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Review

Vitamin E supplementation and lifespan in model organisms

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ABSTRACT

We have conducted a comprehensive literature review regarding the effect of vitamin E on lifespan in model organisms including single-cell organisms, rotifers, *Caenorhabditis elegans*, *Drosophila melanogaster* and laboratory rodents. We searched Pubmed and ISI Web of knowledge for studies up to 2011 using the terms "tocopherols", "tocotrienols", "lifespan" and "longevity" in the above mentioned model organisms. Twenty-four studies were included in the final analysis. While some studies suggest an increase in lifespan due to vitamin E, other studies did not observe any vitamin E-mediated changes in lifespan in model organisms. Furthermore there are several studies reporting a decrease in lifespan in response to vitamin E supplementation. Different outcomes between studies may be partly related to species-specific differences, differences in vitamin E concentrations and the vitamin E congeners administered. The findings of our literature review suggest that there is no consistent beneficial effect of vitamin E on lifespan in model organisms which is consistent with reports in human intervention studies.

1. Introduction

The term vitamin E is used to describe a group of eight lipid soluble substances with a chromanol ring and a saturated (tocopherols) or unsaturated (tocotrienols) carbon side chain (see Fig. 1). Depending on the methyl groups found at the chromanol groups these compounds are referred to as α-, β-, γ- or δ-tocopherols and tocotrienols (Kamal-Eldin and Appelqvist, 1996). As a free radical scavenger and lipophilic molecule, vitamin E may protect the membranes from oxidative damage by reacting with fatty acid peroxides via electron transfer (Traber and Atkinson, 2007). Additionally, vitamin E may regulate gene expression (Azzi, 2007; Rimbach et al., 2002, 2010). Over the last few decades a possible influence of vitamin E on longevity has been studied in animals and humans. However, it remains unclear whether this group of antioxidants can prolong or, on the contrary, decrease lifespan. In this review, we summarize the studies on vitamin E supplementation and lifespan in model organisms of increasing biological complexity, thereby addressing the question if and to what extent vitamin E increases the lifespan of single cell organisms and rotifers, nematodes, flies, mice and rats.

2. Vitamin E supplementation in different model organisms

2.1. Single-celled organisms and rotifers

The effect of vitamin E on single-cell organisms and rotifers was examined in five studies (Enesco and Verdone-Smith, 1980; Lam et al., 2010; Minogue and Thomas, 2004; Sawada and Enesco, 1984; Thomas and Nyberg, 1988). Of these studies, four reported an increase in lifespan with vitamin E and one, on *Saccharomyces cerevisiae* (Lam et al., 2010), showed a reduction in lifespan. These findings are summarized in Table 1.

2.1.1. Rotifer Philodina

The first study to investigate the influence of vitamin E on the lifespan of rotifers was conducted by Enesco and Verdone-Smith in (1980) on the rotifer *Philodina*. In this study, DL-α-tocopherol at a concentration of 0.05 μl/ml (solubilized in Tween 80) was added to the medium in which the rotifers were grown. The rotifers were transferred to new medium every 24 h and checked for vitality. Vitamin E treatment significantly increased mean lifespan compared to both the solvent control and the non-solvent control (by 1.9 days (10.2%) and 1.7 days (9.2%) respectively), whilst maximum lifespan was unaffected. Furthermore, the average number of

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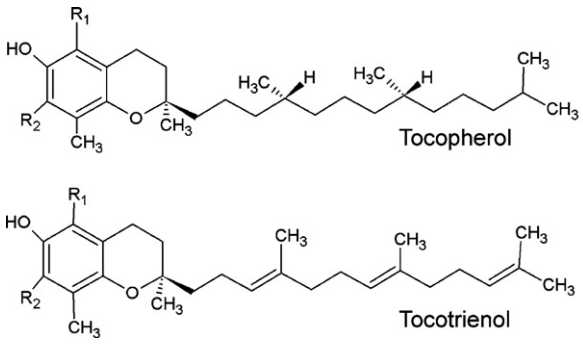

	R_1	R_2
α-	CH_3	CH_3
β-	CH_3	H
γ-	H	CH_3
δ-	H	H

Fig. 1. Chemical structures of tocopherols and tocotrienols.

Table 1
Vitamin E lifespan studies on single-cell organisms and rotifers in chronological order.

Species	*n*	Form of vitamin E	Dose	Initiation of treatment	Mean lifespan	Maximum lifespan	Study
Rotifer philodina	96	DL-α-Tocopherol	0.05 μg/ml	After birth	+1.9 days (10.2%)	No effect	Enesco and Verdone-Smith (1980)
Asplanchna brightwelli	24	DL-α-Tocopherol	25 μg/ml	0–3 h prior to birth	+1.1 day (+17%)	–	Sawada and Enesco (1984)
Paramecium tetraurelia	64 lines	DL-α-Tocopherol	25 μg/ml 25 μg/ml	Day 0 Day 9	+8.5 days (n.s.) +18.3 days	+23 days (n.s.) +80 fissions (n.s.) –	Thomas and Nyberg (1988)
Paramecium tetraurelia	96	α-Tocopherol	10 μg/ml 100 μg/ml 1000 μg/ml 10,000 μg/ml	Day 0 Day 0 Day 0 Day 0	−7.5 days (n.s.) +30 days (n.s.) +115 days +216.5 days	– – – –	Minogue and Thomas (2004)
Saccharomyces cerevisiae	90–110 per plate	α-Tocopherol	20, 50, 80, 120, 150 μM	–	–	–	Lam et al. (2010)

–, data is not available; n.s., not significant.

offspring per rotifer was significantly increased by 10% compared to both control groups.

2.1.2. *Asplanchna brightwelli*

Sawada and Enesco examined the effect of vitamin E on another rotifer, *Asplanchna brightwelli*. DL-α-Tocopherol solubilized in Tween-80 was used at concentrations ranging between 5 and 100 μg/ml. They demonstrated that 25 μg/ml of DL-α-tocopherol extended mean lifespan (±S.E.M.) of the rotifers from 5.5 days (±0.13) to 6.4 days (±0.17) compared to the control, whilst lower concentrations had little effect. Interestingly, the highest concentration used, 100 μg/ml, caused a reduction in lifespan. Based on these initial results, they used α-tocopherol at a concentration of 25 μg/ml in subsequent experiments aimed at elucidating the exact timing of the effects. They solubilized 25 μg/ml α-tocopherol in either Tween-80 or ethanol and found that the increase in mean lifespan was similar with both solvents. By separating the rotifers into pre-reproductive, reproductive and post-reproductive periods, they found that lifespan was only significantly increased during the pre-reproductive period. The number of offspring was unaffected in this experiment (Sawada and Enesco, 1984).

2.1.3. *Paramecium tetraurelia*

In 1988, Thomas and Nyberg were the first to investigate the effects of vitamin E on the lifespan of a single-celled organism, *Paramecium tetraurelia*. The effect of 25 μg/ml DL-α-tocopherol was examined in eight different genotypes, of which only one (that with the shortest mean lifespan) showed a significant increase in mean lifespan, measured in days and fissions. The supplemented subgroups demonstrated a bulk increase in maximum lifespan by 17.6% and in mean lifespan by 14.1% compared with controls (supplementation 58.5 ± 16.6 vs. control 50.5 ± 10.6). Subsequent experiments investigated the impact of switching the organisms from control to vitamin E (25 μg/ml) media early in life at days 0, 1, 9, 17 or 25. Only those subgroups transferred at day 9 showed a significant increase in mean lifespan in days and fissions. Those that were transferred to α-tocopherol-containing media after 9 days of clonal lifespan showed only minor, inconsistent effects on lifespan extension.

Table 2
Maximum clonal lifespan in days and fissions of *P. tetraurelia* supplemented with different amounts of α-tocopherol.

	Control	25 μg/ml	100 μg/ml	1000 μg/ml
Maximum clonal lifespan in fissions	237	260	271	330
Maximum clonal lifespan in days	66	68	74	141

Differences are not statistically significant ($p > 0.05$) (modified from Thomas and Nyberg, 1988)

This study also evaluated the effects of higher concentrations of α-tocopherol (100 and 1000 μg/ml) on maximum clonal lifespan. An increase in the maximum lifespan was observed with increasing doses of α-tocopherol, yet these data were non-significant. Table 2 summarizes the results of this experiment.

Interestingly, however, when comparing controls to organisms given 25 μg/ml of α-tocopherol and organisms given 100 μg/ml

to those given 1000 μg/ml of α-tocopherol, the subgroups that received the higher concentrations of α-tocopherol showed a slower increase in death rate despite a higher initial mortality, indicating that an adaptive process may have occurred. The same pattern was observed for fission rates. Furthermore 25 μg/ml of α-tocopherol increased the survival of non-dividing organisms beyond their clonal lifespan from 1.05 days to 1.49 days when compared to controls (Thomas and Nyberg, 1988).

Minogue and Thomas provided further evidence of the effects of higher doses of vitamin E on *P. tetraurelia*. The authors observed that high concentrations of α-tocopherol (100, 1000 and 10,000 μg/ml) dose-dependently enhanced mean lifespan, despite lower concentrations modestly reducing lifespan compared to controls with or without medium. 1000 μg/ml of α-tocopherol extended mean lifespan by 126.5 fissions (49.5%) and 115 days (151.3%) compared to controls (mean lifespan in days ± S.E.M.: supplementation 191 ± 16 vs. control 80 ± 3; mean lifespan in fissions ± S.E.M.: supplementation 382 ± 4 vs. control 278.5 ± 11) whilst 10,000 μg of α-tocopherol increased lifespan by 83.5 fissions (32.7%) and 216.5 days (234.9%) (mean lifespan in days ± S.E.M.: supplementation 292.5 ± 8.5 vs. control 80 ± 3; mean lifespan in fissions ± S.E.M.: supplementation 339 ± 13 vs. control 278.5 ± 11). Maximum lifespan was not reported. Minogue and Thomas also found that the survival curves of *P. tetraurelia* supplemented with 10 and 100 μg/ml of α-tocopherol were no different to controls, displaying little mortality in the early phase of lifespan with a sudden and strong reduction in survival later in the lifespan. However, organisms treated with 1000 μg/ml of α-tocopherol had a high mortality rate in the first 4 weeks with only 75% surviving. Mortality in this group then slowed until survival dropped again in the late stages of their lifespan. Those organisms administered 10,000 μg/ml of α-tocopherol demonstrated a strong initial decline in survival such that only ~50% survived until 5 weeks. Survival then plateau'd until 15 weeks when the death rate slowly increased again. Death rates confirmed the survival curves with the high dose treatments showing a consistently elevated death rate indicating a cytotoxic effect of α-tocopherol (Minogue and Thomas, 2004).

2.1.4. S. cerevisiae

More recently, Lam et al. (2010) assessed the effect of α-tocopherol on the lifespan of the baker's yeast, *S. cerevisiae*. The organisms of the K6001 strain were grown in the linear phase in glucose medium with vitamin E concentrations ranging between 20 and 150 μM. They found that α-tocopherol dose-dependently decreased replicative lifespan (estimated by measuring the optical density of the medium). However, cell viability was unaltered by these α-tocopherol concentrations. To elucidate the molecular properties of α-tocopherol responsible for the observed effects they used α-tocopherol acetate, which lacks antioxidant activity, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMC) and trolox, two synthetic analogs of vitamin E which lack the phytyl tail. Only PMC decreased the replicative lifespan in the same dose-dependent manner as α-tocopherol. However, PMC also decreased cell viability indicating a toxic effect. Thus, Lam et al. concluded that both the antioxidant capacity and lipid solubility of α-tocopherol are responsible for its effects on *S. cerevisiae*. In contradiction to its antioxidant activity, vitamin E was found to increase ROS production and thus oxidative stress when administered for 20 h. Combining α-tocopherol with coenzyme Q_{10}, another antioxidant which may potentially restore α-tocopherol, was also unsuccessful; replicative lifespan was similarly decreased (Lam et al., 2010).

2.2. Nematodes

The most commonly used nematodes to test the responses to tocopherols and tocotrienols are *Caenorhabditis elegans* (Adachi and Ishii, 2000; Harrington and Harley, 1988; Ishii et al., 2004; Zou et al., 2007; Zuckerman and Geist, 1983), *Caenorhabditis briggsae* (Epstein and Gershon, 1972) and *Turbatrix aceti* (Kahn and Enesco, 1981).

Vitamin E, with few exceptions, has been shown to extend the lifespan of nematodes (see Table 3). One study showed that α-tocopherol treatment increased both mean and maximum lifespan (Epstein and Gershon, 1972) whereas others only demonstrated an effect on mean lifespan (Harrington and Harley, 1988; Ishii et al., 2004; Kahn and Enesco, 1981; Zuckerman and Geist, 1983) or showed no effect at all (Adachi and Ishii, 2000; Zou et al., 2007).

2.2.1. C. briggsae

Epstein and Gershon in 1972 were the first to investigate the effects of vitamin E on aging in nematodes, specifically in *C. briggsae*. α-Tocopheryl-quinone at 400 μg/ml had no effect on the length of their reproductive period nor on the number of progeny. Continuous supplementation with 400 μg/ml α-tocopheryl-quinone increased the median lifespan by 11 days (31%) and the maximum lifespan by 13 days (23%) compared to medium and solvent controls. Administering the α-tocopheryl-quinone on days 1, 10, 20 or 30 revealed that only an early initiation of the treatment prolonged median and maximum lifespan; starting treatment at days 20 or 30 had no effect. The duration of α-tocopheryl-quinone treatment correlated with the increase in the nematodes lifespan; treating for 6 days after hatching prolonged median lifespan by 5 days (16%) and maximum lifespan by 6 days (10%), treating for 10 days prolonged median lifespan by 11 days (31%) (supplementation 46 ± 2 days vs. control 35 ± 2 days) and maximum lifespan by 14 days (25%) (supplementation 69 ± 4 days vs. control 56 ± 3 days). α-Tocopheryl-quinone treatment was found to delay the accumulation of lipofuscin, suggesting that it has antioxidant properties (Epstein and Gershon, 1972).

2.2.2. T. aceti

Following on from the work of Epstein and Gershon, Kahn and Enesco found that DL-α-tocopherol at a concentration of 100 μg/ml significantly increased the mean lifespan (±S.D.) of the nematode *T. aceti* by 15.3 days (33.7%) (supplementation 60.7 ± 30.6 days vs. control 45.4 ± 29.5 days) and increased the maximum lifespan by 8 days (7%) compared to the control. This increase in lifespan occurred in the early phase, before day 30, and in the late phase, after day 72. In subsequent experiments, nematodes were initially grown in treatment or control medium and then transferred to treatment or control medium, yielding four supplementation patterns (control–treatment, control–control, treatment–control and treatment–treatment). The results demonstrated that early treatment with α-tocopherol (i.e. within the first 24 h after hatching) significantly increased mean lifespan by up to 46.2%. Transferring the nematodes 96 h after hatching had no significant effect on lifespan. Together, this study indicates that α-tocopherol exerts its positive effects on nematodes in the early phase of their lifespan (Kahn and Enesco, 1981).

2.2.3. C. elegans

2.2.3.1. Influence of time and dose of α-tocopherol supplementation. Zuckermann and Geist evaluated whether there is an age-dependent effect of α-tocopherol on lifespan. Parental *C. elegans* were grown in an α-tocopherol (200 μg/ml)-supplemented or solvent control medium and newly born nematodes transferred to either an α-tocopherol or control medium at hatch or day four. The maximum lifespan and the time point at which 50% of the worms were still alive were measured. These survival rates showed that there seems to be no carryover effect from the paternal generation to the offspring as the progeny from vitamin E supplemented and control parents showed no difference regarding lifespan. However, if the nematodes hatched from egg masses and stayed in

Table 3
Vitamin E lifespan studies on nematodes in chronological order.

Species	*n*	Form of vitamin E	Dose	Initiation of treatment	Mean lifespan	Maximum lifespan	Study
Caenorhabditis briggsae	100	α-Tocopheryl quinone	400 μg/ml	Day 1	+11 days #	+13 days	Epstein and Gershon (1972)
				Until day 6	+5–6 days #	+6 days	
				Until day 10	+11 days #	+13 days	
Turbatrix aceti	288	DL-α-Tocopherol	100 μg/ml	Day 1	+15.3 days	+8 days (n.s.)	Kahn and Enesco (1981)
				Day 4	n.s. effect	n.s. effect	
				Day 30	n.s. effect	n.s. effect	
Caenorhabditis elegans	47	DL-α-Tocopherol	200 μg/ml	At hatching	+7 (n.s.) #	+8 days (n.s.)	Zuckerman and Geist (1983)
Caenorhabditis elegans	24	DL-α-Tocopherol	100 μg/ml	At hatching	+2 days (n.s.)	+3 days (n.s.)	Harrington and Harley (1988)
			200 μg/ml	At hatching	+5 days	+7 days	
			200 μg/ml	Until day 3	+4 days	+5 days	
			400 μg/ml	At hatching	+1 day (n.s.)	+2 days (n.s.)	
Caenorhabditis elegans	400	α-Tocopherol acetate	80 μg/ml	At adult stage	+0.5 days (n.s.)	–	Adachi and Ishii (2000)
		Tocotrienol mix.	8 μg/ml	At adult stage	+1.5 days	–	
			80 μg/ml	At adult stage	+3.2 days	–	
C. elegans		α-Tocopherol	400 μg/ml	At adult stage			Ishii et al. (2004)
Wild type	~200				+11%	–	
mev-1	~200				n.s. effect	–	
Caenorhabditis elegans	~400	α-Tocopherol	20 μg/ml	Young adults	+0.5 days (n.s.)	n.s. effect	Zou et al. (2007)
fem-1			200 μg/ml		−1.8 days (n.s.)	n.s. effect	
		γ-Tocopherol	20 μg/ml		+2.1 days	n.s. effect	
			200 μg/ml		+2.6 days	n.s. effect	
		α-toc + γ-toc	20 μg/ml		+2 days (n.s.)	n.s. effect	
			200 μg/ml		+0.3 days (n.s.)	n.s. effect	

–, data is not available; n.s., not significant; #, data are given as median.

vitamin E supplemented medium their lifespan increased by 7 days compared to those without vitamin E supplementation (supplementation 54 days vs. control 47 days). Later supplementation did not significantly affect the 50% survival rate but increased the maximum lifespan (supplementation 74 days vs. control 66 days). These results suggest that α-tocopherol exerts its effects in the early stages of the lifespan of nematodes. Zuckermann and Geist also demonstrated that high concentrations of α-tocopherol (400 μg/ml and 800 μg/ml) had detrimental effects on *T. aceti*, reducing their size and the number of offspring compared to controls and lower concentrations of α-tocopherol (200 μg/ml). These results again suggest that α-tocopherol is toxic at higher concentrations (Zuckerman and Geist, 1983).

2.2.3.2. Effects of α-tocopherol on development and aging. Harrington and Harley examined in more detail the distinct effects of α-tocopherol on development and aging in *C. elegans*. The addition of 200 μg/ml DL-α-tocopherol after hatching (levels were depleted after 3 days) increased maximum lifespan by 5 days (17%) (supplementation 34 days vs. control 29 days), median lifespan by 3 days (16.6%) (supplementation 21 ± 1 days vs. control 18 ± 2 days) and mean lifespan by 4 days (20%) (supplementation 24 ± 1 days vs. control 27 ± 1 days), resulting in an overall significant increase in the lifespan of *C. elegans* by 17% compared to the solvent control. Continuous administration of DL-α-tocopherol increased lifespan by 22%. Administration of α-tocopherol at 100 and 400 μg/ml slightly, but not significantly, increased the nematodes lifespan by 9% and 4%, respectively. They found that when 200 μg/ml α-tocopherol was administered to the nematodes from day 4 of life until death, mean lifespan was significantly increased from 18 to 21 days and maximum lifespan increased from 29 to 31 days compared to the solvent control. At concentrations of 200 and 400 μg/ml, DL-α-tocopherol significantly decreased the number of offspring per animal by 23–30% and significantly delayed the onset of their reproductive period by 19–28%. The authors concluded that higher doses of vitamin E can lead to growth retardation or developmental delay (Harrington and Harley, 1988).

2.2.3.3. Supplementation with tocopherol and tocotrienols. To compare the influence of tocotrienols and tocopherols on the lifespan of *C. elegans*, Adachi and Ishii used DL-α-tocopherol acetate and an extract of palm oil (referred to as "tocotrienol mixture") containing 95% of vitamin E composed of 22% α-tocopherol, 24% α-tocotrienol, 37% γ-tocotrienol and 12% δ-tocotrienol. When given in the adult stage, the tocotrienol mixture at doses of 8 and 80 μg/ml medium significantly increased the mean lifespan of *C. elegans* by 1.5 days (8.7%) and 3.2 days (19.8%) (supplementation 18.7 ± 1.9 and 20.6 ± 1.6 days vs. control 17.2 ± 1.8 days), respectively, while maximum lifespan was unaffected. In contrast, 80 μg/ml of α-tocopherol acetate did not significantly alter lifespan (Adachi and Ishii, 2000). The tocotrienol mixture (at 80 μg/ml) significantly decreased the levels of carbonylated proteins by one third in 15 day old animals, indicating that the mixture has antioxidant properties. The tocotrienol mixture (80 μg/ml) was also able to abolish the significant reduction in lifespan caused by irradiation with ultraviolet light when given after the irradiation, whereas α-tocopherol acetate (80 μg/ml) offered only minimal protection which was non-significant. Furthermore, when treatment was initiated before the irradiation insult, the tocotrienol mixture significantly extended the lifespan by 2.1 days (12.1%) compared to untreated, non-irradiated controls (Adachi and Ishii, 2000). This is in accordance with a study showing that the lifespan increased

in *C. elegans* that were fed γ-cyclodextrin tocotrienol complexes (Kashima et al., 2012). Complexation with γ-cyclodextrin has been shown to enhance the absorption of lipid-soluble substances and may be used as a strategy to improve tocotrienol bioavailability (Ikeda et al., 2010).

2.2.3.4. Wild-type and mev-1 mutant. Another study investigating the effects of vitamin E on longevity in nematodes was conducted by Ishii et al. in 2004. They treated wild-type *C. elegans* and the mev-1 mutant that has increased superoxide production and is highly susceptible to oxidative damage, with Coenzyme Q_{10} and α-tocopherol. Mev-1 encodes a succinate dehydrogenase cytochrome b subunit. The mev-1 mutant has a defective electron transport in complex II and is therefore characterized by an increased superoxide production and is highly susceptible to oxidative damage. A mutation in the succinate dehydrogenase cytochrome b causes oxidative stress and aging in nematodes (Ishii et al., 1998, 2004). α-Tocopherol (400 μg/ml) significantly increased the lifespan of wild-type nematodes by 11% compared to untreated controls, yet the lifespan of the mev-1 mutant remained unaltered. However, they found that, unlike Coenzyme Q_{10}, vitamin E failed to diminish levels of superoxide anion production in both wild-type nematodes and the mev-1 mutants (Ishii et al., 2004).

2.2.3.5. Influence of α- and γ-tocopherol. Zou et al. studied the effects of 20 and 200 μg/ml α-tocopherol or γ-tocopherol on the sterilized *C. elegans* mutant, fem-1, in adulthood. Their findings revealed opposing effects of the two tocopherols on lifespan. 200 μg/ml α-tocopherol marginally and non-significantly decreased mean lifespan and did not affect maximum lifespan. Contrastingly, γ-tocopherol significantly extended mean lifespan to 14.7 days (16.7%) at 20 μg/ml and to 15.2 days (20.6%) at 200 μg/ml compared to controls with a mean lifespan of 12.6 days. A combined administration of α- and γ-tocopherol in equal proportions at 20 and 200 μg/ml did not significantly affect the lifespan of *C. elegans*. The authors hypothesize that this might be due to α-tocopherol negating the positive effects of γ-tocopherol (Zou et al., 2007).

2.3. Flies

The available data relating to the effects of vitamin E on the lifespan of flies originates from five studies, of which four were conducted in *Drosophila melanogaster* (Bahadorani et al., 2008; Driver and Georgeou, 2003; Miquel et al., 1973; Zou et al., 2007). Kakkar et al. (1996) investigated lifespan in the fruit fly *Zaprionus paravittiger* and Zou et al. (2007) also examined the effects on the Mexican fruit fly, *Anastrepha ludens*. In addition, the effects of α-tocopherol on the housefly *Musca domestica* were tested in a study by Sohal et al. (1985). The main results of these studies are summarized in Table 4.

2.3.1. M. domestica

Sohal et al. fed flies sucrose containing 0.5% and 2% α-tocopherol. The lower dose of α-tocopherol did not alter mean lifespan, whereas the higher dose significantly decreased lifespan by 5.4 days (26.1%) (supplementation 15.3 ± 4.2 days vs. control 20.7 ± 5.9 days). The metabolic rate, measured as oxygen consumption per time per weight of flies, was not affected by the treatment at either concentration. However, SOD activity was significantly decreased in flies given 2% α-tocopherol at 6, 9 and 12 days of age by between 20.3% and 24.9% compared to controls. Interestingly, the SOD activity of α-tocopherol-treated flies remained almost constant with age, while SOD activity in control flies increased from days 3 to 9 then slightly decreased on day 12. This indicates an age-related variation in SOD activity that is abolished by α-tocopherol. The authors suggest that the administration of exogenous antioxidants may cause a compensatory depression of endogenous defenses. On the other hand, α-tocopherol treatment did not alter catalase activity at both concentrations. The glutathione content of flies supplemented with both concentrations of α-tocopherol was also not statistically different from controls. Hydrogen peroxide levels were dose-dependently increased by α-tocopherol, reaching statistical significance at 2% α-tocopherol. The appearance of fluorescent material (e.g. lipofuscin) which is believed to accumulate in the aging organism upon free-radical induced damage was not affected by the α-tocopherol treatment (Sohal et al., 1985).

2.3.2. Z. paravittiger

α-Tocopherol was administered to male and female fruit flies of the species *Z. paravittiger* at concentrations ranging between 1 and 50 μg/ml of medium. On the one hand, median and maximum lifespan were significantly increased by α-tocopherol supplementation at concentrations of 1 μg/ml (median lifespan mean ± S.D. male/female in days, 37.4 ± 0.1/43.3 ± 0.2; maximum lifespan ± S.D., 83.3 ± 1.2/85.0 ± 1.0), 5 μg/ml (median lifespan mean ± S.D. male/female in days, 46.9 ± 0.3/48.8 ± 0.5; maximum lifespan, 88.7 ± 1.2/99.3 ± 1.2) and 10 μg/ml (median lifespan mean ± S.D. male/female in days, 43.3 ± 0.7/46.4 ± 0.4; maximum lifespan, 85.7 ± 1.5/94.3 ± 1.5) in comparison to the control (median lifespan mean ± S.D. male/female in days, 35.1 ± 0.7/41.7 ± 0.1; maximum lifespan, 76.7 ± 1.2/82.0 ± 2.0). On the other hand, α-tocopherol at concentrations of 25 and 50 μg/ml decreased median and maximum lifespan by 33.7–54% and 16.1–35.5%, respectively (median lifespan mean ± S.D. male/female in days, 23.3 ± 0.2/23.8 ± 0.3 and 16.8 ± 0.1/19.2 ± 0.2; maximum lifespan, 64.3 ± 0.6/65.0 ± 1 and 50.3 ± 1.2/53.7 ± 2.3). Supplementation of α-tocopherol at 5 μg/ml decreased TBARS levels in fly homogenates at all ages, measured at 7-day intervals during a period of 43 days starting from day 1. Furthermore, this concentration of α-tocopherol ameliorated the age-dependent decrease in the activity of the antioxidant enzymes catalase and peroxidase (Kakkar et al., 1996).

2.3.3. D. melanogaster

Miquel et al. fed ~3000 adult male flies of the species *D. melanogaster* a medium containing 0.06%, 0.12% or 0.25% α-tocopherol acetate. All three concentrations resulted in a significant increase in the flies' lifespan with the highest dose having the largest effect, increasing the mean and maximum lifespan by 8–15% and by 12% compared to controls, respectively (Miquel et al., 1973).

Driver and Georgeou supplemented male flies with vitamin E solubilized in ethyl acetate at concentrations of 3, 20, 100 and 200 μg/ml. The lifespan of control flies was 79 days (±6) whilst that of flies administered 3, 20, and 200 μg/ml vitamin E was 86 (±6), 92 (±6) and 76 days (±2), respectively. As such, only the 20 μg/ml dose of vitamin E significantly elevated lifespan compared to controls. When given in conjunction with paraquat, a chemical that acts as a redox cycler and generates a superoxide anion radical when metabolized in mitochondria, vitamin E at a concentration of 200 μg/ml was able to extend lifespan and abolish the detrimental effects of paraquat. When flies were administered vitamin E at a concentration of 250 μl/ml for the first 6 weeks of life, their lifespan significantly decreased by 12.6 days (13.6%; supplementation 11.6 ± 0.31 vs. control 13.4 ± 0.3). Furthermore, the authors found that vitamin E supplementation altered the circadian rhythm of flies, causing a significantly different pattern and length of their diurnal activity cycle when the lighting conditions were changed from a 12 h dark, 12 h light cycle to continuous light (Driver and Georgeou, 2003).

Zou et al. investigated potential sex-specific differences in responses to α- and/or γ-tocopherol at concentrations of 20, 100 or

Table 4
Vitamin E lifespan studies on flies in chronological order.

Species	*n*	Form of vitamin E	Dose	Age at start of treatment	Mean lifespan	Maximum lifespan	Study
Drosophila melanogaster	~3000	α-Tocopherol-acetate	0.06%, 0.12%, 0.25%	Adult	+8–12%	+15%	Miquel et al. (1973)
Musca domestica (male)	1600	α-Tocopherol	0.5%	–	n.s. effect	–	Sohal et al. (1985)
			2%		−5.4 days	–	
Zaprionus paravittiger	4500 (male/female)	α-Tocopherol	1 μg/ml	–	+2.2/1.7 d. #	+6.6/3 d.	Kakkar et al. (1996)
			5 μg/ml		+11.8/7.2 d. #	+12/17.3 d.	
			10 μg/ml		+8.1/4.7 d. #	+9/12.3 d.	
			25 μg/ml		−11.8/17.9 d. #	−12.3/17 d.	
			50 μg/ml		−18.3/22.5 d. #	−26.3/28.3 d.	
Drosophila melanogaster	–	–	3 μg/ml	–	+7 days (n.s.)	–	Driver and Georgeou (2003)
			20 μg/ml		+13 days	–	
			100 μg/ml		–	–	
			200 μg/ml		−3 days (n.s.)	–	
Drosophila melanogaster	~900	α-Tocopherol	20 μg/ml	–	n.s. effect	–	Zou et al. (2007)
			100 μg/ml		n.s. effect	–	
			200 μg/ml		−1.9 days	–	
	~900	γ-Tocopherol	20 μg/ml	–	n.s. effect	–	Zou et al. (2007)
			100 μg/ml		n.s. effect	–	
			200 μg/ml		−1.6 days	–	
Anastrepha ludens	~8800	α-Tocopherol	100 μg/ml	–	n.s. effect	–	Zou et al. (2007)
		γ-Tocopherol	100 μg/ml		n.s. effect	–	
Drosophila melanogaster	600	α-Tocopherol	0.005, 0.05, 0.5, 5, 25 IU/ml		n.s. effect	n.s. effect	Bahadorani et al. (2008)

–, data is not available; n.s., not significant; d, days; # data are given as median.

200 μg/ml in canton-S wild-type flies of the species *D. melanogaster*. At 100 μg/ml, α-tocopherol modestly, yet significantly, increased the mean lifespan of female flies by 1.7% but had no affect on males. In contrast, the same dose of γ-tocopherol as well as the two highest doses of α- and γ-tocopherol significantly reduced the mean lifespan of female flies by 1.3 days (9.9%), 1.6 days (6.9%) and 2.9 days (12.5%), respectively. The authors also reported that 100 μg/ml of α- or γ-tocopherol did not significantly affect the lifespan of flies of the species *A. ludens*, with mean lifespan even slightly decreasing in male flies (Zou et al., 2007).

Bahadorani et al. examined the effects of α-tocopherol in male SOD 1-deficient and wild-type *D. melanogaster* both under normal and elevated oxygen partial pressures. Food intake was found to be unaltered by treatment compared to controls. α-Tocopherol was tested under normal oxygen conditions at concentrations of 0.005, 0.05, 0.5, 5.0 and 25.0 IU/ml and was not found to significantly alter longevity. In addition to the lifespan extension in SOD 1-deficient flies, α-tocopherol significantly increased the antioxidant capacity of these animals, measured as trolox equivalents, by about 32% compared to the control (Bahadorani et al., 2008).

Taken together, the studies examining the effects of α-tocopherol in flies are inconsistent and do not show a uniform effect of vitamin E in these insects. Two studies reported a decrease in the lifespan of flies supplemented with vitamin E (Sohal et al., 1985; Zou et al., 2007), whilst Zou et al. found no significant effect of vitamin E on the lifespan of *A. ludens*. Similarly, Bahadorani et al. (2008) and our own experiments showed no significant effects on wild-type *Drosophila*. Both Driver and Georgeou as well as Kakkar et al. observed differing effects on lifespan according to the dose of vitamin E used (Driver and Georgeou, 2003).

2.4. Rodents

As vertebrates and mammals, rodents are far more complex organisms than the others included so far in this review. A number of studies have been conducted in rodents to elucidate the effects of vitamin E on lifespan; one in rats (Porta et al., 1980), and seven in mice (Blackett and Hall, 1981; Hsieh and Lin, 2005; Ledvina and Hodanova, 1980; Lipman et al., 1998; Morley and Trainer, 2001; Navarro et al., 2005; Selman et al., 2008). The main results of these eight studies are discussed below and summarized in Table 5.

2.4.1. Mice

Lipman et al. investigated whether initiating antioxidant supplementation during the late stage of a rodent's life is beneficial in terms of longevity. Eighteen month old mice were given DL-α-tocopherol acetate at a concentration of 500 μg/g or a control diet containing 30 μg/g of vitamin E. They found that the vitamin E group showed an increase in bodyweight of 0.07 mg/kg day^{-1} (based on the average food intake of the mice). However, whilst serum levels of α-tocopherol were significantly higher in supplemented mice, this did not lead to an improvement in longevity (Lipman et al., 1998).

Selman et al. induced oxidative stress in C57BL/6 mice by decreasing room temperature (7 °C). Four month old male and female mice were supplemented with α-tocopherol at either 22 μg/g or 550 μg/g. Supplementation had no effect on the rate compared to controls but significantly increased both the median and maximum lifespan in both sexes of mice. Median lifespan of α-tocopherol-supplemented mice was increased from 682 to 785 days (15.1%) compared to the control. Maximum lifespan,

Table 5
Vitamin E lifespan studies on rodents in chronological order.

Species	*n*	Form of vitamin E	Dose	Age at initiation of treatment	Mean lifespan	Maximum lifespan	Study
Mouse (female)	52	DL-α-Tocopherol acetate	4400 μg/g	6.5 weeks	+13.8 days (n.s.)	+267 days (n.s.)	Ledvina and Hodanova (1980)
Rat (Wistar, male)	376	DL-α-Tocopherol	2000 μg/g	After weaning	n.s. effect	n.s. effect	Porta et al. (1980)
Mouse (CH_3/LAF_1)	192	DL-α-Tocopherol	2500 μg/g	5 weeks	–	n.s. effect	Blackett and Hall (1981)
Mouse (C57BL/6)	153	DL-α-Tocopherol acetate	470 ppm	18 months	n.s. effect #	n.s. effect	Lipman et al. (1998)
Mouse (Balb/c)	399	–	20 μg/g 400 μg/g 4000 μg/g	Before birth	n.s. effect # n.s. effect # n.s. effect #	n.s. effect n.s. effect n.s. effect	Morley and Trainer (2001)
Mouse (male)	90	DL-RRR-α-Tocopherol	5000 μg/g	28 weeks	+168 days #	+140 days	Navarro et al. (2005)
Mouse (MRL/lpr)	88	All-rac-α-tocopherol acetate	50 μg/g 250 μg/g 375 μg/g 500 μg/g	3 months	No effect +36 days −20 days −15 days	– – – –	Hsieh and Lin (2005)
Mouse (C57BL/6)	87	α-Tocopherol	550 μg/g	4 month	+103 days #	–	Selman et al. (2008)

–, data is not available; n.s., not significant; #, data are given as median.

determined as the age of the oldest 10% in each cohort, was significantly increased from 834 to 945 days (5.7%) in the α-tocopherol group compared to the control. The content of α-tocopherol in the liver was significantly increased in supplemented animals but, in accordance with the findings from Hsieh and Lin (2005) (see below), the levels of TBARS and the amount of oxidative damage to DNA were not affected by the higher amount of α-tocopherol. Additionally, decreasing the temperature in this experimental setting could also have induced incidents other than oxidative stress.

Macroarray analysis revealed that the expression of several hepatic genes was elevated in α-tocopherol-treated animals, including seven genes encoding cytochrome p450 enzymes and genes encoding enzymes associated with phase II detoxification. However, Selman et al. found no significant increase in the expression of genes encoding enzymes involved in cellular DNA repair or antioxidant defense, such as SOD and catalase (Selman et al., 2008).

Ledvina and Hodanova conducted experiments in female mice of the C3 strain. α-Tocopherol acetate solubilized in 1 ml of ether and 1 ml of ethanol was added to the diet at a concentration of 4.4 mg/g. The diet also contained 13.6 mg/g of sunflower oil. A second experimental subgroup was fed on a diet containing sunflower oil with an approximately equal iodine number of 127–136, at a concentration of 12.0 mg/g and solubilized in the same manner as the α-tocopherol diet. Animals commenced these diets at 46 days of age. Food intake and body weight were significantly altered by the treatments. Up to day 130, the mice ingested about 15.4 mg of α-tocopherol acetate, dropping to 13.6 mg from day 131 onwards due to decreased food consumption. Mean and maximum lifespan were higher in the α-tocopherol supplemented animals compared to control subgroups, yet this did not reach significance. Mean and maximum lifespan for the α-tocopherol supplemented subgroup, the sunflower oil subgroup and the control were 704.2 ± 209 and 1200 days, 620.4 ± 217.2 and 1078 days as well as 690.4 ± 168.1 and 933 days, respectively (Ledvina and Hodanova, 1980).

A comparative approach on the effects of vitamin E on the lifespan of two strains of mice, the CH_3H/He and LAF_1 strains, was taken by Blackett and Hall. These two strains differ in their predisposition for tumor development; the CH_3H/He strain having a higher tendency than the LAF_1 strain. The mice were supplemented with 2500 μg/g DL-α-tocopherol starting at 5 weeks of age. Maximum longevity in α-tocopherol-supplemented mice of both strains was not significantly altered compared to control animals. However, there was a significant increase in mean lifespan of mice administered DL-α-tocopherol compared to the control, mainly caused by a decreased death rate prior to 24 month of age. α-Tocopherol treatment also decreased the number of fatal tumors by 33.3% compared to the control. Furthermore, the predisposition of the CH_3H/He strain to develop more tumors was abolished in the supplemented subgroup. Furthermore, the content of lipofuscin in the murine heart muscles was decreased by α-tocopherol by 28% in CH_3H/He mice and by 36% in LAF_1 mice compared to the respective controls, indicating an antioxidant effect of DL-α-tocopherol in these mice at the high concentration of 2500 μg/g (Blackett and Hall, 1981).

Morley and Trainer evaluated the effects of supplementing with vitamin E straight after conception in Balb/c mice. Although serum concentrations of vitamin E increased dose dependently with 20, 400 and 4000 μg/g of vitamin E supplementation, the median lifespan values did not significantly change with different vitamin E concentrations or differ from controls (804, 830 and 802 days for female offspring supplemented with 20, 400 and 4000 μg of vitamin E per g of food, respectively) (Morley and Trainer, 2001).

Another study involved continuously supplementing adult CD-1/UCadiz mice with 5000 μg/g DL-α-tocopherol from 28 weeks of age. The mice were monitored for their lifespan, neuromuscular and cognitive performance (tightrope and maze tests). The control diet contained 29 μg/g of DL-α-tocopherol. Median and maximum lifespan of supplemented male mice increased significantly from 61 ± 4 to 85 ± 4 weeks (39.3%) and from 116 ± 4 to 136 ± 4 weeks (17.2%), respectively. Median and maximum lifespan of female mice also increased from 78 ± 4 to 88 ± 5 weeks (12.8%) and 148 ± 4 to 155 ± 4 weeks (6.9%), respectively, but these changes were not significant. Furthermore, α-tocopherol-supplemented male mice demonstrated significantly enhanced neuromuscular and cognitive performance at 50 and 76 weeks of age. The levels of protein carbonylation products and TBARS in the liver and brain

were significantly decreased in α-tocopherol-supplemented mice at 76 weeks of age. α-Tocopherol also improved the age-associated reduction in activity of several protective enzymes, including nitric oxide synthase and SOD, in aging mice (Navarro et al., 2005).

Hsieh and Lin supplemented MRL/lpr mice that suffer from an autoimmune disease and are susceptible to die within the first 6 month of age with all-rac-α-tocopherol acetate at concentrations of 250, 375, and 500 μg/g. Control food contained 50 μg/g all-rac-α-tocopherol acetate. α-Tocopherol supplementation had no effect on feeding behavior and body weight of mice. They found that mice fed 250 μg/g of all-rac-α-tocopherol acetate lived significantly longer than mice supplemented with 500 μg/g all-rac-α-tocopherol acetate (213 ± 76 days vs. 157 ± 49 days, respectively), and also longer than control animals (177 ± 45 days) and those fed 375 μg/g all-rac-α-tocopherol acetate (162 ± 57 days). The higher doses of all-rac-α-tocopherol acetate led to significantly increased levels of the vitamin E in the liver, kidney and plasma but did not reduce the levels of TBARS in these tissues (Hsieh and Lin, 2005).

2.4.2. Rats

Porta et al. studied the effects of DL-α-tocopherol on the lifespan of male rats that were fed a diet enriched with 15% of either coconut oil (rich in saturated fatty acids), safflower oil (rich in unsaturated fatty acids) or both oils combined from weaning. DL-α-Tocopherol was added at a concentration of either 20 μg/g or 2000 μg/g but, due to the different amounts of vitamin E in the oils, the final concentration was increased by 0.7 μg/g in the coconut oil diet, 5.2 μg/g in the safflower oil diet and by 3.4 μg/g in the oil combination diet. Rats fed on the coconut oil-containing diet had significantly reduced body weights soon after initiation of the diets. The mean and maximum lifespan of rats were not significantly affected by the different amounts of unsaturated fatty acids in the diets or by DL-α-tocopherol supplementation. The 50% survival time (the day at which only 50% of a subgroup was still alive) was significantly increased in the group receiving 2000 μg/g of DL-α-tocopherol and safflower oil compared to all other experimental subgroups and the control (median lifespan in days ± S.E.M. 594.7 ± 35.6 vs. control 600.1 ± 30.6; mean lifespan ± S.E.M. 739.5 ± 34.1 vs. 724.7 ± 29.3; maximum lifespan ± S.E.M. 1013 ± 50.0 vs. 1037 ± 63.1). This beneficial effect was related to a delay in the onset, as well as a reduction in the incidence, of malignant neoplasms. The 50% survival time was also increased in rats fed on the combined oil diet supplemented with 20 μg/g DL-α-tocopherol compared to the subgroups supplemented with the same concentration of vitamin E but in different oils (Porta et al., 1980). This indicates that the amount of unsaturated fatty acids in the oils may influence longevity.

Taken together, these studies in rodents show inconsistent effects of vitamin E on longevity. Whilst three studies demonstrated that vitamin E supplementation improves lifespan (Blackett and Hall, 1981; Navarro et al., 2005; Selman et al., 2008), one study failed to show significant lifespan effects but did reveal a significant increase in the time at which 50% animals were still alive (Porta et al., 1980). Another study found divergent effects with different doses of vitamin E with a low concentration increasing lifespan and a higher concentration reducing it (Hsieh and Lin, 2005). Three other studies found no significant effect of vitamin E administration on longevity (Ledvina and Hodanova, 1980; Lipman et al., 1998; Morley and Trainer, 2001).

3. Discussion

The potential benefits of vitamin E on health and lifespan have been intensely investigated and this review article focuses on the studies carried out in model organisms. This appraisal clearly highlights that vitamin E has inconsistent effects on longevity in single-celled organisms, rotifers, nematodes, flies and rodents.

In accordance with the studies in model organisms described herein, studies in humans have yielded similarly inconsistent results regarding the beneficial effects of vitamin E, with randomized clinical trials reporting positive, negative and no effect depending on the outcome measured (Abner et al., 2011; Clarke et al., 2008; Dietrich et al., 2006; Mustacich et al., 2007; Traber and Atkinson, 2007). Because of its properties as a lipid-soluble antioxidant and membrane protector, vitamin E was assumed to ameliorate or prevent arteriosclerosis and thereby promote healthy aging. However, many well-designed randomized placebo-controlled studies with sufficient power to detect clinical events, such as HOPE, GISSI and PPP, have demonstrated a null effect of vitamin E in the treatment or prevention of coronary heart disease (Kritharides and Stocker, 2002; Mindlen et al., 1996; Valagussa et al., 1999). However, two secondary prevention studies, CHAOS and SPACE, suggest that vitamin E supplementation may be beneficial to coronary artery disease in certain sub-populations, although these studies were small with relatively short follow up (Boaz et al., 2000; Stephens et al., 1996). In the Women's Health Study on vitamin E prevention of cardiovascular disease it was shown that in spite of slightly decreasing cardiovascular mortality, vitamin E supplementation could not lower total mortality (Lee et al., 2005). A large meta-analysis by Miller et al. (2005) even comes to the conclusion that vitamin E supplementation at high doses may elevate the all-cause mortality. However, whilst the authors state that the high-dose trials were often carried out in patients with chronic diseases thereby making the analysis of the mortality data more complicated, a more recent meta-analysis concludes that vitamin E supplementation including high doses does not affect overall mortality (Abner et al., 2011). The inconsistent results from large and smaller scale human studies investigating the potential benefits of vitamin E supplementation clearly warrant the studies in model organisms in which confounding factors can be much more easily controlled. Yet this review highlights that the discrepant results prevail in studies using model organisms.

These inconsistencies are likely to be attributable to a number of factors. For example, there may be species–species differences and the complexity of the model organism may be an important determinant of the function of vitamin E. However, a number of distinct studies in the same species, including rodents and humans, have reported contradictory results. The dose of vitamin E used in the studies differs greatly and many studies have shown a beneficial effect of vitamin E at lower concentrations with higher doses exhibiting adverse effects on lifespan (Adachi and Ishii, 2000; Driver and Georgeou, 2003; Hsieh and Lin, 2005; Kakkar et al., 1996; Lam et al., 2010; Sawada and Enesco, 1984; Zuckerman and Geist, 1983). Unfortunately, when using non-mammalian model organisms it is difficult to categorize the amounts used according to high and low doses because of a lack of daily dose recommendations making it difficult to deduce a dose-dependent effect of vitamin E on lifespan in flies and worms. Nevertheless, very high vitamin E concentrations have been used in many human studies which may account for the lack of beneficial effect observed (Kritharides and Stocker, 2002).

3.1. Vitamin E as an antioxidant

Deducing a possibly life-prolonging effect of vitamin E because of its antioxidant properties seems to have been a too rapid conclusion. Recently we have shown that in rodents, both vitamin E in the liver and biomarkers of oxidative stress increased with age, arguing against the hypothesis that a higher vitamin E status in older organisms could attenuate oxidative damage (Bayram et al., 2012). Additionally, although oxidative stress accompanies the aging

process it is not necessarily its underlying cause and promoting the organism's stress resistance rather than simply lowering ROS levels seems to be the better strategy to slow down aging (Schneider et al., 2011). Schulz et al. demonstrated that increased mitochondrial ROS formation, as a consequence of reduced glucose availability, induces catalase activity and enhances oxidative stress resistance in *C. elegans*. Their results provide evidence for the mitochondrial hormesis (or mitohormesis) theory of aging, which leads to an increase in overall life expectancy. Indeed, they showed that treatment of nematodes with various antioxidants and vitamins had the unexpected effect of decreasing lifespan (Schulz et al., 2007). Consistent with a counteracting role in ROS-induced hormesis, the antioxidant vitamin E prevented the health-promoting effects of exercise in young men (Ristow et al., 2009). It is well known that exercise, in spite of promoting healthy aging and health in general, leads to increased ROS formation (Powers and Jackson, 2008). In line with the "mitohormesis" concept (Schulz et al., 2007), exercise-induced mitochondrial ROS led to induction of endogenous ROS defense. However, with vitamin E supplementation this up-regulation of the anti-oxidative resistance was blocked (Ristow et al., 2009). Due to its antioxidant properties, vitamin E may abolish this increase in stress resistance (such as the induction of endogenous antioxidant enzymes), accounting for the reduced lifespan observed in many of the studies in model organisms described above.

Although in mice the antioxidant properties of vitamin E were shown to be independent of transcriptional events (Li et al., 2012), vitamin E seems to induce Nrf2 (Feng et al., 2010). Nrf2 in mammals (or its homologue SKN-1 in *C. elegans*) is a well known longevity-promoting transcription factor (Tullet et al., 2008) with its target genes encoding for antioxidant proteins and molecules that are involved in xenobiotic metabolism (Copple et al., 2008). Vitamin E could induce Nrf2 directly through increased Nrf2 expression and translocation to the nucleus as suggested by (Feng et al., 2010) or more indirectly by protecting the cell from Nrf2 inhibitors. This mechanism was elucidated in another study with an asthma model in which allergens caused inflammation and suppressed Nrf2 in alveolar macrophages from asthmatics. Administering tocopherol to these patients could attenuate the allergen-induced Nrf2 inhibition (Dworski et al., 2011). It is also possible that vitamin E activates other transcription or longevity factors. The deacetylase sirt1 was shown to have a beneficial effect on aging (Corbi et al., 2012). Sirt1 is a regulator of FoxO transcription factors (Brunet et al., 2004) that are the mammalian homologues of daf-16, a well known longevity gene in *C. elegans* (Lin et al., 1997). While there are still very few available data on the effect of vitamin E on sirtuins or FoxOs, a vitamin E analog, α-tocopheryl succinate was shown to activate FoxO1 (Valis et al., 2011). In the light of a possible effect of vitamin E on lifespan and the emerging evidence of its influence on gene expression (Azzi, 2007), further studies on the interplay of vitamin E and its cellular targets are needed to understand how this vitamin acts in the organism.

3.2. Opposing functions of the tocopherol isoforms and interaction with vitamin K

A further possible reason for the different outcomes observed in the studies in this review may be due to the opposing regulatory functions of the different vitamin E isoforms. Indeed, as described above, Zou et al. reported that whilst low dose α-tocopherol had a positive effect on the lifespan of flies of the species *D. melanogaster*, the same dose of γ-tocopherol had the opposing effect by reducing lifespan (Zou et al., 2007). In fact, other studies have also reported contradictory effects of the two isoforms of tocopherols in vitamin E. Some of these studies showed that tocopherols can act as pro- or anti-inflammatory agents in endothelial cells which could explain the different findings in vitamin E trials on cardiovascular disease. On the one hand, Jiang et al. (2000) describe that γ-tocopherol, in contrast to α-tocopherol, decreased the synthesis of pro-inflammatory prostaglandin E2 by inhibiting the cyclooxigenase 2 (COX-2). On the other hand, Bernikovs et al. found α-tocopherol as inhibiting inflammation by regulating endothelial cell signals during leukocyte recruitment. At a 10% γ-tocopherol/α-tocopherol ratio which is similar to the concentrations physiologically found in humans, γ-tocopherol could abolish this anti-inflammatory effect (Berdnikovs et al., 2009). Dietary vitamin E sources contain mostly α- and γ-tocopherols with γ-tocopherol being more present in US-American diets than in European diets (Wagner et al., 2004). In the body, tocopherol is transported from the liver via very low density lipoproteins (VLDLs). Incorporation into the lipoproteins happens via the α-tocopherol transfer protein (α-TTP) which has a higher affinity to α-tocopherol albeit transferring around 10% γ-tocopherol (Traber and Kayden, 1989; Wolf, 2006). Therefore α-tocopherol supplementation is likely to decrease γ-tocopherol bioavailability and the extent up to which the α-/γ-tocopherol rates change also depends on the type of vitamin E that is consumed with the diet.

As a side effect of vitamin E supplementation, increased bleeding has been reported. Farley et al. gave a possible explanation for this phenomenon by showing that menaquinone (vitamin K) levels are decreased by high vitamin E tissue concentrations (Farley et al., 2012). In the context of lifespan extension it is especially interesting to note that vitamin K can extend lifespan in *C. elegans* (Hunt et al., 2011). A putatively negative effect on longevity with vitamin E supplementation could therefore be due in part to vitamin E ousting the lifespan-prolonging vitamin K from the tissues.

Unfortunately, in most studies the vitamin E levels in the model organisms were not studied and so the amount of the supplemented vitamin E which was bioavailable cannot be given. This is especially relevant in the case of tocopherol acetate supplementation because the acetate needs to be hydrolyzed in vivo to exert anti-oxidative effects. Therefore, an analysis of vitamin E tissue levels should be conducted in future studies.

3.3. Adverse effects of vitamin E by induction of drug metabolism

α-Tocopherol with γ-tocopherol and α-tocopherol with vitamin K interactions do not seem to be the only type of interactions for vitamin E. Similar to many prescription drugs, all forms of vitamin E are metabolized by Phase I cytochrome P 450 (CYP) enzymes in the liver (Sontag and Parker, 2002) and seem to be conjugated by Phase II enzymes before Phase III excretion (Brigelius-Flohe, 2007). In mice, α-tocopherol was shown to induce Phase I CYP expression (Kluth et al., 2005). Contrarily, another report states that there is no up-regulation of CYP enzymes in vivo and no increased mRNA expression of known CYP drug metabolizers in vitro after administration of α-tocopherol (Hundhausen et al., 2005). In a further study, varying results were found with regard to vitamin E induced CYP up- or down-regulation (Traber et al., 2011). However, it seems that Phase II conjugation enzymes (Feng et al., 2010) and Phase III hepatic transporters ABCB1b and ABCG2 (Traber et al., 2011) are induced upon vitamin E supplementation. Considering the finding that vitamin E is eliminated from the organism like xenobiotics, it seems possible that it decreases the plasma levels of co-administered drugs by inducing their enzymatic degradation (Brigelius-Flohe, 2007). This concern is highly relevant considering the fact that in many studies the participating individuals suffer from diseases and are accordingly on medication (Kritharides and Stocker, 2002; Mindlen et al., 1996; Stephens et al., 1996; Valagussa et al., 1999). Under such circumstances vitamin E could interfere

with the treatment of these diseases and thereby negatively affect the lifespan of the patients.

4. Conclusion

Taken together, these studies in model organisms described herein together with published studies in humans have failed to provide compelling evidence for either a beneficial or a detrimental effect on vitamin E on lifespan. However, the interactions of vitamin E with other vitamins and xenobiotics, the possible counteraction against beneficial, ROS-induced hormesis by the antioxidant and the question whether administration of vitamin E decreases oxidative damage in healthy humans are arguments against high dose vitamin E supplementation until the scarce knowledge about the mechanisms of the action of vitamin E in general and of its distinct isoforms has been further elucidated. Moreover, considering the similarly contradicting data in model organisms and human trials, it is tempting to speculate that studies in model organisms could serve as anticipation platforms for human trials with vitamin E.

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References

Abner, E.L., Schmitt, F.A., Mendiondo, M.S., Marcum, J.L., Kryscio, R.J., 2011. Vitamin E and all-cause mortality: a meta-analysis. Current Aging Science 4, 158–170.

Adachi, H., Ishii, N., 2000. Effects of tocotrienols on life span and protein carbonylation in *Caenorhabditis elegans*. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 55, B280–B285.

Azzi, A., 2007. Molecular mechanism of alpha-tocopherol action. Free Radical Biology & Medicine 43, 16–21.

Bahadorani, S., Bahadorani, P., Phillips, J.P., Hilliker, A.J., 2008. The effects of vitamin supplementation on *Drosophila* life span under normoxia and under oxidative stress. Journals of Gerontology Series A: Biological Sciences and Medical Sciences 63, 35–42.

Bayram, B., Nikolai, S., Huebbe, P., Ozcelik, B., Grimm, S., Grune, T., Frank, J., Rimbach, G., 2012. Biomarkers of oxidative stress, antioxidant defence and inflammation are altered in the senescence-accelerated mouse prone 8. Age, http://dx.doi.org/10.1007/s11357-012-9448-0 (Epub ahead of print).

Berdnikovs, S., Abdala-Valencia, H., McCary, C., Somand, M., Cole, R., Garcia, A., Bryce, P., Cook-Mills, J.M., 2009. Isoforms of vitamin E have opposing immunoregulatory functions during inflammation by regulating leukocyte recruitment. Journal of Immunology 182, 4395–4405.

Blackett, A.D., Hall, D.A., 1981. Vitamin E – its significance in mouse ageing. Age and Ageing 10, 191–195.

Boaz, M., Smetana, S., Weinstein, T., Matas, Z., Gafter, U., Iaina, A., Knecht, A., Weissgarten, Y., Brunner, D., Fainaru, M., Green, M.S., 2000. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. Lancet 356, 1213–1218.

Brigelius-Flohe, R., 2007. Adverse effects of vitamin E by induction of drug metabolism. Genes & Nutrition 2, 249–256.

Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., Hu, L.S., Cheng, H.L., Jedrychowski, M.P., Gygi, S.P., Sinclair, D.A., Alt, F.W., Greenberg, M.E., 2004. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303, 2011–2015.

Clarke, M.W., Burnett, J.R., Croft, K.D., 2008. Vitamin E in human health and disease. Critical Reviews in Clinical Laboratory Sciences 45, 417–450.

Copple, I.M., Goldring, C.E., Kitteringham, N.R., Park, B.K., 2008. The Nrf2–Keap1 defence pathway: role in protection against drug-induced toxicity. Toxicology 246, 24–33.

Corbi, G., Conti, V., Scapagnini, G., Filippelli, A., Ferrara, N., 2012. Role of sirtuins, calorie restriction and physical activity in aging. Frontiers in Bioscience (Elite Edition) 4, 768–778.

Dietrich, M., Traber, M.G., Jacques, P.F., Cross, C.E., Hu, Y., Block, G., 2006. Does gamma-tocopherol play a role in the primary prevention of heart disease and cancer? A review. Journal of the American College of Nutrition 25, 292–299.

Driver, C., Georgeou, A., 2003. Variable effects of vitamin E on *Drosophila* longevity. Biogerontology 4, 91–95.

Dworski, R., Han, W., Blackwell, T.S., Hoskins, A., Freeman, M.L., 2011. Vitamin E prevents NRF2 suppression by allergens in asthmatic alveolar macrophages in vivo. Free Radical Biology & Medicine 51, 516–521.

Enesco, H.E., Verdone-Smith, C., 1980. Alpha-tocopherol increases lifespan in the rotifer Philodina. Experimental Gerontology 15, 335–338.

Epstein, J., Gershon, D., 1972. Studies on ageing in nematodes IV. The effect of anti-oxidants on cellular damage and life span. Mechanisms of Ageing and Development 1, 257–264.

Farley, S.M., Leonard, S.W., Labut, E.M., Raines, H.F., Card, D.J., Harrington, D.J., Mustacich, D.J., Traber, M.G., 2012. Vitamin E decreases extra-hepatic menaquinone-4 concentrations in rats fed menadione or phylloquinone. Molecular Nutrition & Food Research 56, 912–922.

Feng, Z., Liu, Z., Li, X., Jia, H., Sun, L., Tian, C., Jia, L., Liu, J., 2010. Alpha-tocopherol is an effective phase II enzyme inducer: protective effects on acrolein-induced oxidative stress and mitochondrial dysfunction in human retinal pigment epithelial cells. The Journal of Nutritional Biochemistry 21, 1222–1231.

Harrington, L.A., Harley, C.B., 1988. Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*. Mechanisms of Ageing and Development 43, 71–78.

Hsieh, C.C., Lin, B.F., 2005. Opposite effects of low and high dose supplementation of vitamin E on survival of MRL/lpr mice. Nutrition 21, 940–948.

Hundhausen, C., Bosch-Saadatmandi, C., Augustin, K., Blank, R., Wolffram, S., Rimbach, G., 2005. Effect of vitamin E and polyphenols on ochratoxin A-induced cytotoxicity in liver (HepG2) cells. Journal of Plant Physiology 162, 818–822.

Hunt, P.R., Son, T.G., Wilson, M.A., Yu, Q.S., Wood, W.H., Zhang, Y., Becker, K.G., Greig, N.H., Mattson, M.P., Camandola, S., Wolkow, C.A., 2011. Extension of lifespan in *C. elegans* by naphthoquinones that act through stress hormesis mechanisms. PloS One 6, e21922.

Ikeda, S., Uchida, T., Ichikawa, T., Watanabe, T., Uekaji, Y., Nakata, D., Terao, K., Yano, T., 2010. Complexation of tocotrienol with gamma-cyclodextrin enhances intestinal absorption of tocotrienol in rats. Bioscience, Biotechnology, and Biochemistry 74, 1452–1457.

Ishii, N., Fujii, M., Hartman, P.S., Tsuda, M., Yasuda, K., Senoo-Matsuda, N., Yanase, S., Ayusawa, D., Suzuki, K., 1998. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394, 694–697.

Ishii, N., Senoo-Matsuda, N., Miyake, K., Yasuda, K., Ishii, T., Hartman, P.S., Furukawa, S., 2004. Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. Mechanisms of Ageing and Development 125, 41–46.

Jiang, Q., Elson-Schwab, I., Courtemanche, C., Ames, B.N., 2000. Gamma-tocopherol and its major metabolite, in contrast to alpha-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. Proceedings of the National Academy of Sciences of the United States of America 97, 11494–11499.

Kahn, M., Enesco, H.E., 1981. Effect of alpha-tocopherol on the lifespan of *Turbatrix aceti*. Age 4, 109–115.

Kakkar, R., Bains, J.S., Sharma, S.P., 1996. Effect of vitamin E on life span, malondialdehyde content and antioxidant enzymes in aging *Zaprionus paravittiger*. Gerontology 42, 312–321.

Kamal-Eldin, A., Appelqvist, L.A., 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31, 671–701.

Kashima, N., Fujikura, Y., Komura, T., Fujiwara, S., Sakamoto, M., Terao, K., Nishikawa, Y., 2012. Development of a method for oral administration of hydrophobic substances to *Caenorhabditis elegans*: pro-longevity effects of oral supplementation with lipid-soluble antioxidants. Biogerontology 13, 337–344.

Kluth, D., Landes, N., Pfluger, P., Muller-Schmehl, K., Weiss, K., Bumke-Vogt, C., Ristow, M., Brigelius-Flohe, R., 2005. Modulation of Cyp3a11 mRNA expression by alpha-tocopherol but not gamma-tocotrienol in mice. Free Radical Biology & Medicine 38, 507–514.

Kritharides, L., Stocker, R., 2002. The use of antioxidant supplements in coronary heart disease. Atherosclerosis 164, 211–219.

Lam, Y.T., Stocker, R., Dawes, I.W., 2010. The lipophilic antioxidants alpha-tocopherol and coenzyme Q10 reduce the replicative lifespan of *Saccharomyces cerevisiae*. Free Radical Biology & Medicine 49, 237–244.

Ledvina, M., Hodanova, M., 1980. The effect of simultaneous administration of tocopherol and sunflower oil on the lifespan of female mice. Experimental Gerontology 15, 67–71.

Lee, I.M., Cook, N.R., Gaziano, J.M., Gordon, D., Ridker, P.M., Manson, J.E., Hennekens, C.H., Buring, J.E., 2005. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. JAMA: The Journal of the American Medical Association 294, 56–65.

Li, G., Lee, M.J., Liu, A.B., Yang, Z., Lin, Y., Shih, W.J., Yang, C.S., 2012. The antioxidant and anti-inflammatory activities of tocopherols are independent of Nrf2 in mice. Free Radical Biology & Medicine 52, 1151–1158.

Lin, K., Dorman, J.B., Rodan, A., Kenyon, C., 1997. DAF-16: an HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. Science 278, 1319–1322.

Lipman, R.D., Bronson, R.T., Wu, D., Smith, D.E., Prior, R., Cao, G., Han, S.N., Martin, K.R., Meydani, S.N., Meydani, M., 1998. Disease incidence and longevity are unaltered by dietary antioxidant supplementation initiated during middle age in C57BL/6 mice. Mechanisms of Ageing and Development 103, 269–284.

Miller 3rd, E.R., Pastor-Barriuso, R., Dalal, D., Riemersma, R.A., Appel, L.J., Guallar, E., 2005. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Annals of Internal Medicine 142, 37–46.

Mindlen, F., Nordaby, R., Ruiz, M., Zavala, A., Guzman, L., Martinez, F., Diaz, R.R., Mackey, C., Marino, M., Romero, G., Zapata, G., Cuneo, C., Kawamura, T., Coelho, O., Massayochi, O., Braga, J., Labrunie, A., Bodanese, L., Manenti, E., Vitola, D., Nicolau, J., Kennedy, J.W., et al., 1996. The HOPE (Heart Outcomes Prevention Evaluation) Study: the design of a large, simple randomized trial of an angiotensin converting enzyme inhibitor (ramipril) and vitamin E in patients at high risk of cardiovascular events. Canadian Journal of Cardiology 12, 127–137.

Minogue, P.J., Thomas, J.N., 2004. An alpha-tocopherol dose response study in *Paramecium tetraurelia*. Mechanisms of Ageing and Development 125, 21–30.
Miquel, J., Binnard, R., Howard, W.H., 1973. Effects of DL-alpha-tocopherol in life span of *Drosophila melanogaster*. Gerontologist 13, pp. 37–37.
Morley, A.A., Trainer, K.J., 2001. Lack of an effect of vitamin E on lifespan of mice. Biogerontology 2, 109–112.
Mustacich, D.J., Bruno, R.S., Traber, M.G., 2007. Vitamin E. In: Litwack, G. (Ed.), Vitamin E: Vitamins and Hormones Advances in Research and Applications. , pp. 1–21.
Navarro, A., Gomez, C., Sanchez-Pino, M.J., Gonzalez, H., Bandez, M.J., Boveris, A.D., Boveris, A., 2005. Vitamin E at high doses improves survival, neurological performance, and brain mitochondrial function in aging male mice. American Journal of Physiology: Regulatory Integrative and Comparative Physiology 289, R1392–R1399.
Porta, E.A., Joun, N.S., Nitta, R.T., 1980. Effects of the type of dietary fat at two levels of vitamin E in Wistar male rats during development and aging. 1. Lifespan, serum biochemical parameters and pathological changes. Mechanisms of Ageing and Development 13, 1–39.
Powers, S.K., Jackson, M.J., 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiological Reviews 88, 1243–1276.
Rimbach, G., Minihane, A.M., Majewicz, J., Fischer, A., Pallauf, J., Virgli, F., Weinberg, P.D., 2002. Regulation of cell signalling by vitamin E. The Proceedings of the Nutrition Society 61, 415–425.
Rimbach, G., Moehring, J., Huebbe, P., Lodge, J.K., 2010. Gene-regulatory activity of alpha-tocopherol. Molecules 15, 1746–1761.
Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., Kiehntopf, M., Stumvoll, M., Kahn, C.R., Bluher, M., 2009. Antioxidants prevent health-promoting effects of physical exercise in humans. Proceedings of the National Academy of Sciences of the United States of America 106, 8665–8670.
Sawada, M., Enesco, H.E., 1984. Vitamin E extends lifespan in the short-lived rotifer *Asplanchna brightwelli*. Experimental Gerontology 19, 179–183.
Schneider, S.A., Schrader, C., Wagner, A.E., Boesch-Saadatmandi, C., Liebig, J., Rimbach, G., Roeder, T., 2011. Stress resistance and longevity are not directly linked to levels of enzymatic antioxidants in the ponerine ant *Harpegnathos saltator*. PloS One 6, e14601.
Schulz, T.J., Zarse, K., Voigt, A., Urban, N., Birringer, M., Ristow, M., 2007. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metabolism 6, 280–293.
Selman, C., McLaren, J.S., Mayer, C., Duncan, J.S., Collins, A.R., Duthie, G.G., Redman, P., Speakman, J.R., 2008. Lifelong alpha-tocopherol supplementation increases the median life span of C57BL/6 mice in the cold but has only minor effects on oxidative damage. Rejuvenation Research 11, 83–95.
Sohal, R.S., Allen, R.G., Farmer, K.J., Newton, R.K., Toy, P.L., 1985. Effects of exogenous antioxidants on the levels of endogenous antioxidants, lipid-soluble fluorescent material and lifespan in the housefly, *Musca domestica*. Mechanisms of Ageing and Development 31, 329–336.
Sontag, T.J., Parker, R.S., 2002. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. The Journal of Biological Chemistry 277, 25290–25296.
Stephens, N.G., Parsons, A., Schofield, P.M., Kelly, F., Cheeseman, K., Mitchinson, M.J., Brown, M.J., 1996. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 347, 781–786.
Thomas, J., Nyberg, D., 1988. Vitamin E supplementation and intense selection increase clonal life span in *Paramecium tetraurelia*. Experimental Gerontology 23, 501–512.
Traber, M.G., Atkinson, J., 2007. Vitamin E, antioxidant and nothing more. Free Radical Biology & Medicine 43, 4–15.
Traber, M.G., Kayden, H.J., 1989. Preferential incorporation of alpha-tocopherol vs gamma-tocopherol in human lipoproteins. The American Journal of Clinical Nutrition 49, 517–526.
Traber, M.G., Labut, E.M., Leonard, S.W., Lebold, K.M., 2011. Alpha-tocopherol injections in rats up-regulate hepatic ABC transporters, but not cytochrome P450 enzymes. Free Radical Biology & Medicine 51, 2031–2040.
Tullet, J.M., Hertweck, M., An, J.H., Baker, J., Hwang, J.Y., Liu, S., Oliveira, R.P., Baumeister, R., Blackwell, T.K., 2008. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. Cell 132, 1025–1038.
Valagussa, F., Franzosi, M.G., Geraci, E., Mininni, N., Nicolosi, G.L., Santini, M., Tavazzi, L., Vecchio, C., Marchioli, R., Bomba, E., Chieffo, C., Maggioni, A.P., Schweiger, C., Tognoni, G., Barzi, F., Flamminio, A.V., Marfisi, R.M., Olivieri, M., Pera, C., Polidoro, A., Santoro, E., Zama, R., Pagliaro, L., Correale, E., Del Favero, A., Loi, U., Marubini, E., Campolo, L., Casari, A., Di Minno, G., Donati, M.B., Galli, M., Gattone, M., Garattini, S., Mancini, M., Marino, P., Santoro, G.M., Scardulla, C., Specchia, G., Cericola, A., Di Gregorio, D., Di Mascio, R., Levantesi, G., Mantini, L., Mastrogiuseppe, G., Tucci, C., Mocarelli, P., Baldinelli, R., Ceriotti, F., Colonna, A., Cortese, C., Fortunato, G., Franzini, C., Gonano, F., Graziani, M.S., Investigators, G.I.-P., 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 354, 447–455.
Valis, K., Prochazka, L., Boura, E., Chladova, J., Obsil, T., Rohlena, J., Truksa, J., Dong, L.F., Ralph, S.J., Neuzil, J., 2011. Hippo/Mst1 stimulates transcription of the proapoptotic mediator NOXA in a FoxO1-dependent manner. Cancer Research 71, 946–954.
Wagner, K.H., Kamal-Eldin, A., Elmadfa, I., 2004. Gamma-tocopherol – an underestimated vitamin? Annals of Nutrition & Metabolism 48, 169–188.
Wolf, G., 2006. How an increased intake of alpha-tocopherol can suppress the bioavailability of gamma-tocopherol. Nutrition Reviews 64, 295–299.
Zou, S., Sinclair, J., Wilson, M.A., Carey, J.R., Liedo, P., Oropeza, A., Kalra, A., de Cabo, R., Ingram, D.K., Longo, D.L., Wolkow, C.A., 2007. Comparative approaches to facilitate the discovery of prolongevity interventions: effects of tocopherols on lifespan of three invertebrate species. Mechanisms of Ageing and Development 128, 222–226.
Zuckerman, B.M., Geist, M.A., 1983. Effects of vitamin E on the nematode *Caenorhabditis elegans*. Age 6, 1–4.