Dietary levels of vitamin E and polyunsaturated fatty acids and plasma vitamin E¹, ², ³

Lloyd A. Witting,⁴, ⁵ Ph.D. and Lok Lee,⁶ M.S.

ABSTRACT Seventeen daily diets (breakfast, lunch, and dinner) were analyzed from a 35-day menu cycle fed to students, under contract in the University dining halls. This 35-day menu cycle was repeated 6.6 times over the course of two 15-week semesters and registration and final examination periods. The average 2,500 kcal diet collected during the sixth and seventh menu cycles contained 96 ± 26 g fat of which 19.5 ± 1.8% was linoleate and 28.7 ± 14.2 mg total tocopherol of which 7.5 ± 3.5 mg was RRR-α-tocopherol. Blood samples obtained from 26 female undergraduate student volunteers contained adequate levels of plasma total vitamin E, 1.09 ± 0.25 mg/100 ml, despite the observation that 71% and 65% of the diets analyzed did not meet the value tabulated in the eighth edition of “Recommended Dietary Allowances” for adult females in terms of RRR-α-tocopherol or total vitamin E activity, respectively. These data emphasize the importance of the average long-term consumption of this fat-soluble vitamin rather than daily intake. Am. J. Clin. Nutr. 28: 571–576, 1975.

Since the mid-1950’s there has been a national trend toward increased consumption of polyunsaturated fatty acids, PUFA. The requirement for vitamin E in body tissues is related to the PUFA content of the tissue lipids (1–4). Because the fatty acid composition of the tissue lipids can be affected by the composition of the dietary fat, it follows that the vitamin E requirement of man has also increased during this period (5). Modification of the tissue lipids of the 70-kg man by the 10–30 g PUFA ingested per day may require a period of months or years to reach equilibrium, depending on the magnitude of the change (1, 2, 6, 7). During a period of continuing and accelerating change in the Nation’s eating habits it is difficult to set a rational recommended dietary allowance, RDA, for vitamin E. Indeed, the eighth edition of “Recommended Dietary Allowances” (8) stated that it is not possible to establish a fixed RDA and that in contrast to the RDA’s for other vitamins there can be no single recommended value for each age and sex group.

Reliable data on the vitamin E (9, 10) and PUFA content of various foodstuffs has only become available in recent years. The values of Harris and Embree (11) for normal intakes of PUFA and vitamin E were based on foodstuffs available for consumption rather than foods consumed. Other pertinent data are based on analyses of foods (12), a limited number of meals in the NIH cafeteria (13), a composite Canadian diet (14) or meals fed to British hospital patients (15).

The present study was undertaken to evaluate the PUFA and vitamin E intake and plasma tocopherol levels of a group of young women consuming a repetitive series of diets over a period of approximately 9 months.

¹ From the College of Nutrition, Textiles, and Human Development, Texas Woman’s University, Box 23975, TWU Station, Denton, Texas 76204.
² Supported in part by Institutional Research Funds, Texas Woman’s University.
³ Taken in part from a thesis submitted by Lok Lee in partial fulfillment of the requirements for the MS degree.
⁴ Associate Professor Nutrition and Food Sciences.
⁵ Present address: 2514 Emerson Lane, Denton, Texas 76201.
⁶ Research Assistant.

Material and methods

Over the course of two 15-week semesters with registration and examination periods in the 37 weeks between September 1, 1972 and May 17, 1973, female undergraduate students, aged 20–22 years, purchasing meals under contract in the dining halls of this University received a series of 35 daily menus repeated 6.6 times. At each meal there was a choice of two entrees and several different side dishes were available. A variety of salad dressings was provided at noon and in the evening. One of us (L.L.) ate regularly in the dining halls during this period to observe students’ food preferences. Information on the consumption of various foods was also provided by the food service supervisor. A sampling bias was known to have occurred since a relatively high proportion of the volunteers were enrolled in the College of Health, Physical Education and Recreation. Complaints regarding food quality and outbreaks of diarrhoea were largely discounted when it was found that both the athletically involved and sedentary students were routinely exceeding the RDA for energy (8) for females 20–22 years of age. Since the vitamin content of foodstuffs may be adversely affected by improper handling techniques, it should be noted that some evidence, i.e., iridescent green roast beef, of poor control was seen.

Meals (breakfast, lunch, and dinner) were selected to provide 2,500 kcal/day for 10 days during the sixth menu cycle and for 10 days during the seventh menu cycle. Two sets of meals were collected on the same days (days 1 and 17) in both menu cycles to evaluate variations in diet constituents. Diets were numbered according to the repetition and day of the menu cycle on which they were collected, i.e., 6–1, 7–1, 6–17, and 7–17 were the diets collected on the 1st and 17th days of both the sixth and seventh menu cycles. On the 19th day of the seventh menu cycle fasting blood samples were obtained from 26 female volunteers.

The three meals comprising a day’s diet were refrigerated as collected, mixed, homogenized in a 1 gallon Waring Blender with 100 mg butylated hydroxytoluene, and stored at –15°C until analyzed. One-fourth of each diet was lyophilized, homogenized and a 50-g aliquot was extracted twice with 500 ml chloroform:methanol (2:1 v/v). When a shortage of chloroform occurred samples were extracted with methyl:ethanol (4:1 v/v) (16). Solvent was removed under vacuum, the residue taken up in petroleum ether and washed with water. Total fat was determined gravimetrically by taking appropriate aliquots to dryness. Fatty acid composition was determined by gas–liquid chromatography of the methyl esters (17) using an ethylene glycol succinate polyester liquid phase and a Bendix flame ionization detector. Butylated hydroxytoluene has a retention time similar to methyl myristate on this column and special care in peak assignment is required.

Aliquots of dietary fat each representing approximately 5% of the total fat were saponified in 30% ethanolic KOH containing 2% pyrogallol (18). Tocopherols in the nonsaponifiable fraction were determined (19) after one-dimensional thin-layer chromato-
6.5, a deviation.

5-week Diets clustered averaged graph 2-3 5.4 25% 1.1

Approximately ± 10 a-, levels 15.3

RRR-a-tocopherol.

The 71% level of this diet contained vitamin E and diet was underrep-

resented with a single low value while week 1 was overrepresented with three low values and three high values.

Plasma total tocopherol levels ranged from 0.73 to 1.76 mg/100 ml (Fig. 2). Thin-layer chromatography of pooled residues of plasma extracts revealed the presence of small quantities, approximately 8–12% of the total, of γ-tocopherol.

Discussion

The 17 diets and 2 replicates analyzed are believed to comprise a representative sample of the actual food intakes of the female undergraduate student volunteers over a period of approximately 9 months. In view of the random selection of menus there is no reason to believe that the other 18 menus not analyzed differed greatly from the 17 menus actually analyzed. After 6.6 repetitions of the 35-day menu cycle these 17 diets were presented on 44% of the days (113 days) over a 37-week period. No constructive information is available on food consumption between semesters and holidays (less than 11% of this period) when the dining halls were closed. No freshmen and only two sophomores were included in this study. Essentially all of the volunteers had, therefore, consumed meals prepared according to this 35-day menu cycle for major portions of the preceding 3–4 years.

Blood samples were obtained 9 weeks after the last previous holiday during the final week of the Spring semester to ensure that the diets had been proffered the maximum number of times. In view of the tissue storage of vitamin E and the protracted periods required to produce mild decreases in plasma tocopherol levels it was considered essential to evaluate the adequacy of an average intake which had prevailed over a prolonged period.

Daily RRR-α-tocopherol intakes, while quite variable, essentially duplicated the range (2.6–15.4 mg) and average (7.4 mg) reported by Bunnell et al. (12) and agree well with the range (4.4–12.7 mg) and average (9.0 mg) reported by Bieri and Evarts (13). A composite Canadian

FIG. 1. RRR-α-tocopherol content of 17 diets. The value above the bar graph is the average ± standard deviation.

FIG. 2. Plasma total tocopherol levels in mg/100 ml for 24 female volunteers. The value above the bar graph is the average ± standard deviation.

25% α-, and 12% δ-tocopherol. RRR-α-tocopherol values were discontinuous in a manner best illustrated by a bar graph (Fig. 1). Approximately 71% of the values were clustered unsymmetrically around an average of 5.4 ± 1.1 mg/day. The other diets contained 2–3 times this level of RRR-α-tocopherol. Diets representing weeks 3, 4, and 5 of the 5-week menu cycle had similar average values, 6.5, 7.2, and 6.6 mg/day. Week 2 was underrep-

[In recent years there have been extensive international discussions regarding the nomenclature of the tocopherols. It seems possible that the designation RRR-α-tocopherol may eventually prevail for the naturally occurring isomer.]
diet providing 2,780 kcal was reported to contain 6.4 mg RRR-α-tocopherol (14). Smith et al. (15) found less than 5 mg α-tocopherol in the meals ingested by British hospital patients. Since the patients' age and/or health resulted in relatively low caloric intakes, the published analyses correspond to approximately 5 mg/2,500 kcal.

The eighth edition of “Recommended Dietary Allowances” (8) differs drastically from the seventh edition (21) in suggesting that vitamers other than α-tocopherol may supply 20% of the vitamin E activity of the diet. After completion of this study Bieri and Evarts (22) presented data indicating that γ-tocopherol has slight activity in the rat, chick and hamster. This activity is related to excellent absorption but very rapid loss from the tissue (23, 24).

Tissue shortage of RRR-α-tocopherol makes it practical to consider the average daily intake of this vitamer. Data from animals fed a constant level of γ-tocopherol may not be applicable to the variable intake encountered in the human diet. The rapid turnover of γ-tocopherol may require that the minimum sustained level of intake be considered. Ingestion of approximately 2.5 times as much γ-tocopherol as α-tocopherol in the human diet results in only 8–12% of the plasma tocopherol being derived from the γ-tocopherol. By the criterion of maintaining plasma levels, γ-tocopherol might be considered to have only 3–5% of the activity of RRR-α-tocopherol.

Separate recovery studies were not conducted in this investigation for tocopherols other than α-tocopherol. It was considered pointless to refine the measurement of vitamers not considered (21) to have significant activity in man. The chromatographic separations were excellent but there is no question that the recoveries of the individual tocopherols differed if only because of their relative concentrations. Detailed data have not, therefore, been presented for β, γ, δ- and total tocopherols. Based on the high value for γ-tocopherol of 10% of the vitamin E activity of RRR-α-tocopherol a 25% error in the determination of this vitamer would be required to produce a 5% error in the total vitamin E activity of the diets.

Draper (25) has tabulated studies of plasma or serum tocopherols in population groups in the United States between 1941 and 1969. If the data of Herting and Drury (26) which are at considerable variance with all other data are deleted, the 18 groups studied had average total tocopherol levels of 1.07 mg/100 ml. The wide fluctuations in plasma tocopherol values described as “typical” by Christiansen and Wilcox (27) were (personal communication) probably related to a failure to evaluate recoveries despite the use of small (approximately 2.5 μg) samples. Recoveries in this range are always low but are highly variable.

Evidence of mild vitamin E deficiency, sensitivity of the erythrocyte to hemolysis in an in vitro test (28), does not become detectable until the level of plasma total tocopherol falls below approximately 0.5 mg/100 ml. By this criterion and the tabulation by Draper (25) the women in the present study were apparently ingesting “adequate” levels of vitamin E and had “normal” (1.09 ± 0.25 mg/100 ml) plasma levels of tocopherol. It is important to emphasize that these “normal” plasma tocopherol levels were observed after prolonged consumption of a repetitive series of diets for approximately one-half of which analytical data have been provided.

Using the accepted conversion factor of 1.0 mg RRR-α-tocopherol = 1.49 IU, the average daily intake of 7.5 mg corresponds to 11.2 IU of vitamin E activity. The alternative conversion factor proposed by Ames (29) is not supported by published data. If as suggested by Bieri and Evarts (22) and the eighth edition (but not the seventh edition) of “Recommended Dietary Allowances” (8, 21) some allowance is made for the vitamin E activity of the other tocopherols, a value of approximately 14 IU/day is obtained. These levels bracket the currently tabulated RDA (12 IU) for adult females. Reference is made to the tabulated RDA since such values are extensively cited regardless of the limitations and disclaimers included in the text. The importance of the average level of intake, rather than daily intake, is apparent in the observation that 71% and 65% of the diets analyzed did not meet the tabulated RDA in terms of RRR-α-tocopherol or total vitamin E activity, respectively.

Harris and Embree (11) suggested that a ratio of 0.6 mg α-tocopherol/g PUFA might be useful in evaluating the adequacy of vitamin E in diets. The limitations of an E:PUFA ratio were previously discussed in detail in this Journal (30). The ratio of approximately 0.4 mg RRR-α-tocopherol/g linoleate found in the present study is in agreement with the E:PUFA
ratio of 0.43 reported by Bieri and Evarts (13), the ratio suggested by the eighth edition of "Recommended Dietary Allowances" (8), and is somewhat less than the ratio of 0.53 reported by Thompson et al. (14). Reference is made to the E:PUFA ratio merely to emphasize that not only have dietary vitamin E levels been similar in various studies but also on the average the different diets have had a similar balance of vitamin E and PUFA.

If vitamin E is to continue to be included in the "Recommended Dietary Allowances" it is pointless to state that a fixed value cannot be established and then include a series of specific values in the tabulation of RDA’s. The E:PUFA ratio of Harris and Embree (11) has basic limitations and has been extensively criticized (14, 30–33). This ratio is not a satisfactory basis for formulation of a variable RDA for vitamin E. Constructive work is needed to develop a relatively simple means of expressing a variable RDA for vitamin E related to the actual requirements of diverse populations rather than to the daily composition of the diet.

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