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THE VITAMIN E STATUS OF HEMODIALYSIS PATIENTS

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ABSTRACT

THE VITAMIN E STATUS OF HEMODIALYSIS PATIENTS

By

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Serum total vitamin E, serum total vitamin E:serum total lipid ratio, and erythrocyte hemolysis were used to compare the vitamin E status of 20 patients with chronic renal failure being treated with hemodialysis and 20 healthy, age and sex matched controls. Subjects showed no evidence of nephrotic syndrome, diabetes or androgen therapy and were within ±15% of ideal weight. Two 24-hour dietary records for each subject were evaluated for intake of α-tocopherol and polyunsaturated fat. Values for serum total vitamin E and the serum total vitamin E:serum total lipid ratio were within normal limits and there was little difference between patients and controls. The erythrocyte hemolysis test did not appear to be a valid indirect assessment of vitamin E status in hemodialysis patients, probably because of interference by uremic toxins. Positive correlations were shown between serum total vitamin E and serum total lipids and between intakes of α- tocopherol and polyunsaturated fat. There was little correlation between dietary intake of α-tocopherol and serum total vitamin E. From this study it appears that the vitamin E status of individuals with chronic renal failure treated with

hemodialysis is normal as assessed by absolute serum levels of total vitamin E and the serum total vitamin E:serum total lipid ratio. The results suggest that the vitamin E requirement of these hemodialysis patients can be met by their usual dietary intakes without the use of supplements.

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INTRODUCTION

The nutritional status of a patient with chronic renal failure who is being treated with hemodialysis plays a major role in predicting the outcome of the illness. Consequently there is considerable interest in determining abnormalities in nutrient parameters which are secondary to the disease process or its treatment. The nutritional status of hemodialysis patients has been studied with respect to several vitamins, while other vitamins, such as vitamin E, have received little attention. Until recently there was no reason to expect abnormalities in the fat-soluble vitamins and research centered around the removal of the water soluble vitamins in the dialysate fluid. With the discovery of abnormalities in the metabolism of vitamins A and D in chronic renal failure it is possible that the metabolism of other fat-soluble vitamins is altered in the hemodialysis patient.

The function of vitamin E in humans cannot be stated with certainty but may involve its possible antioxidant properties or metabolic regulating activities. It is possible that these functions may have implications for the hemodialysis patient. There are a number of complications accompanying uremia and the hemodialysis process, such as

lipid abnormalities, anemia, and a number of nutrient imbalances that may have some relation to vitamin E status.

The experiment described herein was designed to study the effect of selected dietary and serum components on the vitamin E status of free-living adults with chronic renal failure being treated with hemodialysis.

REVIEW OF LITERATURE

Vitamin E

Function

The function of vitamin E at the metabolic level especially in man remains an enigma. After many years of work in the area, investigators cannot agree on the function of vitamin E. Some researchers contend that the vitamin is primarily an antioxidant for polyunsaturated fatty acids (PUFA) in tissues. Others argue that it must be a component of an enzyme or transport system and is therefore primarily metabolic in function (Scott, 1978). The action of vitamin E will continue to be the subject of future research.

The antioxidant theory hypothesizes that unsaturated lipids are under attack by free radicals and when oxygen is present the lipids undergo peroxidation. Vitamin E reacts with the free radicals in vivo and inhibits peroxidation. If vitamin E is not present in sufficient amounts the peroxidation becomes uncontrolled and leads to damage of intracellular membranes and enzymes. Therefore the diverse effects of vitamin E deficiency may be secondary to lipid peroxidation (Green, 1972).

Because of the technical difficulties in measuring the minute amounts of actual peroxides in tissues, the basis of proof of the antioxidant theory rests on the in vitro tests quantifying the susceptibility of red blood cells (RBC) to hemolysis and malonyldialdehyde (MDA) formation from the degeneration of lipid peroxides (Green, 1972). The crux of the matter is if lipid peroxidation in vitro can be considered to be representative of peroxidation in vivo.

The role of vitamin E and selenium in the peroxidation of lipids is often confused because selenium has been effective in treating vitamin E responsive diseases in animals (Burk, 1976). However, a requirement of selenium in some species has been shown even when adequate vitamin E is supplied in the diet. The functional difference is that selenium is part of the enzyme, glutathione peroxidase (Rotruck et al., 1973), which reduces lipid peroxides to hydroxy acids (Christophersen, 1968a, 1968b), while the proposed antioxidant function of vitamin E is to prevent the formation of lipid peroxides. The biochemical properties of selenium are similar to those of sulfur (Li and Vallee, 1980). In vitamin E deficiency glutathione peroxidase detoxifies in vivo lipid peroxides. Therefore the selenium in this enzyme spares vitamin E by preventing the decomposition of peroxides to free radicals that could reinitiate peroxidation. Some signs of vitamin E deficiency that are dependent on dietary PUFA do not respond to

selenium, such as erythrocyte hemolysis, perhaps because the aqueous enzyme cannot reach the cellular site of peroxidation or the peroxides of PUFA may decompose quickly before the enzyme can interfere. If glutathione cannot be formed in RBC, hemolytic anemia will result. Vitamin E therapy has been successful in improving erythrocyte survival in deficiencies of glutathione synthetase (Spielberg et al., 1979). Therapy with vitamin E in a neonate decreased the symptoms of glutathione peroxidase deficiency without altering the levels of the enzyme (Boxer et al., 1979).

The RBC hemolysis test has been utilized to demonstrate the peroxidation of RBC lipids in numerous studies, some of which will be reviewed later. Horwitt et al. (1968) has also attempted to verify the antioxidant theory by showing that adult men receiving 3 mg of tocopherol per day demonstrated increasing RBC hemolysis as the fat content of their diet progressed from 30 gm of lard to 30 gm of oxidized corn oil and finally to 60 gm of oxidized corn oil over a period of six years. During that time plasma tocopherol concentrations gradually declined displaying an inverse relationship to the RBC hemolysis.

Further support for the antioxidant theory is found in the work of Folkers (1974) who showed epoxidation of lipoidal coenzyme Q in vitamin E deficient rabbits thus suggesting that vitamin E protects the highly unsaturated lipid,

coenzyme Q, from oxidation.

Lucy (1972) hypothesizes that vitamin E inhibits the oxidation of non-heme iron-containing proteins found in the mitochondria and smooth endoplasmic reticulum of rat livers. Diplock (1974) demonstrated that oxidation-labile non-heme iron was present in rat livers only when both vitamin E and selenium were available in the diet. The function of the non-heme iron-containing proteins in microsomal electron transfer systems which are responsible for drug metabolism was investigated. The demethylation of drugs was altered in vitamin E deficient rats. Diplock proposed that during vitamin E deficiency the selenium protein may be replaced by a sulfur protein which would change the kinetics of the associated demethylase system. In related work Carpenter and Howard (1974) investigated the effect of vitamin E and androgens on the enzyme systems bound to the endoplasmic reticulum in rat livers. They showed that the effect of vitamin E on hepatic microsomal hydroxylations of drugs is independent of the androgen status of the rat.

The lack of evidence of lipid peroxides in tissues has led to research which openly questions the antioxidant theory and consequently has resulted in many interesting

discoveries of proposed metabolic functions. Vitamin E is thought to play a role in heme synthesis (Nair, 1972) by controlling the induction and repression of the rate determining enzymes aminolevulinic acid synthetase and aminolevulinic acid dehydratase. The heme protein, catalase, may catalytically scavenge peroxides created by the oxidation of lipids. Caasi et al. (1972) support this idea with their work on the effect of vitamin E deficiency on heme proteins, which showed that catalase activity decreased significantly when rats were fed a vitamin E deficient diet.

Many other metabolic functions for vitamin E have been postulated recently. Kawai et al. (1974) showed that the addition of vitamin E to brain cell enzyme preparations inhibited cell membrane ATPases in vitro. Their results suggest the possibility of a vitamin E enzyme function because the antioxidant, BHT, did not produce a similar ATPase inhibition when it was added to the cell preparations. Tocopherol has been shown to promote larger body sizes of rotifers when included in the culture medium (Gilbert, 1974). This was the first report of a vitamin E stimulated growth response. The conversion of vitamin B_{12} to its coenzyme form may be inhibited by a vitamin E deficiency (Pappu et al., 1978). Olson (1974) has tested the hypothesis that vitamin E regulates the synthesis of specific proteins necessary for muscle function and found

that the turnover of creatinine phosphokinase was twice the normal rate in vitamin E deficient rabbits.

Another function of vitamin E relates to its interaction with vitamin A. Green (1972) suggests there are four ways for vitamin E to exhibit a sparing effect on vitamin A: 1) protect vitamin A in the gut from oxidation, 2) increase vitamin A absorption, 3) increase vitamin A utilization, and 4) increase storage of vitamin A. The effects of this complex relationship depend on dosage of the vitamins, diet, and the length of the experimental period. In studies with rats fed a diet deficient in vitamins A and E, Sondergaard (1972) showed that the addition of vitamin E to the diet maintained approximately 90% of liver stores of vitamin A. When other antioxidants were given with the vitamin A deficient diet this effect was not seen. In situations where the intakes of both vitamins A and E are excessive, vitamin E tends to alleviate the toxicity of vitamin A (McCuaig and Motzok, 1970; Jenkins and Mitchell, 1975). The latter authors have also shown an interaction between vitamin A and E when given in excess amounts such that each appears to cancel the negative effects of the other.

While it appears that there is a sizable body of literature against the antioxidant theory, new research makes a strong argument to renew the controversy. With the availability of a new technique, expired air from a subject can be analyzed by gas chromatography for ethane and

pentane which evolve from the peroxidative decomposition of linolenic and linoleic acids (Riely et al., 1974). This is the first in vivo method for determining peroxidation of lipids. Hafeman and Hoekstra (1977a) have validated the method by showing that supplements of vitamin E, selenium, or methionine when added to a diet deficient in all three will protect against peroxidation as evidenced by ethane evolution when rats are injected with carbon tetrachloride (CCl_4). In continued research (1977b) these authors have shown similar effects with vitamin E and selenium deficient diets without the use of CCl_4.

Deficiency

A deficiency of vitamin E is rarely seen in humans except for premature infants and children or adults with failure to absorb fats. However, deficiency states can be easily induced in animals producing a variety of symptoms (Bieri, 1975). The contrast between man and animal can be explained by their respective nutritional intakes. Animals are restricted in the variety of foodstuffs consumed as compared to man. In addition, animals can be raised from infancy in the vitamin E deficient state, which would never be considered in humans. It is highly unlikely to be able to induce a vitamin E deficiency in healthy human adults because of the tissue stores of this fat-soluble vitamin.

Fitch (1972) contends that there may be three mechanisms involved in the etiology of the anemia seen in

conjunction with vitamin E deficiency, depending on the species involved. The mechanisms are: 1) blood loss, possibly by effusion of blood into tissues that may occur in poultry, 2) oxidant hemolysis as demonstrated by the RBC of premature human infants, and 3) ineffective erythropoiesis as seen in bone marrow abnormalities of rhesus monkeys.

The increased susceptibility of RBC hemolysis in vitamin E deficiency is easily quantified and is reported frequently in the literature. The RBC hemolysis test involves the incubation of washed erythrocytes in solution with an oxidant such as hydrogen peroxide or dialuric acid. The resulting hemolysis is quantitated by measuring colorimetrically the hemoglobin released and comparing it to that released in distilled water by red blood cells from the same sample. The results are expressed as a percentage. Erythrocytes incubated in buffered saline may be incubated also and used as controls (Sauberlich et al., 1974).

Leonard and Losowsky (1967) compared the results of RBC hemolysis and plasma vitamin E levels in 233 ill and healthy subjects and found that approximately 90% of the subjects with hemolysis greater than 10% had plasma vitamin E levels of less than 0.3 mg/100 ml. In addition to documenting the effects of hydrogen peroxide (H_2O_2) concentration, its rate of addition, time, and temperature on the

RBC hemolysis test, Horwitt et al., (1956) showed when 19 human males were fed a vitamin E depleted diet over a 20 month period that there was an inverse relationship between plasma vitamin E and the percent of RBC hemolysis. Stocks and Dormandy (1971) examined the autoxidation of RBC from healthy adult humans by measuring the formation of malonyldialdehyde (MDA), a product of polyunsaturated fat oxidation. This in vitro technique has been criticized for not being representative of in vivo reactions; however, the same criticism can be made for the RBC hemolysis test. Their results showed that the concentration of specific antioxidants such as α-tocopherol or BHA had only a partial effect on MDA formation when RBC were exposed to oxidative stress and that therefore there may be a multitude of intra- or extra-cellular factors affecting the susceptibility of cells to autoxidation.

One of these other factors that has been examined in detail is the effect on RBC hemolysis of fatty acid composition, both within the erythrocyte and as taken in the diet. When rats were fed either corn, cottonseed, or hydrogenated coconut oils as 30% of the diet or fat-free diets each with or without tocopherol supplements, the rats consuming the unsupplemented corn oil, hydrogenated coconut oil, or fat-free diets demonstrated RBC hemolysis greater than 50% (Alfin-Slater et al., 1969). Brin et al. (1974) studied the effect of erythrocyte fatty acid levels

on erythrocyte hemolysis in rats and rabbits. They showed that in RBC membranes arachidonate is oxidized preferentially over linoleate and because rabbits have lower levels of arachidonate than rats, the RBC of rabbits were relatively more resistant to RBC hemolysis.

Gyorgy et al. (1952) have shown that in the normal newborn infant, some hemolysis (approximately 30%) is seen in the first few days of life, but the levels of hemolysis begin to approach normal by the fifth day. In the premature infant, the supplementation of 25 to 150 mg daily of α-tocopherol acetate at 2 to 4 weeks of age changed the results of the RBC hemolysis test from strongly positive to negative (Gordon and deMetry, 1952). Gordon et al. (1955) in further studies, examined erythrocyte hemolysis levels in full term and premature infants at birth and 7 weeks of age. There was no significant difference in the two groups at birth, however at 7 weeks the full term infants, especially those that had been breast fed, had significantly less hemolysis than the premature infants.

Because iron catalyzes the autoxidation of unsaturated fatty acids (Smith and Dunkley, 1962) its routine use in the treatment of the anemia of premature infants was investigated by Melhorn and Gross (1971). Premature infants were divided into 4 study groups as follows: vitamin E supplemented, iron supplemented, vitamin E and iron supplemented, or no supplementation and followed for 16 weeks.

The lower the birth weight the more pronounced was the effect of increasing RBC hemolysis in any of the four groups. The group receiving iron supplements had the greatest degree of hemolysis followed by the group receiving no supplements, followed by the group receiving vitamin E and iron supplements, and followed by the group receiving vitamin E supplements in order of decreasing hemolysis. Those infants receiving only vitamin E demonstrated the highest serum vitamin E levels, especially those with the lowest birth weights. This research was extended by Williams et al. (1975) who examined the fatty acid composition as well as the iron levels in formulas being fed to premature infants. High and low levels of both PUFA and iron were given in four combinations. Those infants receiving high levels of both PUFA and iron were the only group to demonstrate hemolytic anemia as evidenced by RBC hemolysis. The use of intramuscular vitamin E in the premature infant has been observed to prevent high levels of RBC hemolysis in most cases if given in sufficient amounts (Graeber et al., 1977).

In addition to the anemia associated with the vitamin E deficiency in premature infants, other conditions may be related to the vitamin deficiency in infants. Retrolental fibroplasia, a disease of growing blood vessels in the eye, occurs in premature infants as their retinal vessels (which are sensitive to oxygen tension) are not yet mature.

Johnson et al. (1974) have reported that treatment with intramuscular vitamin E appears to decrease the incidence and severity of retrolental fibroplasia when compared with a placebo treatment. Bronchopulomonary dysplasia is the toxic result of exposure of high concentrations of oxygen to the lungs which may occur with the acute respiratory distress syndrome of premature infants. Intramuscular injections of vitamin E were reported to have no statistical effect in decreasing the severity of the disease (Ehrenkranz et al., 1978), but the infants receiving vitamin E generally required less respiratory therapy.

Melhorn and coworkers (1971) have demonstrated that anemias which are considered hemoltyic in nature, such as sickle cell disease, autoimmune hemolytic anemia, erythroblastosis fetalis, and iron deficiency will give abnormal values for RBC hemolysis. Anemias due to bone marrow abnormalities, however, such as aplastic anemia, juvenile pernicious anemia, folic acid deficiency anemia, and thalassemia are associated with minimal or no RBC hemolysis. Serum vitamin E levels were reported to be within the normal range for all anemias in both classifications.

Other workers are in disagreement, however, having found lower levels of serum vitamin E in some of the anemias that Melhorn's group classified as hemolytic. Fujii and Shimizu (1973) found significantly lower values for serum vitamin E, although still within the normal

range, in subjects with iron deficiency anemia when compared with controls. Natta and Machlin (1979) using a plasma tocopherol criteria of 0.8 mg/gm of total lipid found that 75% of sickle cell anemia patients were deficient in vitamin E as compared to none of the controls. These deficient patients responded to oral supplementation of DL-α-tocopheryl acetate. Additional work (Natta et al., 1980) showed that supplementation of α-tocopherol increases plasma levels of the vitamin and decreases by approximately one half the percentage of circulating irreversibly sickled cells. Therefore vitamin E may exhibit a stabilizing effect on the erythrocyte membrane.

Cystic fibrosis is associated with malabsorption of fats that may result in vitamin E abnormalities. This hypothesis was investigated by Farrell and coworkers (1977) who found that cystic fibrosis (CF) patients who were not supplemented with vitamin E were indeed deficient as evaluated by plasma α-tocopherol and RBC hemolysis levels. The percent of hemolysis was related to α-tocopherol concentration in an inverse, sigmoidal manner. In vivo hemolysis was assessed by measuring the decreased survival of ^{51}Cr labeled erythrocytes in the CF patients. Oral supplementation of α-tocopheryl acetate restored plasma concentrations of the vitamin to within normal limits and improved the lifespan of labeled erythrocytes.

Vitamin E requirement

The vitamin E requirement of a species depends on numerous variables including: efficiency of fat absorption, age, dietary levels of pro-oxidants, antioxidants, selenium, PUFA, sulfur amino acids and other fat-soluble vitamins (Scott, 1978). Various authors have hypothesized recommendations for vitamin E requirements, usually in relation to dietary fat. It has been recommended that the intake of tocopherol should be 1 mg for every 60 mg of PUFA consumed (Harris and Embree, 1963). Witting (1972) has criticized this level as impractical as it is difficult to attain in normal mixed diets, although he did agree that the vitamin E requirement is related to the tissue PUFA content. With the trend to replace vegetable fat for animal fat in the American diet, Bieri and Evarts (1973) found that γ-tocopherol was the predominate form of tocopherol in typical American intake. This was due to the increased consumption of corn and soybean oils which are excellent sources of γ-tocopherol. They suggested that more than the α-content of diets needed to be considered in the requirement. At the time the γ-form was considered to be poorly utilized, but shortly thereafter the same authors (1974) investigated the absorption of the γ-form in rats and saw no marked difference between its absorption and that of the α-form. However, they did note that its rate of tissue turnover was twice as great as that for α-tocopherol.

The four tocopherols and four tocotrienols have been considered as food sources of vitamin E, although α-tocopherol has the highest vitamin E activity in man (Bauernfiend, 1977). Biological activity of the different vitamers is difficult to assess in humans due to a lack of technology, so tests with animals are generally accepted as indicators of activity. Different species will give a variety of different values (McLaughlin and Weihrauch, 1979).

According to the Food and Nutrition Board's (FNB) current Recommended Dietary Allowances (RDA) of 1980 the correct designation for intake of vitamin E is now milligrams of α-tocopherol equivalents. This term is derived as the sum of: the milligrams of α-tocopherol, the milligrams of β-tocopherol multiplied by 0.5, the milligrams of γ-tocopherol multiplied by 0.1, and the milligrams of α-tocotrienol multiplied by 0.3. These are the only vitamers believed to have significant biological activity which are present in American diets. If only the α-tocopherol content is known, the value should be increased by 20% to account for the other vitamers, resulting in an estimate of α-tocopherol equivalents.

Horwitt (1974) proposed a formula which recommends that individuals should receive 4 mg of α-tocopherol equivalents per day even if they consume no PUFA to compensate for tissue synthesis of peroxidizable compounds. Horwitt's formula for the requirement of vitamin E in α-tocopherol equivalents is:

4 α-tocopherol + 0.25 (% PUFA of total lipids + gm of PUFA) equivalents

This formula is based on earlier research of Horwitt (1962) where the levels of linoleate in depot fats tended to approach the percentage in the diet if it was fed over a sufficiently long period. Witting and Lee (1975a) suggest that dietary requirement should be related to tissue linoleate levels which can be easily obtained and measured. Based on the 1974 Recommended Dietary Allowances (RDA) a ratio of 0.6 mg of dietary vitamin E activity per gm of linoleate in 100 gm of adipose tissue fatty acids would result in adequate vitamin E nutriture. The current RDA (1980) are given as 8 and 10 mg of α-tocopherol equivalents for adult females and males, respectively.

Intake of Vitamin E and Fat

It is often difficult to assess the vitamin E content of foods if direct analysis is not available and one must rely on tables of food composition because there is a lack of published information on this vitamin. In addition, tables of composition will demonstrate considerable variability in the nutrient content of different samples of the same food depending on soil composition, degree of maturity, trimming of edible portion, variety or strain, length of storage, milling and other processing such as canning or cooking (Watt, 1980). In a study of vitamin E intakes of subjects in the United Kingdom, poor correlation was found between values of intakes estimated

from food tables and values from actual analysis of the food (Smith et al., 1971).

Recent analyses have updated the literature of vitamin E content of foods especially for the various tocopherols in vegetable oils (Yuki and Ishikawa, 1976), canned and vended foods (Koehler et al., 1977), as well as a wide variety of food stuffs (Bauernfeind, 1977; McLaughlin and Weihrauch, 1979).

Bunnell and coworkers (1965) have analyzed the α-tocopherol content of a typical daily intake of food that might be consumed and found it to range from 2.6 to 15.4 mg with an average of 7.4 mg. Bieri and Evart (1973) analyzed cafeteria meals for alpha, gamma, delta and total tocopherols, fat, PUFA, and calories. They reported daily values of 9.0 mg α-tocopherol, 38.1 mg total tocopherol, 118 gm fat, 21.2 gm PUFA, and a α-tocopherol:PUFA ratio of 0.42. In a survey of food intakes of dentists and their wives, the men consumed an average of 45 α-tocopherol equivalents (converted) with a range of 4 to 146 equivalents while the women consumed 41 α-tocopherol equivalents with a range of 3 to 143 equivalents as judged by food frequency data compiled by computer (Cheraskin et al., 1974). Witting and Lee (1975b) analyzed typical meals served in the cafeteria of a women's university and found average intakes of 7.5 mg α-tocopherol, 28.7 mg total tocopherols, 96 gm fat, and 19.9 gm PUFA for a typical 2500 kcal daily

intake.

Intake does not necessarily reflect absorption. Losowsky et al. (1972) in studies utilizing rats and humans showed that vitamin E deficiency, amount of fat intake, or vehicle of administration did not affect absorption of α-tocopherol, although the percent of absorption did decrease with increasing doses of the vitamin.

Analysis of Vitamin E

To preface the section on vitamin E status, a discussion of the various methods of analyzing the vitamin E concentration in blood will be presented. The Emmerie-Engel (1938) colorimetric method later modified by Quaife et al. (1949) has been used for many years. Fluorometric methods have been utilized by others (Duggan, 1959; Hansen and Warwick, 1966; Thompson et al., 1973; and Taylor et al., 1976). Most recently high performance liquid chromatography (HPLC) techniques have appeared in the literature (Hatam and Kayden, 1979; and Bieri et al., 1979). The Emmerie-Engel reaction depends on the reduction of Fe^{3+} which may be non-specific if other reductants are present. The fluorometric methods have the advantages of sensitivity and specificity but are not applicable to differential analysis of tocopherols. When compared with the Emmerie-Engel reaction, Duggan (1959) found the fluorometric method to give slightly lower results. Thompson compared the fluorometric method with both colorimetric and column

chromatography methods and also reported the fluorometric method gave slightly lower results than the Emmerie-Engel reaction. However, the chromatography method indicated that both the colorimetric and fluorometric methods had a tendency to overestimate the concentration of vitamin E. With the advent of HPLC techniques the separation of the tocopherols is possible in a method that is claimed to be rapid, free from interferring impurities and useful with small samples (Bieri et al., 1979).

Vitamin E Status

There are numerous reports in the literature of blood vitamin E and lipid levels in American subjects. In 197 adult subjects of both sexes Harris et al. (1961) found a mean total plasma tocopherol level of 1.05 mg/100 ml ± 0.32 with a range of 0.36 to 1.80 mg/100 ml using a modified Emmerie-Engel method. Bieri and coworkers (1964) reported mean serum tocopherols of 1.06 ± 0.25 mg/100 ml for 71 men and 1.04 ± 0.27 mg/100 ml for 61 women by the Emmerie-Engel reaction where one third of the subjects were Black and two thirds were Caucasian. Rubenstein et al. (1969) investigated serum vitamin E and various serum lipid components in 27 normal subjects and reported mean vitamin E levels of 1.14 ± 0.30 mg/100 ml with no significant difference between sexes. The vitamin E values showed a correlation of $r = 0.85$ with serum total lipids. In plotting the previously mentioned values for patients with hyperlipemia,

the authors concluded that a serum vitamin E level exceeding 2.3 mg/100 ml usually indicates an elevated serum total lipid level. Using identification of α-tocopherol by combined gas liquid chromatography, mass spectrometry, and infrared spectroscopy, Siakotos and coworkers (1974) found a mean level of 0.49 ± 0.08 mg/100 ml α-tocopherol with a range of 0.39 to 0.64 mg/100 ml in nine normal unsupplemented adults. Plasma α-tocopherol determinations by thin layer chromatography showed mean values of 0.96 mg/100 ml with a range of 0.66 to 1.50 mg/100 ml in twelve normal subjects (Chow, 1975), while the mean total vitamin E value was 1.15 mg/100 ml. Using a fluorometric method, Hansen and Warwick (1966) reported mean values of total tocopherol of 1.85 ± 0.53 with a range of 1.00 to 3.10 mg/100 ml for adults less than 40 years of age and 1.45 ± 0.37 with a range of 0.90 to 2.30 mg/100 ml for adults older than 65 years. Blood samples from 26 female college students demonstrated total plasma tocopherol levels of 1.09 ± 0.25 mg/100 ml with a range of 0.73 to 1.76 mg/100 ml when analyzed by thin layer chromatography (Witting and Lee, 1975). In another study of college women (Yeung, 1976) a mean value of 0.87 ± 0.07 mg/100 ml of vitamin E was seen in twelve subjects using the spectrofluorometric method of Thompson et al. (1973).

Horwitt et al. (1972) examined the nature of serum tocopherol:serum total lipid relationships. After comparing

the plots of serum tocopherol with serum cholesterol, triglycerides, phospholipids, and total lipid levels, they concluded that the plot of total tocopherol versus total lipid produced the most useful indicator of vitamin E status. A ratio less than 0.8 mg of total tocopherol per gm of total lipid in serum should be considered as an indicator of inadequate vitamin E nutriture. In a sample of 22 healthy, young adults (21 to 35 years of age), Farrell and coworkers (1978) found concentrations of 0.79 ± 0.04 mg/100 ml for total tocopherols by a modified Emmerie-Engel technique, 549 ± 14 mg/100 ml for total lipids by a turbidimetric method and a tocopherol:lipid ratio of 1.38 ± 0.08 mg/gm. Twenty adult males on a neurology ward demonstrated serum α-tocopherol levels of 1.17 ± 0.10 mg/100 ml by liquid chromatography, serum total lipid levels of 685 ± 46 mg/100 ml by a turbidimetric method, and a tocopherol:lipid ratio of 1.71 ± 0.13 mg/gm (Vatassery and Chiang, 1979).

Status and Requirement of Vitamin E in Rats

The National Academy of Sciences (Board on Agriculture and Renewable Resources, 1978) has recommended the requirement for vitamin E in the rat can be met by 30 mg of DL-α-tocopheryl acetate (40.5 mg D-α-tocopheryl acetate) per kilogram of diet with a linoleic concentration of up to 5% when adequate sulfur containing amino acids and selenium are available in the diet.

Plasma levels of tocopherol in the rat vary depending on the amount and type of fat in the diet in addition to the intake of tocopherol. Rats fed soybean oil containing 15 and 108 mg/100 ml of α- and γ-tocopherols, respectively, had plasma concentrations of 0.17 and 0.10 mg/100 ml of α- and γ-tocopherols, respectively (Bieri and Evarts, 1975a). Stock rats from the same laboratory had plasma α-tocopherol concentrations of 0.2 to 0.4 mg/100 ml. The ingredients of the diet for the stock rats were not reported. Female Zucker rats that had been vitamin E depleted for two weeks and then repleted with 40 mg of DL-α-tocopheryl acetate/kg of diet had plasma α-tocopherol levels of 0.71 mg/100 ml (Bieri and Evarts, 1975b). Organs that had high concentrations of tocopherol were the liver, heart, and lungs. Rose and Gyorgy (1952) have shown that female Sprague-Dawley rats are sufficiently depleted after two weeks on a vitamin E deficient diet to show positive results for the erythrocyte hemolysis test.

Therapy with Vitamin E and Use of Excessive Doses

In addition to the previously mentioned uses of vitamin E for retrolental fibroplasia, cystic fibrosis, bronchopulmonary dysplasia, sickle cell anemia and hypervitaminosis A, it has been shown effective in improving walking tolerance and arterial blood flow to the lower legs of patients suffering from intermittant claudication (Haeger, 1974). In a double blind study investigating the effects of

megavitamin E therapy on angina pectoris, however, no significant effects were seen (Anderson and Reid, 1974).

The possibility of negative effects of megavitamin E doses have been questioned for some time. Recent studies continue to show inconclusive results. In vitro tests of leukocyte activity have shown depressed results when human subjects received 300 mg of DL-α-tocopheryl acetate for three weeks while in vivo tests have shown no suppression (Prasad, 1980). In a large double blind study with 200 subjects, half of whom received 600 mg of DL-α-tocopheryl acetate daily, there were no positive or negative effects of supplementation on general health, although there was a significant reduction of thyroid hormone levels and an increase in triglyceride levels in females (Tsai et al., 1978). In another study (Farrell and Bieri, 1975) 28 subjects who had been ingesting 100 to 800 mg per day of vitamin E for an average of three years showed no evidence of toxicity as assessed by a battery of twenty standard clinical blood assays.

Chronic Renal Failure

Overview

Chronic renal failure (CRF) is a major disease process characterized by acid-base disturbances, anemia, abnormal calcium-phosphorus metabolism, electrolyte and fluid imbalances, catabolism, and anorexia due to the reduced capacity

of the kidney to perform its functions. Kidney function is impaired by glomerular, tubular, vascular or interstitial damage to the organ (Geschickter and Antonovych, 1971). When an individual with chronic renal failure has less than 10% of his kidney function remaining, transplantation of a kidney or dialysis must be instituted to prevent death. As of 1978 there were approximately 35,000 individuals in the United States receiving dialysis treatments (Wineman, 1978). Glomerulonephritis is the primary disease leading to dialysis in 42% of the population. Other major causes are cardiovascular disease and hypertension, 14%; urinary tract disease, 11%; congenital abnormalities, 8%; diabetes mellitus, 7%, kidney infections, 6%; and unknown causes, 12%. In recent years the age of the typical dialysis patient has been gradually increasing due to the tendency to transplant younger patients as well as broadened patient acceptance criteria for dialysis patients. In many dialysis centers the mean age exceeds 50 years of age.

Anemia

As early as the 1950's the dual mechanism of the anemia of CRF was recognized. Desforges and Dawson (1958) noted mild abnormalities in RBC fragility, decreased RBC survival time, and decreased plasma iron turnover in CRF patients and concluded that both RBC destruction and the inability of bone marrow to compensate for it contributed to the

anemia. Eklund et al. (1971) saw a degree of hemolysis in uremics, especially those with chronic pyelonephritis and active urinary tract infections, when compared with healthy controls. In examining iron kinetic data, they saw depressed erythropoiesis which resulted in a larger circulating iron pool which was not utilized. Wallner et al. (1976) have tested for the presence of a serum factor toxic to erythropoiesis in CRF patients and found that in a system utilizing dog bone marrow cells that CRF sera but not normal sera or sera from anemias of other chronic diseases inhibited heme synthesis. Addition of urea or creatinine did not impair the marrow system activity; consequently these authors conclude that there is a factor in the serum of CRF patients that inhibits erythropoiesis in vitro. Two explanations for the hemolytic nature of the anemia of uremia of CRF are given by Jacob and coworkers (1975) whose results show that in 25% of the dialysis patients studied, the hexose monophosphate shunt of RBC was inhibited and that chloramine (a powerful oxidant) from unpurified water appeared to induce hemolysis. The mechanical trauma that RBC receive during the in vitro dialysis treatment may also contribute to the anemia. Reitman et al. (1970) have shown with in vitro studies that when RBCs were exposed to mechanical trauma (such as a rotary pump) that RBCs from rabbits injected intraperitoneally with vitamin E were less susceptible than those from normal rabbits to

hemolysis induced by H_2O_2 at concentrations greater than 1.5%. The effect was due to vitamin E rather than the trauma, however, as control RBCs (not pumped) displayed hemolysis similar to normal RBCs. Another contributing factor to the anemia of CRF is iron deficiency, which may be due to frequent blood sampling; limited iron consumption and absorption from a diet restricted in protein and, indirectly, in ascorbic acid; occult blood loss; and blood loss during hemodialysis treatments. Compared to serum iron or transferrin levels, serum ferritin is the best indicator of bone marrow iron stores (Aljama et al., 1978). Consequently serum ferritin should be a useful indicator of total body iron stores in uremic subjects.

Lipid Metabolism

In contrast to the hypercholesterolemia seen in nephrotic syndrome, CRF is characterized by hypertriglyceridemia (Feldman and Singer, 1974). The mechanism of the hypertriglyceridemia is controversial. It has been hypothesized that an accumulation of insulin antagonists is responsible for the lipid disorder. One study showed no correlation between insulin and triglyceride concentrations in either uremics or controls. While the concentrations of insulin were similar for both groups, the uremic subjects had elevated concentrations of triglycerides not seen in control subjects (Kaye et al., 1973). Glucose intolerance is a frequent feature of uremia presumably because of

tissue insensitivity to insulin, which would result in elevated levels of serum insulin. Heuck et al. (1978) have reported elevated levels of insulin in uremic rats. When the rats were fed diets rich in either carbohydrate, fat or protein, triglycerides were most elevated in the group fed a carbohydrate-rich diet prompting the authors to recommend low carbohydrate diets for uremic patients because of the risk of coronary heart disease.

In addition to the insulin-related theories, it has been suggested that a defect in the removal of triglycerides from plasma exists in patients with uremia. A significant relationship has been reported between plasma triglyceride levels and both very low density lipoprotein (VLDL) secretion rate and post-prandial insulin response in uremic patients, 50% of whom were receiving hemodialysis treatments (Reaven et al., 1980). The majority of these patients also demonstrated decreased removal of VLDL from plasma, higher triglycerides at any given VLDL secretion rate than controls, and lower triglycerides at any given post prandial insulin response than controls, indicating a decreased ability of insulin to stimulate VLDL secretion, however, they did not examine the uptake of triglycerides. Changes in dietary carbohydrate of these patients affected post prandial insulin response, triglyceride levels and VLDL secretion showing a strong influence of diet on this mechanism.

Chronic hemodialysis may further aggrevate uremic hypertriglyceridemia. Dialysate glucose levels may be an additional source of carbohydrate for the uremic patient. Dombeck et al. (1973) saw no effect of a glucose-free dialysate on lipid levels, but they did note that one half of their non-dialyzed uremic patients already exhibited hypertriglyceridemia. Swamy et al. (1977) however, reported triglyceride concentrations were lowered in 25% of patients receiving a glucose-free dialysate for 6 months.

Other factors such as type of primary disease, nutritional status, body weight, and medications may also effect hypertriglyceridemia. Gutman et al. (1973) found hyperlipidemia in 70% of dialyzed and nondialyzed uremic patients and it correlated with sufficient caloric intake and good nutritional status in both groups. Dombeck and coworkers (1973) reported that administration of an androgen to male dialysis patients resulted in marked elevations of triglyceride concentrations in 75% of the patients.

Nutrition

Patients that have been treated with hemodialysis for a period of time show the combined effects of the treatment and the disease process. Many of these effects are nutrition related. Previously nutritional therapy was a conservative treatment for CRF, used to prolong life. Today, however, it is an integral component of the therapeutic regimen of maintenance hemodialysis which allows the

patient a near normal mode of existence (Burton, 1977). The balance between the patient's renal excretory capacity and nutritional intake will determine the patient's health status. Therefore the goal of nutritional therapy is to minimize the symptoms of the toxicity and other metabolic disorders of CRF while promoting optimal nutritional status (Kopple, 1978).

In CRF many metabolic disturbances require adjustments in nutritional therapy. Some examples are: retention of nitrogenous wastes requiring a restriction of dietary protein; decreased ability to excrete sodium, potassium, magnesium, and phosphorus necessitating their restriction in the diet; decreased intestinal absorption of calcium requiring dietary restriction of phosphorus and possible supplementation of 1,25 dihydroxycholecalciferol; deficiency or decreased activity of some vitamins requiring supplementation; and a tendency toward tissue wasting indicating a need for careful nutritional monitoring (Kopple, 1978). Successful nutritional therapy requires a strong team effort by the physician, dietitian, nurse, and social worker to reinforce the patient and his family.

Nutritional studies are different from other types of medical research due to a number of factors including: whole body response, body stores, bioequivalency of some nutrients, multiple functions of nutrients, and divergence of results of short- and long term studies (Burton and

Wineman, 1978). Nutritional studies in CRF are further complicated by the type, stage, and progression of the disease. Under ideal conditions, studies should be done at a time when the patient is in a steady state (Kopple et al., 1975). However, with the progressive nature of CRF the patient is always displaying a gradual, but over time, significant change in excretory capacity and consequently metabolic and endocrine function.

Vitamins

The many vitamin abnormalities seen in hemodialysis patients are due to: 1) decreased consumption of nutrients because of dietary restrictions, 2) removal of water-soluble vitamins in the dialysate during hemodialysis, and 3) altered excretory or metabolic functions which may affect absorption from the gut, excretion from the body, or activity in the body. Altered metabolic functions may be due to drug-nutrient interactions (Kopple, 1975). The practice of reducing the potassium content of vegetables by leaching may also leach water soluble vitamins which would compound the decreased intake of vitamins due to dietary restrictions (Burge, 1974).

The literature regarding vitamin status in CRF is not extensive. In many cases the number of subjects studied was small. Other frequent problems include no indication of the levels of dietary intake of nutrients, no reference to recent transfusions, no mention if supplements were

being given, and no discussion of possible interferring drugs.

Lasker et al. (1963) reported normal to elevated levels of vitamin B_{12}, biotin and pantothenic acid, normal to decreased levels of thiamin, decreased levels of folacin and niacin in a study of four patients. Stone et al. (1975) examined plasma pyridoxal-5-phosphate, plasma glutamic-oxaloacetic transaminase and erythrocyte glutamic-oxaloacetic transaminase and found lower concentrations in fourteen hemodialysis patients than in thirteen normal control subjects. Ascorbic acid concentrations have been shown to decrease markedly during hemodialysis of sixteen subjects (Sullivan and Eisenstein, 1970).

Ito et al. (1971) have shown hemodialysis patients in Japan to have elevated levels of riboflavin. The same authors reported a mean plasma vitamin E concentration of 1.22 ± 0.91 mg/100 ml for six hemodialysis patients as compared to 2.13 ± 0.73 mg/100 ml of total vitamin E for 10 controls ($p<0.05$). However, the value for the patients would still be considered within the normal range for populations in the United States.

Vitamin A metabolism is abnormal in CRF possibly due to increased amounts of free retinol binding protein (RBP). The RBP has been shown to have an extended half-life in CRF (Vahlquist et al., 1973) which may indicate that it is normally catabolized by the kidney. Yatzidis and coworkers

(1975) reported serum vitamin A concentrations two to five times the normal mean value. They hypothesized that a decrease in the excretion of vitamin A derivatives might be possible.

The literature as reviewed indicates the importance of nutrition in CRF and the need for more studies of vitamin status with larger numbers of subjects, more emphasis on the evaluation of the amount of nutrients consumed, and more critical selection of a homogenous patient population. In as much as the previously mentioned reference related to vitamin E status in CRF was only in the form of a letter to the editor, there is a need for more research in this area to confirm and expand upon the results.

In the vitamin E literature there are implications for possible interactions between the vitamin and some conditions present in renal failure. Previous research has shown a correlation between serum vitamin E and serum lipids. Absolute serum vitamin E levels have been reported to be normal in hemodialysis patients. Many CRF patients are also known to have elevated levels of triglycerides. If the serum vitamin E levels are considered in relation to total serum lipids is there the possibility that the ratio may be below the levels suggested for adequate nutriture? Other questions may be raised. Is there a relationship between the iron status of CRF patients and serum levels of vitamin E? What is the relationship between dietary

intake and serum vitamin E in this condition? Is the hemolytic anemia of CRF related to vitamin E status? The present experiment was designed to examine the vitamin E status of adults with CRF being treated with hemodialysis and to study the relationship of vitamin E status and relevant parameters.

MATERIALS AND METHODS

Subject Recruitment

Twenty individuals with chronic renal failure were recruited from the outpatients receiving hemodialysis treatment at the Michigan Nephrology Center at Borgess Medical Center in Kalamazoo, Michigan. Patients that met the following criteria were invited to participate in the study:

1) No evidence of nephrotic syndrome, diabetes, or severe anemia (hematocrit <20%)
2) Body weight within ±15% of desirable weight
3) No androgen therapy
4) At least 18 years of age
5) Treated with hemodialysis for a minimum of two months
6) History of compliance to medical treatment regimen

Twenty apparently healthy control subjects that matched the patients in sex and age (±2 years) were recruited from the staff of the Michigan Nephrology Center and from the faculty, staff, and students at Michigan State University. Control subjects met basically the same criteria as above with the exception of hemodialysis and medical treatment.

Informed Consent

The project was reviewed and approved by the University Committee on Research Involving Human Subjects at Michigan State University and the Human Research and Clinical Investigation Committee at Borgess Medical Center. All subjects that participated were fully informed of the nature of the study and of their involvement in it. Separate consent forms were prepared for the patients and controls because of different blood collection procedures (Appendices A-1 and A-2). The consent forms explained the possible risks, stated that the subject could withdraw from the study at any time without penalty, and verified that the results would be kept in strict confidence.

Subject Characteristics

Subjects ranged in age from 18 to 82 years (mean age: 60 years); 55% of the subjects were male (mean age: 67 years) and 45% were female (mean age: 52 years); see Tables 1 and 2. There were one male and three female Blacks in the patient group. The remainder of the subjects were Caucasian. The patients had been on hemodialysis from 2 to 75 months (mean: 26 months) at the time of the blood collections. They were receiving hemodialysis treatments for 8 to 20 hours per week (mean: 13 hours). As seen in Table 1 the patients had a variety of underlying kidney diseases. The primary diseases leading to dialysis in this population were glomerulonephritis, 35%; cardiovasular

Table 1. Summary of patient characteristics

Subject number	Sex	Age	Height (cm)	Weight (kg) Pre Treatment	Weight (kg) Post Treatment	Type of Renal Disease	Length of time on dialysis (mo)	Hours of dialysis per week (hr)	Vitamin and mineral supplements[1,2]
19[3]	F	18	146.1	46.3	44.5	Pyelonephritis	51	18	III,IV,V
17[3]	F	39	157.5	66.1	64.5	Glomerulonephritis	21	16	III,V
20	F	50	157.5	59.6	58.6	Hypertensive nephrosclerosis	26	12	III,V
1	F	53	155.6	61.5	60.3	Glomerulonephritis	75	18	III,V,VII
8	F	57	166.4	64.2	61.6	Glomerulonephritis	63	18	III,V,VII
5	F	59	148.6	46.0	44.4	Etiology unknown	25	12	
6	F	60	159.4	62.8	62.6	Glomerulonephritis	2	12	II,V
4	F	60	154.9	54.8	53.4	Interstitial nephritis	13	12	II,V,VII
7	F	70	152.4	48.3	48.0	Polycystic disease	18	15	II,V
14	M	48	185.4	74.0	73.1	Glomerulonephritis	13	12	II,V,VII
3[4]	M	53	177.8	66.2	66.2	Polycystic disease	72	20	I,II,IV
11	M	63	170.8	56.7	55.0	2° Atherosclerosis	2	8	III,V
12	M	65	172.7	75.6	74.1	Glomerulonephritis	31	15	III,V
10	M	65	174.6	69.0	69.6	Hypertensive nephrosclerosis	4	8	III,V,VII
16	M	69	171.5	68.6	66.6	Pyelonephritis	35	15	III,IV,V,VIII
13	M	70	172.7	65.1	64.8	Etiology unknown	12	15	III,V
15	M	72	176.5	76.2	75.4	Glomerulonephritis	40	16	II,IV,V
18	M	73	165.1	57.8	57.8	Hypertensive nephrosclerosis	17	8	III,V
9	M	77	168.9	66.2	65.8	Etiology unknown	4	8	VI,V
2	M	82	175.3	69.0	67.9	Pyelonephritis	2	8	

[1]Composition of vitamin supplements is listed in Appendix C

[2]
I Ascorbic acid 500 mg
II Becotin
III Becotin with vitamin C
IV Rocaltral 0.25 ug
V Folic acid 1.0 mg
VI Albee with vitamin C
VII Imferon 2 cc per week
VIII $FeSO_4$ 10 grain

[3]Previous kidney transplant

[4]Bilateral nephrectomy

Table 2. Summary of control subject characteristics

Subject number	Sex	Age (yr)	Height (cm)	Weight (kg)	Vitamin and mineral supplements[1]
119	F	19	165.1	50.5	Abdol with minerals
105	F	38	157.5	55.7	
102	F	51	158.8	65.1	One A Day, vitamin C, vitamin E 1000 IU[2]
106	F	52	158.1	60.1	
103	F	57	160.7	59.9	
104	F	59	160.7	55.5	Vicon Forte[2]
117	F	60	162.6	61.9	
109	F	61	154.9	62.0	
116	F	68	165.1	62.0	D∝-tocopherol 400 mg[2], vitamin C 500 mg, vitamin B complex 50 m
108	M	48	191.1	84.2	Vitamin C 200 mg[2]
121	M	54	175.3	78.0	
107	M	61	170.2	82.7	
123	M	63	170.2	73.9	
113	M	67	171.5	63.5	
118	M	67	172.7	74.7	
120	M	68	174.6	72.6	Vitamin C 500 mg[2], High Potency B Complex with Minerals
122	M	70	174.0	73.6	
115	M	75	179.1	75.7	D∝-tocopherol 400 mg[2], vitamin C 500 mg, vitamin B complex 50 m
112	M	78	182.9	86.4	
114	M	81	170.2	75.7	

[1]Composition of vitamin supplements is listed in Appendix C

[2]Not taken on a daily basis or not taken recently

and hypertensive disease, 20%; kidney infections (pyelonephritis and interstitial nephritis), 20%; unknown etiology, 15%; and congenital diseases (polycystic diseases), 10%. Approximately 90% of the patients received some form of vitamin B complex supplementation, including 1.0 mg of folic acid (Table 1). Vitamin supplements consumed by the controls are noted in Table 2.

The heights and weights of the subjects were measured at the time of the blood sample collection. The weights of the patients ranged from 84 to 111% of the desirable weight/height for men and from 93 to 125% of the desirable weight/height for women according to Bray (1975). For controls, the men ranged from 97 to 129% of the desirable weight/height and women ranged from 88 to 124% of the desirable weight/height. Weight was measured on a beam balance in light clothing without shoes. Height was measured with the subjects in stocking feet, eyes straight ahead, and backs straightened on a beam balance height bar.

Nutrient Intake

All subjects were asked to record on a prospective basis their total dietary intakes for two consecutive 24 hour periods. The subjects were given Food Diary sheets (Appendix B) on which to record the food consumed. Subjects were instructed to record: all items actually consumed (including beverages), methods of preparation, and

amounts consumed. The items recorded on the Food Diary sheets were coded and analyzed by the Michigan State University Nutrient Data Bank computer program (1979) (adapted from the Highland View Hospital - Case Western Reserve University Nutrient Data Base).

Collection of Blood Samples

Blood samples were collected from mid March through early May in 1980. All blood samples were collected following a 12 hour overnight fast. Blood was taken from the arterial circulation of patients during the initiation and during the termination of a routine dialysis treatment. Blood was taken from the controls by venipuncture. All samples were drawn into a dry syringe and separated into two aliquots. Approximately 0.5 ml of whole blood was placed in a polypropylene tube for immediate use in the erythrocyte hemolysis test and the remainder of the blood drawn was centrifuged in a non-fluorescent glass tube. The serum was divided into two portions, the first of which was refrigerated pending total serum lipid analysis within 48 hours. The second portion was placed in a glass vial, had the atmosphere within replaced with nitrogen, and was frozen for later vitamin E analysis. Some patient samples were known to contain the Australian antigen which is associated with serum hepatitis B; therefore infectious material precautions were exercised when handling samples.

Blood Analysis

Routine Blood Analysis. As part of their routine medical care, the patients included in this study have the following blood tests performed at least every six months: hematocrit, blood urea nitrogen, and serum creatinine, triglycerides, cholesterol, vitamin A, ferritin, and ascorbic acid. Means and individual values for each of these parameters are listed in Appendix D.

RBC Hemolysis. The method utilized in the current study was modified from the recent method of Farrell (1977), as described in Appendix E-1. The erythrocyte hemolysis test was used in this study to determine if it is a valid indicator of vitamin E deficiency in hemodialysis patients in the presence of the dual anemia of CRF. Two concentrations of hydrogen peroxide, 2% and 3.8%, were used in this study.

Total Lipids. Total serum lipids were determined by the sulfo-phosphovanillin method of colorimetric analysis which was first described by Chabrol and Charonnet in 1938 and modified by Zollner and Kirsch in 1962 (Appendix E-2). Good correlation is seen between this procedure and gravimetric methods (Postma and Stroes, 1968). Knight et al. (1972) have described the reaction as having three steps: (1) a carbonium ion is formed when concentrated sulfuric acid reacts with unsaturated lipids, (2) an aromatic phosphate ester is formed when phosphoric acid reacts with

vanillin, resulting in increased carbonyl group reactivity, and (3) the carbonyl group of the phosphovanillin reacts with the carbonium ion to form a colored complex. This reaction requires a carbon to carbon double bond, but compounds with multiple double bonds are affected by steric hinderance.

Vitamin E. The fluorometric assay for vitamin E of Thompson et al. (1973) in which serum proteins are precipitated with ethanol and the vitamin E is extracted into hexane was modified for this study (Appendix E-3). The major changes in the method included: increasing sample size from 0.2 to 0.3 ml, increasing the ethanol:water ratio during the precipitation from 1:1 to 2:1, and increasing the extraction mixing from 1 to 3 minutes. Thompson has shown good correlation between this fluorometric assay and the method utilizing the Emmerie-Engel reaction.

All vitamin E determinations were done within a 72 hour period. A separate standard curve was determined for each of the six assays run during this period. For any patient, the pre- and post-treatment samples and the sample from the age and sex matched control were analyzed in the same run. The coefficient of variation of the concentration value for the pooled control serum used for each of the six runs was 2%; therefore the standard curves were not adjusted. Recoveries of α-tocopherol standards that were added to rat and human sera averaged 97.5%.

Rat Study

The rat study was performed as part of this study to confirm the reliability of the erythrocyte hemolysis test as performed in this study. At the onset of the study it could not be foreseen if any of the subjects would demonstrate a vitamin E deficiency and hence a positive erythrocyte hemolysis test. In addition there is no practical standard for this test due to the fragile nature of RBC. Consequently rats were depleted of vitamin E to verify the results of the RBC hemolysis test.

Five male Sprague Dawley rats weighing 225-250 gm were obtained from Spartan Research Animals, Inc., Williamston, Michigan. For 13 weeks rats were fed ad libitum a vitamin E deficient purified diet with the following composition (gm/100 gm): dextrose, 69; vitamin-free casein, 20; partially hydrogenated soybean oil, 5; vitamins 0.16; and minerals 5.27 (Appendix E). The soybean oil had been heated to approximately 375^{o}F for 80 hours over a two week period when it was used for frying in a foodservice facility. Four rats were killed after consuming the diet for thirteen weeks. The fifth rat was killed in the eleventh week because of respiratory problems. Blood samples were collected from the intra-orbital sinus with a heparinized capillary pipet after the rats had been anesthetized with ether. Some samples were collected from the heart at the time the rats were killed. Assays were performed for erythrocyte

hemolysis and serum vitamin E on samples collected during weeks 10 to 13. Blood samples were treated in the same manner as described for human subjects.

Statistical Methods

Pre- and post-treatment serum levels of vitamin E were analyzed by paired t test. Correlations were computed for the two levels of hydrogen peroxide and the two treatment groups. A multiple regression utilizing generalized least squares was computed using the patients' serum vitamin E values and selected routine blood analyses. A randomized block analysis with 3 covariates was applied to the multiple regression computed for the serum vitamin E levels and dietary and serum parameters (Gill, 1978). All regressions were done on a Cyber 750 computer utilizing the Statistical Package for Social Sciences (Vogelback Computing Center, 1979).

RESULTS

Nutrient Intake

The daily intake of calories, carbohydrate, protein, total fat, polyunsaturated fat, total tocopherol, α-tocopherol, other tocopherols, total vitamin A, iron, selenium, and ascorbic acid was tabulated for each subject and the average intake for the two-day period calculated. The mean intakes of these nutrients as well as values for the ratio of α tocopherol:polyunsaturated fat and the estimated α-tocopherol equivalents were determined (Table 3). The average polyunsaturated fat intake values ranged from 0.8 to 17.6 mg per day. Average tocopherol intakes ranged from 1.1 to 39.7 mg per day for total tocopherol, 0.5 to 14.7 mg per day for α-tocopherol, and 0.1 to 16.0 mg per day for other tocopherols.

The patients participating in this study follow dietary restrictions for protein, sodium, potassium, and fluid which are reflected in their lower intakes of protein, total vitamin A, iron, and ascorbic acid as compared to controls. These dietary restrictions also contribute, along with other factors, to the limitation of total energy intake in the patient group.

Table 3. Average daily dietary intakes of subjects[1,2,3]

	Males		Females	
	Patients (n=11)	Controls (n=11)	Patients (n=9)	Controls (n=9)
Total calories (kcal)	1637 ± 96	2245 ± 145	1404 ± 126	1690 ± 105
Total carbohydrate (gm)	189 ± 16	261 ± 19	152 ± 15	196 ± 19
Total protein (gm)	60 ± 4	92 ± 6	52 ± 4	70 ± 4
Total fat (gm)	72 ± 4	98 ± 9	67 ± 7	67 ± 5
Polyunsaturated fat (gm)	9.6 ± 1.2	13.6 ± 2.3	9.0 ± 1.4	8.7 ± 1.0
Total tocopherol (mg)	9.8 ± 1.9	15.1 ± 2.9	10.7 ± 2.0	13.0 ± 3.6
Alpha tocopherol (mg)	4.0 ± 0.9	6.0 ± 1.3	3.7 ± 0.7	4.2 ± 0.8
Other tocopherols (mg)	5.1 ± 1.1	6.9 ± 1.6	5.5 ± 1.1	5.7 ± 1.2
Estimated alpha tocopherol equivalents (mg)[4]	4.8 ± 1.0	7.2 ± 1.5	4.4 ± 0.8	5.1 ± 0.9
Alpha tocopherol (mg): polyunsaturated fat (gm)	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Estimated retinol equivalents (ug)[5]	1558 ± 507	3677 ± 1310	661 ± 100	2220 ± 242
Iron (mg)	10 ± 1	17 ± 2	9 ± 1	14 ± 3
Selenium (ug)	14 ± 7	10 ± 2	7 ± 4	13 ± 4
Ascorbic acid (mg)	61 ± 12	143 ± 19	42 ± 7	153 ± 22

[1]Analyzed using the Michigan State University Nutrient Data Bank Computer Program.

[2]Mean ± SEM.

[3]Vitamin or mineral supplements are not included.

[4]Calculated by multiplying 1.2 times the two day average value of α-tocopherol intake for each subject (FNB, 1980).

[5]Calculated by multiplying 0.3 times the two day average value of total vitamin A (IU) for each group (FNB, 1980).

Blood Analysis

Vitamin E and Total Lipids. The mean values for serum total vitamin E, serum total lipids, and the ratio of serum total vitamin E:serum total lipids are shown in Table 4. The values for total vitamin E ranged from 0.8 to 1.9 mg/100 ml for female patients, 1.0 to 1.7 mg/100 ml for female controls, 0.8 to 1.5 mg/100 ml for male patients, and 0.9 to 1.6 mg/100 ml for male controls. Total lipids ranged from 353 to 589 mg/100 ml for female patients, 423 to 603 mg/100 ml for female controls, 384 to 770 mg/100 ml for male patients, and 424 to 680 mg/100 ml for male controls.

Separate multiple regressions for each sex were computed with serum total vitamin E as the dependent variable, patient-control pairs and patient-control groups as covariates, and the total serum lipids, the intake of polyunsaturated fats, and the intake of α-tocopherol as independent variables. For both males and females, only serum total lipids had a significant relationship with serum total vitamin E levels ($p<0.05$). There was little statistical evidence of a difference between patient and control groups for serum total vitamin E.

Using values for patients alone, multiple regressions were computed for males and females with serum total vitamin E as the dependent variable and serum triglycerides, serum cholesterol, serum total lipids, serum ferritin, months of hemodialysis, and hours of hemodialysis per week as

independent variables. For the males, serum total lipids, serum ferritin, serum triglycerides, and serum cholesterol contributed significantly to the regression equation at the level of $p<0.05$. Serum triglycerides, cholesterol, and total lipids levels showed a high degree of correlation with serum vitamin E levels. Ferritin was not correlated with serum vitamin E. For the females, none of the independent variables were significant in the regression equation. However, the sample size for females dropped from nine to six due to some missing values and with such a small sample and six variables, statistic significance is difficult to achieve.

On the same day that patient samples were collected at the beginning of a hemodialysis treatment, a blood sample was also collected at the termination of the treatment for determination of serum total vitamin E. Mean values ± SD for the post-dialysis samples were 1.09 ± 0.38 mg/100 ml for the female patients and 1.07 ± 0.32 mg/100 ml for the male patients. A paired t test showed no significant change between pre- and post-treatment values for females. For males, however, a slight mean decrease of 0.07 mg/100 ml was statistically significant at $p<0.05$. For both sexes separate multiple regressions were done with the difference between the pre- and post-treatment serum total vitamin E value as the dependent variable and months of hemodialysis treatment, hours of hemodialysis per week, post hemodialysis weight,

and the difference between pre- and post-hemodialysis body weight as the independent variables. For both males and females, none of the variables had a significant effect on the difference between the pre- and post-treatment vitamin E values in the regression equation. However, there was a slight negative correlation ($r = -.40$ to $-.55$) when the difference between pre- and post-treatment serum total vitamin E was compared with the difference between pre- and post-hemodialysis body weight. There is no apparent explanation for the slight mean decrease in serum total vitamin E following hemodialysis as seen in the male patients.

Erythrocyte Hemolysis

Table 4 shows the mean values[1] for erythrocyte hemolysis. None of the erythrocyte hemolysis values for the controls at either concentration of hydrogen peroxide exceeded 20%, the maximum value considered to reflect normal vitamin E status (Interdepartmental Committee on Nutrition for National Defense, 1963). At the 2% concentration of hydrogen peroxide, two male patients had erythrocyte hemolysis values greater than 20%, while four patients of each sex had values of more than 20% at the 3.8% concentration of hydrogen peroxide. However, all subjects, both patients and controls, had normal values for serum total vitamin E and serum total vitamin E:serum total lipid ratio. Because the erythrocyte

[1] One value of 31% for a female control was removed from the data on the basis of the Grubbs and Beck test for outlier values (Gill, 1978).

Table 4. Erythrocyte hemolysis, serum total vitamin E and total lipids[1,2]

	Males		Females	
	Patients (n=11)	Controls (n=11)	Patients (n=9)	Controls (n=9)
Total vitamin E (mg/100 ml)	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
Total lipids (mg/100 ml)	538 ± 24	540 ± 17	500 ± 27	550 ± 19
Total vitamin E (mg)/ total lipids (gm)	2.1 ± 0.1	2.3 ± 0.2	2.2 ± 0.2	2.4 ± 0.1
2% H_2O_2 erythrocyte hemolysis (%)	8.8 ± 5.1	0.4 ± 0.3	2.6 ± 1.2	1.0 ± 0.5
3.8% H_2O_2 erythrocyte hemolysis (%)	21.3 ± 5.6	3.1 ± 1.1	21.6 ± 5.7	1.6[3] ± 0.5

[1]Blood samples were collected following a 12 hour fast. Samples from patients were collected at the beginning of a hemodialysis treatment.

[2]Mean ± SEM.

[3]n=8

hemolysis test is an indirect test, factors other than vitamin E deficiency may have influenced the results.

After separating the data by group and sex, preliminary correlations were computed for erythrocyte hemolysis test values from assays with 2% or 3.8% solutions of hydrogen peroxide. The test results for the two solutions were not strongly correlated, although the relationship was positive. Because of this lack of correlation results for the two solutions could not be combined; therefore for each of the concentrations of hydrogen peroxide, correlations were computed comparing the erythrocyte hemolysis values with the serum total vitamin E values with separate correlations for each subject group (patients vs. controls), each sex, and each of the two concentrations of hydrogen peroxide for a total of eight correlations. There was little evidence ($p<0.05$) of correlation between the erythrocyte hemolysis values and the serum total vitamin E values in the patients or controls regardless of sex or hydrogen peroxide concentration, except for the female controls at the 2% level of hydrogen peroxide. These subjects showed a positive correlation. It does not appear that the erythrocyte hemolysis test is useful in hemodialysis populations, probably because of interference related to the hemolytic anemia of uremia.

Rat Study

The vitamin E content of the soybean oil used as the source of fat in the rat diet was analyzed by the same method used for serum. There was a mean content of 0.35 mg/100 ml ± 0.03 of vitamin E in the oil, so by calculation there was 0.02 mg of vitamin E per 100 gm of rat diet, or 0.2 mg per kilogram of diet. The diet was not analyzed for vitamin E content.

The rats consumed an average of 20 to 25 gm of diet per day and four of the animals weighed an average of 510 gm when they were killed in the thirteenth week. Due to illness the fifth rat had been killed in the eleventh week: it had lost weight and weighed 350 gm.

The values of erythrocyte hemolysis for the rats ranged from 60 to 74% at 2% hydrogen peroxide and 33 to 43% at 3.8% hydrogen peroxide. All samples at the 3.8% level displayed an uncharacteristic green color, possibly due to contamination in used test tubes. All assays for erythrocyte hemolysis in the human subjects utilized new test tubes. In any case, all assays in the rat showed results well in excess of 20% hemolysis. The serum values of total vitamin E in the rats ranged from 0.03 to 0.12 mg/100 ml, which is well below the minimum values associated with adequate nutriture. Consequently, the erythrocyte hemolysis test in this study appears to be an accurate predictor of vitamin E deficiency in the rat.

DISCUSSION

Subject Characteristics

The patient population that was sampled in this study appears to be similar to the national population described by Wineman (1978) with regard to age and the primary disease leading to dialysis. The patients in the present study tended to be older because only patients being treated during the day shift were selected for participation. This arrangement was necessary in order that the twelve hour fast required for the total lipid test would occur overnight. It was considered an undue hardship to require a patient to fast for twelve hours during the day. Consequently younger patients who held day-time jobs were excluded from the study because they received their hemodialysis treatments in the evening.

Nutrient Intake

One day dietary records may show large fluctuations in variability of nutrients consumed (Garn et al., 1978) when compared with seven day records. It is misleading to use one day records to ascertain intakes for individuals. However, Garn et al. (1976) stated they are useful in deriving information from population samples if the

dietary records are carefully taken and analyzed. He cautioned that these one day records should not be used to rank individuals or to estimate the proportion of individuals at nutritional risk. One day food records were compared with diet histories for a group of 35 obese, pregnant women by Van Den Berg and Mayer (1954). Their results show that significantly higher intakes of calories, carbohydrate, protein, and fat were reported with the diet history than with the one day record. They reasoned that an obese population may be reluctant to list their true intakes and that the fact of recording the food eaten acts as a check on food intake.

It was not feasible to collect seven day dietary records from the hemodialysis patients if they were to be interviewed regarding their intake following completion of the records. It was possible to collect two day dietary records from all subjects. Most subjects were interviewed briefly when they turned in their records.

The Michigan State University Nutrient Data Bank contains values for approximately 70 nutrients and 3,500 food items. However, the values for all nutrients are not complete for each food item. For the nutrients analyzed in this study the values for the tocopherols, selenium, and polyunsaturated fat may be incomplete for some food items. Therefore the values shown in Table 3 may be somewhat lower for those nutrients than actual intake because of

missing values.

In general the control subjects consumed greater amounts of nearly all of the nutrients compiled than did the patients. Sullivan and Eisenstein (1970) along with Kopple and Swendseid (1975) have noted that dialysis patients have decreased intakes of those nutrients found in potassium and protein rich foods. The control subjects consumed at least 100% of the RDA (FNB, 1980) for protein, retinol equivalents, and ascorbic acid. The RDA for iron was met by all control subjects, except the females under the age of 50 years who consumed 70% of the allowance. None of the subjects, either patients or controls, appeared to consume 100% of the RDA for tocopherol equivalents. The intakes of tocopherol equivalents ranged from 40 to 70% of the allowance. The low intakes of tocopherol probably reflect the limitations of the data bank due to missing values. The patients consumed 100% of the RDA for protein and male patients consumed 100% of the RDA for retinol equivalents, iron, and ascorbic acid. Female patients consumed the following percentages of the RDA: retinol equivalents, 85%; iron, 55%, and ascorbic acid, 70%.

The values for typical daily intakes of α-tocopherol, total tocopherol, and polyunsaturated fat cited in the literature by Bieri and Evarts (1973) and Witting and Lee (1975b) are considerably higher than those seen in this

study. Aside from the limitations of the nutrient data bank, it is important to note that in the two studies mentioned the investigators did not evaluate food actually consumed by individual subjects, but rather analyzed typical cafeteria menus. Cafeteria food items may not resemble those cooked at home or in other types of food services in type and method of preparation. Although the average daily energy level of the diets evaluated in those studies was 2,400 to 2,500 kcal which is considerably more than the energy intake of the subjects in this study, it is not enough to account for the differences in tocopherol levels. In this study there was a positive correlation between the intakes of α-tocopherol and polyunsaturated fat.

Blood Analysis

Vitamin E. The values seen for total vitamin E in this study are similar to values analyzed by the Emmerie-Engel method cited in the literature for subjects of similar age. Researchers using a variation of this spectrophotometric method found mean values of 1.05 mg/100 ml of vitamin E in studies of 197 and 132 subjects ranging in age from 17 to 64 years (Harris et al., 1961; Bieri et al., 1964). Using a similar spectrophotometric method, Farrell et al. (1978) found mean levels of 0.78 mg/100 ml in 22 subjects 21 to 35 years of age. Rubenstein et al. (1969) found slightly higher levels than the previously mentioned researchers of 1.14 mg/100 ml using a variation of the

Emmerie-Engel method. In twelve females, 19 to 26 years old, Yeung (1976) reported mean vitamin E values of 0.87 mg/100 ml using the same fluorometric method that was modified for the present study. The value cited by Yeung is lower than reported here but the age range of his subjects tended to be much younger. Ito et al. (1971) reported plasma total vitamin E levels of 1.2 mg/100 ml for patients in Japan receiving maintenance hemodialysis which compare very favorably with the levels reported in this study.

Serum Lipids. Based on assays using the same colorimetric method as used in this study, Postma and Stroes (1968) have shown that serum lipid values for a large number of serum samples will have a range of 500 to 1800 mg/100 ml with most values falling between 500 and 1000 mg/100 ml. In studies conducted to evaluate vitamin E status, mean values for serum total lipids of 549 mg/100 ml for young adults (Farrell et al., 1978) and 685 mg/100 ml for adult males (Vatassevy and Chiang, 1979) were reported using the turbidimetric method. In a study of patients with CRF, Dombeck et al. (1973) reported that 50% of eighteen predialysis patients with uremia had abnormal total lipid concentrations and that the percentage increased on dialysis if androgens were given. Although serum cholesterol and triglyceride concentrations are frequently reported in CRF (Kaye et al., 1973; Dombeck et al., 1973; and Reaven et al., 1980) serum total lipids are rarely evaluated, probably

because of the availability of other methods for the diagnosis of hyperlipoproteinemia.

The results of the present study show very little difference between the total lipid values of patients and controls especially for females. This was unexpected as the hypertriglyceridemia associated with CRF would be expected to contribute to an increase in total lipids. The subjects for this study were screened very carefully for factors such as nephrotic syndrome, diabetes, obesity, and androgen therapy which are known to contribute to the elevation of lipids. In past reports of lipid abnormalities these contributing factors may not have been well controlled. Gutman et al. (1973) in examining triglycerides did restrict his sample to non-nephrotic subjects. There also were no diabetic patients included in the study although it did not appear they had been deliberately omitted. Gutman defined obesity as greater than 20% over the ideal weight and included one slightly obese patient in his sample. Use of androgen therapy was not evaluated. In the previously mentioned study by Dombeck, only glucose in the dialysate and androgen therapy were evaluated in relation to the lipid status of the subjects. In the present study the control subjects may have had a tendency to exceed their ideal weight by a slightly greater degree than the controls which might account for the lack of difference between lipid values for patients and controls.

Vitamin E:Total Lipid Ratio. Because there was little difference in the values for vitamin E and total lipids between patients and controls, it is logical that there would be little difference between the total vitamin E: total lipid ratio. The ratio computed for this study compares favorably with the minimum ratio of 0.8 mg/gm suggested by Horwitt et al. (1972) as indicating adequate vitamin E nutriture. Other authors (Farrell and coworkers, 1978; and Vatassevy and Chiang, 1979) have reported vitamin E: total lipid ratios of 1.38 and 1.71 mg/gm respectively in normal subjects.

Erythrocyte Hemolysis

The erythrocyte hemolysis test has been used as an indirect indicator of vitamin E deficiency since 1948 (Gyorgy and Rose). Rose and Gyorgy (1952) were the first to use hydrogen peroxide for the oxidizing agent in this colorimetric method. Others (Gordon and deMetry, 1952; Horwitt et al., 1956) have refined the method. The erythrocyte hemolysis test is not specific for vitamin E deficiency, however, but also is positive in those hemoglobinopathies where peripheral RBC destruction plays a major role (Melhorn et al., 1971). The test is normal in states where the anemia is primarily the result of ineffective erythropoiesis. The anemia of CRF is due to both increased hemolysis (Desforges and Dawson, 1958; Eklund, 1971) and a defect in erythropoiesis (Wallner et al., 1976).

An inverse relationship would be expected between erythrocyte hemolysis values and serum total vitamin E (Horwitt et al., 1968; Leonard and Losowsky, 1967). In the case of the patients, erythrocyte hemolysis was not inversely related to serum vitamin E levels probably because the hemolysis is aggrevated by factors unrelated to vitamin E status such as uremic toxins or metabolic defects (Jacob et al., 1975; Desforges and Dawson, 1958). However, in the control group, an explanation for the absence of an inverse relationship between erythrocyte hemolysis and serum vitamin E levels was not readily apparent.

Rat Study

In contrast to the results seen in human subjects, the values for erythrocyte hemolysis and serum vitamin E seen in five rats demonstrated the expected inverse relationship. The rats had mean serum vitamin E levels of 0.07 mg/100 ml and erythrocyte hemolysis values exceeding 30%. The diet consumed by the rats contained no vitamin E in the vitamin mix and no other major contributing source of the vitamin except the oxidized soybean oil. Analysis of the oil showed that it contributed 0.2 mg of vitamin E per kg of diet. In comparison, rats fed 17 mg of α-tocopherol/kg of diet, which is below the 30 mg of DL-α-tocopheryl acetate/kg recommended to meet the requirement of rats, had plasma α-tocopherol levels of approximately 0.4 mg/100 ml after eight months on the diet (Yang and Desai, 1977).

Rats that had been fed at the level of 200 mg of DL-α-tocopheryl acetate per kg of diet, however, had plasma tocopherol levels of 1.3 mg/100 ml (Machlin et al., 1977).

Statistical analysis failed to show a significant relationship between serum vitamin E and intake of PUFA or α-tocopherol. Because of the storage potential of vitamin E as a fat soluble vitamin, dietary intake would be expected to have little effect on circulating plasma levels unless there had been a long term deficient intake.

Examining patient data only, serum vitamin E for both males and females was not related to the number of months on dialysis or the number of hours of treatment per week. Those variables were chosen as indicators of the severity and length of the disease process. In the small sample of female patients serum vitamin E was also not related to serum levels of total lipids, cholesterol, triglycerides, or ferritin. For males the serum lipid components, cholesterol, triglycerides, and total lipids, in addition to ferritin were significantly related to serum vitamin E with serum lipids being the most prominent. The relationship between serum vitamin E and the serum lipid components has been demonstrated by others such as Horwitt et al. (1972). Serum ferritin may be related to vitamin E status because of the oxidation promoting activities of iron.

There was no significant difference between pre- and post-dialysis levels of vitamin E for female patients.

The change in concentration of vitamin E in males from pre- to post-treatment was very slight, 0.07 mg/100 ml; however, it was statistically different. Because vitamin E is fat soluble, it would not be expected to cross the membrane during dialysis. Its molecular weight is small enough to allow it to pass if it were free and not bound to low density lipoproteins. The difference between the pre- and post-treatment levels of vitamin E was not related to the amount or length of hemodialysis treatment, body mass, or weight loss during hemodialysis for both males and females. Body mass is somewhat representative of plasma volume and weight loss during dialysis is due partially to plasma volume lost during treatment. The decrease in serum vitamin E which was observed in males might not be seen if a larger number of subjects were evaluated.

CONCLUSIONS

The vitamin E status of individuals with CRF in this study was normal as assessed by absolute serum levels and the serum vitamin E:serum lipid ratio. The erythrocyte hemolysis test did not appear to be an accurate predictor of vitamin E status in patients with CRF receiving hemodialysis. The nutrient intake for a two day period as compiled by the Michigan State University Nutrient Data Bank also did not have any relation to the vitamin E status of these patients.

This study indicates that the vitamin E requirements of the hemodialysis patients studied are being met by their current dietary intakes without the use of vitamin E supplements. These results, however, should not be interpreted as showing that the requirements of hemodialysis patients are the same as normal individuals. The vitamin E requirements of CRF patients on hemodialysis have not been addressed in this study, only the vitamin E status has been examined. Additional research would be needed to determine the requirement for vitamin E. Because the function of vitamin E is not well understood in normal individuals, it would be difficult to assess the requirement

in a chronic disease state such as CRF.

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APPENDICES

Appendix A-1

CONSENT FORM FOR HEMODIALYSIS PATIENTS

DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION

MICHIGAN STATE UNIVERSITY

EAST LANSING, MICHIGAN

I, _____, agree to participate in a study of the status of vitamin E (tocopherol) in renal patients on hemodialysis being conducted by Pat Smith Brown, R.D. (M.S.U.) under the direction of Dr. J.A. Green (Borgess Hospital), Dr. H.S. Park (Borgess Hospital) and W. Chenoweth, Ph.D. (M.S.U.). I understand that the purpose of this study is to determine if the levels of serum tocopherol are low, normal, or high when compared to a control group. I understand the relationship of vitamin E with serum lipids (fats), and erythrocyte hemolysis (red blood cell breakage) will also be examined. I understand that I will be asked to keep a 2 day food diary listing everything I eat. I realize that on one day only, following a 12 hour fast, a 7 ml sample of blood will be taken at the beginning of a dialysis treatment and a 3 ml sample of blood will be taken at the end of the same dialysis treatment. I have been told that the following tests will be performed on the blood samples: serum tocopherol, total serum lipids, and erythrocyte hemolysis. I know that the potential risks are minimal since the blood will be drawn in conjunction with a dialysis treatment and will not require any additional venipunctures. I understand the only risk will be the loss of 10 ml of blood which is approximately equal to 2 teaspoons. I understand that the results of this study will provide information concerning the effects of hemodialysis and renal disease on vitamin E and its relationship with serum lipids and erythrocyte hemolysis which will be of benefit to hemodialysis patients in general. I understand that there are no guaranteed benefits to me as an individual. I give my consent to have my medical records released to the researcher to obtain information concerning results of laboratory tests, length of dialysis treatment, body weight and height, and medications. I have had all my questions regarding this study answered. I know that I may withdraw from this study at any time without penalty or jeopardy to my medical treatment. I understand that all information gathered in this study from me, my medical records, or assays (tests) of my blood will be kept in strict confidence and that I will remain anonymous in all reports of the results. A summary of the results will be provided to me at my request.

DATE _____

RESEARCHER/PHYSICIAN_____

SIGNED_____

WITNESS_____

Appendix A-2

CONSENT FORM FOR CONTROL SUBJECTS

DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION

MICHIGAN STATE UNIVERSITY

EAST LANSING, MICHIGAN

I, _____, agree to participate in a study of the status of vitamin E (tocopherol) in renal patients on hemodialysis being conducted by Pat Smith Brown, R.D. under the direction of Dr. J.A. Greene, M.D., Dr. H.S. Park, M.D. and W. Chenoweth, Ph.D. I understand the purpose of this study is to determine if the levels of serum tocopherol are low, normal, or high when compared to a control group. I understand the relationship of vitamin E with serum lipids (fats), and erythrocyte hemolysis (red blood cell breakage) will also be examined. I understand that I will be asked to keep a 2 day food diary listing everything I eat. I realize on one day only, following a 12 hour fast, a 7 ml sample of blood will be taken. I have been told that the following tests will be performed on the sample: serum tocopherol, total serum lipids, and erythrocyte hemolysis. I know that the potential risks are those associated with venipuncture such as: fainting, minor pain, or a small purplish swelling at the site of venipuncture, and the loss of 7 ml of blood which is approximately 1½ teaspoons. I understand that the results of this study will provide information concerning the effects of hemodialysis and renal disease on vitamin E and its relationship with serum lipids and erythrocyte hemolysis which will be of benefit to hemodialysis patients in general. I understand there are no guaranteed benefits to me as an individual. I have had all my questions regarding this study answered. I know that I may withdraw from this study at any time. I understand that all information gathered in this study from me or assays (tests) of my blood will be kept in strict confidence and that I will remain anonymous in all reports of the results. A summary of the results will be provided to me at my request.

DATE_____

RESEARCHER/PHYSICIAN_____

SIGNED_____

WITNESS_____

I wish to have the results of these tests sent to my physician: __yes __no

Name of physician _____

Address _____

_____

Appendix B

FOOD DIARY

Please write down everything you eat and drink for the next two days. Be sure to put down how the food was prepared (For example, if you ate potatoes write down if they were boiled, mashed, fried or however they were fixed.) Try to guess how much you ate in common household measurements such as measuring cups, tablespoons and teaspoons. If you have sandwiches write down how many slices of bread and whether or not you used butter, margarine, mayonnaise or anything else. Don't forget to write down what you drink.

Time	Food (how was it prepared?)	Amount
Morning		
Afternoon		
Evening		

APPENDIX C

Table 5. Composition of vitamin and mineral supplements per capsule/tablet[1]

Nutrient	Becotin	Becotin with vitamin C	One-A-Day	Allbee with vitamin C	Abdol with minerals[2]	Vicon forte
Thiamin HCl (mg)	10	10	2	15	2.5	10
Riboflavin (mg)	10	10	2.5	10.2	2.5	5
Pantothenic acid (mg)	25	25		10	2.5	10
Niacin (mg)	50	50	20	50	20	25
Pyridoxine (mg)	4.1	4.1	1	5	0.5	
Ascorbic acid (mg)		150	50	300	50	150
Cyanocobalamin (ug)			1		1	10
Vitamin A (IU)			5000		5000	8000
Vitamin D (IU)			400		400	
Vitamin E (IU)						40
Zinc sulfate (mg)					0.5	80
Magnesium sulfate (mg)					1	70
Manganese chloride (mg)					1	4
Iron (mg)					15	
Folic acid (mg)					0.1	1

[1] Composition was not available for High Potency B Complex with Minerals manufactured by Michigan Pharmical as it has been discontinued for several years.

[2] Also contains: iodine, 0.15 mg; potassium, 5 mg; copper, 1 mg; calcium, 44 mg; and phosphorus, 34 mg.

APPENDIX D

Table 6. Routine blood analyses for patients[1,2]

Subject number	Hematocrit (ml/100 ml)	Blood urea nitrogen (mg/100 ml)	Serum creatinine (mg/100 ml)	Serum triglycerides (mg/100 ml)	Serum cholesterol (mg/100 ml)	Serum vitamin A (ug/100 ml)	Serum ferritin (ng/ml)	Serum ascorbic acid (mg/100 ml)
Females								
19	24	89	11.4	208	216	109		0.7
17	26	58	11.1	148	239	124	628	0.5
20		122	19.2		128		1813	
1	50[3]	93	11.4	93	204	154	555	3.6
8	31[3]	88	10.9	275	201	45	333	1.4
5	31[3]	72	11.0	122	270	47	468	0.8
6	30[3]	71	7.7	58	192			
4	31[3]	57	9.7	186	244	161	357	4.0
7	34[3]	86	11.9	106	175	475	>500	3.8
Mean	32	82	11.6	150	208	159	661	2.1
± SEM	±3	±7	±1.0	±25	±14	±60	±196	±0.6
Males								
14	33[3]	75	13.5	165	215	563	>500	0.4
3	20	48	6.0	183	200	197	1524	2.9
11	20[3]	80	7.8	195	173	142	509	13.4
12	36[3]	77	15.2	188	242	149	93	2.1
10	30[3]	81	10.1	85	102	78	103	0.6
16	32	78	11.8	291	307	77	312	1.3
13	30[3]	82	15.8	105	185	422	400	0.2
15	36	71	11.2	134	172	110	288	0.4
18	36[3]	92	8.4	131	189		293	1.4
9	32[3]	105	13.9	538	223	85	369	1.9
2	29[3]	66	7.1	96	140	63	184	1.7
Mean	30	78	11.0	192	195	189	416	2.4
± SEM	±2	±4	±1.0	±39	±16	±53	±118	1.1
Normal range[4]	M 41-51 F 37-47	8-20	0.6-1.5	30-180	140-330	30-65	20-300	0.7-2.0

[1] Determinations made over a six month period

[2] Values were determined on pre-dialysis samples unless specified otherwise

[3] Post-dialysis value

[4] From the clinical laboratory at Borgess Medical Center

Appendix E-1

Erythrocyte Hemolysis Determination

Reagents

1. 1% sodium citrate in 0.9% saline

2. Phosphate buffered saline (PBS) made with equal parts of 0.9% saline and phosphate buffer

   Phosphate Buffer
   250 ml of 0.2M potassium phosphate, monobasic (KH_2PO_4)
   197 ml of 0.2M sodium hydroxide (NaOH)
   Distilled water to make 1000 ml
   Adjust with 1.2N sodium hydroxide to pH 7.4

3. 30% hydrogen peroxide (H_2O_2) diluted with PBS to concentrations of 2% and 3.8% (made fresh each time)

Procedure

1. Add approximately 0.5 ml of fresh whole blood to 0.2 ml of 1% sodium citrate in a capped, calibrated, 15 ml, polypropylene, conical, centrifuge tube and gently shake to mix.

2. Incubate the tube at 25°C for 2 hours.

3. Add 10 ml of PBS at 37°C. Invert gently several times to mix the contents.

4. Centrifuge the tube at 1800 rpm for 10 minutes and then remove the supernatant.

5. Dilute the remaining red blood cells (RBC) to a 2.5% solution with PBS at 37°C. Gently invert the tube several times.

6. Centrifuge the tube at 1800 rpm for 10 minutes and then remove the supernatant.

7. Dilute the remaining RBCs to a 5% solution with PBS at 37°C. Gently invert the tube several times.

8. Label new, disposable, polypropylene, 12x75 mm culture tubes with caps from 1-10 for each sample.

9. Pipet 0.3 ml aliquots of the 5% RBC/PBS solution with a plastic pipette tip into each of the 10 labeled tubes.

10. Add 0.3 ml of freshly made 2% H_2O_2 to tubes 1-3 with a plastic pipette without contacting the sides of the tube.

11. Add 0.3 ml of freshly made 3.8% H_2O_2 to tubes 4-6 with a plastic pipette without contacting the sides of the tube.

12. Add 0.3 ml of PBS to tubes 7 and 8.

13. Add 3.8 ml of distilled water to tubes 9 and 10.

14. Cap all tubes, invert twice and incubate at 37°C for 3 hours.

15. Add 3.5 ml of PBS to tubes 1-8.

16. Centrifuge all tubes at 1800 rpm for 10 minutes.

17. Transfer the supernatant to cuvettes.

18. Read the absorbance of the supernatant at 577 nm on a spectrophotometer.

19. Average the values for tubes 7 and 8. Subtract this average from all other values. Average the values for tubes 1-3 and tubes 4-6 and divide by the average of tubes 9 and 10 to get the percent of hemolysis with the two concentrations of H_2O_2.

Appendix E-2

Total Serum Lipid Determination[1]

Reagents

1. 1.3×10^{-2}M vanillin in 11.8M phosphoric acid
2. Total Lipid Standard (750 mg/dl) - 2.09×10^{-2}M oleic acid. 3.22×10^{-3}M palmitic acid, and 2.63×10^{-3}M stearic acid in specially denatured alcohol
3. Concentrated sulfuric acid
4. Absolute ethanol

Procedure

1. Prepare dilutions of the standard as follows:

Tube Number	750 mg/dl Standard (ml)	Absolute Ethanol (ml)	Value (mg/dl)
1	0.5	1.0	250
2.	1.0	0.5	500

2. In duplicate pipet: 0.1 ml water into a 15 ml pyrex test tube
   0.1 ml serum into an identical tube
   0.1 ml of the standards into 3 identical tubes (250, 500, and 750 mg/dl total lipid)
3. Add 5 ml concentrated sulfuric acid to each tube. Seal with parafilm and mix thoroughly using a mechanical mixer for 15 seconds.
4. Place all tubes in a boiling water bath for 10 minutes.
5. Remove and cool to room temperature in a cool (running) water bath.
6. Pipet 0.2 ml aliquots of the surfuric acid digests into appropriately labeled dry 18x150 mm test tubes.
7. Add 3 ml of the vanillin-phosphoric acid solution to each tube.
8. Mix thoroughly on a mechanical mixer for 30 seconds.

9. Place tubes in the dark at room temperature for 45 minutes (±15 minutes).

10. Transfer to cuvettes and tap to remove bubbles.

11. Read absorbance at 525 nm against the water blank set for zero absorbance.

12. A linear regression equation was prepared from the absorbance and concentration values of the 3 total lipid standards and used to calculate the concentration of the serum from its absorbance value.

---

[1] The phosphovanillin reagent and the method used are those specified in the package insert, CH44-4921E, revised 1/79, from the Dade Division of the American Hospital Supply Corporation. The method was first described by Chabrol, E. and R. Charonnet. Presse med. 45:1713, 1937 and was later modified by Zollner, N. and K. Kirsch. Zeitschrift fur die Gesamte Experimentelle Medizin 135:545, 1962.

Appendix E-3

Vitamin E Determination

Reagents

1. UV Grade Hexane, glass distilled

2. KOH pellets, reagent grade

3. Ethanol 95%. Distill over KOH pellets (ca 50 g/ liter)

4. Quinine sulfate U.S.P. Prepare a 1% solution of quinine (10 mg/ml) by dissolving 1.05 gm of the salt in 100 ml 0.1N H_2SO_4. Dilute with 0.1N H_2SO_4 to prepare a quinine solution containing 0.1 ug/ml.

5. Vitamin E. Dissolve D-α-tocopherol (Eastman Kodak Co.) in ethanol to give a solution containing approximately 100 ug/ml. Check the concentration by reading in a spectrophotometer at 292 mu
   ($E^{1\%}_{1cm} = 72$).
   Dilute with ethanol to make standard dilutions with the following concentrations: 40 ug/ml, 20 ug/ml, 10 ug/ml, 5 ug/ml, and 2.5 ug/ml.

6. Acid Wash. Combine one liter of 3N HCl in distilled water with one liter of 50% ethanol in distilled water.

Procedure

1. All glassware to be used in the preparation for or in the actual determination should be prewashed in the acid wash.

2. In triplicate, dilute 0.3 ml of each of the following: serum, water, and the five tocopherol standards with 1 ml of distilled water in 12 ml conical glass stoppered centrifuge tubes and mix on a mechanical mixer for 5 seconds.

3. Add 2 ml of ethanol and mix on a mechanical mixer for 10 seconds.

4. Add 5 ml of hexane, stopper the tubes, and mix thoroughly for 3 minutes with a mechanical mixer.

5. Centrifuge the tubes for 10 minutes at 1000 rpm.

6. Keep the tubes stoppered and protected from light until the hexane extracts can be read in a spectrofluorometer.

7. Using a Varian SF 330 spectrofluorometer set the following controls:

Read Mode	0.25 sec	Excitation	295 mv
Time Constant	1	Emission	320 mv
Selector	10	Slits	10 mv excitation
Sensitivity	1		20 mv emission

8. Insert the 0.1 mg/ml quinine sulfate solution first and adjust the variable control so that the quinine solution reads 2.5.

9. Do not alter the controls after this point.

10. Read the fluorescence of the water blank, standards and serum.

11. A linear regression equation is prepared from the fluorescence and concentration values of the tocopherol standards and used to calculate the concentration of the serum from its fluorescence.

APPENDIX F

Table 7. Composition of purified rat diet

Ingredients	g/8 kg diet
Dextrose	5565.00
Casein, vitamin free	1600.00
Soybean oil, oxidized[1]	400.00
Monosodium phosphate	160.00
Calcium carbonate	160.00
Potassium chloride	40.00
Iodized sodium chloride	40.00
Choline chloride	12.00
Vitamin mix[2]	21.60
Mineral mix[3]	0.53

[1]Heated to approximately 375° for 80 hours over a two week period.

[2]Composed of (in mg/kg of diet): vitamin A (500,000 IU/gm), 10; vitamin D_3 (40,000 IU/gm), 7.5; menadione, 0.4; thiamin hydrochloride, 3; riboflavin, 3; pyridoxine hydrochloride, 3; calcium pantothenate, 6; niacin, 30; folic acid, 1; and vitamin B_{12} (0.1% in mannitol), 2.

[3]Composed of (gm/100 gm of mineral mix): magnesium sulfate, anhydrous, 73.82; zinc sulfate ($ZnSO_4 \cdot 7H_2O$), 19.66; manganous sulfate, monohydrate, 5.73; cupric sulfate ($CuSO_4 \cdot 5H_2O$), 0.73; potassium iodate, 0.06; and ferrous sulfate, 0.56.