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ERRATUM

This article was originally published in Volume 26, Number 6, Pages 565-583 without the accompanying tables due to a printing error. The article is reprinted below in its entirety and we apologize for any inconvenience.

COMMENTARY

Will the 'Good Fairies' Please Prove to us that Vitamin E Lessens Human Degenerative Disease?

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Recent research about the role of free radical derivatives of oxygen and nitrogen in biological systems has highlighted the possibility that antioxidants, such as vitamin E, that prevent these processes *in vitro* may be capable of carrying out a similar function in living organisms *in vivo*. There is increasing evidence that free radical reactions are involved in the early stages, or sometimes later on, in the development of human diseases, and it is therefore of particular interest to inquire whether vitamin E and other antioxidants, which are found in the human diets, may be capable of lowering the incidence of these diseases. Put simply, the proposition is that by improving human diets by increasing the quantity in them of antioxidants, it might be possible to reduce the incidence of a number of degenerative diseases. Of particular significance to these considerations is the likely role of the primary fat-soluble dietary antioxidant vitamin E in the prevention of degenerative diseases such as arteriosclerosis, which is frequently the cause of consequent heart attacks or stroke, and prevention of certain forms of cancer, as well as several other diseases. Substantial evidence for this proposition now exists, and this review is an attempt to give a brief account of the present position. Two kinds of evidence exist; on the one hand there is very substantial basic science evidence which indicates an involvement of free radical events, and a preventive role for vitamin E, in the development of human disease processes. On the other hand, there is also a large body of human epidemiological evidence which suggests that incidence of these diseases is lowered in populations having a high level of antioxidants, such as vitamin E, in their diet, or who have taken steps to enhance their level of intake of the vitamin by taking dietary supplements. There is also some evidence which suggests that intervention with dietary supplements of vitamin E can result in a lowered risk of disease, in particular of cardiovascular disease, which is a major killer disease among the developed nations of the world. The

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intense interest in this subject recently has as its objective the possibility that, by making some simple alterations to dietary lifestyle, or by enhancing the intake of vitamin E by fortification of foods, or by dietary supplements, it may be possible to reduce substantially the risk of a large amount of common, highly disabling human disease. By this simple means, therefore it may be possible to improve substantially the quality of human life, in particular for people of advancing years.

Keywords: Vitamin E, human degenerative disease

I. INTRODUCTION

The current year, 1997, is the 70th anniversary of the publication by Herbert Evans and his associates of their seminal work[2] that brought together the results of their researches at Berkeley, California, which described the discovery of vitamin E.[3,4] Speaking at a Symposium held in his honour in Zurich in 1961, Evans described the early days of their discovery and observed that 'good fairies attended every phase of the advent and early history of vitamin E'.[1] He failed to mention, with his characteristic modesty, that the discovery, using of course the relatively unsophisticated techniques of the time, involved a huge amount of effort, and the conviction on his part that the missing factor in his rat diets, which could prevent in female animals the phenomenon of gestation resorption, was indeed a dietary factor. This was followed soon after by the discovery that lettuce, and then a lipid-soluble extract of lettuce, could prevent the condition; this was only one step away from the discovery of vitamin E itself. The subsequent isolation of a pure specimen of vitamin E, the demonstration of its structural formula by, in Evans' own words, 'the young genius chemist of outstanding ability, Erhard Fernholtz,[5,6] and the first synthesis of the vitamin by Paul Karrer,[7] are all matters of history to which Evans refers.[1] Evans' choice of the name tocopherol, with its Greek derivation τοχο–πηεροσ, owed more apparently to his lunching with the Professor of Greek at Berkeley at that time, than to his own classical scholarship. The lesson for today from the work of Herbert Evans is surely that his meticulous attention to the constituents of the diet that was given to his rats, coupled with his masterly histological examination of the pathology of the condition he observed, led to his classic observation, and that in these rushed and hurried days such attention to scientific detail is frequently sacrificed on the altar of the expedient need to publish quick results.

Following the bright period of close attention by the 'good fairies', research on vitamin E proceeded at a very slow pace for about 40 years, probably chiefly because it could not be shown that the vitamin is an essential nutrient for man, so that research funding was not easily attracted by those wishing to pursue investigation of the cellular and biochemical modes of action of α-tocopherol. During this period there was great controversy as to the molecular mode of action of vitamin E and two leading theories emerged which were, to some extent at least, mutually incompatible. The first hypothesis was that vitamin E had a specific role as an enzyme co-factor in a similar manner to the mode of action of some B-vitamins, and there was some evidence suggesting that α-tocopherol functioned in the mitochondrial electron transfer chain.[8] The second hypothesis, which was first proposed by the Nobel Laureate Henrik Dam[9] was that vitamin E simply functioned as an intracellular lipid antioxidant which prevented 'rancidity' of polyunsaturated fats *in vivo* as well as within the gastrointestinal tract. The principal later protagonist of this hypothesis was Al Tappel and he developed it into a sophisticated theory in which α-tocopherol was capable of interacting with vitamin C, which itself underwent oxidation-reduction chemistry by interaction with glutathione and thence with glucose via hexose monophosphate shunt-derived NADPH.[10] This far-sighted view, which Tappel pursued with almost crusading zeal, is the foundation of what we know today to be the cellular mode of action of vitamin E. The basis of the establishment of present day understanding of the biological role

of vitamin E lies, as so often happens in science, in another field altogether. During the early 1970's the late Trevor Slater at University College, London was studying the damage caused to liver cells by halogenoalkanes like carbon tetrachloride. Similar work was being undertaken by others at the same time Recknagel[11] and Comporti, reviewed later.[12] Slater began to emphasise the then startling hypothesis that free radical reactions were involved in liver smooth endoplasmic reticulum of rats intoxidated with carbon tetrachloride, which gave rise to a number of sequelae chief among which was the induction of peroxidation of membrane phospholipid polyunsaturated fatty acids.[13-15] The significance of these observations to research on vitamin E was that Slater showed[14] that free radical scavengers such as vitamin E and other lipid-soluble antioxidants, were capable of exerting a powerful modulating influence on the peroxidative damage induced by carbon tetrachloride. At about the same time work in our laboratory at Vitamins Limited had however shown[16,17] that carbon tetrachloride caused very little destruction *in vivo* of added ^{14}C-labelled vitamin E, which indicated that the vitamin must be very efficiently regenerated from whatever radical species it became following quenching of the $CCl_3\cdot$ radical. A marvellous symposium of about six of us was held when Richard Recknagel visited London at about this time, which began in Richard's hotel lobby and ended in Trevor Slater's tiny office at University College with Trevor declaiming in his usual modest but enthusiastic way his conviction of the rightness of the new ideas. Poor Ken Rees who shared the office with Trevor, was consigned to the corridor! This work laid the ground for the current so-called free radical theory of disease; it is common knowledge that work in this area has expanded exponentially since these initial observations so that at the present time there is intensive interest in the field, chiefly because free radical events have been shown to be involved in the aetiogenesis of a number of human disorders, among which are the degenerative diseases arteriosclerosis and some forms of cancer. There is accumulating evidence that antioxidants, particularly vitamin E, are capable of lowering the risk of such diseases in human populations, because the vitamin is capable of preventing the free radical-driven events that lie at the heart of of the pathogenesis of these conditions.

This short review seeks to discuss the evidence, both from the viewpoint of basic science, and from medical epidemiology, which suggests that vitamin E may have a highly significant role in the prevention of human degenerative diseases and possibly even in their therapy.

II. BASIC SCIENCE

1. Origins of Free Radicals

It is customary to think of oxygen as a benign substance upon which animals depend to oxidize their food and release the energy contained within it. This ignores the potentially dangerous role of oxygen in the generation of harmful free radicals, and does not address the involvement of free radicals in many processes that are beneficial to the body. Free radicals are defined as atomic or molecular species that have one or more unpaired electrons; they are in many instances highly reactive because the unpaired state is thermodynamically unstable. It is however, important to emphasise that not all reactions in living systems that involve free radicals are damaging,[18] and that several normal biological processes *in vivo* depend on free radicals. Bacterial killing in phagocytic cells (neutrophils, monocytes, macrocytes and eosinophils) involves a respiratory burst in which oxygen is reduced to the superoxide radical.[19,20] Nitric oxide, which is involved in the regulation of vascular tone,[21] is a free radical. Formation of products of the arachidonic acid cascade, prostaglandins and prostacyclins, involves the generation of free radicals. Detrimental effects of oxygen radicals are thought to depend in the first instance on the failure of con-

trol of the generation and further metabolism of simple derivatives of molecular oxygen; control of these processes depends on the antioxidant nutrients. Detailed consideration of these fundamental questions has been undertaken.[22,23]

The overall scheme for the reduction of molecular oxygen to water does not reveal that a series of intermediates can be involved such that the addition of electrons is sequential, and electrons derived randomly may be used to drive the formation of intermediates in an uncontrolled fashon. It is this uncontrolled random formation of oxygen radicals that lies at the heart of the concept of the involvement of oxygen radicals in the genesis of disease. Superoxide anion radicals may be formed by the addition of a single electron to oxygen and the electron deficiency of the superoxide radical may be made up by the addition of a further electron to form the peroxide anion O_2^{--}, which interacts with two hydrogen ions to form H_2O_2. Further reduction of hydrogen peroxide to water involves the addition of two more electrons which can occur *in vitro* by so-called Fenton chemistry, in which the electrons are provided by the oxidation of ferrous ions, Fe^{2+}, to ferric ions, Fe^{3+}. It is attractive to suppose that such reactions are responsible *in vivo* for the formation of hydroxyl radicals, but this is highly controversial at present because the Fenton reaction is driven by low-molecular weight chelated or free iron (or copper) and these cations are known to only be present under normal non-pathological conditions in biological systems tightly bound to proteins. However, following initiation of oxidative stress an immediate consequence may be the release of low-molecular weight ions which can then drive Fenton chemistry. Since the metal acts only as a catalyst, the amount of the cation required is very small and this amount could be provideed by a very small pool of free iron or copper.

2. Targets for Attack by Free Radicals *In Vivo*

Primary radical species differ in their reactivity, and their potential for damage to living cells depends on their chemical reactivity. Of greatest potential significance as an initiator of damage is the hydroxyl radical, $OH^{\bullet}$, since it reacts with most biological macromolecules with rate constants of 10^{-10} or more. The damage caused by $OH^{\bullet}$ is likely to be very local to the point at which it is generated, so that, for example, highly vulnerable DNA may be damaged. Antioxidants are therefore of the essence in affording protection at the site of damage Secondary radicals or radical products may however be produced which can move within the intracellular, or even intercellular environment, and which may be severely detrimental at sites far removed from the point of the initial radical attack.

There are three principal targets for attack by free radicals in living cells; these are DNA, proteins and polyunsaturated fatty acids (PUFA).

DNA

From measurements of the production *in vivo* of modified purine and pyrimidine bases, which are thought to be derived from the processes of DNA excision and repair, it has been estimated that oxygen radicals cause about 10,000 DNA base modifications per cell per day,[24] but this may be an overestimate. It is clear therefore that the potential for damage by radical species that may lead to mutagenesis and carcinogenesis is of major significance. An excellent recent account of the effect of free radicals on DNA (1993)[25] suggests that damage to DNA may be of significance in the aetiology of cancer, by causing direct mutagenic effects, by being involved in the promotion of transformation of mutated cells, and through expression of genes that may be important in the same context. Both DNA damage and mitogenesis, caused by agents that increase the rate of mitosis, are thought to be of significance in the cancer process. Four endogenous processes that lead to significant DNA damage are oxidation, methylation, deamination and depurination, and repair mechanisms exist in most

cellular systems for these processes.[26] The measurement of DNA adducts shows that oxidation is likely to be the most significant endogenous damage; since vitamin E acts in the lipid phase it is not readily apparent how it can prevent this process *in vivo* but vitamin E may be fundamental to control of the cancer process by preventing DNA modification by cytotoxic aldehydes.[27,28] Mitogenesis could cause oxidative damage to DNA by higher oxygen consumption and by consequent exposure of DNA to endogenous oxidants. Rapidly dividing cells are much more susceptible to mutation than non-dividing cells and lowering the rate of mitogenesis causes a greatly lowered incidence of cancer. The chemistry of attack by free radicals on DNA is very complex; lesions in chromatin include damage to bases, sugar lesions, single strand-breaks, abasic lesions and DNA-nucleoprotein cross-links.[29]

The question of what activated oxygen species is capable of reacting directly with DNA *in vivo*, and what is its origin, has not been clearly resolved. It is clear that hydroxyl radicals and singlet oxyen produce several types of damage to DNA, and alkoxyl radicals have also been shown to cause DNA damage.[30] The reactivity of oxygen species demands that they must be generated close to the DNA and it is unlikely that migration of such reactive species can account for direct damage to DNA. Free iron and copper may exist in the nucleus,[31] and the relatively stable compound hydrogen peroxide, which can migrate freely within cellular structures, may give rise to hydroxyl radicals *in situ* in the nucleus by Fenton-type reactions. It may be of particular significance in the mutagenic and carcinogenic process that activated phagocytes are in close proximity to the site of initiation of tumorigenesis, or in sites of inflammation, because the oxygen burst associated with phagocytosis is a source of both hydrogen peroxide and the superoxide anion radical. It should also be borne in mind that damage by oxygen radials to DNA repair and replication enzymes may occur[32] which will also have significant consequences for maintenance of DNA housekeeping.

An important potential relationship also exists between lipid peroxidation and the causation of mutagenesis and carcinogenesis; this is the interaction of aldehyde metabolites of lipid hydroperoxides with DNA which may have consequences for the cell cycle.

Unsaturated Fatty Acids

Attack by free radicals on unsaturated fatty acids has been the subject of extensive study. Studies of the basic chemistry involved were of interest in the area of oxidative rancidity of fats which for many years has been of key importance to the oil industry. There are two aspects of this process that are pertinent to the question of the significance of free radical damage in the aetiogenesis of disease. The first is the possibility of peroxidation of polyunsaturated fatty acids in biological membranes; the second is the peroxidation of polyunsaturated fatty acids within circulating lipoproteins. Although mechanistically these are similar, their biological effect is quite different.

Lipid Peroxidation in Membrane Polyunsaturated Fatty Acids (PUFAs)

The mechanism of attack by free radicals on polyunsaturated fatty acids is well understood. In arachidonic acid, for example, attack by a radical, such as the hydroxyl radical, abstracts a hydrogen atom from the fatty acid to produce a carbon-centered radical. Following an intermolecular rearrangement which yields a mixture of two products, either the 9- or the 13-carbon-centered radicals, there is attack by these radicals on molecular oxygen which yields either a 9- or a 13-peroxyl radical. These new lipid peroxyl radicals can react with another molecule of unsaturated fatty acid to yield a lipid hydroperoxide and a further lipid radical which will enter the reaction sequence again so that a chain reaction is set up. The chain may be broken by a lipid antioxidant such as α-tocopherol, from which is formed an α-tocopheroxyl radical. A more detailed des-

cription of this reaction sequence is given by Cheeseman.[33] It is presumed that a similar sequence of reactions occurs within the structure of biological membranes, although spatial constraint of the polyunsaturated fatty acid within the phospholipid in the lipid bilayer lamella structure will impose constraints on the progress of peroxidation, as well as interception of radicals by membrane proteins with consequent protein damage.

Lipid hydroperoxides are not stable end-products under physiological conditions; they may be exposed to transition metal ions which catalyse the formation of further reactive lipid radical species. In addition there is the possibility that complete degradation of the peroxidized lipid may give rise to products that are biologically active and cytotoxic. Cleavage of carbon-carbon bonds within lipid hydroperoxides formed during peroxidation leads to several different types of product which may be placed in three main classes which are:

(i) Alkanals, typified by malondialdehyde,[34] which are highly reactive compounds that can cause considerable intracellular damage by a variety of reactions, which include reaction with protein thiols and cross linking of amino groups of proteins.

(ii) Alkenals, typified in particular by 4-hydroxynonenal.[35] The biological activity of these compounds has been explored in some detail and there is no doubt that these compounds are of major significance in cytotoxicity and cytostasis, which are caused by specific effects on the cell cycle. Several reviews of these actions of hydroxyalkenals have appeared.[36] The chemical reactivity of hydroxyalkenals is due to the fact that they are strongly electrophilic and will therefore react with a range of biological nucleophiles. Among these the reaction with glutathione (GSH) is undoubtedly of key importance because it may also explain the depletion of GSH assocated with lipid peroxidation. The cytotoxicity of 4-hydroxynonenal has been demonstrated in a wide range of different cell types and the LD_{50} of HNE varies from 5μM to 1 mM.[33,28] The mechanism of HNE cytotoxicity is however not fully understood.

(iii) Alkanes, such as pentane, which is produced as the end product of the oxidation of linoleic and arachidonic acid, and ethane which is derived from linolenic acid.[34]

Oxidation of Plasma Lipoproteins

The key role played by low density lipoprotein (LDL) oxidation in the early events that lead up to atherogenesis, and the development of coronary heart disease, has become accepted as the principal most likely explanation of a key stage in this complex multifactorial process. The subject has been reviewed in detail.[37] The physical arrangement of the lipids and protein within the LDL particle has been the subject of much study; the particle consists of a central core of cholesterol esters arranged in a lamellar fashion around which cholesterol, phospholipids and apoproteins are distributed. High-affinity receptors for LDL are found on the plasma membrane of most cell types and this receptor is recognized by the $apoB_{100}$ lipoprotein. The primary function of LDL is transport of cholesterol within the body and the function of the receptor is the removal of cholesterol from the circulation, so that it is delivered to extra-hepatic tissues. Since up to 70% of the total cellular receptor activity is located in liver cells, this also provides the mechanism for the return of cholesterol to the liver. The apoprotein of LDL contains ε-lysine residues which are the main target of reactive aldehydes formed as degradation products of polyunsaturated fatty acid hydroperoxides. Oxidatively modified LDL, which has thus been changed by ε-lysine modification, is taken up specifically at high rates by macrophage scavenger receptors which differ from the receptors that are responsible for normal LDL metabolism. These scavenger receptors are responsible for the enhanced uptake of cholesterol into macrophages that is observed prior

to the formation of foam cells in the atherogenic process. The oxidation of PUFAs within the phospholipids and cholesterol esters of LDL appears to be the first significant process that occurs, and degradation of the resultant lipid hydroperoxides to aldehydic products leads to the formation of Schiff Base complexes with ε-lysine residues within the $apoB_{100}$ lipoprotein. This modification of the LDL $apoB_{100}$ lipoprotein, which occurs within the arterial intima, leads to avid uptake of the oxidized LDL by macrophage scavenger receptors and thus to the beginning of fatty streak formation.

Proteins

Understanding of the importance of protein modification as a manifestation of free radical damage has only recently been emerging. Much pioneering work was done in this area by Tappel[38] and Stadtman has reviewed the subject in detail recently.[39,40] In addition to the posibility of attack by primary radical species, proteins may also be the target of attack by secondary radicals in particular those derived from lipid peroxidation, and also from the degradation products that are derived from the process. These may react with proteins located in the immediate vicinity of the lipid-derived radicals and products of lipid peroxidation such as malonaldehyde or 4-hydroxynonenal may form stable cross-linked products with specific amino acids such as lysine. This mechanism is of particular importance in considering the oxidative modification of LDL that is thought to be of importance in the atherogenic process. Proteins may thus be important targets for free radical attack and amplification of these effects may occur because the protein is an enzyme, so that its catalytic effect may become modified by one quite simple amino acid modification at the active site of the enzyme. Alterations in membrane function caused by structural protein modification may also have widespread detrimental consequences such as disturbance of ionic balance within cells. Oxidative modification of proteins may also lead to their increased susceptibility to proteolytic attack, so that increased hydrolysis of protein at the site of damage is important in the pathological process.

3. Prevention of Free Radical Attack by Antioxidants: The Role of Vitamin E

The prevention of free radical damage by antioxidants is vital to present assessment of disease prevention by vitamin E and the likely consequences of intervention with the vitamin in human populations. For the purposes of this discussion, the term vitamin E is used in the nutritional sense to express the vitamin E activity of the constituent tocopherols in the food that is being consumed. The tocopherols differ widely in their biopotency (for further explanation, see[41]), but in general the most significant biologically active component of food is α-tocopherol. Antioxidants can act at several different points in the sequence of reactions that lead to the formation of oxidized products and multiple mechanisms of action are likely to occur. With respect to lipid peroxidation, which is the most important manner of intervention by vitamin E in the free radical-driven processes that lead to disease, there are at least five different mechanisms by which antioxidants may bring about their effect.[22] These are:

(i) decreasing localized oxygen concentrations so that oxidation by molecular oxygen is less likely to occur;
(ii) prevention of chain initiation by scavenging initiating free radicals;
(iii) binding of transition metal ion catalysts to prevent generation of initiating free radicals;
(iv) decomposition of peroxides so that they cannot be converted to further active radical species and thus act as initiators;
(v) chain breaking to prevent continued hydrogen abstraction by active free radicals.

Antioxidant protection consists of three main levels of defence which can most clearly be

seen in the case of lipid peroxidation. The first largely enzymatic level of defence involves trace amounts of the minerals Mn, Cu, Zn and Se which function through their involvement at the active site of mineral-dependent enzymes; they control the formation and proliferation of primary radical species. The second level of defence involves vitamin E, which may act in concert with vitamin C, and it may also involve the carotenoids as well as dietary polyphenolic substances; it is concerned with the prevention of proliferation of secondary radicals in the chain reactions which occur in lipid peroxidation, initiated by primary radicals. The third level of protection is provided by enzymatic removal of lipid hydroperoxides preventing the formation of secondary radicals and aldehydic products from chain-terminated metabolites, and it enables removal of such molecules from the possibility of metal-catalysed reactions which might initiate further damage. This protective mechanism, viewed as a whole, depends on the continuing availability, from outside the organism, of a supply of certain specific nutrients which include minerals (Se, Mn, Cu), vitamins C and E and also possibly a range of carotenoids. It is thus also capable of being compromised by the failure to supply, through the diet, one or more of these nutrient substances. These nutrients have become known as the 'antioxidant nutrients', and it is the question of the need for a continuous supply of these nutrients in the diet which is at the centre of the present debate as to the possibility that human disease risk, or incidence, might be lowered by improvement in the quantity of these nutrients in the diet, or supplied by supplemental means. Furthermore, the possible role of non-nutrient polyphenols, if they are absorbed and distributed to the tissues, is a topic of great current interest.

The control of secondary radicals involves vitamin E in conjunction with vitamin C. Lipid antioxidants such as α-tocopherol can act as a chain-breaking antioxidant by donating a hydrogen atom to a lipid peroxyl radical. This is the presumed function *in vivo* of vitamin E which is the principal, or only, biological lipid antioxidant. The molecular structure shows that it is amphipathic and it has been suggested that it functions by becoming orientated within biological membranes so that the polar head group is localized at the lipid water interface and the non-polar side chain is located among the fatty acyl chains of membrane phospholipids.[42] Following donation of the hydrogen atom from α-tocopherol it is thought that the resultant α-tocopheroxyl radical may interact, before it can be further oxidised irreversibly to α-tocopherylquinone, with ascorbic acid (vitamin C), or possibly directly with GSH. Dehydroascorbate thus formed is regenerated by reduction by NADPH which is in turn derived from pentose phosphate glucose metabolism. The biological functions of vitamin E and vitamin C are thus interdependent, although vitamin C also has other functions.[43] This postulated interaction between the two vitamins has been questioned because it was shown[44] that guinea pigs deprived of vitamin C appear to be able readily to repair the α-tocopheroxy radical. Present research also suggests that other non-nutrient antioxidant substances may be absorbed by humans and may also be capable of exerting this tocopherol-regenerating process; likely candidates for this are dietary polyphenolic substances.

Lipid hydroperoxides are formed in biological membranes and in LDL as a consequence of the chain-breaking activity of α-tocopherol, as well as from uncontrolled peroxidative chain reactions. Lipid hydroperoxides present a further possible source of oxidative damage; this is because catalysis by divalent transition metal cation complexes may cause the re-formation of lipid peroxyl radicals, or of other radical species such as the alkoxyl radical. It follows therefore that the removal of lipid hydroperoxides is essential to prevent re-formation of radicals and this is achieved by enzymatic degradation involving three enzymes; selenium-containing glutathione peroxidase (GSHPx), selenium-containing phospholipid hydroperoxide glu-

tathione peroxidase (PLHPGSHPx) and some forms of glutathione-S-transferase (GST), which act in this instance as a peroxidase.

4. Free Radicals, Antioxidants and the Development of Human Disease

Oxidative damage is of great importance in the aetiology of many human diseases. There are many unsubstantiated claims in the literature, but there are nevertheless several disease states in which the involvment of oxidants, and free radicals, is becoming well established. These include atherosclerosis,[37,45,46] some forms of cancer,[25,33,47] cataract[48-50] and other eye disorders,[51-53] connective tissue disorders[54] which may involve the inflammatory response,[55] and some aspects of central nervous system injury.[56] Only atherosclerosis and cancer will be considered here.

Cancer

Carcinogenesis is a multi-factorial process that takes place over a considerable period of time. The stages of initiation and promotion leading to establishment of transformed cells involve a number of stages at which free radical-involving processes might be involved; the possibility also exists that the involvement of free radicals in other cellular processes may also have an indirect effect on the cancer process. The investigation of free radical-involving or -derived processes is of critical importance to the possibility of cancer prevention by antioxidants. There is a great potential for supposing that relatively straightforward molecular alterations in DNA structure could lead to mutagenesis and eventual carcinogenesis. However, this is an overly simplistic view and a number of potential mechanisms of carcinogenesis must be considered.

The question of the involvement of free radicals in cell proliferation has been reviewed by Burdon,[47] and the involvement of lipid peroxides in the carcinogenic process has been considered by Cheeseman[33] and by Morrero and Marnett.[57] Mechanisms for regulation of mammalian cell proliferation are now becoming understood more clearly. The fundamental controlling agents, which are specific growth factors present in serum that provide external signals for cellular proliferation interact with cell surface receptors to trigger intracellular second messenger systems which activate genes, including protooncogenes, which express key biochemical timing devices required for the proliferation process. Study of these complex events indicates that free radical-related events are of considerable importance as modulators of growth regulation. There have been many observations that show an inverse relationship between levels of lipid peroxidation and rates of cellular proliferation[58]) and the bursts of DNA synthesis in the S-phase of regenerating rat liver are accompanied by a rise in α-tocopherol level and a lowered susceptibility to lipid peroxidation.[59] Another mechanism by which peroxidation may affect the rate of cell proliferation is through the aldehyde products of LOOH breakdown. It has been shown above that a variety of carbonyls, particularly hydroxyalkenals like 4-hydroxynonenal, may have profound effects on cell proliferation, mediated through effects on adenyl cyclase and phospholipase C activities, as well as effects on protooncogene expression.[47] These effects may have considerable significance in the cancer process but the role of these processes in the overall causation of cancer remain to be established. The multifactorial nature of carcinogenesis necessitates consideration also of the role of free radicals, and of lipid peroxides in particular, in chemical carcinogenesis. There is a large literature, reviewed by Morrero and Marnett,[57] on the metabolic activation of carcinogens by peroxyl radicals and there is now a complete picture emerging of the the role of peroxyl radicals in the metabolic activation of polycyclic hydrocarbons and of their DNA conjugates. The role of vitamin E in modulating these processes remains to be established but it seems highly likely that this may also be a point at which vitamin E is capable of influencing the cancer process.

It is clear from the foregoing that there are many possible ways in which free radical-related events in cellular metabolism may impinge on the cancer process; this must provide an excellent rationale for a part of the disease process, from which the potential role of vitamin E and other antioxidants as cancer preventing agents may eventually emerge.

Atherosclerosis

Coronary artery disease is the chief cause of death in Europe and in the USA. The basic science rationale which explains the probable role of free radical-mediated events in arteriosclerosis, and the inhibitory function of the antioxidants, has been discussed earlier in this article. The primary cause of coronary heart disease, and also of stroke and other vascular diseases, is the condition known as atherosclerosis which is characterized by thickening and other pathological degeneration of the arterial intima. As indicated earlier, it is now thought to be highly likely that the formation of foam cells, which is a very early event in arteriosclerosis, involves oxdative changes in LDL which is capable of being inhibited by the antioxidant nutrients. It is however clear that the events that lead to atherosclerosis are extremely complex and require a detailed knowledge of the biological and biochemical processes involved. Early studies on the oxidation of LDL in isolated cultured cells demonstrated that the recognition of native LDL by high affinity macrophage receptors was lost and that oxidised LDL (LDL_{ox}) was taken up instead by the scavenger receptors on the macrophage surface. Oxidation of LDL caused oxidation of the PUFAs as indicated above, formation of lysolecithin and a modification of the apolipoprotein B_{100} which involved the loss of available lysine residues that is responsible for the change in receptor specificity, so that the LDL_{ox} was taken up by the scavenger receptors.[60] The suggestion that aldehydes released from the oxidised PUFAs might bind to the ε-amino groups of lysyl residues within the apoprotein has subsequently been confirmed. Detailed consideration of the significance and mechanism of LDL oxidation is given in a review.[45] The proposal[61] concerning LDL oxidation is of crucial significance to the atherogenic process and this detailed hypothesis was summarized as follows.

(i) Traces of lipid hydroperoxides, which may be preformed or formed enzymatically by the action of lipoxygenase, are decomposed to reactive alkoxyl or peroxyl radicals by trace amounts of Fe^{2+} or Cu^{2+}. These radicals act as initiators within the LDL particle so that the PUFA of the LDL is oxidised to lipid hydroperoxides (LOOH).
(ii) LOOH provide a continuous source of new alkoxyl and peroxyl radicals in the presence of transition metal ions and the initial event is thus amplified.
(iii) Chain scission decomposition of alkoxyl radicals leads to the formation of a range of reactive aldehydes, such as malonyldialdehyde, hydroxyalkenals and alkanals; the apoprotein B may also be partially degraded.
(iv) The lipophilic nature of the aldehydes causes them to remain associated with the LDL particle, to diffuse to the apoprotein B and to react there with amino acid side chains which causes an increase in the negative surface charge of the apoprotein B resulting in its recognition by the macrophage scavenger receptors.

The control of the process of LDL oxidation by vitamin E is thus central to the role played by LDL oxidation in the aetiology of atherosclerosis and the possible preventive function of the vitamin. Around 30–50% of the plasma vitamin E is located in the LDL fraction, and other antioxidants such as carotenoids are present in much lower amounts. It is therefore reasonable to suggest that the enhancement by dietary means, or by the use of dietary supplements, of LDL vitamin E content could be expected to result in a slowing of the

process of LDL oxidation and therefore of the arteriosclerotic disease process. The question of regression of existing arteriosclerotic plaque is also an important one in the present context. Although there is now ample evidence that regression occurs both in experimental animals[62] and in man[63] there is as yet only a little evidence, which is discussed in a subsequent section, that indicates that antioxidant nutrients may play a part in the regressive process. This is an intensely practical question because the possibility of therapy of patients with established arteriosclerosis with vitamin E has been addressed from time to time in clinical circles.

III. HUMAN STUDIES

5. The Role of Vitamin E and Other *Antioxidants* in Prevention of Human Cancer and Cardiovascular Disease

The principal human diseases in which it has been demonstrated, in epidemiological studies, that a high incidence of disease is associated with a low intake of antioxidant nutrients, are some forms of cancer in specified sites, and cardiovascular disease (heart attacks), and to a lesser extent cerebrovascular disease (stroke). In such human studies it is often difficult or impossible to separate the role of vitamin E from that of other antioxidant nutrients, so that in considering the evidence relating to these diseases some mention of other antioxidants is inevitable.

Cancer

There has been very considerable interest in the possibility that the antioxidant nutrients vitamin E, vitamin C and carotenoids, may together or separately, have a protective effect against a wide range of human cancers. The search for a single protective agent has led to confusion of the evidence that links a high intake of a specified nutrient with lowered risk of a particular cancer, with the very extensive evidence that links a high intake of fresh fruit and vegetables with a lower disease risk. There are several reviews of the epidemiological literature that suggest a protective role for vitamin C[64,65] and beta carotene[66,67] against the incidence of cancer; in both the weight of evidence for a protective function is very considerable. It is however clear that in all those studies, where participants were asked about fruit and vegetable consumption, it was not possible to be certain that the effect that was reported was indeed due entirely to the nutrient in question, and it must be recognized that no allowance could be made for the effect of other factors in the foods. In acknowledging this fact it is nevertheless of the greatest importance to recognize the overwhelming evidence that exists linking low incidence of cancer in many body sites with a high intake of fresh fruits and vegetables. It has often been presumed, perhaps with only limited justification, that the beneficial effect demonstrated has been due to the high content of antioxidant nutrients in these foods. This interrelationship was examined in depth in a large review of all available epidemiological studies in the literature.[68] References to associations with retinol were omitted because the level in the body of retinol is under homeostatic control and is uninfluenced by increased intake of provitamins A (i.e. carotenoid compounds) beyond a certain theshold level; it is therefore generally agreed that the putative anticancer activity of carotenoids probably derives from the action of the carotenoid itself rather than from its first being converted into vitamin A. The review is restricted to case control and prospective epidemiological studies in which both the dietary intake, or blood nutrient level, and cancer status are identified as being in the same individuals. This is a very important point in considering the validity of such epidemiological studies.

Although the methodology of studies in the literature differs somewhat, and the meaning of relative risk, in particular when it is given a numerical value, may also differ, the review[68] uses methodology such that results can be compared between different studies. Infor-

mation about intake of a nutrient through diet was obtained by means of a questionnaire on frequency of consumption of named foods. Respondents were then grouped into those with low, moderate or high intake of individual foods, groups of foods or of nutrients calculated from them. Risk of cancer was expressed as Relative *Risk (RR)* and the risk of cancer in the group exposed to an identified risk factor (such as low fruit and vegetable intake) was expressed as a ratio to the risk in the group not so exposed (those with the highest intake). For practical purposes this means that an RR of > 1.0 indicates an increased risk of disease, so that an RR of 2.0 in a low-consuming group indicates twice the risk of cancer compared with the high-consuming group. Similarly an RR of < 1.0 indicates a lowered risk of disease where the same comparison is being made. Table I, which is derived from the review of Block *et al.* (1992),[68] shows a summary of the results that were obtained in this important study. The data are concerned with studies of established invasive cancer, and precancerous conditions are omitted; all available literature citations to the end of 1991 were included in this study. The statistical significance of the results was based on the results reported by the individual authors: $p < 0.05$ or more, the lower level of the 95% confidence interval at an RR ≥ 1.0. In almost all the studies reviewed adjustment for smoking was made or the effects in smokers were reported separately.

It will be clear from the results presented in Table I. that there is remarkable consistency in the data that link a low level of intake of fresh fruits and vegetables with a higher risk of cancer: indeed it could be said that the evidence is overwhelming. Consideration of the relationship in individual cancer sites, which evidently differ somewhat, were made in the review;[68] reference to the review should be made for more detail. This review demonstrated unequivocally that consumption of diets rich in fruits and vegetables is associated with a lowered risk of subsequent cancer. The study did not however provide any evidence that an identified individual nutrient derived from the fruit- and vegetable-rich diet was the factor that might provide a reason for the remarkable association that was demonstrated. Measurement of blood plasma nutrient levels in subjects who consumed high levels of fruit and vegetables was made in many of the studies, and

TABLE I Summary of epidemiological studies of fruit and vegetable intake and cancer prevention

Site	No. of studies	Protective ($p < 0.05$)	Harmful ($p < 0.05$)	Relative Risk (Range)
ALL	170	132	6	
ALL except prostate	156	128	4	
LUNG	25	24	0	2.2 (1.2–7.0)
ORAL CAVITY/PHARYNX	9	9	0	2.3 (1.7–2.5)
LARYNX	4	4	0	2.0 (2.1–2.8)
OESOPHAGUS	16	15	0	2.0 (0.7–4.8)
STOMACH	19	17	1	2.5 (0.5–5.8)
COLORECTAL	27	20	3	1.9 (0.3–3.3)
BLADDER	5	3	0	2.1 (1.6–2.1)
PANCREAS	11	9	0	2.8 (1.4–6.4)
CERVIX	8	7	0	2.0 (1.2–4.7)
OVARY	4	3	0	1.8 (1.1–2.3)
BREAST	14	8	2	1.3 (1.1–2.8)
PROSTATE	14	4	2	1.3 (0.6–3.5)
MISCELLANEOUS	8	6	0	

Note: in many studies significance levels of $p < 0.01$ or stronger were reported. Significance was defined here as $P < 0.05$ for comparison purposes only. From Block, 1992 [68].

it was possible in many cases to demonstrate correlations between high blood plasma levels of the antioxidant nutrients and lower risk of disease. This does not however establish a cause and effect relationship between, for example high intake, and high plasma level, of vitamin E and low incidence of cancer because the high plasma vitamin E level could have been a marker for other substances in fruits and vegetables which were the actual beneficial agents. It is unfortunate that the association of high vitamin E intake with low cancer incidence has in some instances been taken to imply a cause and effect relationship, without justification of this conclusion. Resolution of this point must await the results of further human intervention studies.

Cardiovascular disease

A confounding factor in studying the relationship between vitamin E and cardiovascular disease is the variation in the blood triglyceride and cholesterol levels of patients with heart disease. Changes in vitamin E levels need to be judged against the background of changes in the level of triglyceride and cholesterol. Thus it was found[69] that, when fasting blood samples from 116 healthy volunteers were analysed for vitamin E and blood lipids, there was a strong association between vitamin E and the serum lipid concentrations in those who did not smoke but that the rise in the serum lipid level in a cohort of smokers was not associated with a corresponding rise in vitamin E level. Similarly[70] when vitamin E, cholesterol and triglycerides were measured in sera from 167 patients with angina pectoris. an increase in the concentration of vitamin E was observed only in patients with lipidaemia, wheras the vitamin E content was similar to that of a control population in patients with hypertension, in smokers and in patients free of hyperlipidaemia. A correlation was found between vitamin E and triglyceride level of the samples ($r^2 = 0.52$). These data also correspond to data from another part of the study in which 224 men and 435 women without ischaemic heart disease were examined. In the men, vitamin E content was found to be correlated with triglycerides ($r^2 = 0.50$) and in the women with cholesterol. The ratio of vitamin E to triglyeride level is thus a more reliable index of vitamin E status in human subjects. It was concluded[71] that 'the plasma status of tocopherol can conclusively be interpreted only in comparison to the level of plasma lipids' and Gey introduced the term "lipid-standardized vitamin E' to indicate the degree of saturation of plasma lipids by vitamin E which may be taken as the only reliable index of the vitamin E status of an individual. This measure which is in line with advice offered by Horwitt some years earlier,[72] has been adopted and used consistently by workers in the field since that time.

Many aspects of the antioxidant hypothesis for disease prevention were brought together for the first time[71] when the available evidence was discussed that showed an association between high intake of aantioxidant nutrients and low incidence of ischaemic heart disease; the following points were made. (i) The accumulation of lipid hydroperoxides within arteriosclerotic plaque is positively correlated with the extent of arteriosclerosis, which highlights the possibility that oxidation of lipid might be a primary event in plaque formation. (ii) Peroxidised diets in many cases lead to toxicity expressed through degenerative heart disease in animals. (iii) Peroxidised low density lipoprotein is cytotoxic to endothelial cells in culture. (iv) Endothelial or smooth muscle cells in culture could generate lipid peroxidation products that could modify apoprotein B of LDL. (v) Experimental myocardial necrosis induced by catecholamines, hyperbaric oxygen or by ischaemia and subsequent reperfusion, is accompanied by lipid peroxidation, inhibited by antioxidants. (vi) Plasma of patients with ischaemic heart disease contains elevated levels of thiobarbituric acid reacting substances. (vii) Marginal deficiency of vitamin E in animals caused functional and morphological alterations in heart muscle and arterial walls.

Early epidemiological studies did not defined the status of antioxidants in the subjects investigated by measurement of their blood nutrient levels. Many studies show a good correlation between low incidence of ischaemic heart disease mortality, and high consumption of fresh fruits and vegetables, and also the ascorbic acid intake calculated from food tables, and the correlation for vitamin C was found to be highest at r = 0.7–0.9. It was assumed that the components of fresh fruits and vegetables that were likely to be of significance were vitamins E and C and the carotenoids.

Data from the pilot WHO/MONICA study[71] showed some inverse correlation, in particular with respect to vitamin C and vitamin E, between the medians of the plasma parameters measured and incidence of coronary heart disease mortality. The main WHO/MONICA study was designed to monitor (hence MONI-) determinants of cardiovascular (hence -CA) disease in 39 collaborating centres in 26 European countries.[73] In the Vitamin Substudy statistical medians of the plasma antioxidant levels in approximately 100 randomly assigned apparently healthy males (40–49 yrs of age) selected at each of 16 study sites were measured and correlated with the established age-specific ischaemic heart disease mortality for men who were 40–59 years of age; the mortality figure used was the established mean value for at least the preceding three years. The ischaemic heart disease mortality differed six- to seven-fold between the lowest as compared to the highest mortality figures[74] and the methodology is described in detail, including particularly the technique of lipid standardization of the levels of vitamin E to common concentrations of cholesterol (5.7 mmol/litre) and triglycerides (1.25 mmol/litre). The measurements of the plasma antioxidant levels were carried out as soon as possible after the blood was drawn so that the samples were only stored for a short period of time during which it had been established by careful control measurements that no deterioration of the samples had taken place.

The classical risk factors, total plasma cholesterol, blood pressure and smoking habits, did not reveal correlations in univariate analysis with ischaemic heart disease mortality risk which itself differed within the twelve groups by six-to seven-fold although there were some variations in the classical risk factors within a small band of normal values and for cholesterol the variation was 5.1–6.2 mmol/litre. This is an important observation because it enabled study of the relationship between blood antioxidant levels in these 12 study populations, and the risk of ischaemic heart disease without the need to allow for confounding effects caused by variation in plasma cholesterol level which is regarded as a classical risk factor. Where it was found that classical risk factors were comparable for all the groups examined, there was a statistically significant inverse correlation (P = 0.002) between the age-specific ischaemic heart disease mortality and the absolute, unadjusted, plasma level of vitamin E which in univariate analysis gave the strong correlation of $r^2 = 0.63$. Lipid standardization of the plasma vitamin E levels improved the significance of the inverse relationship with mortality so that the correlation coefficient was now $r^2 = 0.73$. Four study populations were found to have plasma cholesterol levels that lay outside the band of values (5.1–6.2 mmol/litre) that was chosen for the 12 population subgroup; two of these, from Finland, lay above the chosen band and two, from Italy lay below it. When these study populations with high or low blood cholesterol levels were included using lipid standardized vitamin E levels, there was also a statistically significant inverse correlation and the correlation coeficient was $r^2 = 0.62$. The correlation coefficients and statistical significance for vitamin E and the other parameters measured in the complete, 16 population group, study as well as in the 12 population subgroup are given in Table II, from which it is clear that the only parameter beside vitamin E which shows a significant negative correlation with ischaemic heart disease mortality was vitamin C.

Edinburgh, in Scotland, has been identified as a city with a high incidence of ischaemic heart disease (298/100 000; n = 108).[74] To test the hypoth-

TABLE II Pearson's correlation coefficients in univariate analysis between age-specific mortality from ischaemic heart disease and various measured parameters (MONICA Study)

Parameter	Populations with usual cholesterol (n = 12)		All populations (n = 16)	
	r^2	P	r^2	P
Cholesterol	0.04	0.53	0.29	0.03
Blood pressure, systolic	0.01	0.80	0.19	0.09
Blood pressure, diastolic	0.08	0.36	0.25	0.05
% Smokers	0.002	0.09	0.01	0.65
Vitamin A, Absolute	0.22	0.13		
Vitamin A, lipid std.	0.16	0.19	0.24	0.05
Vitamin E, absolute	0.63	0.002		
Vitamin E, lipid std.	0.73	0.004	0.62	0.0003
Vitamin C	0.41	0.03	0.11	0.22
β-Carotene, absolute	0.21	0.14	0.04	0.48

From Gey *et al.* (1991)[74]

esis that plasma concentrations of antioxidant nutrients might be related to the risk of angina, and to measure the extent to which such risk is independent of classic risk factors for coronary heart disease, a study was conducted[75]; in this case-control study a sample of 6000 men aged 35–54 was surveyed by a postal questionnaire. To avoid the confounding effect of dietary changes in subjects who had been diagnosed as having heart disease and had been advised by their doctor to make dietary changes, only subjects who had had chest pain and had never seen a doctor were included in the study. The 125 angina cases who were identified were compared with 430 healthy subjects without any symptoms of heart disease and complete data were obtained for 110 angina cases and 394 control subjects. Measurement of plasma vitamins and non-fasting lipids, and platelet fatty acid composition, was made at once without storage of the samples; adipose tissue was sampled under local anaesthetic. There was found to be a statistically significant ($P < 0.01$) difference between the controls and the cases with respect to smoking habit (respectively 29% and 46%); no other classical risk factors were found to be of significance including plasma total and HDL cholesterol. Table III shows the plasma antoxidant concentrations in the two groups; there was no significant difference between the vitamin A and unadjusted vitamin E levels, and there was a significantly lower level of carotene ($P < 0.001$), vitamin C ($P < 0.01$) and lipid adjusted vitamin E ($P < 0.01$) in the plasma of the angina cases. The odds ratio for angina pectoris by quintiles of plasma vitamin E concentration with and without adjustment for classical risk factors for coronary heart disease are given in Table IV.[75] In this population case-control study, low plasma concentrations of vitamin E, vitamin C and β-carotene were found to be related to an increased risk of angina pectoris in men. For plasma vitamin E the relationship remained significant after adjustment for age, blood pressure, total and HDL cholesterol, non-fasting triglycerides, relative weight and smoking status and it was concluded that some populations with a high incidence of coronary heart disease might benefit by increasing their intake of antioxidant nutrients, particularly vitamin E.

The results of the Health Professionals Study (The Physician's Study and the Nurses' Health Study) were published in 1993.[76,77] The Physician's Study is a prospective investigation of 51 529 male health professionals who were aged 40–75 years in 1986 when the study began. A number of the subjects were excluded for dietary or health reasons, and the remaining 39 910 men

TABLE III Plasma nutrient concentrations

	Mean (SEM)		
	Controls (n = 394)	Angina cases (n = 101)	P
Vitamin A (mmol/1)	2.32 ± 0.03	2.29 ± 0.05	NS
β-Carotene (mmol/1)	0.49 ± 0.02	0.30 ± 0.03	<0.001
Vitamin C (mmol/1)	35.3 ± 1.1	28.1 ± 2.1	<0.01
Vitamin E (mmol/1)	24.0 ± 0.3	22.7 ± 0.6	NS
Vitamin E (mmol/mmol cholesterol)	3.86 ± 0.04	3.66 ± 0.08	<0.01

From Riemersa *et al* (1991)[75]

were eligible for inclusion in the follow-up study. The 1986 questionnaire enquired about frequency of intake of 131 foods and ten additional questions specifically addressed the current use of vitamin supplements. Case assessment was from records of fatal coronary disease, non-fatal, myocardial infarction, coronary artery bypass grafting and percutaneous transluminal coronary angioplasty. Each participant's follow-up time began with the date of the 1986 questionnaire and continued until the diagnosis of an end-point, death or January 1990 whichever came first. Relative risks were calculated by dividing the incidence rate of coronary disease among the men in each category of antioxidant intake by the rate for the men in the lowest category of disease. The age-adjusted and multivariate relative risks of coronary heart disease according to quintile group for the intake of vitamin E is given in Table V. As compared to the men in the lowest quintile group for vitamin E, the men in the highest quintile group had an age-adjusted relative risk of coronary disease of 0.59 (95% confidence interval, 0.47–0.75; P for trend - 0.001). The total intake was further subgrouped according to dietary or supplemental sources. Quintiles of dietary vitamin E were calculated on the basis of dietary intake without supplements, wheras the dose categories for supplemental vitamin E were those specified in the base-line questionnaire; Table VI gives the result of this calculation. The maximal reduction in risk is shown in men who consumed 100–400 iu/day with no further increased reduction at higher doses. There was also found to be a suggestion of an inverse trend between duration of use of vitamin E and the risk of disease. Men who reported use of vitamin E supplements for 10 or more years had a relative risk of 0.65 (95% CI 0.46–0.92) compared to non-users. The multi-

TABLE IV Odds ratios for angina pectoris by quintiles of plasma Vitamin E concentration (with and without adjustment for CHD risk factors)

	μmol/1	Odds ratio (95% CL)
Quantile 1	<18.9	
Unadjusted		2.51 (1.24–5.10)
Adjusted		2.68 (1.07–6.70)
Quintile 2		
Unadjusted	19.0–21.8	1.04 (0.44–2.44)
Adjusted		1.69 (0.72–4.00)
Quintile 3	21.9–24.2	
Unadjusted		1.00 (0.43–2.35)
Adjusted		1.18 (0.49–2.81)
Quintile 4	24.3–28.1	
Unadjusted		1.63 (0.77–3.43)
Adjusted		1.64 (0.76–3.51)
Quintile 5	>28.2	
Unadjusted		1.0
Adjusted		1.0

Linear trend after adjustment in logistic regression was statistically significant (P = 0.02)
From Riemersma *et al.* (1991)[75]

TABLE V Relative risk of coronary heart disease according to quintile group of vitamin E intake among 39 910 male health professionals

VARIABLE	QUINTILE GROUP					P FOR TREND
	1	2	3	4	5	
Vitamin E Median intake iu/day	6.4	8.5	11.2	25.2	419	
Coronary Disease cases	155	140	130	127	115	
Age-adjusted Relative risk	1.0	0.88	0.77	0.74	0.59	0.001

(From Rimm et al, 1993)[76]

TABLE VI Relative risk of coronary heart disease according to quintile group for dietary vitamin E intake and category of supplemental vitamin E

VARIABLE	QUINTILE GROUP FOR DIETARY INTAKE				
	1	2	3	4	5
Dietary vitamin E (IU/day)	1.6–6.9	7.0–8.1.	8.2–9.3	9.4–11.0	11.1
CHD (No. cases)	79	89	90	79	56
Relative risk	1.0	1.10	1.17	0.97	0.79
Vitamin E supplement (IU/day)	0	<25	25–99	100–249	>250
CHD (No cases)	406	120	40	17	84
Relative risk	1.0	0.85	0.78	0.54	0.70

(From Rimm et al, 1993)[76]

variate relative risk of coronary heart disease among men taking specific vitamin E supplements (i.e. not multivitamin supplements) was 0.75 (95% CI 0.61–0.93) as compared with nonusers. Among men who took vitamin E supplements in doses of at least 100 iu/day for two or more years the relative risk was 0.63 (95% CI 0.47–0.84). The reduction in relative risk of coronary artery disease associated with the highest quintile group for total vitamin E intake was slightly less among current smokers (RR 0.67, 95% CI 0.34–1.31) compared to those who had never smoked (RR 0.52, 95% CI 0.34–0.78).

The Nurse's Health Study[77] began in 1976 with 121 700 female registered nurses. A total of 87 245 women remained in the study following the usual exclusions. Similar criteria and questionnaires as those used in the men's study (see above) were employed to obtain information as to possible relationships between vitamin E intake and the relative risk of coronary heart disease. During 679 485 person-years of follow-up from 1980 to 1988, 552 cases of major coronary heart disease were identified and documented.

TABLE VII Age-adjusted relative risk of major coronary heart disease according to quintile group for total vitamin E intake and intake from dietary sources

VARIABLE	QUINTILE GROUP FOR VITAMIN E INTAKE					P FOR
	1	2	3	4	5	TREND
Total intake (Total)						
Median (iu/day)	2.8	4.2	5.9	17	208	
Range (iu/day)	1.2–3.5	3.6–4.9	5.0–8.0	8.1–21.5	21.6–1000	
Age-adjusted RR	1.0	0.90	1.0	0.68	0.59	
95% CI		0.70–1.16	0.78–1.27	0.52–0.89	0.45–0.78	<0.001
RR adjusted*	1.0	1.0	1.15	0.74	0.66	
95% CI		0.78–1.28	0.90–1.48	0.57–0.98	0.50–0.87	<0.001
Dietary intake (Without Supplements)						
Median (iu/day)	2.6	3.6	4.4	5.4	7.7	
Range (iu/day)	0.3–3.1	3.2–3.9	4.0–4.8	4.9–6.2	6.3–100	
Age-adjusted RR	1.0	0.97	0.77	0.98	0.79	
95% CI		0.75–1.26	0.59–1.01	0.77–1.26	0.61–1.03	0.12
RR adjusted*	1.0	1.04	0.97	1.14	0.95	
95% CI		0.80–1.35	0.66–1.14	0.89–1.47	0.72–1.23	0.99

*Adjusted for age and smoking
(From Stampfer et al, 1993)[77]

TABLE VIII Relative risk of coronary heart disease according to the use of multivitamin and vitamin E supplements with adjustments for major coronary risk factors

VARIABLE	VITAMIN E SUPPLEMENTS		MULTIVITAMINS	
	USERS	NONUSERS	USERS	NONUSERS
No cases CHD	49	503	152	400
No. person-years	93 921	585 564	231 310	448 175
	Relative risk associated with vitamin use (95% confidence interval)			
Crude risk, unadjusted	0.61(0.45–0.81)		0.74 (0.61–0.89)	
Adjusted for age and smoking	0.57(0.41–0.78)		0.78 (0.64–0.96)	
Excluding vit E use for <2 yrs	0.54(0.36–0.82)		0.88 (0.70–1.09)	
Excluding vit E use for < 2 yrs and doses < 100iu/day	0.52(0.34–0.89)		0.88 (0.71–1.01)	

(From Stampfer et al, 1993)[77]

There was a statistically significant reduction in risk of coronary disease among women with a high intake of vitamin E compared to those with a low intake; these results are given in Table VII. Adjustment for age and smoking history showed clearly that the lower risk of heart disease was primarily associated with taking a vitamin E supplement. In a multivariate model that controlled for a number of risk factors, the relative risk associated with the use of specific vitamin E supplements was 0.61 (CI 0.45–0.81) (Table VIII) and this was improved further by excluding users of less than 100 iu of vitamin E per day for less than two years (RR = 0.52, 95% CI, 0.34–0.89).

A study in Finnish men[78] found that the titre of autoantibodies to oxidatively modified LDL was a predictor of the progression of carotid atherosclerosis. Antibodies against epitopes of oxidised LDL had previously been shown to recognize material in atherosclerotic lesions in rabbit and in man.[79-81] In this study[78] the titre was measured of autoantibodies to malondialdehyde (MDA)-modified LDL (MDA-LDL), in baseline serum samples from 30 Eastern Finnish men with accelerated two year progression of carotid atherosclerosis and 30 age-matched controls. The men with high atherosclerotic score had a significantly higher titre of MDA-LDL (P = 0.003). Cases also had a higher proportion of smokers (37% vs 3%), higher LDL cholesterol (4.2 mmol/litre vs 3.6 mmol/litre) and a higher serum copper concentration.

The role of vitamin E therapy in retarding, or even reversing, the progression of cardiovascular disease is an exciting possibility. A part of the Physician's Health Study, which has not been published in full, suggested that beta carotene may have a role in retarding the progression of cardiovascular disease;[82] those subjects who received 50mg beta carotene on alternate days had a 44% reduction in all major coronary events defined as myocardial infarction, revascularization or cardiac death (Relative Risk = 0.56, 95% CI 0.44.0.89, P = 0.016), and a 49% reduction in all major vascular events defined as stroke, myocardial infarction, revascularization and cardiac death (Relative Risk = 0.51, 95% CI 0.29–0.88, P = 0.018), after adjusting for age and asprin taking. With respect to vitamin E, a recently published intervention study study shows a clear effect of vitamin E on the reduction of angiographically proven coronary atherosclerosis.[83] Patients (2002) were enrolled following rigorous screening as to their suitability and followed up for a median of 510 days; 1035 were given 800 iu R,R,R-α-tocopherol for the first 546 patients and 400 iu for the remainder; 976 patients received placebos. The primary endpoints were a combination of cardiovascular death and non-fatal myocardial infarction (MI), as well as non-fatal MI alone. The plasma α-tocopherol levels were found to show a considerable rise in the treated groups, but did not change in the placebo groups.

α-Tocopherol treatment significantly reduced the risk of the primary trial endpoint of cardiovascular death and non-fatal MI (41 vs 64 events; RR 0.53, 95% CL 0.34–0.83, p = 0.005), which was due to the reduction in risk of non-fatal MI (14 vs 41 events; RR 0.23.95% CL 0.11–0.47, p = 0.005). There was however a non-significant excess of cardiovascular deaths in the α-tocopherol-treated group (27 vs 23 events). The conclusion was that in patients with angiographically proven symptomatic coronary atherosclerosis, α-tocopherol treatment substantially reduced the rate of non-fatal MI, with beneficial effects apparent after 1 year of treatment. This study raises a number of questions as to the mechanism of the beneficial effect of vitamin E; some authors have commented that it may have been due to a reduction of coronary atherosclerotic lesions; others have suggested that the mechanism may be mediated through thrombotic effects and alteration in blood platelet function. It should also be noted that there has been considerable criticism of this study because some vital information was not given. For example, no details were given of the therapy that was being given to the subjects in the study; it can be presumed that, since they all had clinically established heart disease, they would have all been given a range of different drugs to control their clinical condition, and the question of interactions of these drugs with the vitamin E dosage needs to be clarified.

The evidence reviewed here makes a compelling case for the liklihood that the risk of cardiovascular disease may be lowered by increasing the intake in human populations of vitamin E. Taken together with the biochemical rationale which has been advanced for the mechanism of atherosclerosis there is a very compelling case for the possibility that vitamin E may have a direct preventive function in the development of atherosclerosis and subsequent coronary disease, as well as in having some therapeutic effect in patients with established heart disease. With respect to the epidemiological evidence it is particularly striking that low incidence of cardiovascular disease is negatively correlated with vitamin E levels in three kinds of study. Thus, in the MONICA/WHO cross-cultural comparison of 16 European study populations,[84] in the case-control study of angina pectoris,[75] and in the very large Heath Professionals' Studies which were in the nature of intervention trials although the intervention with vitamin E was self-determined,[76,77] vitamin E was consistently associated with lowered risk of disease. It must however be stressed that epidemiological data cannot establish causal relationships and can only point to relationships which must be tested by other means. It remains therefore a matter of judgment to decide whether the relationship is likely to be a causal one, as Steinberg points out in his excellent Editorial,[85] and "that judgment will be affected by the strength of the relationship, its consistency, its biological plausibility and other criteria". There are two further interrelated burning questions that need to be addressed urgently; the first is "how much vitamin E is needed in the human diet for the optimal promotion of health"? And, secondly, "is the high level of vitamin E that seems to be needed for health promotion a pharmacological activity of the vitamin which is superimposed upon its conventially accepted function as a general lipid free radical scavenger?". At the present time there are no easy answers to either question. More research, of a strictly 'human' kind, is needed that is directed to addressing these areas of uncertainty.

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