Oxidants, Antioxidants And Coronary Artery Disease

*R.B. SINGH**

Introduction:

The major causes of death in developed countries such as cardiovascular disease (CVD) and cancer show markedly different incidence rates in different countries[1-3]. These differences seem to depend on the varied dietary patterns in Europe but the classic lipid hypothesis alone fails to explain the differing rates of coronary artery disease (CAD)[1-5]. There is clear evidence that populations living in Mediterranean countries and Japan enjoy a longer life expectancy than Northern Europeans. Genetic or racial factors do not explain these societal differences as revealed by studies in migrants[3]. New findings from the Seven Countries Study showed that the relative increase in CAD risk with an increase in serum total cholesterol was comparable in different cultures[4]. However the absolute increase was quite different from culture to culture, since absolute level of the CAD risk differed substantially among cultures. It appears that reductions in serum total cholesterol levels are not likely to bring cultures with a high CAD risk, back to a CAD mortality level characteristic for the Mediterranean and Japanese cultures unless other factors are also changed[5]. The Mediterranean and Japanese diets, low in saturated fat and rich in antioxidants, may have beneficial effects both on the oxidizability of low density lipoprotein (LDL) particles and on thrombogenesis apart from an effect on LDL levels per se[6-8].

Coronary Artery Disease in Indians:

The prevalence of CAD varies between 3-4% in rural to 8-10% in urban North and west Indians[9,10]. However in urban south Indians[9] and immigrants to industrialized countries, the prevalence of CAD is about 14%. The relative CAD risk among these cultures depend on varied dietary saturated fat intake and serum cholesterol levels[9]. However absolute risk of CAD differs from culture to culture which emphasizes the role of other factors such as smoking, hypertension, antioxidants, insulin resistance, lipoprotein (a) and sedentary lifestyle in the prevention of CAD[9,10]. Plasma levels of antioxidant vitamins A, E and C and beta-carotene were inversely associated with risk of CAD and coronary risk factors[11-17]

Oxidants, Free Radicals and Coronary Artery Disease:

Experimental studies indicate that the oxidized form of LDL (ox-LDL) is more atherogenic than native LDL[6-8]. The amount of ox-LDL is the result of the balance between the amount of oxidative stress and the antioxidant capacity[6]. The susceptibility of LDL particles to oxidation is related to their fatty acid composition: polyunsaturated fatty acids increase susceptibility to oxidation compared with monounsaturated fatty acids. A iven level of serum total cholesterol will represent different levels of ox-LDL in different cultures depending upon the oxidants and antioxidants present in the tissues. In the Indian foods, common oxidants are linoleic acid, free sugar, cholesterol oxide in Indian ghee, pesticides etc. Tobacco consumption is the most potential oxidant which produces oxidative stress (Table 1).

Free radicals are a chemical species with one or more unpaired electrons such as unpaired oxygen, hydroperoxides, superoxides anion, hydroxyl and paroxyl radicals[11-13]. Free radicals develop widely in mammalian cells but are precisely controlled by several antioxidant protective mechanisms. When oxidants are in excess of antioxidants, excess of free radical are produced which can attack DNA, disrupt the function of vital enzymes and cause peroxidation of polyunsaturated fatty acids in the cell resulting into cell damage[11-13]

Heart Research Laboratory, Medical Hospital and Research Centre, Moradabad, India.

Antioxidants:

The primary enzymatic defences depend functionally on the mineral manganese, copper, zinc and selenium and secondary substances present in the diet (vitamin, E,C and beta-carotene) (Table 2)[11-13].

TABLE 1

Oxidants

Foods	Lifestyle	Environmental
1. Linoleic acid	1. Smoking	1. Paraquat and carbon tetra chloride (dry cleaning)
2. Free Sugar	2. Exercise in excess.	2. Pollutants free copper, iron, lead, cadmium.
3. Oxidized cholesterol in Indian ghee.	3. Alcohol intake.	3. Industrial smoke and gases.
4. Pesticides.		4. Excessive exposure to sun.
		5. Radiation.
		6. Drugs.
		7. Solvents.
		8. Oxygen and Ozone.

An unbalanced excess of free oxygen radicals due to lack of antioxidants may increase the risk of coronary artery disease (CAD)[11-13]. There is evidence that development of atheroma is partly dependent on incorporation of oxidised cholesterol into monocytes and macrophages within the arterial wall because of the receptors which do not recognise native low density lipoproteins (LDL) but do attract oxidised LDL[6-8]. Antioxidants prevent the oxidation of LDL as well as platelet adhesion so important for thrombosis and they may also prevent damage to endothelium and myocardium by the free radicals[6-8]. Diet also contains other antioxidants such as flavonoids and phenolics which are commonly consumed in Japan, France and Mediterranean countries[2-5].

Epidemiologic and Case Control Studies:

Antioxidant vitamins deficiency predisposing to CAD has been observed in several countries including in 33 populations of the WHO Monica study and in 595 elderly subjects from Moradabad[15-20]. (Table 3). In case control studies, interpretability is severely limited because it is not possible to discern whether blood levels were a cause or consequence of the disease. In Scotland, Riemersma et al[18] reported a significant inverse association between plasma levels of vitamin E and angina pectoris detected by questionnaires. In several other studies including our case control study, a significant inverse association has been reported between plasma ascorbic acid and CAD particularly in acute myocardial infarction (AMI)[16]. In a population based case control study, a significant inverse association has been observed between serum beta carotene in frozen samples and subsequent risk of myocardial infarction[12]. However earlier studies from Finland in a sample from Dutch persons showed no significant association between plasma vitamin E and subsequent CVD mortality. In the Peerzada prospective study from north India, comprising 152 adults including 13 patients of CAD, low plasma vitamin E and C were strongly related to CAD[17]. Both low dietary intakes and low plasma vitamin levels have been correlated with populations and groups such as smokers and diabetes who are at higher risk of CAD[11-18].

TABLE 2

Antioxidant defenses in the body

Primary enzymatic defenses	Functional dependence
Superoxide dismintase	Zinc, manganese
Glutathione peroxidase	Selenium
Catalase	Iron
Uric acid	
Secondary stabilising substances	
Beta-carotene	
Vitamin - C	
Vitamin - E	
Vitamin - A	
Carnitine	

Observational Studies:

Of 6000 healthy adults included in the Basel study[11], 1663 subjects had complete laboratory

analysis of blood samples, 132 patients died due to CAD and 31 due to stroke over a follow-up of 10 years. Low plasma level of carotene below quartile 1 and low plasma vitamin A levels were associated with a significantly higher risk of death from CAD. Low carotene in presence of low vitamin C was also related to increased risk of stroke death. In nurses healthy study[21], during a follow-up of 87,245 female nurses upto 8 years there were 437 nonfatal myocardial infarctions and 115 deaths due to CAD. As compared with women in the lowest fifth of the cohort with respect to vitamin E intake, those in the top fifth had a relative risk of major coronary disease of 0.66 (95% confidence interval 0.50 to 0.87, p<0.001) after adjustment for age and smoking. Women who took vitamin E supplements for more than 2 years, had a relative risk of major coronary disease of 0.59 (95% confidence interval 0.38 to 0.91) after adjustment for age, smoking status, risk factors for CAD and use of other antioxidants nutrients including multivitamins. These data suggest that the use of vitamin E supplements is associated with reduced risk of CAD. The overall association of antioxidant vitamin E, C and carotene intake (the relative risk in the highest intake of antioxidant vitamins intake was 0.54, p-trend 0.001) with CAD was significant.

In another study comprising 39,910 male health professionals[22] follow-up after 4 years showed 667 cases of CAD including 360 revascularization, 201 nonfatal MI and 106 fatal MI. It has been observed that after control for age and several coronary risk factors, a lower risk of CAD among men was related with higher consumption of vitamin E (P for trend = 0.003). Men who took at least 100 IU/day for at least 2 years, had a multivariate relative risk of CAD of 0.63 (95% confidence interval, 0.47 to 0.84) as compared with those who did not consume vitamin E supplements. Carotene intake was inversely related with the CAD risk among current smokers and former smokers but not in nonsmokers. A high intake of vitamin C was not associated with a lower risk of CAD. In 1976, 1299 elderly subjects from Massachusetts[12] were studied. After a follow-up of 4 years, a total of 151 CVD deaths were recorded which included 47 fatal MI. After controlling for age, sex, smoking, alcohol consumption, cholesterol intake and functional status, the relative risk of CVD deaths in the highest quartile of beta-carotene intake was 0.57 (p-trend = 0.016). The corresponding relative risk for fatal MI was 0.32 (p-trend = 0.016). In the National Health and Nutrition Examination Survey (NHANESI)[12] during a follow-up of median 10 years, in those with the highest vitamin C intake from diet and supplements, the standardized mortality ratio for CVD deaths compared to American whites was 0.75 for females and 0.58 for males. These studies are compatible with possible benefits of antioxidant vitamins on CVD.

In evaluating more than 34,000 postmenopausal females for the intakes of vitamin A, C and E during 7 years of follow-up, 242 died of CAD. Vitamin E consumption was inversely associated with a risk of death from CAD. Intake of margarine, nuts and seeds and green salads were inversely associated with the risk of CAD death[23]. In one study[24] in 1188 men, 532 women (mean age 63.2 years), higher plasma levels of beta-carotene were associated with lower risk of death from CVD and all causes. Those who received beta-carotene randomly (50mg/day) had no reduction in mortality from all causes and CVD after 4.3 years follow-up. However regardless of number or sample size of such studies and their consistency, observational studies are unable to control for potential effects of confounding variables unknown to the investigators. Therefore it is possible that reliable inferences can result from large scale, randomized trials.

Intervention Trials:

Of the vailable trials in patients with intermittent claudication[12], angina pectoris and the subgroup analysis from the United States Physicians Health Study, in the majority of the earlier trials, the numbers of subjects were small and dropout rates high. While patients with claudication reported benefit with vitamin E, angina patients showed no benefit because duration of treatment was only 6 months which is too less to retard the progression of atherosclerosis. The Physicians Health Study begun in 1982 consisted of 22,071 male physicians aged 40-84 years, aimed to test aspirin (325mg on alternate days) in reducing CVD and beta-carotene (50mg on a alternate days) in decreasing cancer risk. While doctors with prior AMI were excluded, 333 physicians with prior revascularization or with chronic stable angina did enter the trial. During a follow-up for an average

60.2 months, the multivariate relative risk of major coronary events comprising nonfatal MI, fatal CAD and coronary revascularization among those assigned beta-carotene was 0.49, with 95% confidence interval 0.31 to 0.99 (p=0.047). For major vascular events including major coronary events as well as non-fatal and fatal stroke, the relative risk was 0.46 with 95% confidence interval 0.24 to 0.85 (p=0.014). After 12 years of follow-up, this randomized trial showed neither benefit nor harm in terms of neoplasm, CVD and death from all causes[25].

ORS trial is testing probucol in high risk patients with hypercholesterolemia. The Alpha Tocopherol beta-carotene cancer prevention study showed no benefit on cancer or CAD during a follow-up of 5-8 years in Finnish male smokers[26]. The SU. VI. MAX study is testing beta-carotene, vitamin E and vitamin C as well as zinc and selenium in healthy French men and women. The Women Health study would evaluate risks and benefits of beta-carotene, vitamin E and low dose aspirin on CVD and cancer among 40,000 apparently healthy US female nurses[12].

TABLE 3

Plasma Levels of Antioxidant Vitamins in Subject with Coronary Artery Disease and Coronary Risk Factors[15].

Plasma level (umol/L)	Glucose Into-lerance	Diabetes	Smoking	Coronary artery	Without risk factors
			mean ±SD		
Vitamin C	32.6±4.2*	19.4±3.1**	23.6±3.7**	20.3±3.1**	37.8±5.6
Vitamin E	18.1±3.4	14.2±3.1**	17.8±3.6**	15.0±2.6**	20.2±4.2
Vitamin E/cholesterol	3.8±0.67	2.72±0.6**	3.7±0.6**	2.7±0.6*	3.9±0.6
Vitamin A	2.3±0.2	2.0±0.2**	2.1±0.2*	2.0±0.2**	2.3±0.2
Beta-carotene	0.46±0.08	0.3±0.05**	0.3±0.06**	0.3±0.05**	0.5±0.09
Lipid peroxides (nmol/ml)	1.6±0.2	2.6±0.2**	2.7±0.2**	2.6±0.2**	1.5±0.12

Singh et al, 1995.

* = p<0.05, ** = p<0.01

In a secondary prevention trial, approximately 8000 female nurses with history of CVD not included in the above trial have been randomized[12] to treatment with three antioxidant beta-carotene, vitamin E and vitamin C. Currently several primary prevention trials[12], testing antioxidant vitamins or probucol in the prevention of CVD are under way. The probucol

The Indian diet heart study comprising roughly 800 high risk patients and those with CVD showed that antioxidant rich fruits, vegetables, legumes and nuts (600g/day) can cause significant increase in plasma levels of vitamin A, E, C and carotene leading to significant reduction in cardiac end points within 2 years. In many chronic conditions, an-

tioxidant status may be important[27]. In the Indian Experiment of Infarct Survival[28], of 125 patients with suspected AMI that entered the trial, 63 were administered antioxidant vitamin A, E, C and beta-carotene and 62 the placebo for a follow-up period of 28 days. After treatment, the mean infarct size (creatine kinase gram equivalents and MB creatine kinase gram equivalents) reduction was significantly higher in the antioxidant group compared to changes in the control group. Cardiac enzyme lactate dehydrogenase and electrocardiographic QRS score were significantly less in the antioxidant group A compared to placebo group B. Plasma levels of vitamin A, E, C and beta-carotene showed significant increase in group A than group B. Serum lipid peroxides indicating ischaemia induced free radical stress showed significant greater decline in group A than group B. Angina pectoris, ventricular ectopics and left ventricular enlargement and hypertrophy were less common in the antioxidant group compared to control group. In a recent study[27] dietary intakes and plasma levels of antioxidant vitamins were inversely associated with CAD and many chronic diseases. In 505 patients[29] with suspected acute MI, plasma level of vitamin C was lower. Consumption of 600g/day of fruits, vegetables, legumes and nuts was associated with significant reduction in oxidative stress and lower level of cardiac enzyme in the intervention group compared to control group. The Indian Lifestyle and Heart Study in Elderly showed that the consumption of fruits, vegetables and legumes is 50% lower than that prescribed by WHO (400 vs 200g/day).

In a more recent study[30], ascorbic acid 2g or a placebo in 46 patients with significant CAD reversed endothelial vasomotor function in the brachial circulation. In a double blind, placebo controlled trial[31] of 2,002 patients with angiographically proven CAD, 546 patients were given 800 IU of vitamin E per day while 1035 vitamin E supplements received 400 IU/day and remaining 967 patients received a placebo. Vitamin E levels rose to 51.1 umol/L in the 400 IU/day group and 64.5 umol/L in the 800 IU/day group. After one year, treatment with vitamin E was associated with significant reduction in the cardiovascular death and nonfatal MI. Vitamin E and C beta-carotene have also been noted to decrease experimental lipid peroxidation[32].

The Beta-Carotene and Retinol Efficacy Trial[33], involving a total of 18,314 smokers, former smokers and workers exposed to asbestos were administered 30mg/day of beta-carotene and 25,000 IU/day of vitamin A. After an average of 4 years of supplementation, the treatment showed no benefit and may have had an adverse effect on the incidence of lung cancer and on the risk of death from lung cancer, CVD and any cause.

In conclusion, it is apparent from above studies that beta-carotene and vitamin A and E are ineffective in the prevention of CVD and cancer in smokers and in other high risk subjects. However vitamin E and C appear to be protective in the secondary prevention of CAD[34].

Therapeutic Doses:

Animal experiments of vitamin E have shown a lack of adverse effects even at high doses[35]. In humans, the doses of vitamin E may be 100-3200 IU/day and the usual doses are 400-600 IU/day.

Vitamin C is considered free from adverse effects in doses of 500-1000 mg/day. Higher doses may cause diarrhoea. Most experts advise 500mg/day of vitamin C for a beneficial effect. However higher doses may be tried if there is no benefit with optical dose of 500 mg/day.

Carotenoids have been subjected to extensive toxicity testing. The doses of beta-carotene vary from minimum 10mg/day to 50mg/day. Doses higher than 25mg/day may be associated with yellow colouration of skin.

The dose of selenium is 100-300mg/day which is not very well defined. More evidence is needed to define the doses of antioxidants in the prevention of CAD. In smokers, higher doses should be tried before we conclude that the treatment with antioxidant is not effective. It seems that presence of CAD is a good indication for antioxidant therapy. However combined treatment with vitamin E (400mg/day), vitamin C (400mg/day) and beta-carotene (20mg/day) is likely to be more beneficial than single agent in view of the synergestic action of these antioxidants in the prevention of CAD. Until more evidence is