

REVIEW

Vitamin E supplementation and mammalian lifespan

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Vitamin E refers to a family of several compounds that possess a similar chemical structure comprising a chromanol ring with a 16-carbon side chain. The degree of saturation of the side chain, and positions and nature of methyl groups designate the compounds as tocopherols or tocotrienols. Vitamin E compounds have antioxidant properties due to a hydroxyl group on the chromanol ring. Recently, it has been suggested that vitamin E may also regulate signal transduction and gene expression. We previously reported that lifelong dietary vitamin E (α -tocopherol) supplementation significantly increased median lifespan in C57BL/6 mice by 15%. This lifespan extension appeared to be independent of any antioxidant effect. Employing a transcriptional approach, we suggest that this increase in lifespan may reflect an anti-cancer effect *via* induction of the P21 signalling pathway, since cancer is the major cause of death in small rodents. We suggest that the role of this pathway in life span extension following supplementation of vitamin E now requires further investigation.

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1 Introduction

Following the discovery that it reduced foetal re-absorption in female rats in 1922 [1], the potential benefits of vitamin E to health and lifespan have been intensely studied and debated [2–4]. The naturally occurring form of vitamin E constitutes a family of eight stereoisomeric lipid-soluble compounds (vitamers), each possessing a 6-chromanol ring and a 16-carbon side chain, differentially substituted with methyl groups [5]. These vitamers can be sub-divided into two groups depending on saturation of the side chain, the tocopherols (α -, β -, γ -, δ) and tocotrienols (α -, β -, γ -, δ) [4–6]. Tocopherols possess a saturated side chain containing three chiral centres of which the natural form of the vitamer holds configuration R at position 4', 8', and 12', with tocotrienols possessing an unsaturated side chain [4–6]. The anti-oxidant properties are conveyed by a hydroxyl group on the chromanol ring. In terms of dietary requirements and physiological function, the best understood vitamer is α -tocopherol. However, its exact function is currently disputed (for review, see [7]), with

its primary, possibly exclusive, role suggested to be as a potent peroxyl radical scavenger that prevents lipid peroxidation [8]. Alternatively, it has recently been suggested that vitamin E may primarily act as a ligand-regulating signal transduction and gene expression [9].

Both natural and synthetic forms of Vitamin E are commercially available as food supplements. The natural form is supplied as a pure 3R stereoisomeric form (RRR- α -tocopherol), whereas the synthetic formulation, identified as all-rac- α -tocopherol (all-racemic), consists of an equimolar concentration of eight stereoisomeric forms of the vitamer. These eight isomeric forms (RRR, RRS, RSS, RSR, SSS, SRR, SSR, and SRS) are designated depending on the configuration at three chiral centres located on the α -tocopherol molecule [10]. The relative bioavailability of natural *versus* synthetic vitamin E is estimated to be equal at a dosage ratio of 1.36:1 [11], although this figure has been disputed (*e.g.* [12]). Synthetic all-rac- α -tocopherol increases in plasma to only 50% of the level achieved by the natural RRR- α -tocopherol and degradation of all-rac- α -tocopherol is three to four times higher. The higher biological activity of the natural compound may be directly related to its structure, as the relative antioxidant properties of the synthetic stereoisomeric forms, despite their similarity in structure, are not equal. Consequently, potency cannot be determined by their inherent antioxidant potential alone [6].

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Abbreviation: α -TTP, α -tocopherol transfer protein

Initial intestinal absorption shows comparable levels of RRR- α -tocopherol and all-rac- α -tocopherol in chylomicrons, suggesting indiscriminate uptake. Bio-discriminatory activity is mediated by the liver, and driven by the α -tocopherol transfer protein (α -TTP), a 30–35 kDa protein belonging to the SEC14 lipid ligand-binding protein family [4, 13]. This protein is responsible for maintaining plasma levels. The α -TTP has a clear preference for R stereoisomers at the position 2-chiral centre, where the side chain and ring meet. Consumption of synthetic α -tocopherol leads to preferential incorporation of 2R forms into serum lipoproteins [14]. α -TTP appears to mediate the transfer of α -tocopherol from hepatic parenchymal cells to VLDL, although Brefeldin A, an inhibitor of VLDL secretion, had no effect on α -tocopherol secretion [15]. Additional regulatory proteins, including tocopherol-associated protein and tocopherol-binding protein may also help facilitate intracellular transport of vitamin E [16].

2 Impact of vitamin E supplementation on health

While it is estimated that 35 million individuals in the United States take high levels of vitamin E supplements, it is also estimated the majority of the US population consume less than the recommended daily allowance (15 mg) [4, 17]. In addition to nutritional deficiency, several pathologies also cause vitamin E deficiency. For example, the autosomal recessive disorder ataxia of vitamin E deficiency caused by a mutation in α -TTP [4], and more commonly conditions affecting fat malabsorption, including cholestasis, celiac disease, cystic fibrosis, chronic diarrhoea, abetalipoproteinaemia and hypolipoproteinaemia can cause vitamin E deficiency [4, 18, 19]. Classical clinical manifestations of deficiency include peripheral neuropathy, progressive ataxia, erythrocyte haemolysis, and retinitis pigmentosa [4, 19]. In addition, deficiency during pregnancy increases the incidence of asthma in human infants [20], increases anxiety levels in rats following social isolation [21], and increases calcium oxalate crystal formation and tubular damage in rat kidneys following ethylene glycol exposure [22]. Chronic deficiency of α -tocopherol, leading to chronic lipid peroxidation also appears to enhance disease progression in a mouse model of Alzheimer's disease [23]. However, the impact of vitamin E deficiency on oxidative stress *in vivo* appears ambiguous. For example, supplementation increased lipid peroxidation in plasma, liver, and red blood cells of rats [24], increased retinal lipid peroxidation in mice [25] and significantly increased lipid peroxidation (F4-neuroprostane) levels in the cortex and cerebellum of mice [26]. Interestingly, it was also demonstrated in this same experiment that low concentrations of exogenous α -tocopherol enhanced superoxide flux in isolated brain mitochondria of mice, an effect subsequently reversed at higher α -tocopherol concentrations [26].

The effects of vitamin E supplementation on health appear similarly confusing, with randomized clinical trials in humans reporting positive, negative and no effect depending on the particular outcome measured [5, 8, 17, 27, 28]. However, reported positive effects following long-term supplementation in humans include a reduced risk of cardiovascular disease and various cancers (for review, see ([27])), lower risk of death from amyotrophic lateral sclerosis [29], slowing in the progression of Alzheimer's-induced cognitive decline [30, 31] and a delay in the age-related decline in immune function [32]. However, significant ambiguity across human clinical trials [28] has led to the suggestion that such studies have historically been over-optimistic given the confounding effects of inadequate diet and a sedentary lifestyle [4]. In addition, the lack of consensus between vitamin E supplementation and positive effects on health may also be due to differences between trials in dosage and supplement duration, pre-existing endogenous levels of vitamin E and the existence of any potentially confounding environmental and/or pathological conditions [33]. In humans, exercise-induced insulin sensitivity is attenuated by vitamin E (and C) supplementation [34] leading to the suggestion that antioxidant supplementation impedes the exercise-induced reactive oxygen species production required for the improved insulin sensitivity. Polymorphisms in genes associated with uptake and metabolism of vitamin E, and possibly also genes linked to reactive oxygen and nitrogen metabolism, may also explain the high variance between individuals in their response to vitamin E supplementation (for review, see [33]). For example, middle-aged, type-II diabetic patients carrying a haptoglobin 2-2 polymorphism were protected against cardiovascular disease following vitamin E supplementation relative to individuals carrying different polymorphisms [35]. In addition, individual differences in the inflammatory response following vitamin E supplementation in humans appear to be partly mediated by polymorphisms in genes that modulate cytokine production [36]. Systematic reviews of the effects of vitamin E on all cause mortality in humans suggest that the overall impact of high levels of vitamin E supplementation is negative, with a 4% increase in mortality risk relative to placebo [37, 38].

2.1 Impact of vitamin E supplementation on rodent lifespan

The effects of vitamin E supplementation on lifespan are also confusing (Table 1). Recently, we demonstrated that life-long dietary vitamin E (α -tocopherol) supplementation, initiated from 4 months of age, significantly extended median lifespan by about 15% in C57BL/6 mice maintained in the cold ($7 \pm 2^\circ\text{C}$; [39]). Several other studies have reported an increase in median/mean lifespan in rodents following either vitamin E supplementation [40–44] or when

Table 1. The effect of vitamin E supplementation on median/mean lifespan in rodents

Species	Strain	Sex	Age at initiation	Vitamin E form	Dose	Duration	Lifespan effect	Study
Mouse	CD-1/UCadiz	M & F	28 wk	α -Tocopherol acetate	5000 mg/kg	Life-long	Increase/no effect ^{a)}	[42]
	Balb/C	F	3 wk	Vitamin E	400 or 4000 mg/kg	Life-long	No effect	[47]
	C57BL/6	M & F	16 wk	α -Tocopherol	550 mg/kg	Life-long	Increase ^{b)}	[39]
	C57BL/6	M	126 wk	α -Tocopherol acetate	500 ppm	Life-long	No effect ^{c)}	[49]
	C ₃ H/He	NR	5 wk	α -Tocopherol	0.25% w/w	Life-long	Increase	[44]
	LAF ₁	NR	5 wk	α -Tocopherol	0.25% w/w	Life-long	Increase	[44]
	MRL/lpr	F	12 wk	α -Tocopherol acetate	250, 375, or 500 mg/kg	Life-long	Increase/decrease ^{d), e)}	[43]
Rat	Wistar	M	Weaning	α -Tocopherol	2000 mg/kg	Life-long	Increase ^{f)}	[40]

M, male; F, female; NR, gender not reported.

a) Significant increase in male lifespan only, median lifespan in females increased by 14% but effect non-significant.

b) Combined dietary supplementation and life-long cold exposure.

c) Dietary supplementation increased α -tocopherol acetate to 500 ppm compared with 30 ppm in control diet. Supplementation calculated to increase α -tocopherol intake (*per* gram of body weight) by 0.07 mg/day.

d) Dietary supplementation of 250 mg/kg significantly increased lifespan but 500 mg/kg significantly decreased lifespan. 375 mg/kg had no effect on lifespan. Control diet contained 50 mg/kg.

e) Study undertaken using a short-lived mouse model of lymphoproliferative disease.

f) Combined dietary supplementation with safflower oil supplementation increased 50% survival time.

supplemented with a mixed antioxidant diet containing vitamin E [45–46]. However, other rodent studies have reported no such effect on lifespan following vitamin E supplementation or following supplementation with a mixed antioxidant diet [3, 47–50].

There are several potential reasons why dietary vitamin E supplementation does not consistently extend lifespan in rodents. First, the age at which supplementation is initiated and the duration of the supplementation protocol appear critical (Table 1). We showed lifespan extension in mice following life-long supplementation started at 4 months of age [39], although supplementation from 7 months of age also increased lifespan, but only in male, and not female, mice [42]. Mean lifespan in mice supplemented with a mixed antioxidant diet, including α -tocopherol, only increased when initiated at 2 and 9 months, but not when initiated at 16 or 22 months, of age [46].

The specific form and dose of the supplemented vitamin E may also be critical for any lifespan effect, with doses varying by up to one order of magnitude between studies (Table 1). Indeed, it has been reported that a 500 mg/kg dose of α -tocopherol acetate reduced lifespan in mice despite a 250 mg/kg dose, in the same study, increasing lifespan relative to controls [43]. The tissue-specific response to vitamin E may also be dose dependent. Rats supplemented with 5, 30, 60, 250, or 500 mg of α -tocopherol acetate/kg diet, showed a dose response increase in α -tocopherol (but not γ -tocopherol) concentration in liver and plasma, but no further increase in concentration was seen in brain and heart beyond 60 mg/kg of diet [51]. Complex interactions between vitamin E and other experimental variables may

also be critical to any longevity effect. Variations in health status, physical activity, body mass, energy metabolism, and genetic background may all potentially influence the effect of vitamin E on lifespan [4, 33, 39]. In support of this, the studies demonstrating lifespan extension following vitamin E supplementation (Table 1) introduced additional treatment variables, *e.g.* cold exposure [39], exposure to a high fat diet [40], or use short-lived mouse disease models [43]. Gender may also be important, with lifespan extension seen in male but not female mice [42] following vitamin E supplementation, although other studies report no gender effect (*e.g.* [39]). It is also interesting to note that significant gender-specific differences in tissue α -tocopherol levels have been reported [52–54]. We suggest that potential interaction effects between vitamin E supplementation and other experimental variables require further investigation in light of these reported findings.

2.2 Vitamin E, xenobiotic metabolism, P21, and longevity

It is well established that cold exposure increases metabolic rate [55–56], and hence we previously hypothesized [39] that elevated metabolic rate in the cold would help reveal a vitamin E treatment effect on oxidative stress and lifespan in mice, if such an effect existed. However, despite metabolic rate being significantly elevated in the cold and lifespan being extended by vitamin E supplementation, no treatment effect on various DNA and lipid oxidative stress parameters was detected [39], consistent with several other studies (see

[9]). This has led to the controversial suggestion that vitamin E may only act as a potent antioxidant *in vitro* [57]. We observed no treatment effect on oxidative stress-related gene expression in vitamin E-supplemented mice [39], contrary to the reduced antioxidant gene expression seen in cold-exposed female C57BL/6 mice supplemented for 18 months with vitamin C [58].

Vitamin E has been suggested as a key modulator of gene expression [9], although it should be noted that studies examining gene expression using either α -TTP null mice [59–60] or following vitamin E supplementation [61] generally report relatively few gene expression changes. Using a transcriptional approach, we have demonstrated that 2 months of vitamin E supplementation (initiated at 4 months of age) significantly increased the hepatic expression of several cytochrome P450 and xenobiotic metabolism genes in female C57BL/6 mice relative to controls [39]. However, the increased expression of these transcripts appeared transient and disappeared within 18 months of supplementation [39]. In support of our findings, it was recently reported [62] that supplementation over 4 months with α -tocopherol acetate (1000 IU all-*rac*- α -tocopherol/kg diet) increased hepatic expression of several xenobiotic metabolism genes in mice relative to mice maintained on a control diet (35 IU all-*rac*- α -tocopherol/kg diet). Vitamin E is known to undergo side chain degradation, initially metabolised through ω -hydroxylation by the cytochrome P450 system, specifically CYP3A-type cytochromes, followed by five cycles of β -oxidation [63, 64]. Thus hepatic metabolism is suggested to be one of the fundamental regulatory systems by which vitamin concentrations can be endogenously controlled [62, 65], with subsequent conjugation and excretion in urine [66] or bile [67]. The xenobiotic metabolism of vitamin E, particularly if, as we suggest, this response is transient [39], may be a factor in why short-term supplementation, or supplementation initiated in mid/late-life, appears ineffectual in extending rodent lifespan (Table 1). This could be particularly important if this transient enhancement in xenobiotic metabolism of vitamin E produces toxic by-products that might offset any potential benefits.

In mice, following 18 months of supplementation, several genes associated with the cyclin dependent kinase inhibitor 1A (Cdkn1a)/p21(Waf1/Cip1) signalling pathway including p21, mitogen-activated protein kinase and murine double minute 2 were up-regulated [39]. This increased expression of p21 was independent of phosphorylation status of P53, suggesting it was a P53-independent process [39]. P21 is a tumour suppressor, causing cell cycle arrest by interacting with cyclin E/cyclin-dependent kinase complexes to prevent the G1/S transition, and appears to induce apoptosis [68]. However, P21 also shows “antagonistic duality” as it has pro-cancer properties under certain conditions [69]. In agreement with our findings, vitamin E deficiency decreased p21 expression in the liver of rats [70] and mixed tocopherol diets suppressed mammary tumour

growth in female rats while increasing p21 expression levels [71]. In addition, vitamin E-induced apoptosis in colorectal cancer cell lines appears to be mediated through P21 *via* a mechanism involving a CCAAT/enhancer binding protein transcription factor C/EBP β , independent of P53 [72]. P21 can protect cells against oxidative and genotoxic stress, is suggested to integrate the DNA damage response, appears important in endoplasmic reticulum stress signalling and in mitochondrial apoptosis [73, 74] possibly through stabilisation of the NF-E2-related factor-2 protein [73]. Indeed, elevated oxidative stress in mice has been shown to correlate closely with increased tissue p21 expression [75].

In the last 2 years, there has been an explosion of research activity surrounding the potential anti-cancer effects of different vitamin E vitamers. These studies suggest that not only α -tocopherol, but also α -tocopherol and both δ - and γ -tocotrienols might have potent anticancer activities, possibly mediated *via* anti-angiogenic, anti-proliferative, and pro-apoptotic effects [76–86]. These apparent anti-cancer effects appear to be mediated independently of any antioxidant properties, a finding supported by elegant studies using redox-silent forms of tocotrienols [87, 88].

3 Concluding remarks

Our recent research findings demonstrate that life-long vitamin E supplementation, in the form of α -tocopherol, extended lifespan in cold exposed mice [39]. We suggest that when vitamin E is administered over short periods, enhancement of the xenobiotic metabolism response may nullify any beneficial effects. Indeed, short-term administration of vitamin E in humans may actually have a negative impact on all cause mortality when administered at high doses. The observed lifespan effects may not stem solely from specific anti-oxidant activity of vitamin E but may be, at least in part, due to anti-cancer actions, possibly *via* an up-regulation in genes linked to the P21 pathway [39]. Further insights into identifying the possible mechanisms underlying vitamin E-induced lifespan extension will be aided by comprehensive transcriptomic, proteomic and/or metabolomic investigations across several tissues (see [89]) and in both sexes, using experimental designs that minimise the effects of potentially confounding variables. Moreover the time course of amelioration of the P450 response requires further elucidation. The advent of novel genetic approaches, *e.g.* p21-p-luc mice that express luciferase under the control of the p21 gene promoter [90], and various mouse models with global and/or tissue-specific deletions in candidate genes will help inform exactly how vitamin E supplementation ultimately impacts on lifespan and health.

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4 References

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