The Effects of Vitamin E on the Lifespan of *Caenorhabditis elegans* Strains with

Mitochondrial Mutations

By

Danika N. Kusuma

A PROJECT

Submitted to

Oregon State University

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Abstract approved:

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Although assays have been done on the effects of vitamin E alpha-tocopherol (α-tocopherol) on the lifespan of wildtype *C. elegans,* none have been done on the effects of the vitamin on mutant strains with mitochondrial mutations. By using a *mev-1* genetic mutant strain of *C. elegans* with higher levels of reactive oxygen species, the antioxidant effects of vitamin E on lifespan can be further elucidated. N2 wildtype and *mev-1* strains were exposed to 200 µg/mL concentrations of α-tocopherol. They were treated for different exposure times of 2 and 24 hours respectively, and were monitored for lifespan. The *mev-1* strain exhibited a shorter mean lifespan than N2 wildtype in all treatment groups. N2 wildtype exhibited a mean lifespan increase in the 2 hour exposure with α-tocopherol, but the result was not significant ($p=0.89$). The 2 hour *mev-1* treatment group exhibited a 21.7% increase in lifespan compared to the 2 hour *mev-1* control group ($p=0.023$). 24 hour treatment groups showed a general decrease in lifespan compared to the control groups, but the results were not significant. The α-tocopherol appears to have a greater impact on *mev-1* than N2 when the strain is exposed to α-tocopherol for 2 hours. This result further supports a possible link between free radical damage and aging.

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I understand that my project will become part of the permanent collection of Oregon State University,
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Danika N. Kusuma, Author

ACKNOWLEDGEMENTS

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INTRODUCTION

Free Radical Theory of Aging: Free Radicals, Reactive Oxygen Species

First proposed in 1957, the free radical theory suggests that aging occurs due to an accumulation of free radical damage over time (1). Free radicals, also known as reactive oxygen species (ROS), are atoms with unpaired electrons that are often implicated in tissue damage within the body (2). Damage by these oxidative chemical species includes strand breaks in DNA, modifications to lipoproteins, and the inactivation of certain enzymes (3-5). The oxidation processes of free radicals appear largely indiscriminate and ROS react with a wide variety of biological structures (6). Thus, the free radical theory supposes that when free radical damage is left untreated, accumulation of oxidative modifications to important biological structures results in the process known as aging (1). Several studies have shown a correlation between older-age organisms and higher levels of oxidative DNA, protein, and lipid damage (7). Whether oxidative damage plays a role in the aging process cannot be fully ascertained, however, the mechanism by which ROS oxidize biological molecules like lipids is well known (8).

It is believed that the mechanisms by which specific radicals react vary depending on the chemical species (2). ROS are highly reactive atoms and molecules due to the presence of unpaired electrons within their chemical makeup. Free radicals can be positively charged, negatively charged, or chemically neutral, and interact in a variety of

reactions including electron donation, electron acceptance, and addition reactions (9). The end effect of ROS reacting with another molecule is the modification of the aforementioned molecule. Because biological structures within the body such as DNA are highly specific in their chemical formula and arrangement, ROS can damage these structures in such a way that they can no longer carry out their intended functions. Free radical induced damage to DNA, such as the modification of sugars and bases, is well-documented (3,5). Some examples of free radicals include the superoxide anion radical (O_2^{-*}) and the hydroxyl radical (OH^*), which have been implicated in cardiac complications including reperfusion-induced cardiac stunning and reperfusion-induced arrhythmia in rat models (9).

ROS can be derived from both exogenous and endogenous sources. For example, free radicals are created from activities like smoking, from exposure to electromagnetic or ionizing radiation in the environment, and as an inadvertent byproduct of cell processes such as aerobic respiration (11, 2, 12). Many molecules in the human body including constituents of mitochondrial and other electron transport chains directly react with oxygen to create the superoxide radical (2, 13). About 1-3% of oxygen inhaled by a human becomes superoxide, which suggests that ROS are largely unavoidable (2).

Antioxidants

Although the body possesses several mechanisms, including superoxide dismutase enzymes, to combat the destructive effects of radicals (14), additional defenses in the form of antioxidants can also help prevent ROS damage. Antioxidants prevent the effects of free radicals by breaking down the free radical and reacting with the unpaired

electron before it has a chance to do any damage (2). When the antioxidant reacts with the free radical, it creates a stable molecule that will not react with biological structures. Antioxidants exist in many forms, including phenols, vitamin C and vitamin E (15, 16, 17).

Vitamin E

Vitamin E is a fat-soluble vitamin that includes a group of eight antioxidants: alpha-, beta-, gamma-, and delta- tocopherol; and alpha-, beta-, gamma-, and delta- tocotrienol (19). Vitamin E is found naturally in foods like nuts, seeds, and vegetable oils, but is also available as a supplement vitamin (19). Although gamma-tocopherol (ɣ-tocopherol) is the most common type of vitamin E found in the human diet, ɣ-tocopherol's role in the body is relatively unclear (19). This form of vitamin E is thought to have functions that can trap electrophilic mutagens in lipophilic compartments, and generate metabolites that may help natriuresis (18). Of the eight different types of vitamin E, alpha-tocopherol (α-tocopherol) is found in the highest levels within the blood and tissues of the human body (19). Most of the α-tocopherol taken in by the body is preferentially carried by alpha-tocopherol transfer protein (a-TTP) in the liver, which carries and incorporates the α-tocopherol to lipoproteins circulating in the blood (25). Due to the vitamin's hydrophobic nature, vitamin E is absorbed in the body using plasma lipoproteins such as lipid transfer proteins and lipases, receptor-mediated lipoprotein endocytosis, and selective lipid uptake (24). Because vitamin E is fat-soluble, α-tocopherol is best suited to prevent lipid peroxidation that may occur due to the susceptibility of fats to free radical damage (19).

Vitamin E plays an important role as an antioxidant, although it may also have cell-signaling properties that are not as well-known. For instance, alpha-tocopherol was shown to inhibit protein kinase C and the transcription of collagenase (20). Severe deficiency of vitamin E in the human diet appears to cause neurological symptoms including impaired coordination, muscle weakness, and damage to the retina of the eye. Although a deficiency of vitamin E causes deleterious effects, many individuals are recorded to have normal concentrations as low as 20 µmol/L in the blood (19). A higher intake of vitamin E is associated with lower risk of coronary heart disease in men, and skin protection from ultraviolet radiation in mice (21, 22). High dosages of vitamin E more than or equal to 400 IU/d, however, have been seen to increase all-cause mortality (24). Although several studies have been done to explore the effects of vitamin E intake on diseases such as cancer, diabetes, dementia, and cardiovascular disease to name a few, the vitamin's benefit to human health remains a hot debate amongst scientists today (19). Vitamin C has been seen to recover the antioxidant ability of vitamin E after the vitamin has been oxidized by ROS, and possibly plays a role in the pathway of regenerating oxidized vitamin E (23).

The antioxidant effects of α-tocopherol involve intercepting ROS before the radical has a chance to do any damage and have been documented *in vitro* and *in vivo* (26,27). The mechanism by which this takes place is well-known, and involves the reaction of α-tocopherol with ROS to form the relatively more stable tocopheroxyl radical, which can be recycled back to vitamin E or may react with other molecules to create stable products like α-tocopheryl-quinone (27).

The effects of vitamin E on lifespan have been studied in many experiments using a variety of organisms. A vitamin E study done on the model organism *Caenorhabditis elegans* (*C. elegans*) showed that 200 µg/mL vitamin E significantly prolonged *C. elegans* survival (28). Another experiment documented that vitamin E deficiency may cause premature aging in erythrocytes of rats (29). Although many studies have been made to test the effects of vitamin E on many other organisms as well as human diploid cells, the results are inconsistent, with vitamin E possibly increasing lifespan, decreasing lifespan, or having varying effects on the lifespan of different organisms (30-32).

Nematodes as a Model Organism

Caenorhabditis elegans (*C. elegans*) is a microscopic free-living roundworm and an excellent model organism for a variety of reasons. It is a eukaryotic multicellular organism with a genome size of 9.7 x 10^7 base pairs, which is relatively small compared to the human genome of about 3 billion base pairs (33). The *C. elegans* genome is completely sequenced and mapped for transcription factors (34). *C. elegans* are easily maintained in the laboratory cultured on petri dishes. *C. elegans* have a short generation time, and can lay their eggs and produce an entirely new generation of worms in as little as 4 days (35). Most *C. elegans* exist as hermaphrodites although a small, variable percentage of a population can be born male, depending on the strain (35). A hermaphrodite is an organism that produces both sperm and egg. *C. elegans* commonly exist as self-fertilizing hermaphrodites, so only one worm is needed to keep the population going (35). Each hermaphrodite produces about 300 mostly hermaphroditic progeny.

There are four main stages of growth for *C.elegans* that occur over as little as 2.5 days (35). The first stage is the L1 stage, which is the worm stage immediately after hatching from the egg and can be thought of as day 0 in the worm's life cycle. The next two stages, L2 and L3, are occasionally difficult to differentiate microscopically, but should exhibit a larger size in the L3 worms compared to L2 nematodes. When the nematode gets to the L4 stage, a distinct, oval-shaped patch can be seen on the side of the organism at about three-quarters down the worm's length. This patch indicates the differentiation of cells into the worm's ovaries. Finally, the worm reaches the adult stage and egg production, which typically continues for approximately 3 days or until the nematode's sperm and/or eggs are depleted (35). All of these traits make *C. elegans* a notable model organism, but most pertinent to this experiment is the typically short lifespan of 2-3 weeks (35).

Mev-1 Mutant Strain of *C. elegans*

The *mev-1* mutant strain of *C. elegans* has a mutation in the nuclear gene that encodes for the cytochrome b_{560} subunit of complex II in the mitochondrial electron transport chain (ETC) (36). This mutation interferes with the ability of the cytochrome b_{560} subunit to transfer electrons to ubiquinone during the conversion of succinate to fumarate (37). The *mev-1* mutant strain shows an increased production of superoxide anion, which leads to an increased level of ROS (36). The mutant also has a shorter life span than wildtype *C. elegans*, thought to be linked to the overproduction of ROS, although a causative effect cannot be determined (36).

Electron Transport Chains in Mitochondria

The ETC is found in the plasma membranes of bacteria (40,41) and, as mentioned previously, in the mitochondria of eukaryotic species. Regardless of where the ETC is found, it is made up of a series of electron carriers that are arranged according to their redox potentials (38). By passing electrons from a carrier with a higher redox potential to a carrier with a lower redox potential, the ETC creates energy for the cell to use (38).

A mitochondrion is a membrane-bounded organelle found in eukaryotic cells. By oxidizing food that is consumed, the mitochondria produce the energy needed by a cell (42). To do this, the mitochondria employ the aforementioned ETC. At the end of the ETC, the carried electrons are donated to oxygen. In some cases, the electron reacts incorrectly with the oxygen, which converts the oxygen into its radical form (38,39). Succinate, ubiquinone, and cytochrome b_{560} refer to specific electron carriers in the ETC. When the DNA that encodes for electron carriers in the ETC contains a mutation, the electron carriers may not be able to function properly, and may lead to an increase of ROS creation.

Experimental Design

Although assays have been done to explore the effect of vitamin E (α-tocopherol) on the lifespan of wildtype *C. elegans* (28), studies have not been carried out to examine the effects of vitamin E on *mev-1* mutant strains of *C. elegans.* The *mev-1* genetic mutant strain of *C. elegans* is documented to have higher ROS levels than the wildtype strain. By determining the effects of α-tocopherol on the lifespan of the *mev-1* strain, the

antioxidant abilities of α -tocopherol can be further examined. Although a positive correlation between high ROS levels and age has been established in past studies (7), it is still not known if oxidative damage is the cause for aging. If α -tocopherol affects lifespan in *mev-1* differently compared to wildtype *C. elegans*, a further link could potentially be established between ROS levels and lifespan. Additionally, vitamin E has been shown to have variable effects on lifespan (28-32). The experimental data on both N2 and *mev-1* strains of *C. elegans* could potentially be used to further elucidate the effects of α -tocopherol on lifespan.

HYPOTHESES

The results of this experiment expound upon two main hypotheses. The first is based on the free radical theory of aging: high levels of free radical damage result in a decrease in lifespan. This hypothesis is further supported if the *mev-1* strain, which is documented to have higher ROS levels, exhibits a shorter lifespan than N2 wildtype *C. elegans.* The second hypothesis is that vitamin E reduces ROS damage and increases lifespan. If the introduction of antioxidant α-tocopherol to *C. elegans* N2 wildtype and *mev-1* strains increases lifespan, this supports vitamin E's positive effect on lifespan with an emphasis on the vitamin's antioxidative effects.

MATERIALS AND METHODOLOGY

All of the materials in this experiment, except for the α-tocopherol working stock, were prepared according to standard *C. elegans* maintenance protocols as listed on WormBook (43). Brief descriptions of the experimental materials are included here, but for specific recipes, please refer to the appropriate protocol for *C. elegans* maintenance listed on WormBook (43).

Worm Strains and Culture Conditions

The *C. elegans* N2 and *mev-1* genetic mutant TK22 were thawed from Denver lab frozen stocks and left to propagate on a standard OP50-seeded NGM plate for approximately 7 days at 20°C (43). To ensure that all of the worms were the same age for recording lifespan, age-synchronized populations were established using standard bleaching procedures before the start of treatment exposures.

Vitamin E Treatments

A working stock of 2.0×10^5 µg/mL α-tocopherol was used in this experiment. This working stock solution consisted of 0.100 grams of α-tocopherol dissolved in 500 µL of 100% ethanol in a 1.5 mL Eppendorf tube. The tube was covered in aluminum foil to prevent the photo-degradation of α-tocopherol with exposure to light.

Populations of age-synchronized N2 wildtype and *mev-1* genetic mutant strains were treated with α-tocopherol and then monitored for lifespan. Because the α-tocopherol

stocks were suspended in 100% ethanol, a small concentration of 100% ethanol acted as a negative control treatment. For each of the two populations, there were two treatments, with α-tocopherol and with ethanol, and two exposure times, a 2 hour and a 24 hour, for a total of eight experimental groups (Table 1).

The NGM plates of age-synchronized populations of *N2* and *mev-1* strains were washed twice with 6 mL of M9 buffer, for a total of 12 mL per population, and collected into two separate 15 mL Falcon tubes. Next, the treatment tubes were prepared by transferring 3 mL of these worm suspensions and an additional 7 mL of M9 buffer into new 50 mL Falcon tubes, for a total of four tubes per population. Into each tube, 10 µL of a specific treatment were added to create a final concentration of 200 µg/mL α-tocopherol, and 0.1% ethanol.

The treatment tubes were put into a Styrofoam tray and strapped onto a Nutator for 2 hours or 24 hours. After 2 hours elapsed, the corresponding 2 hour exposure tubes were set on ice for ten minutes to pellet the worms at the bottom of the tube. The bottom 200 µL was pipetted onto seeded NGM plates. The same method was carried out for the 24 hour exposure groups.

The post-treatment worms were grown at 20°C and monitored daily until the worms reached the L4 stage of development. Then 24 individual L4 worms from each strain/treatment group were moved onto a fresh seeded NGM plate to grow alone. With 8 different treatment groups and 24 worms per group, a total of 192 individual worms were monitored for lifespan.

Strain	Negative control: 100% ethanol (10 microliters in 10 mL to make 0.1%) 2 hours	200 µg/mL alpha tocopherol 2 hours	Negative control: 100% ethanol (10 microliters in 10 mL to make 0.1%) 24 hours	200 µg/mL alpha tocopherol 24 hours
N2 wild type	24 worms	24 worms	24 worms	24 worms
Mev-1	24 worms	24 worms	24 worms	24 worms

Table 1. 24 worms taken from 8 treatment groups. A total of 192 worms were sampled and monitored for lifespan.

Lifespan Assay

Lifespan for each worm was recorded as the total number of days the worm lived since hatching from its egg, after the bleaching protocol. At the start of the experiment, all of the worms were recorded as day 0 as L1 larvae, the first developmental stage after they have hatched from an egg. Once a day, every one of the 192 individual worms was checked under the microscope for signs of mortality. Each worm was affirmed alive if the nematode exhibited a twitch response to the stimulus of a poke from a platinum wire. If the worm had not moved after ten seconds, the worm was declared dead.

Because *C. elegans* is a hermaphroditic species, the individual treatment worms needed to be separated from their offspring for lifespan monitoring. For approximately one week post- treatment, the target worm was moved away from its offspring and eggs onto a new seeded NGM plate when checking for mortality under a microscope. This was repeated for all 192 worms for as long as there were live offspring present on the plate.

Statistical Tests

Statistical differences between two conditions were first evaluated using t-tests and later ANOVA on Microsoft Excel®. T-tests were plotted with a two-tailed distribution under a two-sample unequal variance, where statistical differences with a p-value less than 0.05 were considered significant.

Analysis of variance (ANOVA) tests are statistical tests used to compare sample means (44). An ANOVA test can be used to infer whether sample distributions are significantly different from one another. The results of an ANOVA can help shed some light on the effects of different independent variables on a specific dependent variable (44).

RESULTS

Average Lifespan

Lifespan of the worms in each treatment group was measured as a number of days lived and then averaged to come up with a mean lifespan for a treatment group. Figures 1-4 plot the average lifespan of the worms in each treatment group. Figures 1 and 2 plot the data for the 2 hour and 24 hour time exposure respectively, and focus on comparing the average lifespan between control groups and vitamin E treated groups. Figures 3 and 4 also plot the data for the 2 hour and 24 hour time exposures respectively, but focus on the comparison between the average lifespan of N2 wildtype and *mev-1*. Significantly different results are denoted by an asterisk (*).
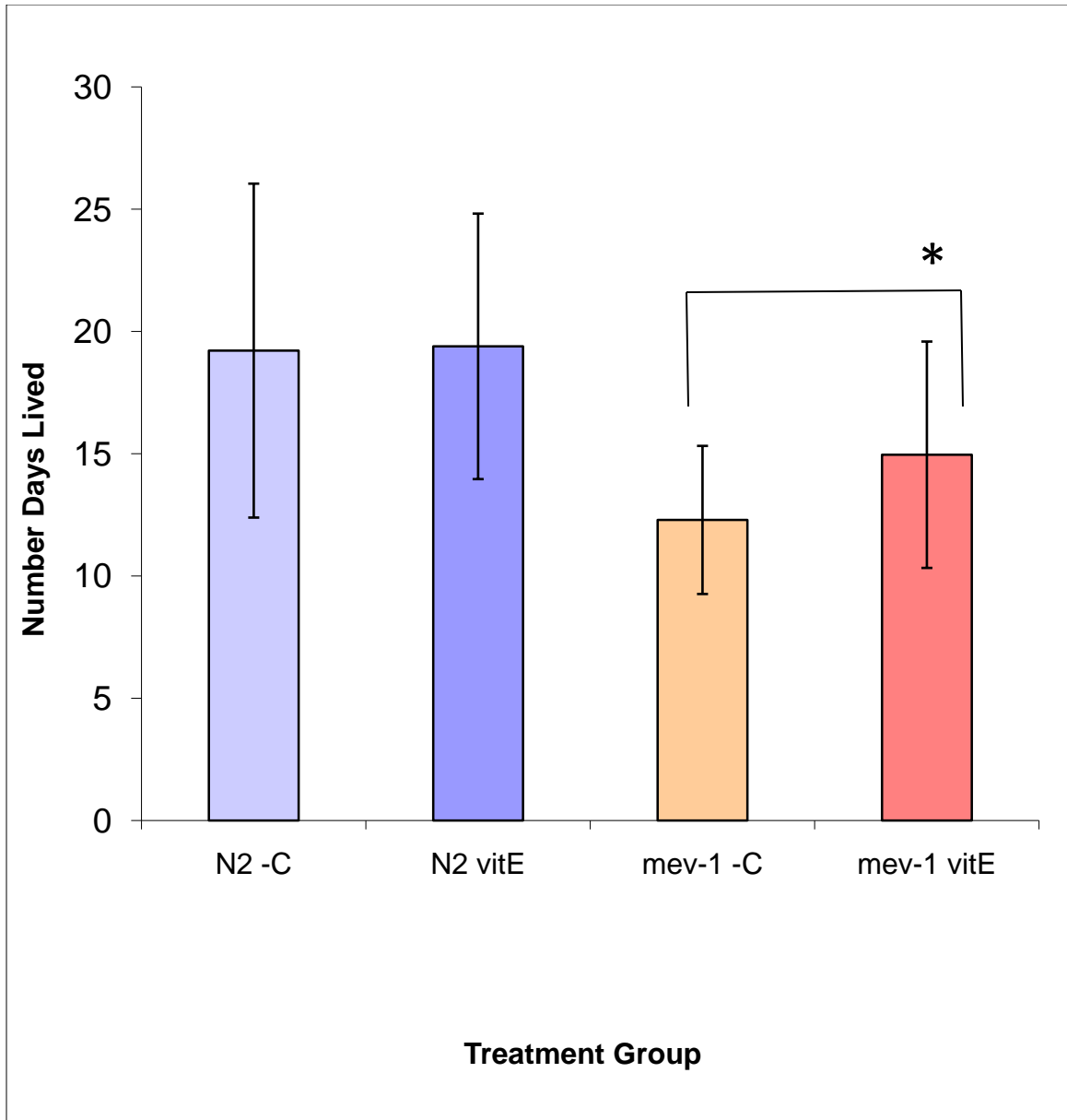
Figure 1. Comparison of mean lifespan between negative control and vitamin E treated within each strain shows an increase in lifespan when treatment exposure is 2 hours. There was a very slight increase in lifespan in the N2 wildtype vitamin E treated group compared to the N2 wildtype negative control, but the means are not significantly different. There is a distinct increase in lifespan in the *mev-1* vitamin E treated group compared to the *mev-1* negative control, and the means are significantly different (p-value<0.05) as denoted by the asterisk (*).
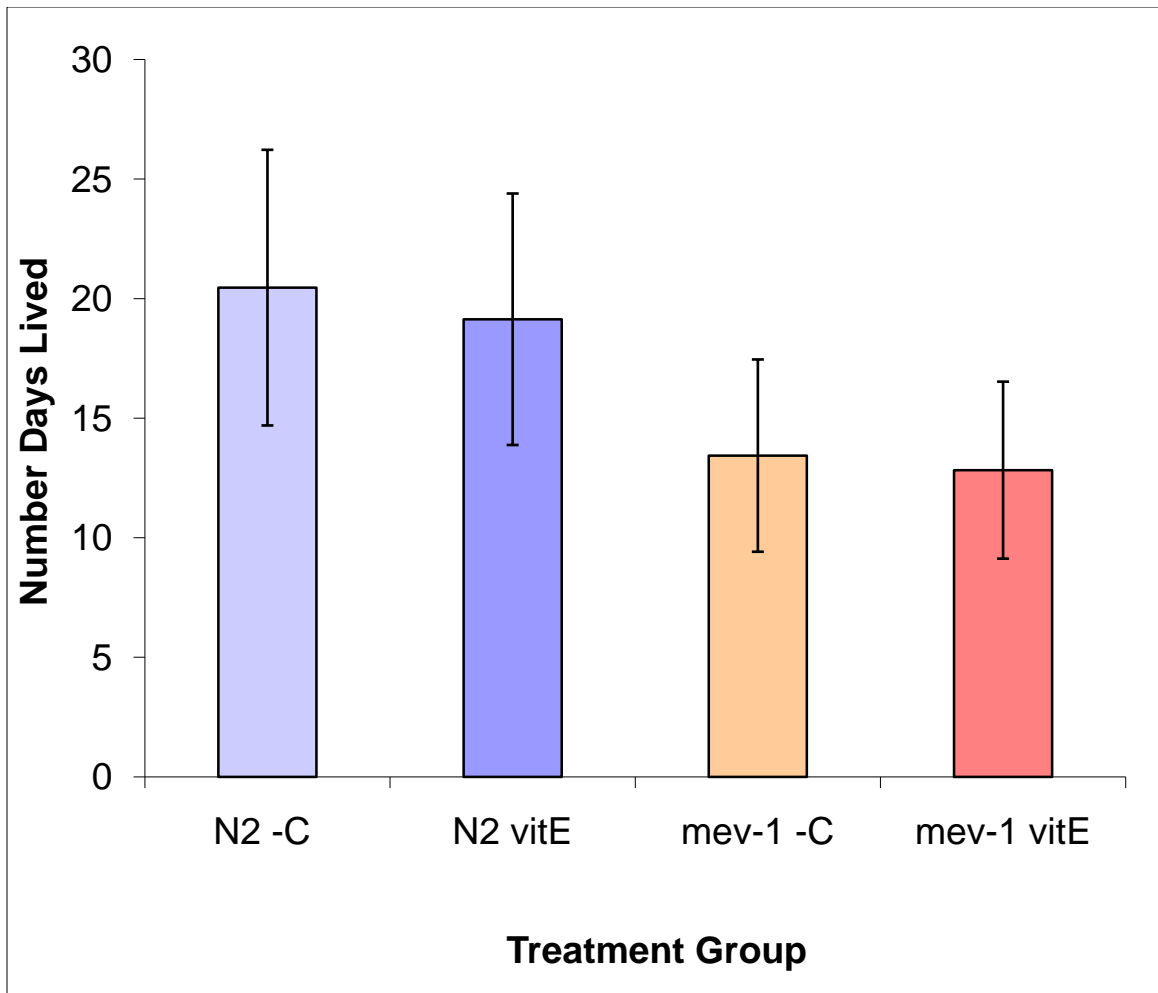
Figure 2. Comparison of mean lifespan between negative control and vitamin E treated within each strain shows a decrease in lifespan when treatment exposure is 24 hours. There is a slight decrease in lifespan in the N2 wildtype vitamin E treated group compared to the N2 wildtype negative control, but the means are not significantly different. There is a decrease in lifespan in the *mev-1* vitamin E treated group compared to the *mev-1* negative control, but the means are not significantly different.
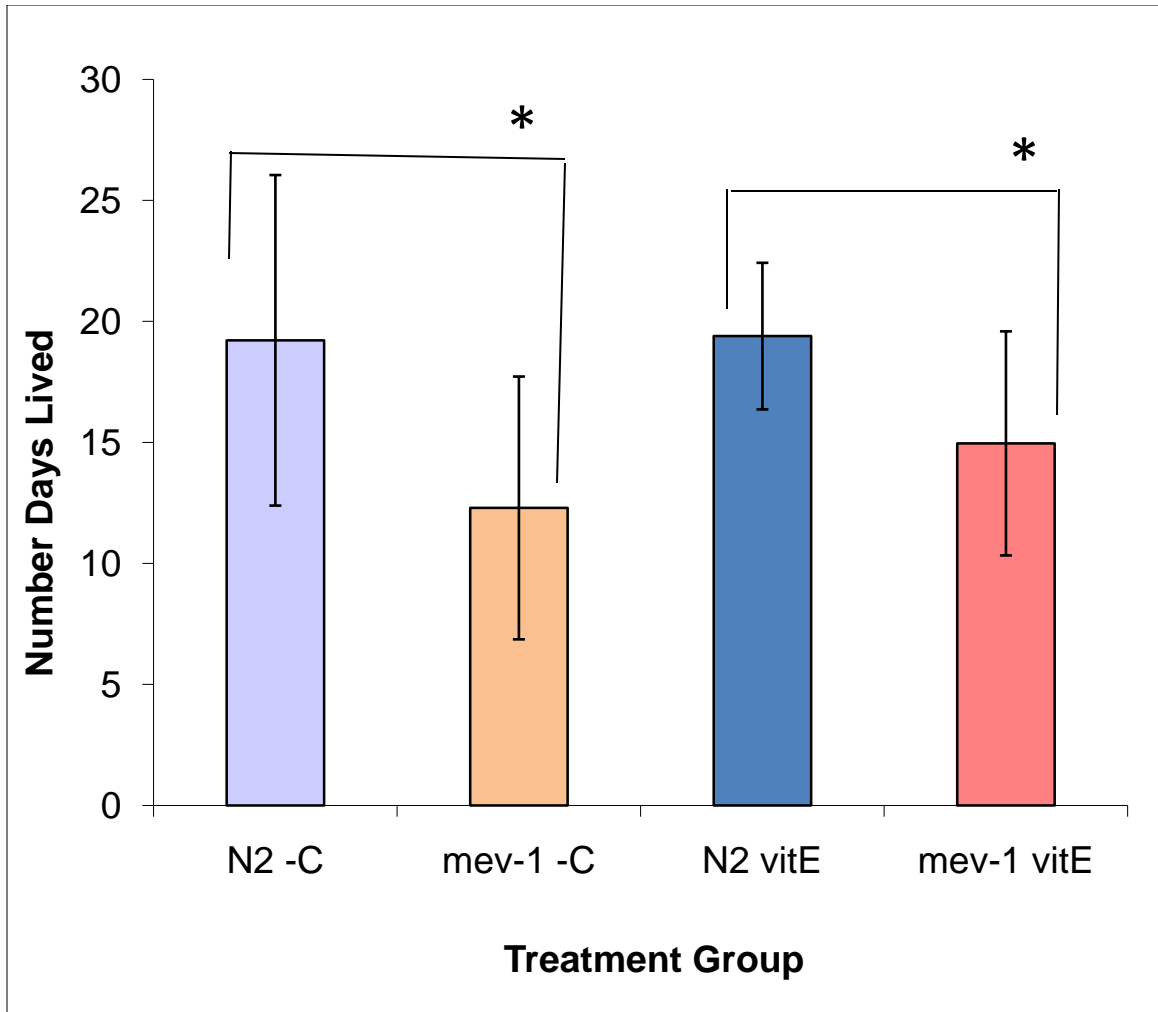
Figure 3. *mev-1* **exhibits a shorter average lifespan than** N2 **wildtype when treatment exposure is 2 hours.** The *mev-1* mutant strain exhibits a shorter lifespan than N2 wildtype in both the control groups and the vitamin E treatment groups. The means between N2 and *mev-1* groups are significantly different in both the negative control, and in the vitamin E treatment sample (p-value<0.05).
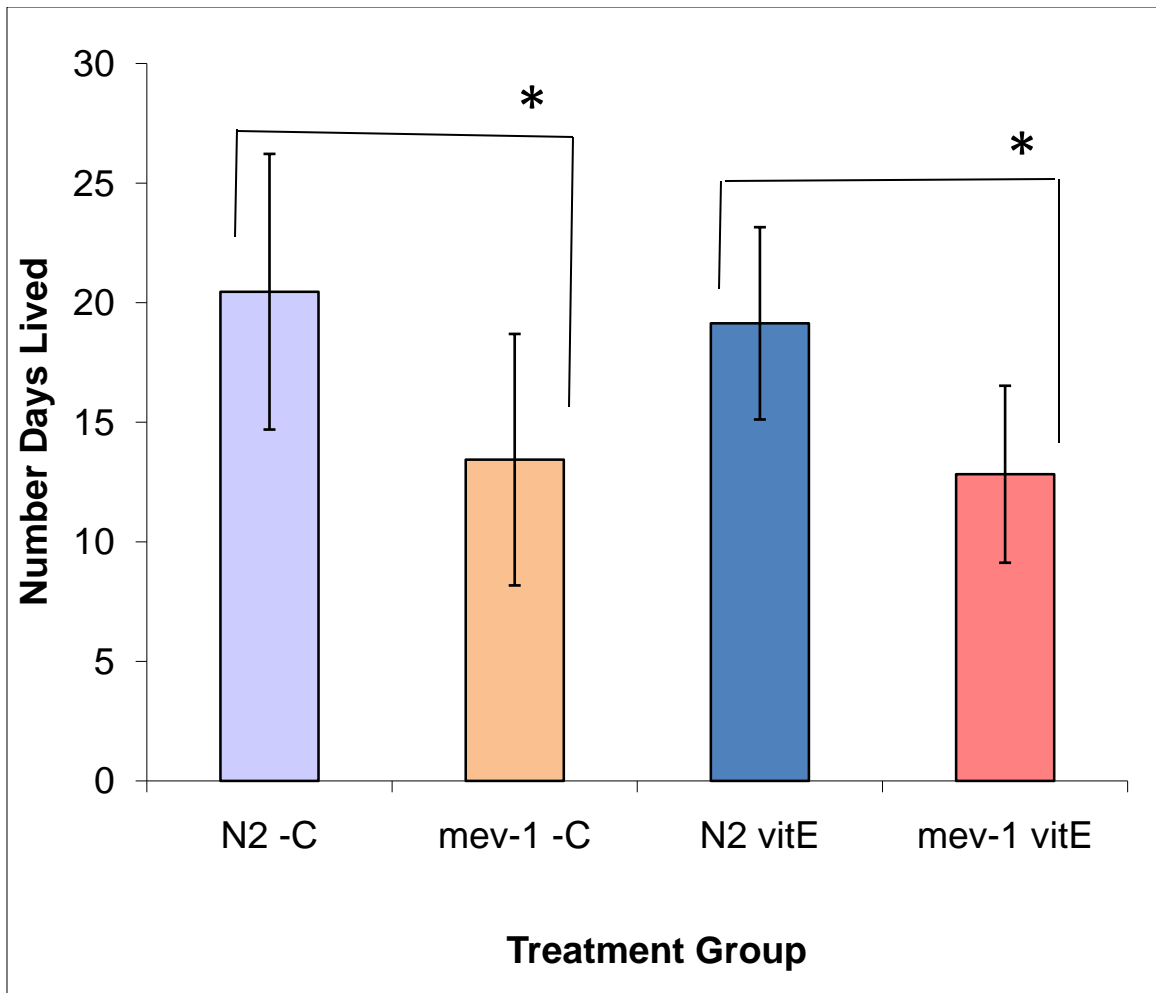
Figure 4. *mev-1* **exhibits a shorter average lifespan than** N2 **wildtype when treatment exposure is 24 hours.** The *mev-1* mutant strain exhibits a shorter lifespan than N2 wildtype in both the control groups and the vitamin E treatment groups. The means between N2 and *mev-1* groups are significantly different in both the negative control, and in the vitamin E treatment sample (p-value<0.05).

For the 2 hour vitamin E exposure (Figures 1 and 3), the mean lifespan for the N2 wildtype control group was 19.22 ± 6.83 days, while the mean lifespan for N2 after treatment with vitamin E was 19.39 ± 5.43 days. For *mev*-1, the mean lifespan for the control group was 12.29 ± 3.03 days, while the mean lifespan for the group treated with vitamin E was 14.96 ± 4.63 days.

For the 24 hour vitamin E exposure (Figures 2 and 4), the N2 wildtype had a mean lifespan of 20.46 ± 5.76 days, while N2 treated with vitamin E exhibited a mean lifespan of 19.14 ± 5.26 days. For *mev-1*, the mean lifespan of the control group was 13.43 ± 4.02 days while the group treated with vitamin E showed an average lifespan of 12.83 ± 3.70 days.

Statistical differences between two conditions were first evaluated using t-tests and later ANOVA on Microsoft Excel®. T-tests were plotted with a two-tailed distribution under a two-sample unequal variance, where statistical differences with a p-value less than 0.05 were considered significant. For the 2 hour exposure (Figure 1), the difference in average lifespan between vitamin E treated N2 and the N2 negative control was not significant (p=0.89), but the difference in mean lifespan of vitamin E treated *mev-1* and *mev-1* negative control was significant (p=0.023). In the 24 hour exposure (Figure 2), vitamin E treated groups and control groups were not statistically different (N2 p=0.42; *mev-1* p=0.59).

In both 2 hour and 24 hour exposure treatments, *mev-1* exhibited a shorter lifespan than the N2 wildtype. For the 2 hour exposure (Figure 3), *mev-1* had a shorter lifespan than N2 in the negative control groups (p=1.1×10^{-4}), and in the vitamin E

treated groups (p=3.4 × 10^{-3}). Similarly in the 24 hour exposure (Figure 4), *mev-1*

exhibited a shorter lifespan than N2 in the negative control groups (p=1.7 × 10^{-5}), and in

the vitamin E treated groups (p=4.2 × 10^{-5}).

ANOVA

In this experiment, an ANOVA was used to help determine whether lifespan

varied significantly between N2 wildtype and the *mev-1* mutant strains, given the type of

treatment or control that the strains were subjected to. Two separate two-way ANOVA

tests were run on the 2 hour treatment group, and the 24 hour treatment group

respectively in Microsoft Excel®. Excel® requires rows of data to be equal in number, so

to compensate for worms that went missing, the raw data closest to the average lifespan

was discarded, so as to not distort the mean calculated with ANOVA. Raw data before

alteration is featured in Appendix A. The results of the ANOVA tests are listed in Table 3

and Table 3 on the following pages.

Anova: Two-Factor With Replication

SUMMARY	Treatment 2 hr	Control 2 hr	Total
N2			
Count	23	23	46
Sum	448	442	890
Average	19.47826	19.21739	19.34783
Variance	30.80632	46.63241	37.87633
mev-1			
Count	23	23	46
Sum	344	283	627
Average	14.95652	12.30435	13.63043
Variance	22.40711	9.58498	17.43816
Total			
Count	46	46	
Sum	792	725	
Average	17.21739	15.76087	
Variance	31.24058	39.6971	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Strain Type	751.837	1	751.837	27.48172	1.08E-06	3.949321
Treatment Type	48.79348	1	48.79348	1.783537	0.18516	3.949321
Interaction	32.88043	1	32.88043	1.201871	0.275939	3.949321
Within	2407.478	88	27.35771			
Total	3240.989	91				

Table 2. ANOVA results for the 2 hour treatment exposure. (n=23) The results of the ANOVA test show that the difference in lifespan between N2 and *mev-1* strains is significant (p-value<0.05). The difference in lifespan between the negative control groups and vitamin E treatment groups is not significant, and there was not a significant interaction between strain type and treatment type that might have affected differences in lifespan.

Anova: Two-Factor With Replication

SUMMARY	Treatment 24 hr	Control 24 hr	Total
N2			
Count	22	22	44
Sum	421	451	872
Average	19.13636	20.5	19.81818
Variance	27.64719	36.2619	31.6871
mev-1			
Count	22	22	44
Sum	282	296	578
Average	12.81818	13.45455	13.13636
Variance	14.34632	16.92641	15.37632
Total			
Count	44	44	
Sum	703	747	
Average	15.97727	16.97727	
Variance	30.7204	38.67389	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	982.2273	1	982.2273	41.27794	7.57E-09	3.954568
Columns	22	1	22	0.924546	0.339044	3.954568
Interaction	2.909091	1	2.909091	0.122254	0.727478	3.954568
Within	1998.818	84	23.79545			
Total	3005.955	87				

Table 3. ANOVA results for the 24 hour treatment exposure. (n=22) The results of the ANOVA test show that the difference in lifespan between N2 and *mev-1* strains is significant (p-value<0.05). The difference in lifespan between the negative control groups and vitamin E treatment groups is not significant, and there was not a significant interaction between strain type and treatment type that might have affected differences in lifespan.

DISCUSSION

Independent Variables

Out of the various forms of vitamin E available, α-tocopherol was used in this experiment for several reasons. α-tocopherol is the most absorbed within the human body, compared to the other seven vitamin E antioxidants available in nature (18,19). Previous experiments found vitamin E α-tocopherol increased average lifespan of wildtype *C. elegans* (2). To establish a means for comparison between the effects of α-tocopherol on N2 wildtype and the *mev-1* mutant strains, this experiment treated both strains with vitamin E α-tocopherol.

Two treatment times, two hour and twenty-four hour exposures, were established for the experiment. The twenty-four hour exposure time was used in past studies of *C. elegans* α-tocopherol exposure (36). A second exposure time of two hours was added because twenty four hours was unusually long for the nematodes to be without food and to be constantly exposed to ethanol.

Mev-1* strain exhibits a shorter lifespan than N2 wildtype *C. elegans

In both the 2 hour and 24 hour exposures, *mev-1* consistently exhibited a shorter lifespan than N2 wildtype (Figures 3 and 4). This result supports the hypothesis that high levels of free radical damage result in a decrease in lifespan, and reconfirms the results of past experiments that have documented shorter lifespan in the *mev-1* strain compared to the lifespan of wildtype *C. elegans* (36,37). Because *mev-1* strains are documented to

have higher ROS levels than wildtype *C. elegans*, this result further supports the

hypothesis that ROS damage may be linked to aging.

2 hour exposures to α-tocopherol may increase lifespan in N2 wildtype and *mev-1*

A slight increase in average lifespan can be seen between the N2 negative control

and the N2 vitamin E treated groups. The means of these two groups, however, were not

significantly different, and thus the increase in lifespan seen in the N2 strain after a 2

hour exposure to α-tocopherol is not a statistically supported result. Increasing the

number of replicates in future experiments might potentially yield more significant

results.

For *mev-1*, a 2 hour exposure to α-tocopherol increased lifespan from

approximately 12.29 ± 3.03 days to 14.96 ± 4.63 days (p=0.023), which is an

approximately 21.7% increase in lifespan. If a human lifespan was increased

equivalently, someone living to 70 years old, might be able to live to 85 years old with a

vitamin E supplement. Based on the results from the worms, however, this would only be

true of humans with *mev-1*-like defects that increase their ROS levels. The discrepancy in

change of lifespan seen between the N2 wildtype and *mev-1* is interesting. The vitamin E

supplement seemed to have an increased positive effect on the lifespan of the *mev-1*

mutant strain compared to the N2 wild-type.

The ANOVA test shown in Table 3 helped to determine whether lifespan varied

significantly between N2 wildtype and the *mev-1* strain given a vitamin E or control

treatment, and if there was an interaction between strain type and treatment type.

Although the ANOVA test determined that there was no significant interaction between strain type and treatment type, the p-value showed some potential (p=0.18516). Because *mev-1* exhibited a greater lifespan increase than N2, there is a possibility that there is some sort of interaction between the strain type and treatment type. If this case were true, it would imply that the *mev-1* mutant strain is more susceptible to a vitamin E treatment.

24 hour exposures to α-tocopherol may decrease lifespan in N2 wildtype and *mev-1*

A 24 hour exposure to vitamin E appears to decrease lifespan in both N2 and *mev-1* (Figure 2), but the results were not significantly different. Although the decrease in lifespan is not significant, this trend could be linked to past studies that have shown that over-exposure to vitamin E may increase all mortality in creatures such as rats (24).

***mev-1* potentially more susceptible to vitamin E treatment, and may support free radical theory of aging and vitamin E's effects to increase lifespan**

Given the *mev-1* strain's overproduction of ROS (36) and vitamin E's role as an antioxidant (19), these results may further support the hypotheses of both the free radical theory and vitamin E's effects in increasing lifespan. The *mev-1* mutant strain is known to have both increased ROS levels and a shortened lifespan. It exhibited the greatest lifespan increase with a 2 hour vitamin E exposure. Because vitamin E is known for its antioxidant properties, it is possible that the vitamin E supplement showed pronounced effects on lifespan in *mev-1* because of the strain's increased ROS. If vitamin E had been acting on the *mev-1* strain's ROS to create that lifespan increase, then it could potentially create another link between oxidative damage and aging lifespan. To do this, it is possible

to measure the level of ROS within the worms before and after the experiment. If the ROS levels have decreased and the worms have exhibited the same lifespan increase, this link can potentially be established.

Future Experiments

Because many of the results were not significantly different, future experiments with increased replication may be needed. Although greater numbers may improve the data, increased replication would be experimentally difficult to achieve on an individual basis. During the course of the experiment, the daily monitoring of each of the 192 worms took one person an average of 3 hours a day. To potentially improve the statistical significance of the data, a replication factor of about 100 worms could be used, but this would be an experimentally difficult task for one experimenter to achieve alone.

Measuring ROS levels before and after treatments is also a plausible future experiment. As mentioned in the previous section, there may be a pronounced effect of vitamin E within the *mev-1* mutant strain compared to N2 wildtype. If ROS levels have decreased after treatment with vitamin E, it may further support a link between increased ROS levels and the process of aging.

Significance of Vitamin E Antioxidant on Lifespan

Vitamin E α -tocopherol appears to have variable effects on the lifespan of *C. elegans mev-1* mutant and N2 wildtype. α -tocopherol increased lifespan with a 2 hour exposure, however, lifespan decreased when the *C. elegans* strains were exposed for 24

hours. The results for the 24 hour time exposure were not significant. In the 2 hour time

exposure, N2 wildtype exhibited a very small increase in lifespan with vitamin E

supplementation, but the increase was not significant. The most promising result is the

increase of lifespan in *mev-1* with a 2 hour treatment of α -tocopherol. This suggests that

antioxidant α -tocopherol increases longevity. Because *mev-1* experienced a greater

increase in lifespan than N2 in the 2 hour treatment, this difference may also imply that

ROS levels may be more linked to lifespan than previously believed. Although the

ANOVA results in Table 3 showed that there was not a significant interaction between

strain type and treatment type, the p-value showed some potential (p=0.18516). Future

experiments with increased replication could potentially improve the significance of the

data, and may help further elucidate the antioxidant effects of α -tocopherol on lifespan.

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APPENDIX A

RAW DATA FOR EACH TREATMENT GROUP

Following this page is a table with the lifespan in days of each of the 24 worms sampled from each treatment group. Treatments are labeled as "Treatment 2hr" or "Treatment 24 hr"if they were exposed to vitamin E for 2 hours or 24 hours respectively. Control groups were exposed to 100% ethanol, not taking into account the total dilution of the sample, for the duration of 2 hours or 24 hours depending on the sample group. Numbers are recorded in days. During the experiment, there were some cases where worms would go missing from the NGM plate: these worms were labeled "MISSING" on the table.

Species	Treatment 2 hr	Control 2 hr	Treatment 24 hr	Control 24 hr
N2	21	15	18	15
	7	12	17	24
	7	12	23	9
	23	17	16	19
	16	24	20	14
	24	25	14	21
	16	30	16	24
	23	16	13	26
	22	35	17	23
	26	28	25	13
	17	17	12	28
	18	9	22	28
	21	29	28	23
	18	10	28	17
	16	22	17	16
	29	14	13	23
	19	20	22	29
	20	19	28	17
	21	14	22	17
	18	19	15	30
	17	23	12	19
	30	17	23	14
	17	15	MISSING	26
	21	MISSING	MISSING	16
mev-1	22	12	8	14
	13	22	12	14
	10	13	9	13
	15	12	14	14
	18	9	14	19
	17	8	17	15
	19	11	17	16
	13	11	8	13
	19	9	12	20
	16	13	15	16
	9	10	22	11
	18	11	12	8
	22	16	8	13
	11	12	15	15
	9	13	8	25
	9	17	14	13
	20	11	16	9
	12	13	13	12
	8	16	11	9
	8	10	13	11
	19	10	14	9
	18	11	7	11
	14	13	16	9
	20	12	MISSING	MISSING