittal CK, Murad F. Activation of guanylate cyclase by superoxide dismutase and hydroxyl radical: a physiological regulator of guanosine 3',5'-monophosphateformation. Proc Natl Acad Sci USA

1977;74:4360-4364.
38. Kau C, Kapoor H. Review: antioxidants in fruits and vegetables – the millennium's health. IJFST. 2001;36:703-725.
39. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev. 2008;16:71-76.
40. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA. 2007;297:842-857.