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Title: DIETARY INTAKE AND PLASMA VITAMIN E IN OLDER SUBJECTS

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The plasma concentrations and the dietary intakes of vitamin E were determined in 20 elderly and ten young subjects. Five subjects in the older group were known to take supplements of vitamin E. The mean concentration of tocopherols (determined chemically) in the plasma of all unsupplemented subjects was 1.12±0.33 mg/100 ml. No significant difference in plasma tocopherols due to age or sex was found. However, the mean concentration in the plasma of the supplemented subjects (1.39±0.26 mg/100 ml) was significantly higher than that of the unsupplemented subjects. The mean alpha-tocopherol activity in the diets (estimated from a three-day diet study) of all subjects was 8.51±3.74 mg. The young group had a significantly greater intake than the elderly group. No significant difference between the sexes was observed. The mean ratio of alpha-tocopherol activity to polyunsaturated fatty acids (E·PUFA) in the diets of all subjects was 1.47±1.17 mg/gm. The significant difference between the

age groups or sexes was observed. A significant linear relationship between plasma tocopherols and dietary alpha-tocopherol activity or the E:PUFA ratio was not found.

Neither age, sex nor dietary intake was related to a significant change in plasma tocopherols. However, subjects using supplements of vitamin E had a significantly higher mean plasma concentration of tocopherols.

Dietary Intake and Plasma Vitamin E of Older Subjects

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INTRODUCTION

Popular literature is filled with suggestions as to possible roles for vitamin E in health and disease. One of the most widely-read authors on nutrition for the layman, Adelle Davis (1970), states that although the primary function of vitamin E is to prevent destruction of fatty acids, large doses can alleviate certain disorders. These disorders include acne and multiple sclerosis (1965) as well as arteriosclerosis, arthritis, bursitis, cancer, diabetes, infertility, and thyroid problems (1970). Another writer on the subject of nutrition, Carlton Fredricks (1965), says of vitamin E:

> Deficiency of this vitamin is easy to incur, and the diet may be more universally inadequate in this factor than any single vitamin . . . (p. 43).

He also refers to alpha-tocopherol as "physiological tranquilizer" (p. 149). Furthermore, according to Fredricks, one ounce of wheat germ per day will protect against coronary disease because of the high content of vitamin E in wheat germ. The spectacular success of Doctors Evan and Wilfred Shute in treating cardiovascular disease with massive doses of vitamin E is described in another popular book (Shute and Taub, 1969).

The claim that vitamin E is particularly effective in the prevention and treatment of degenerative diseases has led to the suggestion

that the vitamin E requirement may increase with age. For example, Dr. Evan Shute, in an interview published in a popular magazine (Trotta, 1972), recommended an intake of 400 I. U. of alpha-tocopherol per day for adult women and 600 I. U. per day for adult men and post-menopausal women. It is important to note that a daily intake of 600 I. U. of vitamin E would be 40 times the dietary intake recommended for healthy adult males by the National Research Council (1974) and that it would be impossible to obtain from natural foods. On the other hand, several scientists (Tappel, 1968; Pryor, 1970) suggest that vitamin E, acting as an antioxidant can prevent the formation of free radicals which damage the structure and function of body cells. Harman and Piette (1966) believe that free radical oxidation is actually responsible for certain symptoms of aging. If this theory is correct, a compound such as vitamin E which prevents free radical formation might slow the process of aging.

As a result of publicity in the lay literature, many older people have been persuaded that their dietary intake of vitamin E is insufficient. Vitamin E in capsule form, providing three to 15 times the recommended daily allowance, is enjoying a brisk business. According to an article in Newsweek (1972), in some areas, sales of vitamin E have increased by 500 percent in one year. Although toxicity has not been reported to result from large intakes of vitamin E, the beneficial effects of megadoses have not been unequivocally

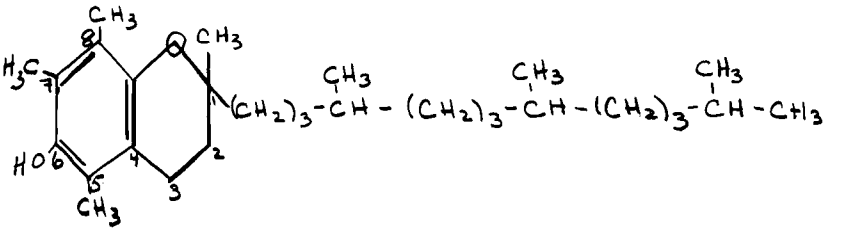
demonstrated in adults. Furthermore, the dangers of self-medication in lieu of medical treatment are obvious.

If, indeed, the requirement of vitamin E increases with age, one would expect to find low concentrations of tocopherol in the plasma of older persons. The hypothesis of this study is that the plasma tocopherol concentrations of aged individuals are not significantly different from those of young adults, so long as the dietary intake of vitamin E is in the range that has been recommended as adequate by the National Research Council.

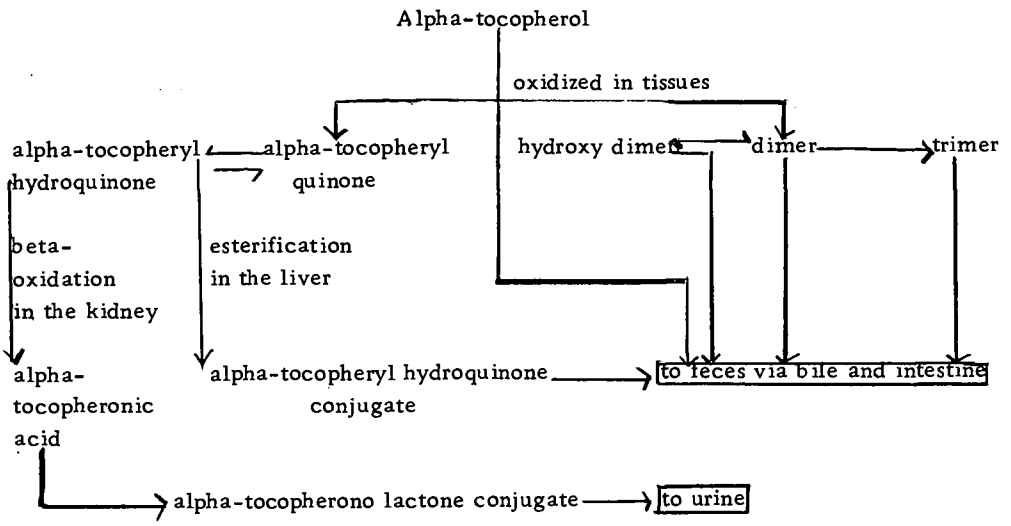
REVIEW OF LITERATURE

Vitamin E

Vitamin E was discovered by Evans and Bishop (1922) and isolated by Evans, Emerson, and Emerson (1936). The name tocopherol (from tokos meaning childbirth, and phero meaning to bear) refers to the fact that animals require this vitamin for successful reproduction. There are eight tocopherols which differ from each other in the position of the methyl group on the ring structure and in the saturation of the side chain. The structural formula for alpha-tocopherol, the most biologically active form, is:



The differences in biological activity of the various tocopherols are related to a greater tissue retention of alpha-tocopherol, not to differences in absorption or tissue uptake (Peake et al., 1972). Tocopherol is a pale yellow, viscous oil which is readily destroyed by ultraviolet light and easily oxidized in alkali but stable in acid (Freed, 1966). Oxidation of tocopherol begins at carbon 6 with the formation of tocopheryl quinone. Draper and Csallany (1969a) have summarized the present information on alpha-tocopherol metabolism as follows:



According to Herting (1966), a deficiency of vitamin E in animals has been found to affect the reproductive system (testicular degeneration and malformation of the embryo), the muscular system (muscular dystrophy, ceroid pigments in smooth muscle), the circulatory system (anemia, exudative diathesis), the skeletal system (incisor depigmentation), and the nervous system (encephalomalacia, lipofuscin pigment). On the other hand, signs of tocopherol deficiency in humans are rare. Majaj et al. (1963) reported a megaloblastic anemia in severely malnourished children which responded only to administration of vitamin E. Nitowsky et al. (1962) found that the creatinuria which occurred in fibrocystic disease, in which vitamin E is poorly absorbed, could be reduced by vitamin E therapy. Leonard and Losowsky (1971) reported impaired red cell survival in adults who suffered from malabsorption syndromes.

The only generally accepted function of vitamin E is that of an

antioxidant, although evidence is accumulating which suggests that this nutrient has more than one role in the body. Phenolic compounds are especially effective as antioxidants because they can form phenoxy radicals with stable resonance hybrids (Altwicker, 1967). Because cellular and sub-cellular membranes are composed of lipoprotein complexes, vitamin E and other antioxidants serve to prevent the destruction of lipids, thus maintaining the cellular and subcellular structure and containment of hydrolytic enzymes. Vitamin E has been implicated in the protection of vitamin A (Moore, 1940) and essential fatty acids (Pritchard and Singh, 1960) from oxidative degradation, maintenance of the integrity of sulfhydryl groups in respiration (Schwarz, 1962), regulation of turnover rates of nucleic acids (Hauswirth and Nair, 1972) and regulation of protein synthesis (Reiss and Tappel, 1973), and even protection from the observed symptoms of vitamin E deficiency (Leonard and Losowsky, 1971 and Majaj et al., 1963).

Vitamin E and Aging

Two theories have been proposed which suggest that vitamin E may participate in the aging process. The best known of the two is based on the ability of tocopherol to act as an antioxidant and prevent the formation of free radicals which can be damaging to the cell's structure and function. The second theory proposes that vitamin E

is active in intermediary metabolism because it has been shown to prevent respiratory decline in specific fractions of liver homogenates.

The Antioxidant Theory

In general, the antioxidant theory states that a deficiency of tocopherol will allow cellular lipids to be peroxidized. Free radicals thus formed will cause tissue damage and ceroid pigment formation. According to work done by Andrews et al. (1965) and Roubal and Tappel (1966b), peroxides of lipids can cause protein alteration by cross-linking. Tappel (1968) reasons that the breakdown of cellular and sub-cellular membranes, rich in polyunsaturated fatty acids, could result in the disruption of the cell's mechanisms for disposing of debris and in the release of hydrolytic enzymes from the lysosomes. The resulting cellular damage can be the precursor of cell death.

The following background to the theory that vitamin E functions as an antioxidant was discussed in a review article by Green and Bunyan (1969). In 1939, at the First International Congress on Vitamin E, Drummond suggested its oxidation-reduction potential, namely, from alpha-tocopherol to alpha-tocopherylquinone, the oxidized form. Davies and Moore in 1941 were the first to suggest vitamin E's role as an in vivo antioxidant. In 1945 Dam and Granados observed that E-deficient chicks and rats had increased levels of

lipid peroxides. Only one year later, Filer, Rumery, and Mason suspected that vitamin E functioned both as an antioxidant and in an as yet undefined more specific role. Harman (1961) has been credited with being the first researcher to suggest that antioxidants can prolong an animal's life span. In 1958 the relationship between polyunsaturated fatty acids and tocopherol was investigated by Christensen, Dam, Prange, and Sondergaard. They discovered high peroxide concentrations in adipose tissue of E-deficient rats fed a diet containing 20 percent cod liver oil, but not with 20 percent lard. Pritchard and Singh (1960) observed a 53 to 75 percent decrease in the concentration of polyunsaturated fatty acids in the heart, liver, adrenals, and plasma of vitamin E-deficient rats.

Draper and Csallany (1969b) reported a definite correlation between the rate of peroxide decrease and the rate of alpha-tocopherol oxidation. The general mechanism for cellular damage involves the interaction of molecular oxygen and lipids to form a free radical intermediate. Since free radicals seek to pair their odd electron, proteins can be attacked, and polymerization will cause the formation of a new protein. Lipid peroxidation can be summarized as follows (Tappel, 1972):

$$L\cdot + O_2 \rightarrow LO\cdot_2$$

$$LO\cdot_2 + LH \rightarrow LOOH + L\cdot$$

where L· represents a lipid radical. Roubal and Tappel (1966b) believe that the crosslinking of protein occurs by the following mechanism:

$$\left.\begin{matrix} LO\cdot_2 \\ \text{or} \\ LO\cdot \end{matrix}\right\} + 2\ PH\ (\text{protein}) \rightarrow \begin{cases} LOOH + P\cdot \\ LOH + P\cdot \end{cases}$$

$$P\cdot + P \rightarrow P\text{-}P\cdot$$

$$P\text{-}P\cdot + P \rightarrow P\text{-}P\text{-}P\cdot \qquad \text{etc.}$$

The amino acid from which the hydrogen was removed was not specified in this article but Roubal and Tappel (1966a) found cysteine, histidine, lysine and methionine to be the most labile. Vitamin E is thought to protect protein against this phenomenon by being preferentially oxidized before lipids (Tappel, 1972). Tappel suggested the following mechanism:

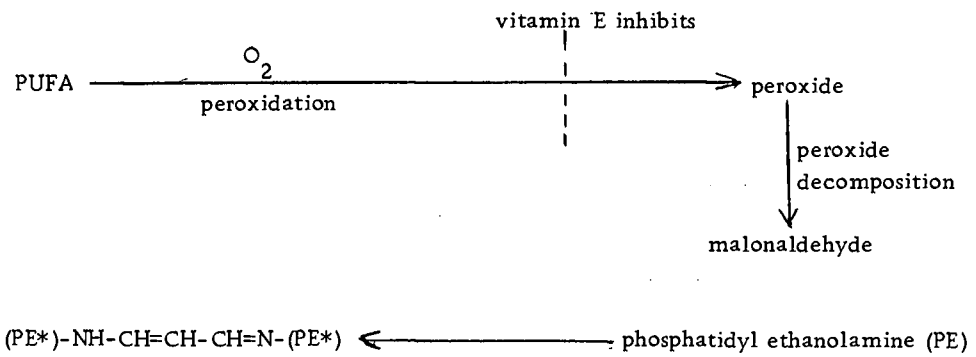
$$LO\cdot_2 + \text{alpha-TH (alpha-tocopherol)} \rightarrow LOOH + \text{alpha-T}\cdot .$$

It can, therefore, be considered to be a radical chain breaker.

The fact that synthetic antioxidants can sometimes replace vitamin E substantiates the theory that tocopherol acts as an antioxidant. Harman (1961) has demonstrated that 2-mercaptoethylamine hydrochloric acid, an antioxidant, extended the half time survival of mice to 18.3 months rather than 14.5 months for the controls. Although this effect was observed in one strain of mice, no difference in the life spans of another strain was observed under the same

experimental conditions. In 1968, Harman again published results of a study that indicated a significant increase in survival time of mice with an antioxidant.

Sulkin and Srivanij (1960) found a greater deposition of lipofuscin pigments in senile rats which were vitamin E-deficient than in younger rats which were deficient. These pigments are lysosomal in origin and can be detected by electron microscopy and histochemical staining. The accumulation of lipofuscin pigments in deficiency states (Reddy et al., 1973; Sulkin and Srivanij, 1960) and the association of these pigments with a decrease in polyunsaturated fatty acids (Witting and Horwitt, 1967) has been cited as additional evidence that vitamin E functions as a protective agent in lipid peroxidation. Reddy et al. (1973) suggest the following pathway for their formation:



where PE* represents phosphatidyl ethanolamine excluding the amino group. Lipofuscin pigments have been implicated in the "clinker" theory of aging because it is possible that the accumulation of abnormal particles can in some way change the cell's activity (Chem. Eng. News, 1962).

The most frequently cited evidence to support the antioxidation theory is the decrease in the concentration of polyunsaturated fatty acids and the subsequent accumulation of peroxides in deficiency states. Witting and Horwitt (1967) found a decrease in all polyunsaturated fatty acids except arachidonate in the muscles of vitamin E-deficient rats. Glavind et al. (1971) found larger amounts of peroxides in the adipose tissue of vitamin E-deficient rats than in those of vitamin E-supplemented rats.

However, the biological antioxidant theory has not gone without being seriously questioned. Bunyan, Diplock and Green (1967) fed rats either a vitamin E-deficient diet or one supplemented with D-alpha-tocopheryl acetate (100 ppm). After 13 months, they found no differences between the groups with respect to polyunsaturated fatty acid content of the liver, kidney, heart, spleen, brain, adrenal gland or adipose tissue. Only when cod-liver oil (eight percent) was added to the diet, were they able to induce a decline in tissue polyunsaturates of the vitamin E-deficient rats, and then only in the leg muscles. Their next step was to assess the effects of antioxidants on peroxides already formed in the tissues. They fed rats cod-liver oil esters for four weeks (Bunyan et al., 1968). Then three different diets were given for the next ten days: a fat free diet, a fat free diet supplemented with vitamin E, and a fat free diet with N, N'-diphenyl p-phenylenediamine, an antioxidant. The peroxide value of adipose

tissue lipids decreased with a fat free diet but the addition of either antioxidant did not increase the rate of disappearance. These researchers suggested that vitamin E decreased the amount of peroxides absorbed rather than preventing the formation of peroxides in vivo. However, Glavind and co-workers (1971) demonstrated no difference in peroxide absorption in vitamin E-deficient or supplemented rats.

Respiratory Decline

A theory which has been suggested as an alternate to the antioxidation theory is based on the ability of tocopherol to prevent respiratory decline. In respiratory decline, there is a decreased in vitro oxygen consumption by the affected tissue. In homogenate liver preparations, Grove, Johnson and Cline (1966) and Schwarz (1962) observed that respiratory decline can be alleviated by both dietary and in vitro administrations of alpha-tocopherol if the mitochondrial membrane is left intact.

There are three fractions used to study respiratory decline--mitochondrial, microsomal and the supernatant. In 1961, Corwin observed that respiratory decline, using alpha-ketoglutaric acid as the substrate, is 26 percent with the mitochondrial fraction and 22 percent with the mitochondrial fraction plus the supernatant. However, when the microsomal fraction was added the decline increased

to 86 and 76 percent, respectively. Grove, Johnson and Cline (1966) also observed that the microsome fraction does have a prominent role in respiratory decline. Electron microscopy of mitochondrial and microsomal membranes from livers of duckling suffering from a vitamin E deficiency has demonstrated that the mitochondria were damaged, not the microsomes (Vos et al., 1972). Analysis of the average number of double bonds per mole of fatty acid revealed that the inner mitochondrial membrane of livers from deficient ducklings had fewer remaining polyunsaturated fatty acids than those from normal ducklings.

There is disagreement as to exactly where vitamin E acts in metabolic pathways. Grove et al. (1965) believe that the Kreb's cycle is affected rather than electron transport. When either beta-hydroxybutyrate or malate was used as the substrate, liver homogenates from vitamin E-deficient rats exhibited respiratory decline. Because NAD+ is a cofactor in the oxidation of both of these compounds, it was suspected that NAD+ was the critical factor in the respiratory decline associated with vitamin E deficiency. However, no decline in the oxidation of succinate or high concentrations of alpha-ketoglutarate was observed. In 1967, Grove and Johnson determined that adding NAD+ before incubation or using microsomes from vitamin E-supplemented rats would prevent respiratory decline.

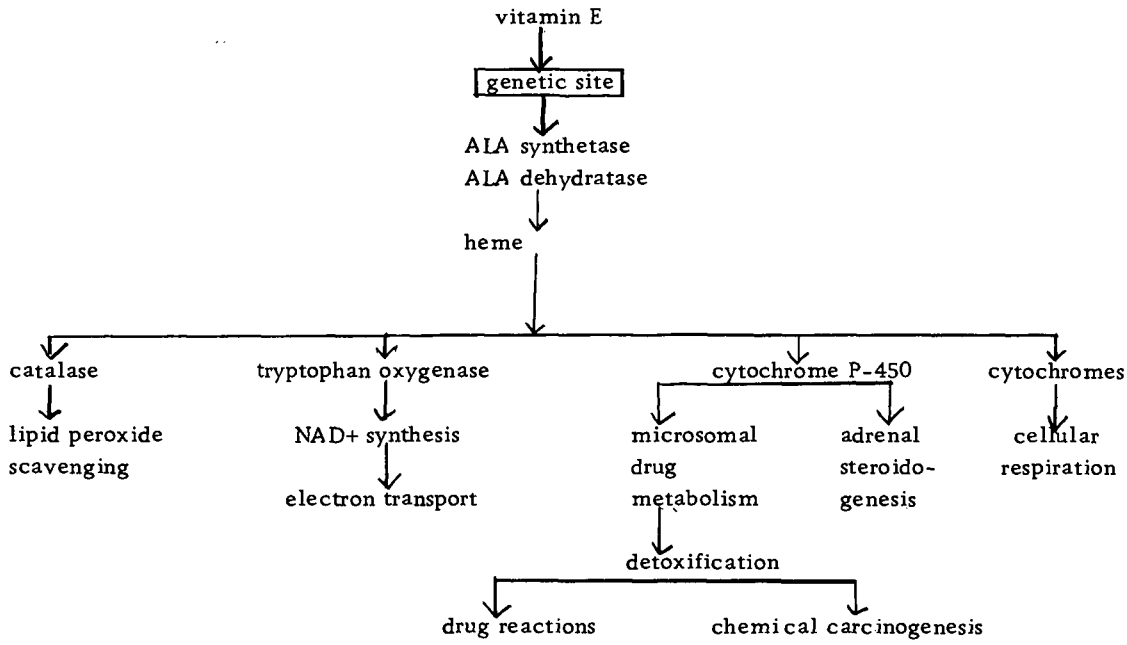
Schwarz (1962 and 1965) believes that a deficiency of vitamin E

affects the dehydrogenase system which connects the Kreb's cycle to the cytochrome chain. He observed that vitamin E prevented a decrease in oxygen consumption when alpha-ketoglutarate or oxalacetic acid was the substrate but not when succinate or isocitrate was the substrate. Because both of the substrates which were associated with respiratory decline in deficiency states are oxidized by systems which involve sulfhydryl groups, Schwarz suggested that vitamin E protects this functional group. Schwarz found no correlation between respiratory decline and peroxide formation.

Green et al. (1961) observed that an intrajugular injection of alpha-tocopherol increased the ubiquinone concentration in the tissues of rats. However, Bieri and Andrews (1963) found that vitamin E had no effect on the ubiquinone concentrations in chick livers. Although the evidence is inconclusive, the similarities in the structures of ubiquinone and vitamin E has led researchers to suspect a possible interrelationship.

Another possible site for the action of vitamin E is heme synthesis. Caasi et al. (1972) found a significant decrease in the activity of hepatic tryptophan oxygenase, catalase, and cytochromes b_5 and P-450 in the livers of rats on a vitamin E-deficient diet for 12 weeks. No difference was found in the activity of non-heme enzymes, however. A similar decrease was also found in heme enzymes in older rats. Schwarz (1972) has related respiratory

failure in liver mitochondria directly to decreased levels of cytochromes. Nair (1972) has postulated that the decrease in heme synthesis can be responsible for the following diverse effects:



Conclusion

Although there is substantial evidence for both of the theories on vitamin E's effect on aging, more research is needed because of contradictory observations. The antioxidation theory of aging bases its assumptions on the discovery of age pigments, the prevention of lipid peroxidation of tocopherol and the ability of other antioxidants to replace vitamin E. However, some experiments have demonstrated no change in lipid content or peroxidation in vitamin E deficiency states. The theory of respiratory decline provides an

interesting alternative to the free radical theory. However, the work completed is not extensive enough at this time to provide a conclusive explanation. The answer might lie in a combination of the two theories or perhaps even in the discovery of a new one.

Vitamin E in Human Plasma

The validity of using plasma levels of tocopherol to determine vitamin E status of humans was demonstrated in a study by Horwitt et al. (1956). These investigators fed a basal diet containing less than 3 mg of tocopherol to an experimental group and a basal diet plus a supplement of 15 mg of tocopherol to a control group. The experimental group originally had a mean plasma tocopherol concentration of 0.99 mg/100 ml but this value dropped to 0.64 mg/100 ml after one year. There was no significant change in the plasma levels of the control group. Significant increases in plasma levels of vitamin E with supplementation were observed by Leonard and Losowsky (1971) and McMasters et al. (1965). According to work done by Bieri (1972) a linear relationship exists between the plasma concentration of vitamin E and the log of the dietary intake in rats.

The Emmerie-Engel reaction is most commonly used for the determination of tocopherols. In this method, ferric chloride is reduced to ferrous chloride and the latter is complexed with alpha, alpha'-dipyridyl. However, there are other reducing

substances in plasma which will also give this reaction, thus causing a higher apparent value. The interfering substance which is present in the highest concentration is carotene, which absorbs at the wavelength used in the reaction. Interference by carotenoids can be eliminated by applying a correction factor based on the measured amount of carotene in plasma or by hydrogenation. Alternatively, tocopherol can be separated by means of column or paper chromatography. Bieri et al. (1964) compared the tocopherol values of 11 sera analyzed by two methods. When a correction factor was used for carotene, the mean tocopherol was 1.174±0.260 mg/100 ml. When tocopherol was isolated by chromatography, the mean value was 1.150±0.230 mg/100 ml. Although Herting and Drury (1965) found good agreement when 36 samples were analyzed after initial separation with paper (0.496 mg/100 ml) or column chromatography (0.502 mg/100 ml), poor agreement was found when 25 samples were analyzed either by using a correction factor for carotene (0.758 mg/100 ml) or after isolation by paper chromatography (0.435 mg/100 ml).

Concentrations of vitamin E in human plasma, as reported by various investigators are presented in Table 1. It can be seen that much higher values were obtained when vitamin E was determined by the Emmerie-Engel reaction without initial separation by chromatographic procedures. This discrepancy is probably due to the presence of other reducing compounds which gave a positive

Table 1. Plasma concentration of tocopherols, as reported in the literature

Reference	Tocopherols (mg/100 ml)		Number of subjects	Methods of determination[a]	Comments
Bieri, J. G. et al. (1964)	mean	1.05	132	EE--corrected for carotene	
	males	1.064	71		
	females	1.035	61		
Chieffi, M. and J. E. Kirk (1951)	mean	0.98	188	EE--carotene removed by hydrogenation	increase with age in men
	males	0.92			
	females	1.01			
Harris, P. L. et al. (1961)		1.05	197	EE--carotene removed by hydrogenation	
Herting, D. C. and E. E. Drury (1965)	Pittsburgh	0.358	37	EE--after isolation by paper chromatography	no trend with age or sex
	Males	0.354	34		
	Females	0.433	2		
	Rochester	0.507	37		
	Males	0.501	25		
	Females	0.520	12		
Leitner, Z. A. et al. (1960)	Mean	1.05	588	EE--corrected for carotene	increase with age
	Males	1.05	348		
	Females	1.06	240		
Lemley, J. M. et al. (1949)	control	1.09	21	EE--carotene removed by hydrogenation	increase with age
	cardiac	0.94	62		
	random	0.92	42		
McMasters, V. et al. (1965)	before supplementation	0.37	19	EE--after isolation by column chromatography	
	after supplementation	1.13			

[a] The Emmerie-Engel reaction (EE) was used in all assays.

Emmerie-Engel reaction.

No evidence was found in the studies listed in Table 1 that concentrations of plasma tocopherols are lower in older individuals. One study (Herting and Drury, 1965) demonstrated no change with age, and three studies (Lemley et al., 1949; Chieffi and Kirk, 1951; and Leitner et al., 1960) demonstrated an increase with age. No consistent difference in plasma vitamin E concentrations between men and women was observed. Although Chieffi and Kirk (1951) found that plasma levels increased with age in men only, females had a slightly higher mean level. Bieri et al. (1964) found a slightly lower level in women. However, when Leitner and co-workers (1960) averaged the values under various age groups they observed that females under 30 years had a significantly higher value than did men ($p \leq 0.02$). Between the ages of 30 and 39, the mean vitamin E concentration of females was significantly lower than that of men. Above the age of 69 the mean value for women was again slightly higher.

Deficiencies of vitamin E in adults are rare except in patients with malabsorption syndromes, such as biliary atresia, celiac disease and cystic fibrosis (Herting, 1966). For example, Leonard and Lòsowsky (1971) found a mean concentration of plasma tocopherol of 0.27 mg/100 ml in six patients with malabsorption syndromes and two alcoholics suffering from malnutrition. Nitowsky et al. (1962) found a range in concentrations of plasma tocopherol of 0 to

0.49 mg/100 ml in 27 males with cystic fibrosis. In two studies reported by Underwood et al. (1972), mean plasma concentrations were 0.175 and 0.242 mg/100 ml in patients with cystic fibrosis as compared to concentrations of 1.161 and 1.311 mg/100 ml in controls.

There is some disagreement as to whether hyper- or hypotocopherolemia is associated with cardiovascular disease. Darby et al. (1949) found higher vitamin E concentrations and Lemley et al. (1949) found lower concentrations in patients suffering from cardiovascular disease. Darby also found a positive correlation between hypertocopherolemia and hypercholesterolemia. This observation was substantiated in a more recent study by Rubenstein et al. (1969).

One test for vitamin E deficiency is the hemolysis of red blood cells in a hydrogen peroxide solution (Horwitt et al., 1956). Bieri and Poukka (1970) found, in rats, that less hemolysis occurred with higher levels of erythrocyte tocopherol and Horwitt et al. (1956) found, in humans, that less hemolysis occurred with greater intakes of dietary tocopherol. According to studies by Herting and Drury (1965) and MacKenzie (1954) hemolysis does not occur when plasma concentrations of tocopherol exceed 0.5 mg/100 ml.

Vitamin E, as a non-specific antioxidant, neutralizes free radicals. Consequently, the chain reactions of lipid peroxidation are stopped and hemolysis does not occur (Younkin et al., 1971). However, the erythrocyte hemolysis tests should be interpreted with

caution. Alfin-Slater et al. (1969a) have stressed that the hemolysis test is an in vitro one and the conditions might differ from those in vivo. These researchers regard the test as a measure of the balance between polyunsaturated fatty acids and vitamin E in the red blood cell rather than an absolute measure of tocopherol alone. Horwitt et al. (1956) have observed that plasma tocopherol levels after vitamin deficiency can return to normal after a few days of supplementation but a return to normal hemolysis values can require months.

Vitamin E in the Diet

Estimates of the alpha-tocopherol content of the average American diet range from 7.4 to 14.9 mg/day (Table 2). It can be seen that values obtained in these studies vary by a factor of over two. This variation is not unexpected because the tocopherol content of foods varies with the method of preservation and storage time (Bunnell et al., 1965), as well as the specific part of the tissue which was analyzed (Booth and Bradford, 1963). For example, Smith et al. (1971) reported that the alpha-tocopherol in four samples of lamb's liver ranged from 0.2 to 2.5 mg/100 gm. The alpha-tocopherol content of two samples of brussels sprouts was 0.3 and 1.1 mg/100 gm, and in seven samples of margarine the alpha-tocopherol ranged from 1.2 to 27.8 mg/100 gm.

Table 2. Average tocopherol content of the American diet, as reported in the literature

Reference	Alpha-tocopherol	Alpha-tocopherol activity	Alpha-tocopherol: PUFA[a]	Alpha-tocopherol activity:PUFA[a]	Method of determination
	mg/day	mg/day	mg/gm	mg/gm	
Bieri, J. G. and R. P. Evarts (1973)	9.0	11.3	0.43	0.54	analysis of cafeteria foods
Bunnell, R. H. et al. (1965)	7.4	9.3			estimated from typical menus
Engle, C. (1949)	15.0	18.8			estimated from diet surveys, The Netherlands
Harris, P. L. and N. D. Embree (1963)	14.9	18.6	0.616	0.770	estimated from National Food Situation, No. 96, p. 19, Washington, D. C., May 1961, U. S. Dept. of Agriculture, Econ. Research Service
Harris, P. L. et al. (1950)	14.0	17.5			estimated from diets from U. S. Dept. of Agriculture, 1949

[a]PUFA = polyunsaturated fatty acids

Although the values in Table 2 are given in milligrams, the amount of vitamin E is often given in international units (I. U.). The conversions are:

1 mg synthetic dl-alpha-tocopheryl acetate = 1.00 I. U. vitamin E

1 mg synthetic dl-alpha-tocopherol = 1.10 I. U. vitamin E

1 mg synthetic d-alpha-tocopheryl acetate = 1.36 I. U. vitamin E

1 mg synthetic d-alpha-tocopherol = 1.49 I. U. vitamin E

Another point of clarification is that although alpha-tocopherol is the most biologically potent tocopherol, the other tocopherols are also active. Bieri and Evarts (1973) suggest that because the activity of gamma-tocopherol ranges from 1 to 22 percent of the activity of alpha-tocopherol, and because gamma-tocopherol is present in significant amounts in foods commonly eaten, the alpha-tocopherol content of the food should be multiplied by 1.25 to allow for the biological activity of gamma-tocopherol.

In 1973 the National Research Council recommended an allowance of 12 I. U. and 15 I. U. of alpha-tocopherol activity per day for average healthy adult women and men, respectively. However, it is difficult to determine the exact vitamin E requirement of a given individual without knowing the amount of polyunsaturated fatty acids ingested. According to Horwitt (1960) the vitamin E requirement increases with the amount of dietary auto-oxidizable compounds. Harris and Embree (1963) recommended an average intake of 0.6 mg

alpha-tocopherol for each gram of polyunsaturated fatty acids (PUFA) in the diet. Herting and Drury (1963) have estimated that cottonseed oil is the only oil which exceeds this ratio. However, according to a rat study by Alfin-Slater et al. (1969b), who fed vegetable oils with wide ranges of polyunsaturated fatty acids to total tocopherol ratios, there is no need for extra vitamin E regardless of the type of vegetable oil ingested. Lewis et al. (1973) found that the growth and health of children receiving a diet with an alpha-tocopherol to polyunsaturated fatty acid ratio of only 0.37 mg/gm was similar to those children receiving an average diet which was assumed to have a higher ratio. Witting (1972) suggested that the vitamin E requirement is related to the polyunsaturated fatty acid content of the tissues and not to the content of the current diet.

PROCEDURE

Subjects

The subjects were chosen from two groups: an older group with ages ranging from 68 to 87, and a younger group with ages ranging from 21 to 27. The older subjects were participants in a regional study of food acceptance and nutritional status, which is being completed at Oregon State University. Five of the older subjects, all women, were chosen because they were known to take vitamin E supplements. Fifteen additional older subjects who did not take vitamin E supplements (seven men and eight women) were chosen to make a total of 20 subjects. A younger group of ten subjects who did not take vitamin E supplements was chosen. Five men and five women were selected to approximate the male:female ratio of the older group who did not take supplements. Specific data on the subjects are given in Table 3.

Preparation of Plasma

Fasting blood samples were drawn at Good Samaritan Hospital for the older group and at the Oregon State University Student Health Center for the younger group. The blood was collected (30 ml from the older group and 5 ml from the younger group in vacutainers containing 12 mg powdered EDTA(Na_2) per 10 ml. The blood was

Table 3. Description of subjects

Subject number	Age	Sex	Vitamin E supplements
5	86	F	-
9	87	M	-
18	69	F	+
22	71	M	-
29	77	M	-
30	68	F	+
32	81	F	-
34	68	F	-
36	71	F	+
39	73	F	-
41	83	M	-
43	69	F	-
47	80	F	-
51	70	F	+
56	86	M	-
58	83	F	+
63	75	F	-
67	75	M	-
69	71	F	-
70	70	M	-
101	23	F	-
102	22	M	-
103	21	F	-
104	23	F	-
105	23	F	-
106	23	M	-
108	27	M	-
109	24	M	-
110	21	F	-
111	25	M	-

centrifuged at 2000 r.p.m. for 20 minutes. The plasma was then separated and held at -10° C until analyzed. Hemolyzed samples were excluded from this study in order to eliminate the possibility of contamination by erythrocyte tocopherol.

Analysis of Blood

Plasma tocopherol was analyzed by the method of Quaife, Scrimshaw and Lowry (1949), as outlined by Bunnell (1967). According to this method plasma proteins are precipitated by alcohol, lipids are extracted into a hydrocarbon solvent, and tocopherol is measured by the Emmerie-Engel reaction. In the Emmerie-Engel reaction, ferric ions are reduced by tocopherol to ferrous ions, which form a red colored complex with alpha, alpha' -dipyridyl. This complex is measured spectrophotometrically at 520 nm. To correct for interference by beta-carotene, the carotene absorbance at 460 nm is determined first. Since carotene absorbance at 520 nm is known to be 29 percent of the absorbance at 460 nm, the factor of $(0.29)A_{460}$ is subtracted from the reading at 520 nm. According to Freed (1966), the problems which can arise with this method are interference by total reducing materials such as chromenols, reduced ubiquinone and other antioxidants and variation in color due to changes in temperature, time and light.

The following is a detailed description of the procedure used

in this study.

Reagents

1) absolute ethyl alcohol

2) xylene (C. P.)

3) alpha, alpha'-dipyridyl in n-propyl alcohol (0. 08 gm/100 ml) which is stored in a dark bottle and refrigerated

4) $FeCl_3 \cdot 6H_2O$ in absolute ethyl alcohol (0. 084 gm/100 ml) which is stored in a dark bottle and refrigerated

5) standard solutions of pure alpha-tocopherol in absolute ethanol (0. 5 mg/100 ml, 1. 0 mg/100 ml, 1. 5 mg/100 ml and 2.0 mg/100 ml)

Equipment

1) test tubes

2) parafilm squares

3) micropipettes (25, 100 and 150 µl)

4) Beckman Model DU spectrophotometer[1] with a microattachment and 1 cm quartz microcuvettes

5) lab stirrer

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[1] Beckman Instruments, Inc., Fullerton, California

Procedure

One hundred μl of distilled water (for the blank), plasma or standard solution and 100 μl of absolute ethanol were pipetted into small test tubes in triplicate and mixed immediately with a stirrer. One hundred and fifty μl of xylene were added and the test tubes were covered with parafilm squares. The mixture was agitated violently for 45 seconds, then centrifuged at 3,000 r.p.m. for ten minutes. One hundred μl of the xylene supernatants were transferred to fresh test tubes and 100 μl of the alpha, alpha'-dipyridyl reagent were added. The mixtures were re-sealed and agitated. One hundred μl of each mixture was pipetted into the microcuvettes of the spectrophotometer and the absorbance was read at 460 nm. Twenty-five μl of the ferric chloride reagent were then added and the cuvettes were rocked back and forth for 30 seconds. Readings at 520 nm were taken exactly 1.5 minutes after the addition of the ferric chloride reagent. The cuvettes were cleaned with acetone and aspirated twice between samples.

Calculations

The daily variation in readings was sufficient to require the use of a complete set of standards and a new regression equation every time a group of samples was run. The regression equations

were calculated by the method of least squares and used to determine the sample concentrations. The value of $(OD_{520} - OD_{blank}) - 0.29(OD_{460} - OD_{blank})$ was used as the corrected absorbance to allow for interference by beta-carotene.

Dietary Evaluation

The subjects were asked to submit a record of all food consumed during a three day period. A computer analysis of the food energy, protein, fat, saturated fatty acids, oleic acid, linoleic acid, carbohydrate, calcium, iron, vitamin A value, thiamin, riboflavin, niacin and ascorbic acid was completed using values from Home and Garden Bulletin No. 72 (1971). The computer analysis listed a daily total and a mean for the three days for each nutrient category.

Vitamin E content of the diets was estimated from data given Bunnell et al. (1965), Engle (1949), Harris et al. (1950) and Herting and Drury (1963). If values for a specific food could not be found in these references, the value for a food which was judged to be the most similar was used.

Statistical Treatment

Two main types of statistical analyses were used--a t-test to determine if a significant difference between means existed, and an F-test and multiple correlation coefficient (R^2) to assess whether two

variables were significantly correlated. The values for the t-test and F-test were calculated by the computer, then compared to values in the tables at levels of significance equal to or less than 0.10. Histograms or scatterplots were requested from the computer for some variables.

RESULTS AND DISCUSSION

Concentration of Vitamin E in the Plasma

The concentrations of total tocopherols for individual subjects are given in Table 4. Group means are presented in Table 5.

The plasma tocopherol concentrations of the unsupplemented subjects ranged from 0.59 to 1.75 mg/100 ml (Table 4). This wide range was seen in the tocopherol concentrations of the younger subjects. In the non-supplemented elderly subjects, plasma tocopherol values ranged from 0.75 to 1.65 mg/100 ml. Mean tocopherol values for the unsupplemented groups (Table 5) were similar to those which have been reported in the literature (Table 1).

No significant differences due to age or sex could be found in the unsupplemented subjects, even at the ten percent level of confidence. The mean tocopherol concentration of the unsupplemented elderly females was greater than that of young females (1.20 mg/100 ml versus 0.99 mg/100 ml). Conversely, the young males had a higher mean of tocopherol level than did the elderly males (1.19 mg/100 ml versus 1.08 mg/100 ml). However, these differences were not significant at the ten percent level.

The subjects taking supplements had significantly ($p < 0.10$) higher concentrations of tocopherol as compared to subjects without supplements in either the elderly or young group of subjects. In this

Table 4. Plasma tocopherol concentrations (mg/100 ml) of individual subjects

Elderly		Young	
Subject	Tocopherol	Subject	Tocopherol
Male		Male	
9	0.75	102	0.67
22	1.38	106	1.00
29	1.00	108	1.07
41	1.35	109	1.51
56	0.80	111	1.70
67	1.25		
70	1.02		
Female		Female	
5	0.99	101	0.81
32	1.10	103	0.93
34	1.39	104	0.59
39	1.09	105	0.89
43	0.82	110	1.75
47	1.06		
63	1.65		
69	1.46		
18[a]	1.66		
30[a]	1.23		
36[a]	1.23		
51[a]	1.14		
58[a]	1.69		

[a]These subjects took supplements of vitamin E.

Table 5. Mean plasma tocopherol concentrations (mg/100 ml) of subjects by groups

	Elderly	Young	Elderly and young
Male--unsupplemented	1.08±0.25	1.19±0.41	1.13±0.32
Female--unsupplemented	1.20±0.28	0.99±0.44	1.12±0.26
Female--supplemented	1.39±0.26*		1.39±0.26*
Male and female--unsupplemented	1.14±0.26	1.09±0.42	1.12±0.33
Total	1.20±0.28	1.09±0.42	1.17±0.33

*Significantly different from all unsupplemented groups ($p \leq 0.10$)

study neither sex or age group influenced plasma concentrations but supplementation had a significant effect.

Correlation of Plasma Vitamin E with Age

No significant difference between the vitamin E concentration in the plasma of the young and that of the elderly subjects was observed. However, the possibility of a trend with aging within the groups was tested. No significant linear relationship existed between the concentration of plasma vitamin E and age in any of the groups. Age correlations (R^2) within each group were as follows:

elderly supplemented females	0.34
elderly unsupplemented females	0.08
elderly males	0.31
elderly unsupplemented group	0.19
young group	0.02

Due to the fact that a significant difference did not exist between the young and elderly subjects, and trends with age within the groups were not significant, age did not influence plasma concentrations of vitamin E in this study.

Dietary Intake of Vitamin E

The average daily nutrient intake of each individual may be found in Table i of the Appendix. The estimated daily dietary intake

of alpha-tocopherol activity (alpha-tocopherol times 1.25) is given in Table 6. These data do not include supplemental vitamin E. Also in Table 6 is presented the ratio of alpha-tocopherol activity to polyunsaturated fatty acids (linoleic acid) in the diet.

The mean alpha-tocopherol activities in the daily diets of these subjects were less than the 8 and 10 mg recommended for women and men by the National Research Council (1974). Furthermore, the total group, the elderly group, and the women had lower mean dietary intakes than any of those reported in the literature (Table 2). The younger group and the men had higher intakes than those estimated by Bunnell et al. (1965) but lower than those of the other four. The daily tocopherol intake of the young group was significantly ($p \leq 0.10$) greater than that of the older subjects. But the intake of the men was not significantly greater than that of the women. There were several individuals whose dietary tocopherol exceeded the recommended allowances. Inspection of their diets shows that they ate larger quantities of mayonnaise, vegetable oils and vegetable oil margarines.

The recommended ratio of 0.6 mg alpha-tocopherol per gram of polyunsaturated fatty acid (or 0.75 mg alpha-tocopherol activity) was exceeded by the total group, as well as by each of the subgroups (Table 6). In fact, only seven of the 30 individual subjects had ratios of less than 0.75 mg/gm. The high ratios appear to be due to the low intake of polyunsaturated fatty acids rather than to a high

Table 6. Average dietary intake of vitamin E

	Elderly			Young			Means	
	Subject	Alpha-tocopherol activity	Alpha-tocopherol activity/PUFA	Subject	Alpha-tocopherol activity	Alpha-tocopherol activity/PUFA	Alpha-tocopherol activity	Alpha-tocopherol activity/PUFA
		mg	mg/gm		mg	mg/gm	mg	mg/gm
Males	9	4.17	0.99	102	13.35	1.09		
	22	5.78	1.38	106	5.90	0.72		
	29	8.95	0.97	108	13.86	0.61		
	41	5.79	0.70	109	16.09	0.71	9.86±4.42	1.09±0.60
	56	7.90	2.82	111	11.42	0.65		
	67	7.83	1.22					
	70	17.28	1.28					
Females	5	5.83	0.87	101	9.19	0.77		
	18	4.74	1.10	103	5.76	1.10		
	30	8.16	1.95	104	6.41	1.23		
	32	4.96	9.94	105	9.38	1.36		
	34	9.60	1.92	110	11.61	0.66		
	36	11.69	1.10				7.60±3.00	1.72±2.14
	39	7.00	1.75					
	43	9.68	1.01					
	47	1.35	0.59					
	51	5.58	0.75					
	58	3.70	1.03					
	63	11.28	0.74					
	69	10.90	3.16					
Means		7.61±3.56*	1.76±2.04		10.30±3.59*	0.88±0.26	8.51±3.74	1.47±1.17

*Young significantly greater than elderly ($p \leq 0.10$)

consumption of vitamin E. The diets of women had higher alpha-tocopherol:PUFA ratios than did those of men, despite the fact that the women's vitamin E intake was lower. Similarly, diets of the elderly had a higher alpha-tocopherol:PUFA ratios than did those of the young, although the mean intake of tocopherol by the elderly was less than that of the young group. In each case, the deciding factor was the dietary content of polyunsaturated fat.

Correlation of Plasma Vitamin E with Dietary Intake

No significant relationship existed between concentrations of vitamin E in the plasma and dietary alpha-tocopherol activity (R^2 = 0.08) or mg alpha-tocopherol activity/gm PUFA (R^2 = 0.00) in the 25 unsupplemented subjects. Scatter plots of plasma vitamin E versus dietary vitamin E and vitamin E/PUFA did not demonstrate any non-random relationship. This lack of correlation was not expected because the supplemented subjects did have greater mean plasma concentrations than the unsupplemented subjects. However, the differences in vitamin E intake among unsupplemented subjects were far less than the differences between supplemented and unsupplemented subjects.

There was no significant relationship between dietary alpha-tocopherol activity and mg alpha-tocopherol activity/gm PUFA.

Correlation of Vitamin E with Carotene, Vitamin A, Vitamin C and Cholesterol in the Plasma

If vitamin E does function as an antioxidant in the protection of carotene, vitamin A and vitamin C, a correlation might be expected between vitamin E in the plasma and these other nutrients. Data were available on the serum ascorbic acid, serum cholesterol, plasma retinol and carotene concentrations of the elderly subjects in this study.[2] A significant ($p \leq 0.05$) linear relationship existed between plasma tocopherols and serum ascorbate ($R^2 = 0.30$). Majaj (1966) also found a correlation between vitamins E and C in the serum of patients suffering from protein-calorie malnutrition. No significant linear relationship was found between plasma tocopherols and carotene ($R^2 = 0.04$) or retinol ($R^2 = 0.01$). These results are in contrast to those of Leitner et al. (1960) who found linear relationships between serum vitamin E, and carotene or vitamin A.

A significant ($p \leq 0.05$) linear relationship existed between plasma vitamin E and serum cholesterol ($R^2 = 0.22$). This relationship is consistent with the correlation between serum tocopherols and cholesterol ($R^2 = 0.42$) found by Rubenstein et al. (1969). This group also observed a correlation between the concentrations of tocopherol and beta-lipoproteins ($R^2 = 0.65$). Both cholesterol and

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[2]Unpublished data from M. S. Lee, G. W. Song and E. S. Yearick.

most of the tocopherols (McCormick et al., 1960; Baker et al., 1967) are associated with beta-lipoproteins in the plasma. Consequently, an increase in plasma lipoproteins could account for concurrent increases in both of these components.

SUMMARY

Relationships between the dietary vitamin E and plasma concentrations of vitamin E have been investigated in 20 elderly and ten young subjects. The total tocopherol concentration in plasma was determined chemically, and the average alpha-tocopherol activity of the daily diet was computed from three-day dietary records. Five of the older subjects were chosen because they were known to take supplements of vitamin E.

The mean concentration of tocopherol in plasma of all unsupplemented subjects was 1.12±0.33 mg/100 ml. No significant differences in plasma concentrations were observed between the various groups of unsupplemented subjects: the elderly versus the young or the men versus the women. The mean tocopherol concentration in plasma of the five supplemented subjects was 1.39±0.26 mg/100 ml. This was significantly ($p \leq 0.10$) greater than that of the 25 unsupplemented subjects. Individual plasma tocopherols showed no significant trend with age either in the supplemented group, the unsupplemented older group, or in the young group.

The mean alpha-tocopherol activity in the daily diet of all subjects was 8.51±3.74 mg. The diet of the younger group contained significantly ($p < 0.10$) more alpha-tocopherol activity than did the diet of the older subjects (10.30±3.59 mg versus 7.61±3.56 mg).

The dietary differences between the sexes were not significant. The diets of both men and women provided somewhat less than the recommended allowances of alpha-tocopherol activity.

The mean ratio of dietary alpha-tocopherol activity to polyunsaturated fatty acids was 1.47±1.17 mg/gm. This exceeds the recommended ratio. No significant linear relationship was found between plasma tocopherols and the dietary content of alpha-tocopherol activity or the E:PUFA ratio.

Significant linear relationships ($p \leq 0.05$) were found between plasma concentrations of tocopherol and ascorbate as well as tocopherol and cholesterol. No relationship was observed between plasma concentrations of tocopherol and vitamin A or carotene.

In this study, neither age nor sex influenced the plasma tocopherol concentration of subjects consuming widely varying amounts of vitamin E. The use of supplemental vitamin E was accompanied by significantly higher concentrations of tocopherol in the plasma.

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APPENDIX

Table i. Dietary Intake (Average of three day - diet study)

Subject number	Food energy (kcal)	Protein (gm)	Fat (gm)	Saturated (gm)	Oleic (gm)	Linoleic (gm)	Carbohydrate (gm)	Calcium (mg)	Iron (mg)	Vitamin A value (I. U.)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Ascorbic acid (mg)
5	1606.4	72.1	66.4	23.5	25.7	6.7	182.9	979.9	10.60	2665.8	.82	1.56	16.5	56.7
9	1383.1	61.6	59.5	23.3	22.9	4.2	157.2	963.4	6.24	3411.2	.73	1.79	7.7	44.0
18	1953.1	107.2	75.6	36.9	27.4	4.3	216.9	2256.5	8.38	6880.0	1.19	3.65	13.0	44.4
22	1754.1	81.5	54.5	22.6	20.4	4.2	227.5	903.4	14.72	3381.6	1.19	1.74	12.4	98.8
29	1888.3	67.3	77.3	23.9	27.3	9.2	244.3	763.8	13.39	3447.6	.99	1.41	13.1	35.0
30	1250.8	59.6	49.9	18.4	18.3	4.2	148.8	607.6	15.43	5069.5	.54	.90	13.3	129.4
32	1817.6	80.1	85.0	39.5	28.7	.5	199.3	1333.9	13.57	30338.7	1.02	3.64	18.1	201.5
34	1690.1	70.7	66.4	26.2	26.4	5.0	214.7	108.8	8.67	10388.0	.89	1.69	13.6	236.6
36	1472.2	49.2	60.7	18.0	22.2	10.6	185.1	421.5	8.10	4071.7	.72	.96	12.4	73.0
39	1175.8	53.7	47.0	17.0	19.3	4.1	136.8	375.6	8.97	2390.6	.68	.80	9.4	131.2
41	2062.3	69.3	72.9	25.0	26.9	8.2	299.7	735.3	18.87	29465.4	.98	2.98	18.6	150.8
43	1590.8	66.8	66.0	22.9	23.3	9.6	189.8	675.5	11.88	9542.1	1.03	1.35	12.3	102.7
47	765.2	47.2	33.4	12.8	13.2	2.3	69.8	204.8	6.49	467.8	.62	.65	9.3	16.0
51	1475.0	65.0	69.2	25.5	27.3	7.4	155.3	562.3	9.99	2143.9	.76	1.24	11.8	20.2
56	1673.9	58.5	55.6	23.6	22.3	2.8	237.2	1028.3	9.45	3845.3	.99	1.77	9.2	63.3
58	953.4	40.2	25.9	8.8	10.8	3.6	142.4	572.6	6.74	3085.7	.51	1.11	7.2	63.1
63	1358.6	50.8	60.7	15.4	19.2	15.3	158.3	351.3	9.48	3717.4	1.04	.87	12.5	177.2
67	2093.5	93.2	91.9	36.9	38.0	6.4	233.4	672.3	13.97	5833.9	1.14	1.65	17.9	149.2
69	1573.3	71.5	52.9	15.8	19.1	6.0	196.1	870.4	10.35	10158.4	1.19	1.69	16.7	170.0
70	2062.6	71.9	73.9	16.9	30.7	13.5	271.7	903.7	13.59	10436.4	1.52	1.94	14.9	204.2
101	1634.4	90.8	75.1	25.4	26.7	11.9	154.6	878.6	13.16	7382.6	1.23	1.88	13.7	132.1
102	2381.4	114.1	128.5	42.7	42.5	12.2	161.6	1149.6	17.04	6390.3	1.04	2.17	13.1	53.7
103	1886.5	93.2	76.3	32.3	26.5	5.2	215.0	1325.9	10.07	4873.7	1.47	2.39	15.6	143.4
104	1227.0	77.0	64.5	24.2	21.1	5.2	88.9	815.9	7.36	3934.2	.63	1.30	12.8	55.3
105	1611.4	48.9	61.0	18.0	20.2	6.9	220.5	479.5	6.77	3014.3	.53	.86	8.9	10.8
106	2364.4	98.4	81.7	30.6	30.8	8.2	277.1	1252.1	13.71	2303.0	1.05	2.37	14.7	71.9
108	2055.6	81.3	107.5	31.1	32.3	22.8	171.4	852.3	14.36	8156.0	1.33	1.75	19.7	150.6
109	2086.3	70.7	105.0	31.3	35.3	23.9	225.0	723.1	11.87	4427.8	1.08	1.49	12.6	100.0
110	1586.0	62.9	77.6	24.1	28.4	17.5	161.5	733.0	8.73	3490.0	.97	1.47	13.2	103.4
111	3549.7	122.3	151.5	54.1	52.8	17.7	419.9	1748.2	16.54	9747.3	1.57	2.96	19.1	195.7

Table ii. Concentrations of carotene, retinol, ascorbate and cholesterol[1]

Subject	Carotene	Retinol	Ascorbate	Cholesterol
	μg/100 ml plasma	μg/100 ml plasma	mg/100 ml serum	mg/100 ml serum
5	86	232.62	0.888	162
9	63	280.86	1.183	229
18	38	258.89	2.025	194
22	140	184.92	1.567	216
29	58	139.09	1.393	167
30	152	127.30	1.389	207
32	200	168.04	1.872	225
34	308	110.15	1.833	211
36	94	92.73	1.805	174
39	69	57.89	1.436	155
41	66	92.73	1.751	171
43	160	115.78	0.610	185
47	76	139.36	1.143	226
51	32	83.08	1.282	203
56	68	78.26	1.693	149
58	63.5	228.87	1.213	218
63	188	101.57	1.981	268
67	244	112.80	1.283	208
69	236	95.40	1.593	238
70	154	107.20	1.300	133

[1]Unpublished data from M. S. Lee, G. W. Song and E. S. Yearick.