

Review Article

Nutrient (zinc and vitamin E)-gene interactions related to inflammatory and antioxidant response in aging and inflammation

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ABSTRACT: Aging is an inevitable biological process with gradual and spontaneous biochemical and physiological changes and increased susceptibility to diseases. Some nutritional factors (zinc and vitamin E) may remodel these changes leading to a possible avoidance of diseases with subsequent healthy aging, because they are involved in improving immune functions and antioxidant defence. The polymorphisms of some genes codifying proteins related to inflammation are predictive on the one hand of longevity, while, on the other hand, they are associated with atherosclerosis. Since the health life span has a strong genetic component, which is in turn also affected by nutritional factors such as zinc and vitamin E, these polymorphisms can be useful tools for establishing the real beneficial effects of zinc or vitamin E in older subjects to prevent or delay as much as possible the appearance of age-related diseases. Therefore, zinc or vitamin E and gene interactions are crucial to healthy aging. (Nutritional Therapy & Metabolism 2008; 26: 118-28)

KEY WORDS: Aging, Antioxidant activity, Cardiovascular diseases, Inflammation, Vitamin E, Genes, Zinc

INTRODUCTION

Aging is an inevitable biological process that is accompanied by gradual and spontaneous biochemical and physiological changes including increased susceptibility to diseases, adverse environmental conditions, and loss of mobility and agility. Alterations in neuroendocrine-immune interactions as well as in antioxidant capacity also play a fundamental role in aging. The inability of an organism to remodel these changes may lead to the appearance of some degenerative age-related diseases. As a result, the remodelling theory of aging has been proposed (1). Various nutritional factors are directly linked with these phenomena, as, for instance, in restoring the neuroendocrine-immune network, metabolic harmony, and the capacity to respond to oxidative stress (2).

Approximately 40 micronutrients (vitamins, essential minerals, and other compounds required in small amounts for normal metabolism) have been reported as essential components in the diet (3). The dietary intake of essential macronutrients and micronutrients is usually inadequate in the elderly (4). Several causes contribute

to this gap. First of all, the poor socioeconomic conditions in which a large part of the older population live may lead to a consumption of inexpensive foods deficient in micronutrients, such as carbohydrates (5). The gap is worsened by loss of appetite, lack of teeth, intestinal malabsorption, and decreased requirements for energy that lead finally to frailty, disability, and mortality (6). Some authors have reported that the deficiency of macronutrients and micronutrients in aging is strictly related to global impairments of immune functions, metabolic harmony, and antioxidant defense by external noxae with subsequent appearance of age-related diseases (7). Indeed, many micronutrients contribute directly or indirectly to the biological activity of some antioxidant enzymes (e.g., SOD, GPx, and catalase) (8), to the efficiency of the immune system (9), and to the maintenance of metabolic function especially in preventing mitochondrial decay (4). In this last context, feeding studies in older rats have shown that mitochondrial metabolites and antioxidants protect neuronal cells from neurotoxin- and oxidant-induced toxicity and oxidative damage, delay the normal senescence of human diploid fibroblasts, and inhibit the oxidant-induced acceleration

of senescence (10). With advancing age, increased oxidative damage to proteins and lipid membranes, particularly in mitochondria, causes a deformation of structure of enzymes, with a consequent decrease in enzyme activity as well as substrate binding affinity for their substrates. An increased level of substrate by micronutrients restores the speed of the reaction as well as mitochondrial function, thus delaying mitochondrial decay and aging (10, 11). In contrast, recent longitudinal studies of dietary daily intake in human nonagenarians and centenarians (successful aging) have shown that an adequate consumption of micronutrients and macronutrients, as well as a satisfactory content of some trace elements within the cells leads to good performance in several immune functions, metabolic compensation, and preservation of antioxidant activity (12, 13). In this context, polyunsaturated fatty acids, highly sensitive to reactive oxygen species, decrease in liver mitochondria from human centenarians, a feature acquired during evolution as a protective mechanism to favor longevity (14). Therefore, nutritional factors may play a pivotal role for healthy aging and longevity. However, the effects of the nutritional factors are strongly influenced by genetic factors, in particular by the genes involved in inflammatory/immune responses. Proinflammatory cytokines such as IL-1, IL-6, and TNF- α ; the antiinflammatory cytokine IL-10; the HSP70 chaperones; and the regulators of trace element homeostasis, metallothioneins (MTs), seem particularly relevant, taking into account that the same genes are involved in the susceptibility to major geriatric disease/disorders such as diabetes, osteoporosis, osteoarthritis, dementia, cardiovascular diseases, and infections (15, 16). Indeed this reemphasizes that up to 25% of the variation in human life span is heritable (17); the rest is due to environmental and lifestyle factors, which impact the aging process, contributing as such to a large interindividual variability. Therefore, one current challenge is to understand how the interaction between genetic factors and nutrients may influence aging and longevity, in view of the high impact on gene expression, protein production, and epigenetic mechanisms implicated in regulating the life span (18). Some dietary patterns with likely different impacts on long-term disease occurrence and survival have been identified. In this context, strong evidence for a beneficial effect of higher conformity with the Mediterranean dietary pattern on causes of death, including those of cardiovascular diseases and cancer, has been reported (19). Anyway, it is also commonly accepted that the complex interactions of multiple polymorphisms play a key role in how individuals may respond to dietary interventions (20). For each nutrient, there is a window of intake between the recommended dietary allowance (RDA),

which is defined as the dietary intake sufficient to meet the requirements of 97% of healthy individuals in a particular stage of life and sex group, and the tolerable upper limit (UL), which is the highest nutrient intake that can be achieved without incurring a risk of adverse health effects for most individuals in the general population (21). Although worldwide research on genetic variations that require a different RDA or UL is still in progress, several genes and alleles have been suggested to affect nutrient utilization, including genes involved in the metabolism of folate and vitamin B₁₂ (22), vitamin E (23), lipids (24), iron (25), and zinc (26). In this last case, it is important to note that the RDA (11 mg/day for men, and 8 mg/day for women) and UL (40 mg/day for adults) for zinc are very close (Food and Nutrition Board and Institute of Medicine, 2001). Without excluding the beneficial effects of all of these nutrients for healthy aging and longevity (27), but taking into account the pivotal role played by zinc and vitamin E in fighting oxidative stress in aging and in some age-related diseases, such as atherosclerosis (23), we herein report the studies carried out on the interrelationships between zinc and some genes related to the inflammatory/immune response (such as MT and IL-6), as well as the interactions between vitamin E and the gene expressions related to inflammation. Clinical trials of zinc and vitamin E are also reported for their possible beneficial effects on longevity or in preventing the initiation and progression of cardiovascular diseases.

ZINC-MT GENE INTERACTION

MTs are essential to intracellular zinc homeostasis by sequestration and release of the metal when it occurs and thereby controlling available free zinc ions (28). The cysteine sulfur ligands in the cluster structure of MT can be reduced (zinc sequestration) or oxidized (zinc release), thus with concomitant changes in the relative amount of bound and free zinc (29). MTs are genetically polymorphous protein families with subfamilies, subgroups, and various isoforms. Humans possess genes for 4 subfamilies (encoded by at least 10 functional MT genes), all located on chromosome 16: the brain-specific MT3, the squamous epithelium-specific MT4, and the ubiquitous MT1 and MT2 (30).

One of the first functions of MT1 and MT2 is to regulate zinc homeostasis and to limit oxidative damage within the cells (28). Following an injurious stimulus, such as a transient inflammation, the subsequent oxidative stress induces the release of zinc from MT via nitric oxide (NO), to promote the activity and expression of antioxidant enzymes, including MT itself, thus reducing

the oxidative damage and the consequences of the injurious stimulus (31). However, the increased expression of proinflammatory cytokines occurring in aging, leads to increased expression of MTs, which in turn sequester considerable amounts of zinc, making it less available for an efficient immune response (32).

If, on the one hand, the reduced zinc ion availability in old age might indicate an excessive sequestration of zinc ions by MTs, on the other, consideration of recent findings on oxidative modification of MTs leading to their loss of function suggests also a mechanism whereby these proteins can also lose their ability to buffer the intracellular free zinc concentration (33). In this case, MTs would be unable either to bind or to consequently release zinc in response to stressors. However, it is still unclear if dysfunctional MTs can be considered a typical alteration associated with specific disease/disorders, such as hyperhomocysteinemia and type 2 diabetes, or if they are a common feature of aging. Anyway, as a consequence of the altered zinc metabolism, a large number of genes that act as zinc sensors and transcriptional activators and/or repressors are also altered in aging (34). This may have a deep impact on the regulation of zinc-dependent transcription factors, because they not only regulate “zinc sensitive” genes but also control their own transcription through positive autoregulatory mechanisms (35).

Taking into account that healthy centenarians display a low MT expression and satisfactory zinc ion availability despite increases in proinflammatory cytokines (IL-6, TNF- α) (36), it may be suggested that this feature reflects the existence of compensatory phenomena able to counteract the effects of inflammation in these exceptional individuals. Moreover, these data suggest that a preservation of zinc homeostasis is an important feature of healthy centenarians. Therefore, the role played by the zinc-gene interaction is pivotal to successful aging and, at the same time, to escape from some age-related diseases. Since, the persistence of inflammatory stimuli over time represents the biological background favoring susceptibility to age-related diseases/disabilities, the absence of specific “robust” gene variants and/or the presence of specific “frail” gene variants might predict, on the one hand, longevity, and on the other, the predisposition to the appearance of the more common age-related diseases, such as infections and cardiovascular diseases. In this context, it has to be considered that the genes selected, because they confer a reproductive advantage early in life, may have dangerous effects in the postreproductive period. In fact, negative selection against these harmful effects fails due to the decline of natural selection with age. This fact means that one gene that is “favorable” in young/adult age may

be “disadvantageous” in aging (the antagonistic pleiotropy theory of ageing (37)).

Following this perspective, the beneficial effect of inflammation, via an optimal MFT-1-MT-zinc-gene interaction, in young and adult age may become detrimental in old age (32). The recent discovery of novel polymorphisms of MT2A and MT1A supports this assumption. Indeed, older subjects carrying the AA genotype for the MT2A polymorphism display low zinc ion bioavailability, chronic inflammation by high IL-6 and altered lipid assessments, with subsequent elevated risk for atherosclerosis and type 2 diabetes (38). By contrast, a polymorphism corresponding to an A/T (asparagine/threonine) transition at the +647 nt position in the MT1A-coding region is the most often involved in female longevity (39).

Such allelic variants may be very useful tools to screen older subjects at risk for zinc deficiency on a genetic basis, taking into account that the actual methodological procedures to test “zinc status” are often misleading and that laboratory investigations to assay zinc ion bioavailability are scarcely reproducible and poorly applicable to clinical practice (40). In this context, a novel reproducible system for testing intracellular zinc ion bioavailability has been developed using a zinc fluorescent probe (Zynpir-1) associated with MT values, with both tests representing valid methods to detect the intracellular zinc status (41).

ZINC-INTERLEUKIN-6 GENE INTERACTION

Interleukin-6 (IL-6) is a pleiotropic cytokine capable of regulating proliferation, differentiation, and activity of a variety of cell types, and it plays a pivotal role in immune response. In particular, the most important function of IL-6 is most likely as a mediator of acute phase inflammatory responses. These include the balance of the proinflammatory/antiinflammatory pathways, lymphocyte activation, and hepatocellular stimulation of acute phase protein synthesis (42). Studies of the effects of aging on inflammatory response show IL-6 to be an important “cytokine for gerontologists”. Recent evidence has shown that abnormally increased concentrations of IL-6 are reliable markers for functional disability and predictors of disability and mortality among the elderly (43). Moreover, IL-6 dysregulation is involved in age-related inflammatory diseases, such as cancer, lymphoma, cardiovascular diseases, osteoporosis, Alzheimer’s disease, diabetes and atherosclerosis (44).

The human IL-6 gene is located on chromosome band 7p21 and consists of 5 exons and 4 introns (45). Genetic studies have identified 4 polymorphisms in the

promoter region of the IL-6 gene (-597G/A, -572G/C, -373A/T, -174G/C) that have a significant effect on IL-6 expression in an in vitro system (46). A variable number of tandem repeat (VNTR) polymorphisms were found in the 3' flanking region of the IL-6 gene (C allele) (47). In humans, a polymorphism in the IL-6 promoter (A/C polymorphism at position -174) altered IL-6 gene transcription rates in vitro (48) and IL-6 levels in vivo (49). In this last paper, people carrying CC or GC genotypes are defined as C+, while those carrying GG genotypes are indicated as C-.

It has been suggested that IL-6 -174G/C locus variability is capable of modulating, on the one hand, individual susceptibility to common causes of morbidity and mortality among elderly, while, on the other hand, it may play a crucial role in longevity (50). Therefore, the genetic variations of this locus of IL-6 gene are fundamental in the elderly population to better understand the intrinsic causes of the longevity. The association of these genetic variations to the possible, different immune responses is an attractive focus in elucidating the molecular mechanisms involved in immunosenescence.

The genetic variations of the IL-6 -174G/C locus have been extensively studied by different groups with, however, contradictory data. Bonafè et al (51) studied IL-6 promoter genetic variability at the -174C/G locus and its effect on IL-6 levels in 700 Italians aged 60 to 110 years, including 323 centenarians. Individuals who are genetically predisposed to produce high levels of IL-6 during aging – i.e., C- men (GG genotype) – at the IL-6 -174C/G locus are disadvantaged for longevity. On the other hand, the capability of C+ individuals (CC and CG genotypes) to produce low levels of IL-6 throughout their life span appears to be beneficial for longevity, at least in men. Women have, conversely, high IL-6 serum levels later in life with respect to men, independently of -174C/G locus polymorphism (51). The inhibitory tone of estrogens on IL-6 gene expression could explain the sex-based difference (52), assuming that its long-term effects last until the extreme limits of the human life span.

The major production of IL-6 in C- subjects for their whole life, including centenarians, has also been confirmed by other in vivo longitudinal studies (53). A more recent study in older and nonagenarian subjects has confirmed that IL-6 production is higher in C- carriers and that these subjects are prone to contract one of the more common age-related inflammatory pathologies such as atherosclerosis (54). Interestingly, in this last study, C- older and nonagenarian subjects also display impaired innate immune response (NK cell cytotoxicity) coupled with increased MT, zinc deficiency, and low zinc ion availability, in comparison with C+ carriers

(54). A functional effect of the IL-6 -174G/C locus on MT expression and zinc-regulated genes has also been confirmed recently by targeted studies with in vitro and in vivo zinc supplementation (55, 56).

These findings clearly suggest that the genetic variations of the IL-6 -174G/C locus play a key role in longevity also at a functional level, and confirm the existence of a higher number of C+ centenarians than C- ones as previously found by Bonafè et al (51). In this context, an intriguing point is the low expression of the signal-transducing component (gp130) of the IL-6 receptor in centenarians, in comparison with the elderly, despite high circulating levels of IL-6 (57), which in turn may be inactive in centenarians (36). Therefore, inflammatory status is not so detrimental in very old age, as, in contrast, occurs in normal aging. However, the discrepancies among all of the genetic studies on the variations of the IL-6 -174G/C locus in relation to longevity in the elderly means that the subject is still unclear. Ethnic, lifestyle, and cultural differences among these populations could also play a role, as well as other undefined factors. Large-scale studies at the European level in many different ethnic populations are needed to clarify this important topic. For this reason, ZINCAGE project (www.zincage.org) has been developed, and the clarification of this point has been one of its tasks. The determination of the genetic variations of the IL-6 -174G/C locus associated with a dietary assessment or with a comprehensive evaluation of zinc status has been reported as an useful strategy to identify older subjects who can benefit from zinc supplementation without health risks (see next paragraph).

ZINC SUPPLEMENTATION IN THE ELDERLY ON THE BASIS OF GENETIC BACKGROUND

One possible cause of the discrepancies existing in the literature on the effect of zinc supplementation upon immune response and antioxidant performance in the elderly (see review in (58)) may be the choice of older subjects who effectively need zinc supplementation in strict relationship with dietary habits and inflammatory status. This fact is supported by the discovery that older subjects carrying GG genotypes (i.e., C- carriers) in the IL-6 -174G/C locus display increased IL-6 production, low intracellular zinc ion availability, and impaired innate immune responses coupled with enhanced MT (56). By contrast, older subjects carrying the GC and CC genotypes (C+ carriers) in the same IL-6 -174 locus display satisfactory intracellular zinc as well as innate immune responses. But, the more intriguing finding is that males carriers of the C+ allele are more likely to

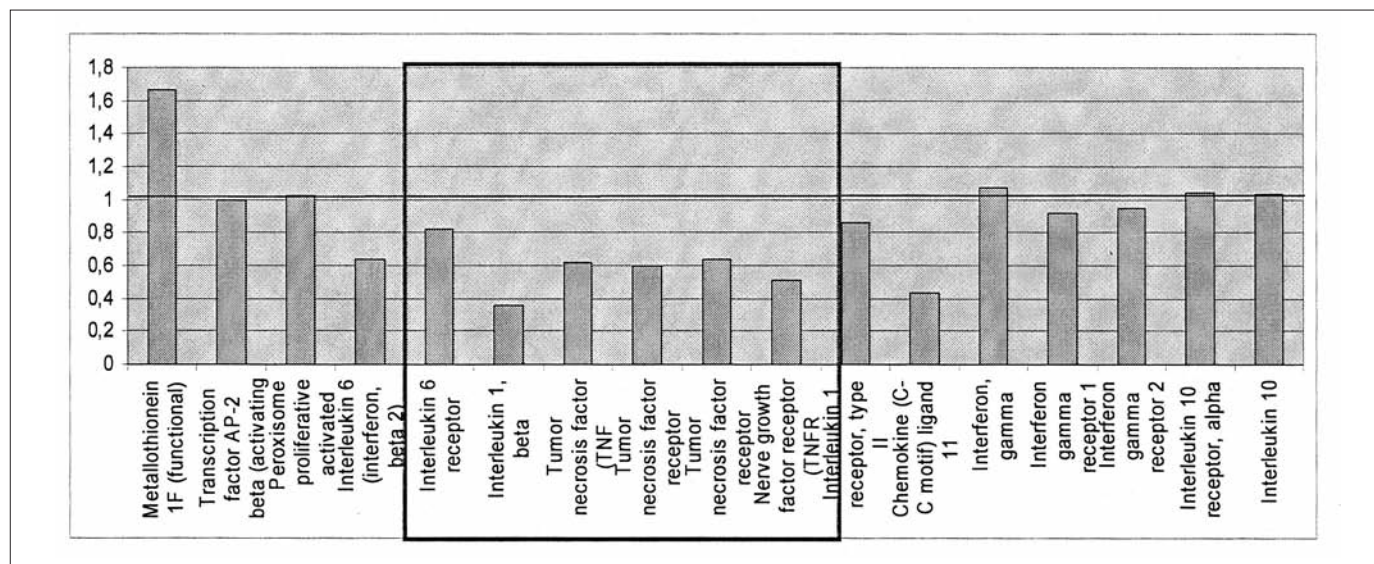


Fig. 1 - *In vitro* zinc treatment (50 μ M) down-regulates proinflammatory cytokine expression (custom array) when referred to the value 1 (baseline control) in human peripheral blood mononuclear cells from young adult donors. The red square represents the proinflammatory cytokines (IL-6, IL-1, and TNF pathways). Data redrawn from the Zincage Project (www.zincage.org) (61).

reach centenarian age than C- carriers. Therefore, older C- subjects are more likely to benefit from zinc supplementation than older C+ carriers. The distribution of the IL-6 -174 genotype is very different among the various European countries, with large differences between northern and southern European countries (58). Zinc supplementation in older C- subjects restores NK cell cytotoxicity to values present in older C+ carriers and considerably improves both zinc status, assessed by the percentage increment of granulocyte Zn (56), and stress response, assessed by the percentage increment of clusterin (CLU) expression and MT protein (58). When the genetic variations for the IL-6 polymorphism are also associated with the genetic variations of MT1A in position +647, the plasma zinc deficiency and the altered immune response are more evident (58), suggesting that the genetic variations of IL-6 and MT1A would be very useful tools for the choice of older people who effectively need zinc supplementation. These results open the hypothesis that the daily requirement for zinc might be different in elderly with a different genetic background. Such a role played by genetic background on the beneficial effects of zinc supplementation is also evident in the better control of proinflammatory cytokine and chemokine production (59), as well as in reducing the expression of genes related to inflammatory status, such as IL-1 and its receptor (55). A very intriguing point is also the beneficial effect of zinc supplementation in the cognitive performance of older individuals

selected on the basis of IL-6 polymorphism (60). In any case, from a microarray analysis *in vitro*, zinc at a dose of 50 mM down-regulates the gene expression of proinflammatory cytokines (Fig. 1), strengthening its antiinflammatory role (56, 58).

VITAMIN E AND GENE EXPRESSION

Alpha-tocopherol, 1 of the 8 isoforms of vitamin E, is the most potent fat-soluble antioxidant known in nature. For years, it was thought that alpha-tocopherol only functioned as a scavenger of lipid peroxyl radicals – specifically, oxidized low-density lipoprotein (oxLDL), thereby serving as a chief antioxidant for the prevention of atherosclerosis (62). Dietary vitamin E is obtained mainly from plant sources including sunflower seeds, olive oil, and almonds, which contain high amounts of alpha-tocopherol, while most other oils and seed oils are rich in gamma-tocopherol. The average dietary intake of vitamin E is currently of about 9 mg/day for men and about 6 mg/day for women (63). Although the majority of Western dietary vitamin E consists of gamma-tocopherol, the alpha-isoform is predominant in vitamin E supplements (62). The alpha-tocopherol isoform of vitamin E inhibits the radical chain propagation within lipid domains by its own conversion into an oxidized product, alpha-tocopheroxyl free radical (64). Alpha-tocopherol reacts with lipid peroxyl radicals at a rate several orders

of magnitude faster than the peroxy radical propagation reaction (62). Following these biochemical characteristics, vitamin E is considered an excellent antioxidant in fighting hydroxy radical formation and chronic inflammatory status, and, as such, in preventing aging and some age-related diseases such as atherosclerosis, cancer, and dementia.

Modulation of enzyme transcription and/or activity by vitamin E has been shown in genes involved in oxidative stress, proliferation, inflammation, and apoptosis. Such genes include SOD, NO synthase, cyclooxygenase-2, nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase), nuclear factor kappa B, phospholipase A2, protein phosphatase 2A, 5-lipoxygenase, activator protein-1, pregnane X receptor, and protein kinase C (23, 62).

Recently, the capacity of alpha-tocopherol to modulate gene expression has been investigated (Tab. I) (23, 65). Inhibition of scavenger receptors SR-A and CD36 expression at the transcriptional level by alpha-tocopherol in aortic smooth muscle cells (66) and monocytes/macrophages (67), followed by a decreased uptake of oxLDL in these cells, can prevent foam cells formation in vitro, and thus inhibit atherosclerosis progression. This hypothesis is supported by the fact that ApoE^{-/-} mice, that are prone to develop atherosclerosis, do not develop atherosclerotic lesions if the CD36 scavenger receptor is absent (68). The expression of CD36 mRNA is correlated with the lipid peroxide content in peritoneal macrophages during mouse aging, and this is ac-

companied by an age-dependent increase in the cellular uptake of oxLDL. Treatment with vitamin E decreased the amount of cellular lipid peroxides and resulted in inhibition of macrophage uptake of oxLDL and in cellular CD36 mRNA expression (69). Inhibiting effects of alpha-tocopherol at the level of gene transcription have been shown for over 30 genes (Tab. I) (23, 65). Many of these genes play an important role in atherosclerosis, such as the cellular adhesion molecules induced by cytokines inside the human vascular endothelium (70), VCAM-1 expressed at the macrophage surface level (71), L-selectin from pulmonary macrophages (71), and Mac-1 (CD11/CD18) induced by oxLDL within monocytes (72).

VITAMIN E SUPPLEMENTATION

Data extracted from cellular and animal models have consistently shown that the gene regulatory effects of vitamin E reduce the levels of O₂ and lipid peroxides, and diminish the number and severity of atherogenic events (23). Excess levels of vitamin E present in the liver activate the pregnane X receptor, a transcription factor that may lead to the expression of drug resistance genes including cytochrome P450, glutathione S-transferase A2, multidrug resistance-associated protein 2 (ABCC2), and hydroxysteroid sulfotransferase (SULT2-40/41) (73-76). During transport via plasma lipoproteins, vitamin E protects the carrier particle from free radical peroxidative

TABLE I - SOME GENES INVOLVED IN INFLAMMATION AND ANTIOXIDANT ACTIVITY AS WELL AS IN APOPTOSIS AND CELL CYCLE MODULATED BY VITAMIN E

Gene Class	Gene	Normal Function	Effect of tocopherols
Scavenger receptors	CD36, SR-BI, SR-AI/II	Uptake of oxLDL	Inhibition by alpha-tocopherol
Extracellular matrix	E-Selectin, L-selectin, ICAM-1, Integrins, Mac-1 Collagen alpha1(1), glycoprotein IIb	Rolling and adhesion of monocytes/macrophages Platelet adhesion	Inhibition by alpha-tocopherol Inhibition by alpha-tocopherol
Inflammatory cytokines	TGF-beta, IL-4, IL-1 beta	Inflammation and chemotaxis of inflammatory cells	Inhibition by alpha-tocopherol
Cell cycle regulation	P27 Cyclin D1, cyclin E	Inhibition of smooth muscle cells proliferation and aortic thickening Induction of proliferation	Induction by alpha- and gamma-tocopherol Inhibition by alpha- and gamma-tocopherol
Apoptosis	CD95L (CD95 APO-1/Fas ligand) Bcl2-L1	Induction of apoptosis Inhibition of apoptosis	Inhibition by alpha-tocopherol Induction by alpha-tocopherol
Transcription	NF-kB	Induction of inflammatory genes	Inhibition by alpha-tocopherol

For single references related to the specific gene class (see reviews in (23, 65)).

damage. This is one of the major and well-characterized modes of alpha-tocopherol antioxidant functions.

However, cellular and preclinical animal models have shown the existence of several “non-antioxidant” functions of vitamin E. Epidemiological as well as animal and clinical studies are indeed inconsistent as to whether vitamin E lowers the risk for atherosclerosis, though some studies show an association between high dietary vitamin E intake and/or its high serum concentrations and lower rates of cardiovascular heart disease (CHD). Other controlled trials examining vitamin E intake in populations with different risks for CHD did not confirm these results (77). Supplementation studies also have not shown any cardioprotective activities of vitamin E (78). The causes for these discrepancies are not clear, and might be due to subject compliance, study design, dosage regime, timing of therapy, and the isoform of vitamin E used. Specific to the elderly, it is not clear whether increased low-density lipoprotein (LDL) modification even occurs *in vivo*, or whether vitamin E supplementation lowers the susceptibility to LDL oxidation in these subjects. For example, while Khalil et al (79, 80) showed that vitamin E was unable to restore the decreased resistance to LDL oxidation in elderly subjects compared with that in young subjects, Cherubini et al (81) suggested that maintaining proper vitamin E status is important to avoid increased risk for atherosclerosis with advanced age. Moreover, whether vitamin E supplementation improves other key parameters related to cardiovascular disease in the elderly is still unresolved. One study with older subjects showed no change in flow-mediated vasodilatation following supplementation with 1,000 IU/day of vitamin E for 10 weeks (82). Another study showed that subjects with low blood concentrations of vitamin E were 2.5 times as likely to have a > 30% narrowing of the carotid artery (83). Relevant to human, and specifically elder, nutrition, most trials show that vitamin E derived from food, but not that from supplements, is inversely associated with mortality from coronary heart disease. For example, Kushi et al (84) and a Finnish trial (85) showed that modifying dietary habits to increase vitamin E intake may be worthwhile in preventing coronary heart disease. To date, the most effective and safe dose of vitamin E, as well as the minimum duration of treatment, are yet to be established, but close attention should be given to studies to establish what dietary intake is necessary to achieve good health (86). Anyway, the beneficial effect of vitamin E may be related to the specific polymorphisms of genes, as occurs for the beneficial effects of zinc supplementation in older people carrying specific polymorphisms for IL-6 and MT1A (56). For example, individuals who carry an allele of the e4 isoform of APOE were shown to benefit

more from vitamin E compared with non-e4 carrying subjects (87).

Several genetic factors may exist that could affect the efficacy of vitamin E in Alzheimer’s disease. Among them, ABCA1 that is a key transporter in the cellular efflux of high-density lipoproteins and influences the age of Alzheimer’s disease onset and cholesterol levels within cerebrospinal fluid (88), and a polymorphism in CYP46A1 that is a central cholesterol catabolic enzyme in the brain associated with Alzheimer’s disease (89). However, more precise studies are required because the precise mechanism for the protective effect of vitamin E against oxidative damage remains elusive (90). It is also important to remember that vitamin E supplementation can be dangerous in specific patient populations, as is suggested for patients on coumarin-based oral anticoagulant therapy (91). Therefore, future research should be aimed also at defining specific reference guidelines for vitamin E supplementation.

CONCLUSIONS

Although some controversial findings exist on the “real” necessity of micronutrient supplementation, the huge amount of observational data clearly suggest that zinc and vitamin E play a pivotal role in healthy aging and longevity. With regard to zinc, although it is a powerful antioxidant, the major problem for zinc supplementation in older people is related to the choice of those who effectively need zinc supplementation, taking into account the fact that excessive zinc is toxic for many organs and systems, including the antioxidant response (16). Simply measuring plasma zinc is not sufficient, because zinc is bound to many proteins. Intracellular zinc ion availability and zinc release by MT, on the other hand, can be used as complementary methods to test zinc status (41). The polymorphisms of IL-6 and MT1A may be of added value to effectively screening older subjects for zinc supplementation in restoring inflammatory/immune response. As a consequence, healthy aging and longevity may be achieved. However, some points require further investigation: first of all, the reason for the limited zinc release in aging and the biochemical mechanism involved, in particular addressing NO-related intracellular pathways. However, IL-6 and MT1A polymorphisms may form a solid rationale for selecting older individuals who effectively need zinc supplementation and not the entire elderly population (see the Zincage Project at www.zincage.org) (61).

With regard to vitamin E, although the studies carried out with cell culture and animal models have shown promising antiatherosclerotic as well as anti-aging ef-

fects, the results of clinical trials are contradictory. It is possible that inadequate subject selection (by sex, vitamin E status, and genetic polymorphism), the presence of advanced lesions, and the dosage and chemical form of vitamin E administered in each of the studies may partly explain the incongruence between the reported data. Individuals with higher risk factors may benefit most from a preventive vitamin E supplementation. This fact is particularly evident in Alzheimer's disease patients with the specific APOE polymorphism. Therefore, the interactions of vitamin E or zinc with some genes related to their beneficial effects are fundamental to the success of clinical trials of these nutrients in aging and in age-related diseases. In any case, a strong degree of caution in both areas has to be considered, taking also into account the recent findings showing excessive vitamin E as a risk factor for mortality in frail people such as the elderly (92). The study of nutrigenetics and nu-

trigenomics will therefore be pivotal for the best use of nutrients as supplements in clinical practice including in parenteral nutrition, during aging and in age-related diseases.

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