African Journal of Biotechnology Vol. 8 (14), pp. 3107-3117, 20 July, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Review

Cardiovascular disease and the potential protective role of antioxidants

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Accepted 17 February, 2009

Epidemiological studies have shown that in the 20th century, cardiovascular disease was responsible for less than ten percent of all deaths globally. However, recent reports indicate that the trend has changed with studies showing that cardiovascular disease currently accounts for about thirty percent of all deaths in the world. It is generally accepted that smoking, high blood cholesterol levels, high triglyceride levels, elevated LDL levels, elevated blood pressure, physical inactivity, insulin resistance, obesity and type 2-diabetes are risk factors for cardiovascular disease. Unfortunately these known risk factors do not provide a full explanation for all cases of heart disease. Recent research has identified what is preferentially termed novel risk factors that may assist to identify persons or populations at risk of developing cardiovascular disease. One such novel risk factor is the presence in the body of free-and hydroxyperoxide radicals. It has been reported that free-and hydroxyperoxide radicals have the potential to damage biological compounds and structures such as proteins, membrane lipids, DNA and carbohydrates and that such damage caused by these radicals are said to be involved in the aetiology and pathogenesis of different diseases such as cardiovascular disease. The link between deficiencies of antioxidants and production of free-and hydroxyperoxide radicals has been reported. This review paper reports on cardiovascular disease, its associated risk factors and the potential protective role of antioxidants in the prevention and management of cardiovascular disease.

Key words: antioxidants, cardiovascular disease, risk factors, preventative, epidemiology

INTRODUCTION AND GENERAL BACKGROUND

In general, cardiovascular disease can be defined as a class of diseases affecting the heart and/or blood vessels. It is frequently associated with any disease that affects the cardiovascular system such as atherosclerosis. This is a condition where the walls of the arteries are damaged and narrowed by plaque deposits consisting of one or more of the following: cholesterol and other fatty substances, calcium, fibrin, and cellular wastes which may eventually lead to blocking of the lumen with subsequent lack of blood flow. Plaque deposits can result in haemorrhage or the formation of a thrombus. When a thrombus blocks the flow of blood through the entire artery, a heart attack or a stroke may occur (Walker, 1994; Walker et al., 2000; Gaziano, 2005; WHO, 2005; Thorogood et al., 2007a; Pieters and Vorster, 2008; White and Dalby, 2008). Epidemiological studies have shown that in the 20th century, cardiovascular disease (CVD) was responsible for less than 10% of all deaths globally. However, according to recent reports, the trend has changed with studies indicating that cardiovascular disease currently accounts for about 30% of all deaths in the world (Gaziano, 2005; British Nutrition Foundation, 2005; Sliwa et al., 2005; Sliwa et al., 2008). Research has shown that cardiovascular disease is now rapidly becoming a leading cause of death worldwide with about 80% of all cases being reported in developing countries.

Reports in South Africa (SASPI Team, 2004; Kahn et al., 2005; Thorogood et al., 2007a; Thorogood et al., 2007b; Li and Wright, 2007) and elsewhere (Gaziano, 2005; British Nutrition Foundation, 2005; Petersen et al., 2005; Li and Wright, 2007; Stein et al., 2008) showed that industrial, technological development as well as econo-

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mic, social and political transformations have resulted in a significant change in the aetiology of illness and death. It has been reported that more than 50 years ago, infectious diseases and malnutrition were the most common causes of death. However, due to improved nutrition and public health measures, many investigators believe that the prevalence of cardiovascular disease now exceeds infectious diseases and malnutrition and is rising to significantly high prevalence in Africa (Kadiri, 1998; WHO, 2002; WHO, 2005). This is a worrying trend which demands urgent attention.

A study by Sliwa and colleagues (2008) noted that there are many threats to the present and future cardiac health in urban African communities with reference to a study which was performed in Soweto, South Africa. This work also reported a high prevalence of modifiable risk factors which may lead to atherosclerotic disease as well as a combination of infectious and non-communicable forms of heart disease, presenting with late clinical manifestations. These investigators concluded that their findings provide strong evidence that the epidemiological transition in Soweto, South Africa has broadened the complexity and spectrum of heart disease in this particular community. In our opinion, the study not only provides an insight into the problem of cardiovascular disease in South Africa in particular, but also provides a general reflection of the problem in Africa and other developing countries.

The WHO (2002) reported that cardiovascular disease accounts for 9.2% of total deaths in Africa. The report also lists hypertension, stroke, cardiomyopathies and rheumatic heart disease as the most prevalent causes of death. The report further observed that the number of disability adjusted for life lost (in years) to cardiovascular disease in sub-Saharan Africa has risen from 5.3 million for men and 6.3 million for women in 1990 to 6.5 and 6.9 million respectively in 2000. Based on this observation, the WHO anticipates that the number may rise to 7.9 and 8.1 million by 2010. Surprisingly and unfortunately, it is now reported that cardiovascular disease has a higher mortality rate in developing countries than in developed countries and it is disturbing to observe that it is affecting younger persons more than ever before (Kadiri, 1998; Reddy and Yusuf, 1998; Davies, 2003; WHO, 2005).

Hypertension is considered to be a major risk factor for CVD and it is also seen to be the most threatening risk factor with national prevalence rates ranging from 15 to 30% in South African adults. Comparative epidemiological studies from Nigeria and elsewhere showed that only 5% of deaths could be as a result of hypertension (Kadiri, 1998; Copper et al., 1998). Other important studies showed that 32.1% of men and 18.9% of women over 30 years of age has a 20% or higher risk of developing cardiovascular disease in the next 10 years in South Africa (Walker et al., 2000; Berger and Marais, 2000; Alberts., 2005; Kahn et al., 2005; Thorogood et al., 2007a). These studies seem to reflect the increased trend in CVD incidences in South Africa, and should perhaps be considered to be a reflection of a global trend.

According to a model developed by Omran (1971), epidemiological transition as it relates to the incidence of cardiovascular disease can be grouped into three stages which include: pestilence and famine, receding pandemics, and degenerative and lifestyle diseases: (a) stage 1 focuses on pestilence and famine exhibiting the following characteristics: predominance of malnutrition and infectious diseases; life expectancy is set at 35 years; dominant forms of cardiovascular disease are rheumatic heart disease, cardiomyopathy due to infection and malnutrition; percentage of deaths due to cardiovascular disease: 5 - 10%; mostly in South-South Asia and other low income countries (Gaziano, 2005). (b) stage 2 is characterised by a predominance of malnutrition and infectious diseases; life expectancy is set at 35 years; dominant forms of cardiovascular disease are rheumatic heart disease, cardiomyopathy due to infection and malnutrition; percentage of deaths due to cardiovascular disease is 5 - 10% of the world's population. Countries in this stage are South-South Asia, parts of all regions excluding high-income regions (Gaziano, 2005). (c) Stage 3 is characterised by an increase in fat and caloric intake; widespread tobacco use; chronic disease deaths exceed mortality due to infections and malnutrition; life expectancy is set at 60 years; dominant form of cardiovascular disease is rheumatic heart disease, stroke (ischaemic and haemorrhagic), percentage of deaths due to cardiovascular disease is greater than 50% of the world's population; life expectancy is set at 35 years and the regions affected are: East Asian Pacific, Latin American countries, and urban parts of the lowest income regions, especially in India (Gaziano, 2005).

RISK FACTORS FOR CARDIOVASCULAR DISEASE

It is generally accepted that smoking, high blood cholesterol levels, elevated blood pressure, physical inactivity, obesity and type 2-diabetes are risk factors for cardiovascular disease. Unfortunately these known risk factors do not provide a complete explanation for all cases of heart disease. Recent research has identified a number of other or what is frequently termed novel risk factors that may assist to identify individuals or populations at risk of developing CVD. Insulin resistance has been reported to be one of these risk factors and this condition occurs when the cells in the body respond slowly/poorly to insulin and may lead to the development of type 2-diabetes (British Nutrition Foundation, 1994; Kahn et al., 2005; Rastogi, 2005; Thorogood et al., 2007a). The insulin resistance syndrome, also known as syndrome X or the metabolic syndrome refers to a combination of health problems which include insulin resistance, abnormal levels of blood lipids, obesity and high blood pressure (SASPI Team, 2004; Connor et al., 2007). It has

been reported that people with the syndrome are said to be three times more likely to develop and die of cardiovascular disease even after control measures have been taken to reduce other risk factors. Scientists generally agree that eating a healthy diet, engagement in physical activity, no-smoking, and moderate drinking of alcohol will assist in avoidance of the characteristics of this syndrome (Department of Health, UK, 1994; Amira et al., 2006; Sliwa et al., 2008; Stein et al., 2008).

Studies have shown that lipid-related cardiovascular disease risk factors include high blood levels of total and low-density lipoprotein (LDL) cholesterol, low levels of high-density (HDL) lipoprotein cholesterol and elevated triglyceride levels. Investigators also observed that although the total amount of fat consumed by an individual for the maintenance of health is an essential factor, laboratory evidence tends to focus on the importance of the types of fatty acid in the diet and the partial replacement of saturated with unsaturated fatty acids. According to the British Nutrition Foundation (2005) and other investigators, high levels of lipoprotein(a) and remnant lipoproteins also appear to increase cardio-vascular disease risk (James et al., 2000; Charltton et al., 2005; Firth and Marais, 2008).

It is believed that the endothelium regulates the normal functioning of blood vessels and plays an integral role in the prevention of cardiovascular disease. It has been observed that abnormalities in its function can increase the risk of heart disease. It has also been reported that dietary factors such as long chain fatty acids, Mediterranean style-diets and some vitamins exhibit beneficial effects on the integrity and function of vessel endothelium (Vorster et al., 1997; Charltton et al., 2005).

Increased levels of some of the coagulation factors such as fibrinogen could possibly predict cardiovascular disease risk (Ridker et al., 2001; Tataru et al., 2001). Overweight, inactivity, smoking and consuming a diet that is high in fat have been documented to increase the risk of blood clot formation. On the other hand, moderate amounts of alcohol and high intakes of long chain fatty acids seem to contribute to reduction in blood cells and platelets to stick together to form a clot (Amira et al., 2006; Connor et al., 2007; Firth and Marais, 2008).

Another risk factor for developing CVD that has been reported is prolonged inflammation within the walls of the arteries. This factor is known to contribute to atherosclerosis which can in turn give rise to an increase in blood pressure and thus increase the risk of cardiovascular disease. Increased levels of some biomarkers of inflamemation for example, C-reactive protein and fibrinogen have been associated with cardiovascular disease risk. Nevertheless, controversies exist whether these factors add significant value when compared with conventional biomarkers of heart disease and remains to be established. Currently, there is insufficient evidence describing the effects of diet on these biomarkers but the polyunsaturated fatty acids and some antioxidant nutrients seem to have anti-inflammatory effects (Ridker et al., 2001; Berton et al., 2003; British Nutrition Foundation, 2005).

Adipose tissue is known to secrete substances that are linked to a number of processes that contribute to the development of cardiovascular disease. One of these substances is leptin which is believed to play a role in appetite control. Weight gain and excess energy intake is associated with a rise in leptin whilst weight loss and exercise is generally associated with a fall in blood levels of leptin (Lee et al., 2005; Walldius and Jungner, 2007; Zahid et al., 2008).

Low birth-weight and low weight gain during infancy are believed to be linked with a high risk of adult cardiovascular disease. The “foetal origins of adult disease” hypothesis proposes that these associations reflect permanent metabolic and structural changes resulting from under-nutrition during critical periods of early development. Another explanation is that reduced foetal growth and cardiovascular disease risk factors have common genetic origins (Wessel et al., 2004).

High blood levels of homocysteine, an amino acid produced in the body, is believed to increase the risk of cardiovascular disease. Several B-complex vitamins are involved in homocysteine metabolism and supplementation with these vitamins, for example folic acid and vitamins B_6 and B_{12} have been shown to reduce homocysteine levels (Chambers et al., 2000; Stublinger et al., 2001; Li et al., 2002; Soinlo et al., 2004).

Free radical damage has been implicated in the development of cardiovascular disease and in order to reduce, a number of antioxidant nutrients are considered to be important for the body’s defence systems. Diets rich in fruit and vegetables, which contain antioxidants such as vitamin C, vitamin E and beta-carotene, are said to be associated with reduced risk of developing cardiovascular disease. It should be noted that in certain cases, human intervention trials using off-the-shelf supplements of these nutrients, have not yielded the expected outcomes. This might be because fruit and vegetables contain some other natural occurring compounds such as flavonoids that are not present in laboratory manufactured supplements (Zock and Katan, 1996; Rao and Agarwal, 2000; Halliwell, 2000; Bounous and Molson, 2003; Stanner, 2005; Oguntibeju et al., 2005; Oguntibeju et al., 2006).

It has been reported that risk factors associated with CVD is multi-factorial, as shown in recent publications which reported that 32.1% of men and 18.9% of women over 30 years had a 20% or higher likelihood of developing cardiovascular disease (Yusul et al., 2001; Bradshaw, 2005; Eze and Ezemba, 2007).

ANTIOXIDANTS AS A DEFENCE SYSTEM

Oxidative stress has been defined as an imbalance between the production of various reactive species and the capacity of the organism’s (human being inclusive) natu-

ral defence mechanisms for coping with these reactive compounds and preventing adverse effects (Nojiri et al., 2004; Reddy et al., 2004). For the purpose of this review, an antioxidant can be defined as a molecule that, when present at low concentration in comparison with those of an oxidizable substrate, can significantly inhibit oxidation of that substrate. Free radicals are known to be produced in the human body during normal metabolic processes and that cigarette smoke, pollution, ultraviolet light, ionizing radiation, ultrasound and chemical shearing forces all contribute to free radical formation (Nojiri et al., 2004; Patel et al., 2007). These radicals include reactive oxygen species (ROS), nitrogen reactive species (NRS), hydroxyl radicals, peroxyl radicals, peroxyl nitrite and singlet oxygen while reactive oxygen species and nitrogen reactive species dominates. It has been reported that free radicals have the potential to damage biological compounds such as proteins, membrane lipids, DNA and carbohydrates and that such damage caused by free radicals have been said to be involved in the aetiology of different diseases such as atherosclerosis and cancer (Napoli et al., 2001; Chen et al., 2003; Loscalzo, 2003). It has also been suggested that the accumulation of cytotoxic products from oxidized LDL on the endothelial wall induces platelet aggregation, release of growth factors, disturbance of eicosanoid homeostasis, and thus promote the formation of atherosclerotic plaque. Laboratory studies revealed that oxidative stress could induce the production of superoxide radicals which may potentially inactivate endothelium-derived nitric oxide (Keaney and Vita, 1995; Ruef et al., 1999; Patel et al., 2007).

Nature is known to be endowed with antioxidants and antioxidant mechanisms that can curtail the potential threat of damage to important biological molecules. The effect of antioxidants can be achieved through three means: (1) the intracellular enzymes such as superoxide dismutase, catalase, glutathione peroxidase and caeruloplasmin. These enzymes catalyze the breakdown of oxidants generated by cellular metabolic processes; (2) anti-oxidant proteins that can remove free transitional metal ions which would facilitate the production of the hydroxyl radical. These proteins include the iron-binding protein transferrin and copper-binding proteins caeruloplasmin and albumin while the third group is richer and probably a more effective mechanism than the abovementioned two mechanisms; these are water-and fat-soluble vitamins. Of the water-soluble vitamins, vitamin C is considered to be the most powerful electron donor and is the first plasma antioxidant to be utilised during exposure to oxidative stress. Its oxidation product, dehydroascorbate can be regenerated to ascorbate within the cells by dismutation and chemical reduction by glutathione or enzymatic reduction by thioredoxin reductase. Unfortunately, in the evolutionary line, man has lost the ability to synthesise the enzyme and is unable to produce vitamin C ***in vivo***. The human therefore must rely on fresh fruits and vegetables as the main source of vitamin C to meet the body's requirement. Uric acid and polyphenols (flavonoids inclusive) are also recognized as a group of antioxidants which are active against free radicals. Their main dietary sources are fruits, vegetables, tea and wine. It is reported that bilirubin may protect human LDL against oxidation by oxyradicals and that thiol groups could act by trapping aqueous peroxyl radicals thereby displaying antioxidant activity (May et al., 1998; Bounous and Molson, 2003; Patel et al., 2007; Oguntibeju, 2008).

In the human, lipid-soluble antioxidants include vitamin E, ubiquinone-10, carotenoids and oxycarotenoids and have been reported as playing a protective role against lipid peroxidation. In terms of quantity, vitamin E is seen as the most abundant antioxidant in the LDL lipid phase and plays an important role by scavenging highly reactive lipid peroxyl and alkoxyl radicals (Zock and Katan, 1996; Halliwell, 2000; Kessler et al., 2003).

NETWORK OF ANTIOXIDANTS

It has been shown that in the reaction between ROS or RNS and an antioxidant, the antioxidant itself is transformed into an antioxidant radical. Although the resulting radical has a reduced ability to react with important cellular macromolecules (lipoproteins, RNA, DNA, cell membranes, cellular proteins and carbohydrates), it can still initiate or cause cellular damage. It is therefore necessary for the antioxidant radical to react with another antioxidant to reduce the reduction potential and to further reduce the reactivity of the antioxidant radical. These antioxidant reactions can continue in a cascade manner, involving many antioxidants, until the antioxidant radical is no longer a threat to the biological macromolecules as it has then been reduced to a product which does not contain enough reduction potential to react with lipids, protein, RNA, DNA and other essential cell macromolecules (Feldman, 2002; Sanchez-Quesada et al., 2004; Bandyyopadhyay et al., 2004; Copper, 2004; Sabu et al., 2007; Akiibinu et al., 2007). Therefore alpha-tocopheroxyl radicals which are formed when alpha-tocopherol reacts with hydroxyl radicals has the reduced ability to be involved in redox reactions (the alpha-tocopheroxyl radical is still reactive). It has been reported that alpha-tocopheroxyl radicals can participate in lipid peroxidation of low density lipoproteins (LDL). It is also believed that the accumulation of alpha-tocopheroxyl radicals or other antioxidant radicals may in fact be one of the reasons for the adverse effects observed in some of the clinical intervention studies that employed antioxidant supplementation (Blomhoff, 2005). However, the alpha-tocopherol can be regenerated by the reaction of alpha-tocopheroxyl radicals with vitamin C, a reaction that generates vitamin C radicals (ascorbyl radical). Therefore, it would appear that cells are better protected by a combination of antioxidants rather than a single source (Buettner, 1993; Packer et al., 2001).

OXIDATIVE STRESS AND THE RISK OF CARDIOVASCULAR DISEASE

For many years the emphasis has been on the relationship between serum total cholesterol levels and the risk of cardiovascular disease. However, the focus has recently shifted to oxidative stress induced by reactive oxygen species (ROS) and nitrogen reactive species (RNS) as important key players in the aetiology and pathogenesis of various chronic diseases, including cardiovascular disease (Ames et al., 1995; Pincemail, 1995; Woo et al., 1997; Rao and Agarwal, 1999). Studies have shown that oxidation of the circulating low-density lipoprotein (LDL) that carry cholesterol into the bloodstream to oxidized LDL (LDL_{ox}) may play a key role in the pathogenesis of atherosclerosis and it is suggested to be the underlying disorder which may lead to heart attack and ischaemic strokes (Parthasarathy, 1998; Heller et al., 1998).

Scientific evidence indicates that ROS generated both ***in vivo*** and in response to diet and lifestyle factors could play a significant role in the aetiology and pathogenesis of atherosclerosis and cardiovascular disease (Ames et al., 1995; Halliwell, 2000; Garibaldi et al., 2002; Walldius, 2007). There are different schools of thought regarding the mechanisms involved in the development of oxidative stress. One such school is of the opinion that oxidation of LDL can be viewed as the primary initial step leading to its uptake by the macrophages inside the arterial wall and the subsequent formation of foam cells and atherosclerotic plaque while others view the deficiencies of antioxidant enzymes, vitamins and trace elements to be another mechanism involved in the aetiology and pathogenesis of atherosclerosis (Agawrwal and Rao, 2000a).

It has been shown that LDL, the major carrier of cholesterol in the body, consists of a large molecular weight-protein, the apolipoprotein B, neutral and polar lipids, and a mixture of lipophilic antioxidants including ß-carotene and vitamin E. Oxidation of LDL is known to take place at different stages starting with native LDL and progressing to seeded LDL, minimally modified LDL (mm-LDL), and finally, the fully oxidized LDL (Parthasarathy, 1998; Agarwal and Rao, 2000b; Karatas et al., 2002). The polyunsaturated fatty acids in the LDL-surface phospholipids may be the first to be oxidized, followed by the core lipids. These oxidative modifications reflect the polyunsaturated fatty acids and oxidation of the cholesterol, phospholipids, and oxidative degradation of the apolipoprotein B itself (Morris et al., 1994; Harrison et al., 2003). As a result of these oxidative modifications of the native LDL molecule, biologically active molecules can be formed, as well as breakdown products of oxidized fatty acids that facilitate the recognition of the modified LDL by the macrophage's scavenger receptors (Kohlmeier et al., 1997).

In addition to inducing the formation of foam cells and plaque in the arterial walls, components of the LDL_{ox} can also influence other events that are related to an increased risk of cardiovascular disease. These include (a) their ability to increase cholesterol accumulation by macrophages, (b) their ability to produce proteins that are chemotactic to monocytes and cytotoxic to a variety of cells causing endothelial injury, (c) causing a change in gene expression in arterial cells leading to increased expression of colony-stimulating factors, (d) increasing expression of adhesion molecules at the endothelial cell surface, (e) inhibiting the endothelium-dependent relaxation factor and promote vasospasm, (f) inhibiting vasodilatation, (g) increasing binding to type 1 collagen, (h) enhance coagulation pathways and platelet aggregation, (i) stimulating the synthesis of auto-antibodies, and their ability to promote migration and proliferation of smooth muscle cells and the formation of foam cells and fatty streaks in the arterial intima which in turn may lead to the eventual rupture of the plaque (Morris et al., 1994; Frei, 1995; Dugas et al., 1998; Cai and Harrison, 2000; Karatas et al., 2002).

Antioxidant nutrients are believed to slow down the progression of atherosclerosis due to their ability to inhibit the damaging oxidative processes (Parthasarathy, 1998; Heller et al., 1998; Karatas et al., 2002; Bandyyopadhyay et al., 2004; Patel et al., 2007). Epidemiological and prospective studies have shown that consumption of antioxidant vitamins such as vitamin E and ß-carotene could reduce the risk of cardiovascular disease (Morris et al., 1994; Harrison et al., 2003). Clinical trials also suggest a reduced risk of cardiovascular disease with vitamin E supplementation (Stephens et al., 1996; Zock and Katan, 1996; Bonetti et al., 2002). The protective effect of vitamin E can be ascribed to its antioxidant properties. Observations that men and women with cardiovascular disease show lower levels of circulating antioxidants have led scientists to support the proposed protective role of antioxidants in the prevention and management of cardiovascular disease (Parthasarathy, 1998; Virtamo et al., 1998). Nonetheless, some large-scale human trials have failed to confirm the protective effect of antioxidants. For instance, the Heart Outcomes Prevention Evaluation (HOPE) study which supplemented with 400 IU/day vitamin E for 4.5 years showed insignificant effects on cardiovascular events in patients at high risk (Ceriello and Motz, 2004).

STUDIES ON THE POTENTIAL PROTECTIVE ROLE OF ANTIOXIDANTS IN CARDIOVASCULAR DISEASE

Several clinical and epidemiological studies suggest that dietary antioxidants may protect the body against cardiovascular disease. For instance, an inverse relationship between serum levels of beta-carotene, other carotenoids and cardiovascular disease have been reported (Howard et al., 1966; Dugas et al., 1998; Kritchevsky, 1999; Arab and Steck, 2000; Nojiri et al., 2001; Nojiri et al., 2004). A study conducted in Israel reported that serum levels of

beta-carotene and lycopene were higher in Israeli men when compared to Czech men. Furthermore, the study also reported that mortality rates, blood pressure and coronary heart disease rates were higher in Czech men than in their Israeli counterparts (Bobak et al., 1999). These investigators concluded that the difference in blood pressure and coronary heart disease of the Czech and Israeli men is related to their blood beta-carotene and lycopene concentrations indicating the positive effect(s) of adequate dietary intake of antioxidants in modulating cardiovascular disease and its resulting consequences. Williams et al. (1992) as well as Keaney et al. (1995) also reported that vitamin E supplementation has beneficial effects. However, Maxwell and Lip (1997) stated that supplementation with vitamin E is ineffective against cardiovascular disease whilst other studies such as that conducted by Nojiri and colleagues (1997) offered opposing results. In their study, on the effects and impact of vitamin C on atherosclerosis, they suggest that vitamin C deficient diets are associated with increased aortic accumulation of cholesterol. A number of studies have reported correlations of coronary heart disease in different populations with dietary intake of antioxidants (predominantly from fruits and vegetables): (a) Armstrong et al. (1975) observed a strong inverse relationship of regional consumption of fresh fruits and vegetables and coronary heart disease in nine United Kingdom regions whilst (b) Feldman (2002) reported scientific evidence for a beneficial health relationship between antioxidant-containing nuts (walnuts) and coronary heart disease. (c) Sabate (1999) observed that consumption of nuts and vegetables with antioxidant properties has a protective effect against ischaemic heart disease. (d) Anderson (2001) noted that fruits containing polyphenolics inhibited ***in vitro*** human plasma LDL oxidation. (e) Dragland et al. (2003) also noted the relationship of fruits or medicinal herbs and protection against cardiovascular diseases. (f) A recent study by Patel et al. (2007) noted that not only did total antioxidant status and total thiol levels predicted overall improved survival in patients with oral squamous cell carcinoma, but it also suggests that this could be beneficial to patients with cardiovascular disease.

The Seven Countries Study Research Group (Finland, Netherlands, Italy, USA, former Yugoslavia, Japan and Greece) which investigated the relationship between food-group intakes and 25-year coronary artery disease mortality in 12, 763 middle-aged men, reported that the average intake of antioxidant flavonoids was inversely related to mortality from cardiovascular disease (Hertog et al., 1995). In a study carried out in Pitkaranta and North Karelia, 93% of the men in Pitkaranta had very low serum vitamin C levels in contrast to just 2% in North Karelia, suggesting that there may be a relationship between vitamin C deficiency and high cardiovascular disease mortality among the men in Pitkaranta compared to the relatively low incidence of cardiovascular disease where high levels of vitamin C were present in serum of men of North Karelia (Matilainen et al., 1996).

In our opinion, the outcome of the Matilainen and colleagues' (1996) study does not suggest an absolute relationship or a cause and effect between vitamin C deficiency and cardiovascular disease since there could be other confounding factors involved. However, the study provides an insight into a possible protective role of antioxidants (in this case vitamin C) against cardiovascular disease. A United States National Health and Nutrition Examination Survey support the view of a beneficial effect of vitamin C on cardiovascular disease (Simon and Hudes, 1999; Simon and Hudes, 2001).

A study carried out by Osganian and others (Osganian et al., 2003) in the Nurses' Health Study, on 73, 286 female nurses over a period of 14 years reported a modest but significant inverse relationship between serum levels of beta-carotene and beta-carotene intake and the risk of cardiovascular disease. Based on the review of 250 research studies, Van't Veer et al. (2000) concluded that fruits and vegetables containing antioxidants are beneficial in the prevention of cardiovascular disease.

Evidence from other studies also provided some insight on the beneficial effects of antioxidants on cardiovascular disease. Nojiri et al. (2001) investigated the association between coronary artery disease and serum anti-oxidative status in a case-controlled study and found in a multiple regression analysis of results that the total antioxidant status negatively correlated with the number of diseased vessels. They also found that high concentrations of carotenoids, glutathione peroxidase and alpha and gamma-tocopherol were significantly related to the number of diseased vessels.

In our opinion, we believe that more scientific evidence-based studies using larger population are required to confirm or refute some of the claims made with particular reference to the effect of antioxidants on cardiovascular disease. However, this does not in any way cast doubt on the importance of antioxidants in the prevention and management of chronic conditions such as cardiovascular disease.

A study in China (Blot et al., 1993) examined dietary vitamin and mineral supplementation in 29, 584 men and women. It was found that there was an improved oxidative status. Also in the Cambridge Heart Antioxidant Study where 2002 patients with coronary atherosclerosis were given vitamin E capsules containing 800 IU daily and followed for 510 days, Stephens et al. (1996) reported the beneficial effects of vitamin E supplementation. It is however also appropriate to note that some other studies have reported no beneficial effects of dietary or vitamin/mineral supplementation on cardiovascular disease (Zock and Katan, 1996; Rapola et al., 1996; Tardiff et al., 2001; Lonn et al., 2001; Ceriello and Motz, 2004).

Even though epidemiological and experimental studies that investigated the possible role of vitamins E and C as well as B-carotene in the prevention of cardiovascular disease in animal and human subjects have been carried

out, only a few of these studies have been performed with consideration of the possible role of lycopene (Agarwal and Rao, 2000b; Ceriello and Motz, 2004).

Lycopene is a naturally occurring carotenoid in tomatoes and tomato products with an open-chain hydrocarbon containing 11 conjugated and two non-conjugated double bonds arranged in a linear array (Tardiff et al., 1997). Due to its high number of conjugated dienes, it is regarded as the most potent singlet oxygen scavenger among the natural carotenoids (Tardiff et al., 1997; Agarwal and Rao, 1998). Recent epidemiological studies have shown an inverse relationship between the intake of tomatoes, serum and adipose tissue lycopene levels and the incidence of cardiovascular disease. A number of ***in vitro*** studies have shown that lycopene can protect native LDL from oxidation and also suppresses cholesterol synthesis (Agarwal and Rao, 1998; Lowe et al., 1999).

Evidence in support of the role of lycopene in the prevention of cardiovascular disease is based on epidemiological observations on normal subjects and populations at risk of developing cardiovascular disease. Fresh fruits and vegetables are considered to be good sources of dietary carotenoids, including lycopene and the antioxidant properties of lycopene have been suggested as being responsible for the beneficial effects of these food products. A Mediterranean diet rich in tomatoes, tomato products, lycopene, and other carotenoids has been reported to be associated with a lower incidence of atherosclerosis and cardiovascular disease (Fuhrman et al., 1997; Dugas et al., 1999).

The strongest population-based evidence in support of the beneficial effects of antioxidants is the result of a study carried out by Kohlmeier et al. (1997). This study evaluated the relationship between adipose tissue antioxidant status and acute myocardial infarction. The study consisted of 662 cases and 717 controls recruited from 10 European countries. Needle aspiration biopsy samples of the adipose tissue were taken shortly after cardiac infarction, and ß-carotenes, lycopene, and alpha-tocopherol levels were determined using the appropriate methodologies. Following the measurement of the beta-carotene, lycopene and alpha-tocopherol levels, adipose lycopene levels expressed as milligrams per gram of fatty acids were reported as significantly high. Adjustments were made for age, body mass index, socio-economic status, smoking, hypertension, and maternal and paternal history of the disease. Interestingly, only lycopene levels were found to be protective against cardiovascular disease with a P-value of 0.005.

Although the epidemiological studies conducted to date have provided some insight into the role of lycopene in cardiovascular disease prevention, it is only suggestive and not evidence of a causal relationship between lycopene intake and the risk of cardiovascular disease. It is therefore essential that more controlled clinical dietary intervention studies should be performed especially in the developing world to confirm the findings of these studies. Unfortunately, our investigation to find supportive evidence indicates that very few such intervention studies have been reported in the literature. In one such study, healthy human subjects consumed a lycopene-free diet for a period of 2 weeks. It was found that their serum lycopene levels decreased by 50% at the end of two weeks and, simultaneously, an increase of 25% ***in vivo*** lipid oxidation was observed (Rao and Agarwal, 1999). In a small dietary supplementation study, six healthy male subjects consumed 60 mg/day lycopene for 3 months. At the end of the supplementation period, a significant 14% reduction in their plasma LDL cholesterol levels was observed (Gomez-Aracena et al., 1997; Agarwal and Rao, 1998).

Other investigators continue to report on different compounds with antioxidant activity and the potential role which these compounds may play in improving oxidative status and improvement of the quality of life of patients. For example, red palm oil has been observed to be a carotenoid rich antioxidant oil and its protective role against oxidative stress has been studied by our group and some collaborators. For instance, Esterhuyse et al. (2005, 2006), Bester et al. (2006), Engelbrecht et al. (2006), Kruger et al. (2007) and Van Rooyen et al. (2008) reported on the protective effect of red palm oil against oxidative stress and ischaemia in a reperfused rat heart model indicating the beneficial effects of antioxidants or antioxidant-containing compounds in this natural product on health status.

The literature indicates that humans have been safely using red palm oil as a nutritious source of edible oil for thousands of years and it is believed that red palm oil contains more nutrients than any other dietary oil. In addition to beta-carotene, alpha-carotene, tocotrienols and lycopene, it contains at least 20 other carotenes along with vitamin E, vitamin K, ubiquinone 10, squalene, phytosterols, flavonoids, phenolic acids, and glycolipids. It is one of the richest natural sources of vitamin E known and contains the highest amount of a super potent form of vitamin E known as tocotrienol. Red palm oil has also been found to contain all of the four known tocotrienols and it is believed that these tocotrienols have up to 60 times the antioxidant activity of ordinary vitamin E. The combination of vitamin E, tocotrienols, carotenes and other antioxidants therefore makes red palm oil an excellent and essential antioxidant source (Fife, 2007). Due to its balanced high levels of nutrients and antioxidants, red palm oil can be seen as a preferential natural dietary supplement for consumption.

Studies have shown that the dietary intake of red palm oil can remove plaque build up in arteries and therefore, reverse the process of atherosclerosis. This has been demonstrated in both animal and human studies. In one such study, 50 subjects diagnosed with atherosclerosis who had suffered at least one stroke were divided into two equal groups. At initiation of the study, the degree of carotid artery blockage ranged from 15 to 79%. Without

any other alteration to their diets or medication, half of the subjects began taking a daily red palm oil supplement. The other half received a placebo (control). The degree of atherosclerosis was monitored using ultrasound scans over an 18 month period. In the group receiving red palm oil, atherosclerosis was halted in 23 of the 25 participants. In seven of these participants, atherosclerosis was not only halted but regressed. In comparison, none of those in the control group showed any clinical improvement and the condition in 10 of them in fact worsened (Tomeo, 1995). This study scientifically indicated that red palm oil is beneficial to human health. Unfortunately, most studies on red palm oil as a good source of antioxidants have been performed on animal models. We strongly advocate for a paradigm shift of the focus from animal studies to humans. Interestingly, the Oxidative Stress Research Unit at the Cape Peninsula University of Technology, Bellville, South Africa is accepting the call to investigate the potential ability of red palm oil to reduce oxidative stress in patients with cardiovascular problems as well as in other chronic diseases such as HIV, TB, diabetes and cancer.

Conclusion and Recommendations

From the scientific reports reviewed by us, it is clear that sufficient epidemiological and clinical evidence exist which support the hypotheses of the role antioxidants play in the prevention and management of cardiovascular disease. However, controversies still exist as regards the role of antioxidants in the prevention of cardiovascular disease. It is known that cardiovascular disease is a progressive disease, therefore it is suggested that most epidemiological studies should not mainly use death due to cardiovascular disease as an end point, but should equally examine the links between early events of the disease such as LDL oxidation. Prevention of the disease is very important since the emphasis in modern medicine has shifted from curative to preventative. Therefore emphasis should be shifted to examine the potential preventative role of natural products with antioxidant activities such as red palm oil, mushrooms and others such as herbals. Studies on antioxidants should be well-controlled as it has been shown that well-controlled clinical and dietary intervention studies provide the most significant information that is required for the proper management of cardiovascular disease.

The use of well-defined participant populations, standardized outcome measures of oxidative stress and disease as well as antioxidant-containing product intake which is considered to be representative of an apparently healthy diet, are important parameters to consider for the useful interpretation of results with regard to therapeutic applications. The duration of the study is also very important and should be considered when designing studies that will address the role of antioxidants in preventing or reducing complications due to cardiovascular disease. Duration should not only be limited to the length in time of supplementation but should also be considered in relation to the age of participating subjects and stage of the disease. Malabsorption or other diseases of the gastrointestinal tract could also affect absorption of antioxidants and consequently the effects of such antioxidants. As a result, investigators are raising relevant questions as per the bioavailability of antioxidants and this should be noted as some of the conflicting epidemiological studies which have been reported may be related to this issue.

We further suggest that the total antioxidant content of an antioxidant-containing product be determined as this could be a helpful instrument for evaluating the antioxidant network hypothesis.

The dosage of the antioxidants, statistical relevance of the study and validity of findings are equally very important factors in this whole concept of antioxidant studies.

Information on genetic susceptibility and environmental interaction including dietary exposure will be important in future studies of cardiovascular disease.

The focus should not be on treating cardiovascular disease with antioxidants, but on the intake of a balanced diet with emphasis on antioxidant-rich fruits, vegetables, nuts, whole grains as has been recommended for the general population by the American Heart Association. It is therefore suggested that serious attention be given to all these factors when designing future studies that are aimed at evaluating the potential role of antioxidants in the prevention of cardiovascular disease.

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