The content of vitamin E in British diets

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1. Analysis of whole daily diets of normal subjects and of diets prepared in hospital to resemble the home diets of ambulant hospital patients showed that the majority of diets in Britain have a vitamin E content less than the lowest recommended intake of 5 mg/d, despite being generally satisfactory in calorie, protein and fat contents.

2. Comparison of measured intake of vitamin E with the intake calculated from food tables showed that the use of such tables may be unreliable.

3. Analyses of selected representative foods and of duplicate whole diets showed that the variations between measured vitamin E content of diets and those calculated from tables may be largely due to the great variability of tocopherol concentration in apparently similar samples of food.

Since the discovery of vitamin E in 1923 by Evans & Bishop (1923), a variety of deficiency states has been described in animals, ranging from sterility in rats to anaemia and muscular dystrophy in monkeys (Dinning & Day, 1957). While no specific syndrome has yet been demonstrated in man, a number of workers have described symptoms and signs in infants which include oedema, colic and failure to thrive, with anaemia, evidence of in vivo haemolysis, increased in vitro susceptibility of erythrocytes to hydrogen peroxide and low plasma tocopherol concentrations (Nitowsky, Gordon & Tildon, 1956; Gordon, Nitowsky, Tildon & Levin, 1958; Majaj, Dinning, Azzam & Darby, 1963; Hassan, Hashim, Van Itallie & Sebrell, 1966; Oski & Barness, 1967, 1968). The latter two measurements have now become established as indicators of vitamin E deficiency. Gordon *et al.* (1958) have suggested that in infants the critical plasma tocopherol concentration for prevention of in vitro haemolysis is 0·5 mg/100 ml, and this also is the concentration suggested by the work of Horwitt (1962) in adults.

Leonard, Losowsky & Pulvertaft (1966) showed that in a high percentage of patients after gastric surgery results of peroxide haemolysis tests were abnormal and plasma tocopherol concentrations were low. Furthermore, they also showed that plasma tocopherol concentrations were low in some patients with peptic ulceration but without operation. In the operated patients, malabsorption may have contributed to the low tocopherol values, but it seems unlikely that this applied to the non-operated group. These patients had, however, on medical advice, changed their diets to depend more on milky foods. This would seem to be a relatively minor change and, if it were responsible for deficiency of vitamin E, it might suggest that the normal diet is only marginally sufficient in tocopherol. The possibility is supported by the work of Desai (1968) who showed that 5·9% of apparently healthy Canadian students

had plasma tocopherol concentrations lower than 0·5 mg/100 ml, and he suggested that it was likely that these low concentrations were a result of poor dietary intake of vitamin E. Recommendations for vitamin E intake have been made in Switzerland (Eidgenössisches Department des Innern, 1957), Germany (Deutsche Gesellschaft für Ernährung, 1965), and America (National Research Council, 1964, 1968); the lowest recommendation for minimum intake is 5 mg vitamin E (as α-tocopherol) per d.

In this study the content of tocopherols in British diets was measured and compared with the content calculated from food tables. The content of vitamin E in individual foods was also measured to assess the variations between samples of a given food and between different types of food and the influence these may have on the dietary intake of the vitamin.

EXPERIMENTAL

Collection of diets and foods

Whole daily diets of forty fully ambulant patients in a metabolic ward, under investigation for suspected malabsorption, were prepared. The diets were as close as possible to the home diets as described by the patients to the member of our team who is a trained dietitian, and this included all such minor items as biscuits, confectionery and drinks. Each diet was prepared and cooked in our own diet kitchen and was presented for analysis in exactly the form in which it would have been eaten. The calorie, carbohydrate, protein and fat contents of the diets were calculated from tables (McCance & Widdowson, 1967). Vitamin E contents were calculated from published values (Bunnell, Keating, Quaresimo & Parman, 1965; Dicks, 1965; McCance & Widdowson, 1967) and from information obtained in our own laboratory (unpublished). Analyses were also performed on duplicates of some of the diets, made up of exactly the same type and quantity of foods but bought, prepared and cooked on a different day, usually within a month of the first diet so as not to be subject to seasonal variation. Diets of ten normal young subjects, all members of medical and nursing staff, were collected at home and also contained a duplicate of every item eaten and drunk.

Individual foods, chosen as examples of meat, vegetable and fat were analysed uncooked. Margarines of different brands were obtained fresh from the manufacturers at the same time of year. Brussels sprouts were bought fresh from the shop at the same time of year and analysed on the day of purchase. Liver samples were obtained fresh, direct from the abbatoir, and analysed on the day of collection.

Assay of tocopherols

Each entire daily diet was homogenized, when fresh, and duplicate 20 g portions of the homogenate were extracted with 200 ml acetone in a Soxhlet apparatus for 2–3 h. The lipids were partitioned into 100 ml light petroleum (b.p. 40–60°) after the addition of 200 ml water, and the solvent was evaporated under reduced pressure at 37°. The residue was taken up in 10 ml ethanol containing 5% (w/v) pyrogallol, and saponified on a boiling water-bath for 15 min with 2 ml 16 N-KOH. Distilled water (20 ml) was added, the unsaponifiable material extracted with 3 × 20 ml diethyl ether (Society for Analytical Chemistry: Analytical Methods Committee, 1959) and

chromatographed two-dimensionally on thin-layer silica gel plates (Bieri & Privel, 1965), the first solvent being benzene–ethanol (99:1, v/v) and the second hexane–ethanol (90:10, v/v). Tocopherol was measured by the method of Emmerie & Engel (1938), the two observed tocopherol spots (corresponding to α-tocopherol and a combination of β- and γ-tocopherol) being eluted directly into 1·5 ml ethanol containing 0·02% dipirydyl. Of this eluant, 1 ml was taken into a cuvette and the colour developed with 0·1 ml of a 0·1% ethanolic solution of ferric chloride. An area of silica equal to that taken with the tocopherol spot was used as a blank. Absorption was measured on a Zeiss PMQ2 spectrophotometer at 520 nm, not less than 30 s and not more than 45 s after addition of the ferric chloride.

The method was standardized against pure α-tocopherol, and total recovery of standard added to diet never fell below 85%. For comparison, 5 μg or 10 μg α-tocopherol were run on each plate. The foods were homogenized and 2 g portions of each homogenate were saponified by the method of Bieri (1969) with 1 ml 11 N-KOH in a 0·75% ethanolic solution of pyrogallol for 15 min at 65–70°. The unsaponifiable material was extracted with 3 × 5 ml hexane. The extract was chromatographed and the tocopherols were determined as above.

RESULTS

The analytical results are shown in Fig. 1. Seven of the ten diets of normal subjects and twenty-nine of the forty diets of patients contained less than 5 mg tocopherol/d. A comparison of the analyses of duplicates of twenty-two diets is shown in Fig. 2. Although there was the same range of content of the vitamin in each group, there were considerable differences in the tocopherol contents of similar pairs.

Both α- and β-tocopherol were measured in eight diets of normal subjects, twenty-four diets of patients and fourteen of their duplicate diets. Ten diets contained as much β- as α-tocopherol, but, because of the lower biological potency of β-tocopherol (30% of that of α-tocopherol, according to Bunyan, McHale, Green & Marcinkiewicz, 1961), it contributed less to the total vitamin E activity. The γ-tocopherol contributed insignificantly to the total vitamin E activity and was therefore not included in the calculations. The α-tocopherol concentration was never greater than 6·3 mg/d, except in one sample. Fig. 3 shows the estimated vitamin E activity of the combined α- and β-tocopherol contents (the β-tocopherol values have been divided by 3 to give approximately equivalent α-tocopherol values in terms of biological activity), compared with the α-tocopherol content alone. Even so, twenty-seven of thirty-eight patients still had diets with a daily vitamin E content below 5 mg and the diets of five out of eight normal subjects contained less.

The analytical results were compared (Fig. 4) with the values obtained by calculating the vitamin E content from food tables. While both occupied the same range of values, Fig. 5 shows that there was no close correlation between measured and calculated values for each diet.

The analyses of liver, brussels sprouts and margarine showed a wide range of tocopherol content for each food type. The vitamin E contents of four apparently

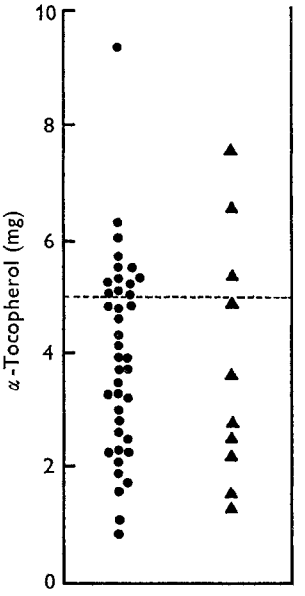

Fig. 1

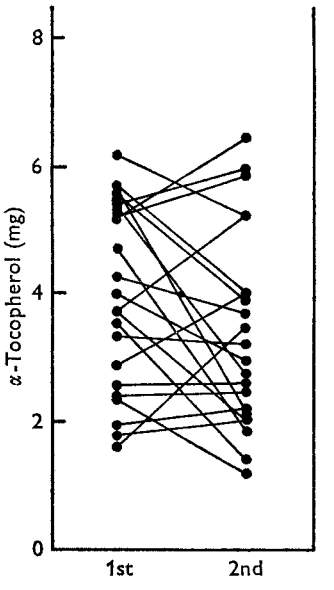

Fig. 2

Fig. 1. α-Tocopherol content of whole daily diets as eaten by patients (●) and normal subjects (▲). The dotted line represents the lowest recommended minimum daily intake.

Fig. 2. α-Tocopherol contents of duplicates of whole daily human diets.

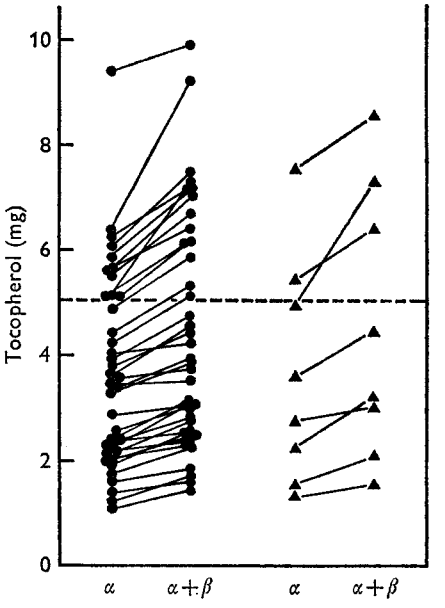

Fig. 3. Vitamin E activity of α- and β-tocopherol contents of whole daily human diets. The β-tocopherol content has been divided by three to give its approximate equivalent activity and added to the value for α-tocopherol. The dotted line represents the lowest recommended minimum dietary intake.

similar samples of lambs' liver ranged from 0·2 to 2·5 mg/100 g, the contents of two samples of brussels sprouts were 0·3 and 1·1 mg/100 g, and seven samples of margarine gave a range of from 1·2 to 27·8 mg/100 g.

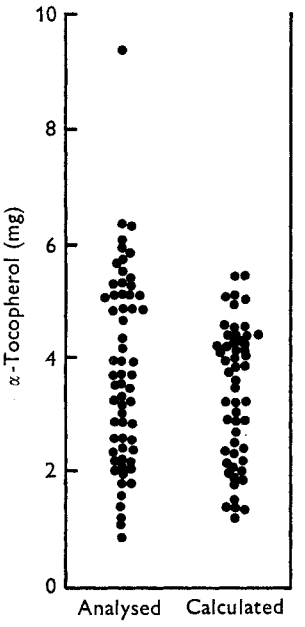

Fig. 4

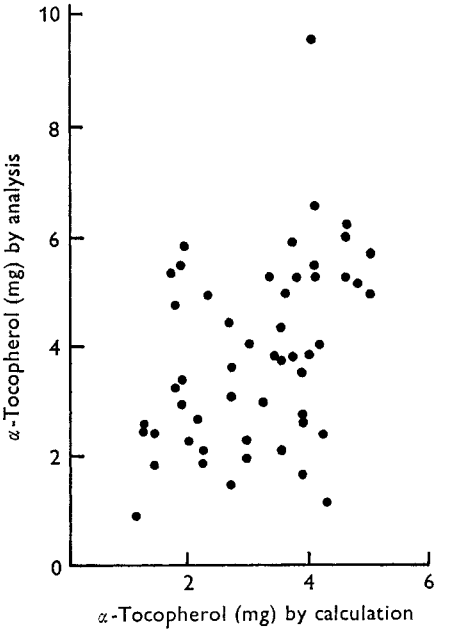

Fig. 5

Fig. 4. α-Tocopherol contents of whole daily human diets as obtained by direct analysis and by calculation from food tables. The two methods gave results covering approximately the same range.

Fig. 5. Relationship between α-tocopherol content of whole daily human diets was determined by analysis or by calculation from food tables. The correlation was poor.

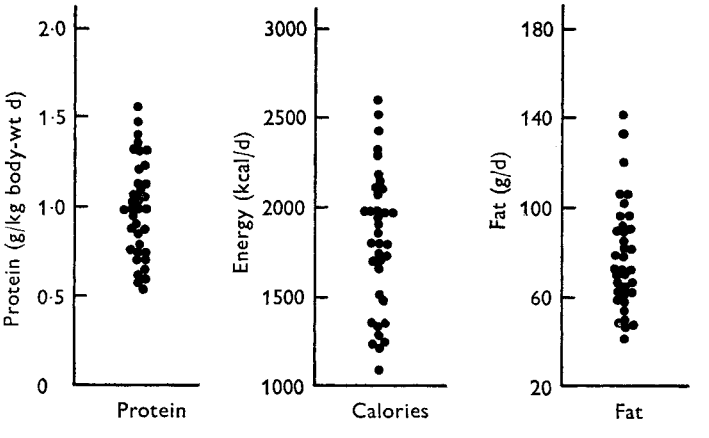

Fig. 6. Daily intake of calories, protein and fat in the human diets studied.

The calorie, protein and fat contents of the patients' diets are shown in Fig. 6. The ranges were 1160–2598 kcal ($4{\cdot}8 \times 10^6$–$10{\cdot}8 \times 10^6$ J), 33–90 g protein (0·58–1·65 g/kg body-weight) and 43–148 g fat (27–56 % kcal) per d.

DISCUSSION

Disease due to deficiency of vitamin E in human infants cannot be doubted, but in adults no specific syndrome has been delineated although there is now good evidence that vitamin E is essential. In patients with low plasma tocopherol concentrations there is decreased red-cell life in vivo (Horwitt, Century & Zeman, 1963; Losowsky & Leonard, 1967) and creatinuria (Nitowsky *et al.* 1956; Losowsky, Leonard, Kelleher & Pulvertaft, 1967), both of which can be corrected by giving α-tocopherol.

It has been suggested that deficiency of vitamin E in man may arise from poor dietary intake (Harris, Hardenbrook, Dean, Cusack & Jensen, 1961; Leonard *et al.* 1966; Desai, 1968), and Horwitt, Harvey, Duncan & Wilson (1956–7) showed that, in normal subjects, diets containing less than 3 mg α-tocopherol/d led to low plasma tocopherol concentrations and increasingly positive in vitro haemolysis tests. Recommendations for minimum intake of vitamin E have been made in other countries. In Switzerland, based on an estimation by the Home Department of the Swiss Confederation (Eidgenössisches Department des Innern, 1957), the daily requirement was set at 10 mg. A range of 5–30 mg vitamin E daily was recommended by the German Association for Nutrition (Deutsche Gesellschaft für Ernährung, 1965). Recommended dietary allowances of the National Academy of Sciences of America were 10–30 mg/d (National Research Council, 1964) in 1964, and in 1968 these were revised to 20–30 mg/d (National Research Council, 1968). The lowest recommended minimum intake is therefore 5 mg/d, and most present recommendations suggest a minimum of 10 mg/d. A number of investigations has been made previously into the daily intake of vitamin E. Harris & Embree (1963), in America, found a range of dietary intake of 5–30 mg/d with a mean of 15 mg, and Bunnel *et al.* (1965), also in America, found a range of 2·16–15·4 mg with a mean of 7·4 mg/d. Both these assessments of dietary intake were based on the summation of the measured content of individual foods, and differences in the two ranges were thought by Bunnell *et al.* (1965) to be due to differences in the preparation of the foods used, together with the greater specificity of their methods compared with those of Harris & Embree (1963). More recently Ikehata, Tanaka & Kamishima (1968), in Japan, found a mean daily content of 5·3 mg in Japanese diets, but this appears to have been based on the analysis of only three diets, and the modes of collection and preparation were not described. Our investigation differs from the above in that it was based on direct analyses of whole daily diets as eaten, and the results include α- and β-tocopherol measured separately. Even if β-tocopherol is included, the majority of diets, both of normal subjects and of patients, contained less than the lowest recommended minimum intake for any country for which a recommendation has been given.

A possible explanation for the low tocopherol content of the diets analysed in this study is that they might be generally inadequate in several nutritional requirements.

Davidson & Passmore (1966) recommended a minimum of 0·85 g protein/kg body-weight and an intake of 1000 kcal/d for basal metabolic and non-occupational activity. There are no specific recommendations for fat intake, but the average British diet contains between 25 and 50% of calories as fat (Davidson & Passmore, 1966). The diets analysed in this study had protein contents above the recommended level in all but nine diets, energy values greater than 1000 kcal/d in all and greater than 1500 kcal/d in all except nine diets, and the fat contents ranged from 27 to 55% of calories. Furthermore, the tocopherol contents in those with relatively low protein and caloric contents were in the same range as in the others, and the ten diets of healthy young, active subjects had the same range of tocopherol content as the diets of the patients. Thus the low contents of vitamin E in these diets cannot be ascribed to their being inadequate in calories, protein and fat.

It is difficult to be sure of the significance of dietary levels of tocopherol considered in isolation. Our preliminary investigations (unpublished) suggest that the content of polyunsaturated fatty acids in British diets is rather lower than in American diets (Gordon *et al.* 1958; Horwitt, 1960; Horwitt, Harvey, Century & Witting, 1961), and thus a somewhat lower intake of tocopherol might be tolerated without producing signs of deficiency. Relationships between vitamin E, selenium and other anti-oxidants have also been described (Horwitt, 1962; Moore, 1962) and may have to be considered.

The usual way of assessing dietary intake of tocopherol is to summate the contents of individual foods, as derived from food tables. Our results suggest that the range of dietary content is similar, whether measured or calculated from tables, but for any individual diet the measured and calculated values do not necessarily correspond. One possible reason for this is that many food tables were compiled when methods for estimating vitamin E were not as specific as those now used, which would, in general, lead to the calculated values being systematically higher, which was not so. A second possibility is that some of the published values may relate to food as purchased, thus not taking account of cooking and preparation, which may be accompanied by large changes in vitamin E content. A third possible reason is that there are probably differences in the vitamin E contents of individual foods due to variations in freshness, method of storage, processing and particularly season of the year (Herting & Drury, 1965; Pingel & Schulze, 1966). Finally, there may be variations in different samples of a foodstuff at the same time of year, even when it is fresh, and when processed and cooked identically.

Our findings of wide variations in vitamin E contents of apparently similar samples of various foods show that the assessment of the dietary intake of vitamin E from food tables can lead to considerable error.

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