Vitamin A and vitamin E in human blood

2.* Levels of vitamin E in the blood of British men and women, 1952–7

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In the first paper of this series (Leitner, Moore & Sharman, 1960) we reported values for the levels of vitamin A and carotenoids found in blood collected between 1948 and 1957 from British men and women. During 1952–7 an investigation, partly overlapping with that on vitamin A, was made on vitamin E. A preliminary paper, based on some of our earlier results, has already been published (Leitner *et al.* 1952). The present communication gives our results in full.

EXPERIMENTAL

Collection of specimens. Blood was collected from volunteers who could be considered normal, apart from reservations already made (Leitner *et al.* 1960). When the amount of blood collected was insufficient for estimating both vitamin A and vitamin E preference was given to vitamin A. The total number of estimations for vitamin E was, therefore, somewhat lower than for vitamin A.

Estimation of vitamin E. Our method resembled that of Quaife & Harris (1944) except that we used a correction for carotenoids instead of the preliminary destruction by hydrogenation used by them. To 2 ml of serum was added the same volume of ethanol, slowly and with constant shaking. Light petroleum, 4 ml, was then added, and the mixture shaken for 10 min. Centrifugation followed, and a 3·5 ml portion of the light petroleum layer was withdrawn. After the estimation of carotenoids the solution was evaporated, under diminished pressure, and the residue was dissolved in 2 ml of hot ethanol. This solution was transferred, with washings to make a total volume of 5 ml of ethanol, into a centrifuge tube, which was placed in a refrigerator for 30 min to allow the separation of sterols. After further centrifugation the supernatant liquid was treated, in 1·6 ml portions, by the procedure of Emmerie & Engel (1938). Thus 0·2 ml of a solution of 2, 2′-dipyridyl, 0·125 % (w/v) in ethanol, was first added, followed by the same volume of ferric chloride, containing 0·125 % (w/v) of $FeCl_3.6H_2O$ in ethanol. The mixture was shaken, and the red colour was measured in the photoelectric absorptiometer (Leitner *et al.* 1960) 2 min later. The green filter used was Wratten no. 55.

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The correction for carotenoids was made on the basis of 1 μg of carotenoid's having the same reducing power as 0·0023 mg of α-tocopherol. Vitamin A has very small reducing powers in comparison with the tocopherols, and was ignored in our estimations. Since it appears that α-tocopherol is the main reducing substance in the blood lipids, our simple method, with a correction only for carotenoids, probably gave more accurate results than might otherwise have been expected.

In our preliminary paper (Leitner *et al.* 1952) results for vitamin E were given for estimations since March 1951. Unexpectedly high results observed during that year, however, have led us to suspect that the routine application of our method for estimating the vitamin was still imperfect. The present paper, therefore, includes only results obtained from 1952 onwards.

RESULTS

Vitamin E levels in men and women. In the period 1952–7, 343 estimations were made on men and 240 on women. For men (mean age 49 years) the mean was 1·05 mg/100 ml serum, and for women (mean age 47) 1·06 mg. The mean for both sexes was 1·05 mg. When all our results were taken together, therefore, there was no evidence of a consistent sex difference, such as found for vitamin A and carotenoids. Examination of the histograms in Fig. 1 confirms this conclusion. For both sexes the commonest arbitrary range was 0·9–1·09 mg/100 ml. We shall see later, however, that sex differences appear when comparisons are made not for all ages combined, but for limited age groups.

Vitamin E in relation to carotenoids. Graphs for both sexes combined, relating the mean vitamin E levels found for arbitrary ranges of carotenoid values, are shown in Fig. 2. It will be seen that two- or three-fold increases in the carotenoid ranges were associated with increases in the vitamin E values of only about 20–30%. We must not conclude, of course, that all the individual vitamin E values fell within this narrow range. Inspection of Fig. 2 clearly indicates a much wider range. If the procedure had been reversed and mean carotenoids had been plotted against vitamin E ranges, four- or five-fold increases in vitamin E values would probably have been associated with only small increases in mean values for carotenoids.

Vitamin E in relation to vitamin A. The graph for this relationship is shown in Fig. 3. Again it will be seen that two- or three-fold increases in vitamin A ranges were associated with increases in the vitamin E mean that never exceeded 30%. It may likewise be mentioned that had this procedure also been reversed four- or five-fold increases in vitamin E values would probably have been associated with only small increases in values for vitamin A.

Vitamin E, sex and age. Vitamin E means for men and women in different age groups are shown in Fig. 4. It will be seen that the graphs diverged considerably, and crossed at two points. Thus for ages under 30 a higher mean was found for women than for men. This difference was statistically significant ($0{\cdot}01 < P < 0{\cdot}02$). For the age group 30–39, however, the relationship was reversed, with the higher mean for men. Again the difference was significant ($0{\cdot}01 < P < 0{\cdot}02$). The means for men remained above those for women in the 40–49 and 50–59 age groups, but both

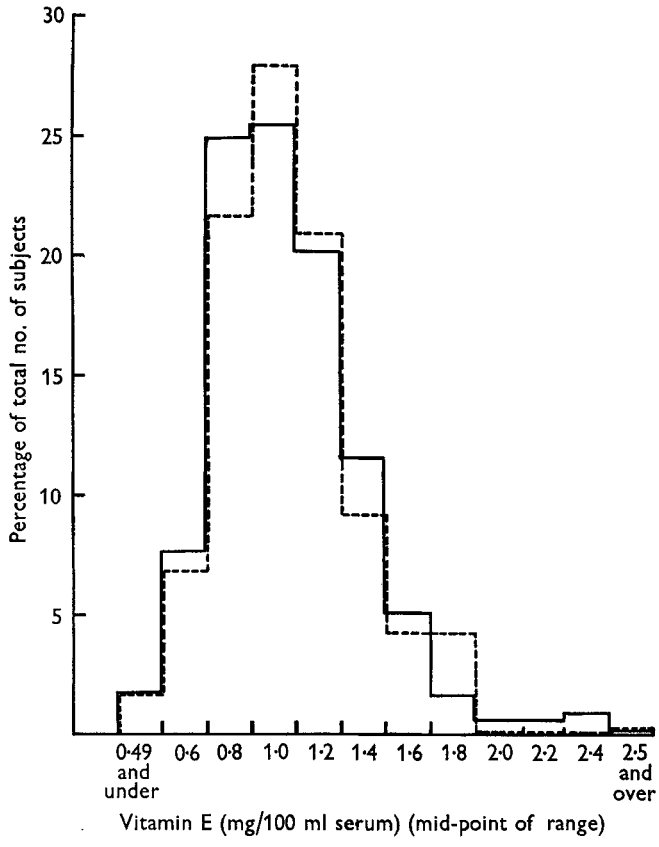

Fig. 1. Distribution of blood vitamin E values among British men and women. ——, males; – – – –, females.

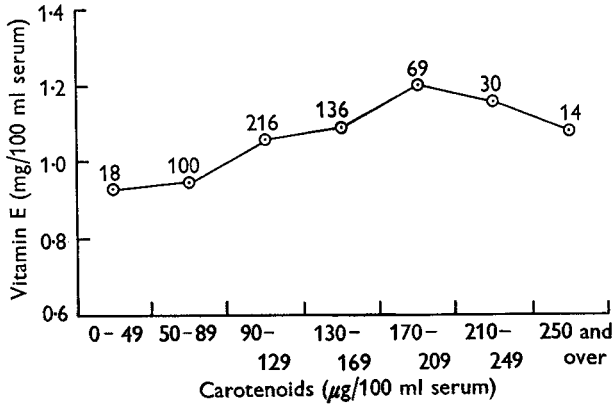

Fig. 2. Relationship between vitamin E and carotenoid content of the blood of British men and women. Values for males and females combined; numbers of subjects are shown beside each point.

sexes had the same mean at 60–69 years. At ages over 69 a higher mean was found for women than for men, but the difference was not significant ($0{\cdot}1 < P < 0{\cdot}2$).

Influence of season. For both sexes combined almost identical levels were found in different seasons, with means of 1·04 mg/100 ml for winter (January–March), 1·06 for spring (April–June), 1·07 for summer (July–September), and 1·04 for autumn (October–December).

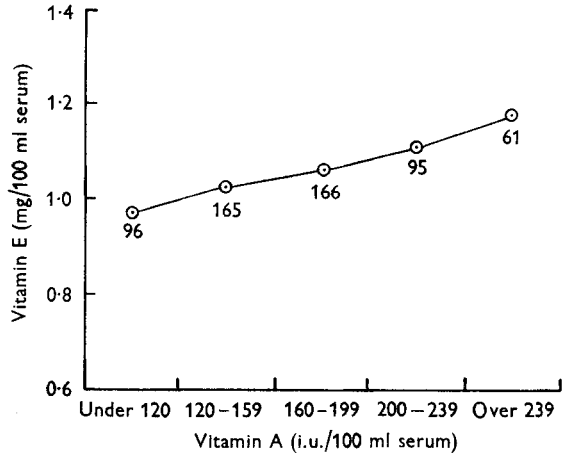

Fig. 3. Relationship between vitamin E and vitamin A contents of the blood of British men and women. Values for males and females combined: numbers of subjects are shown beside each point.

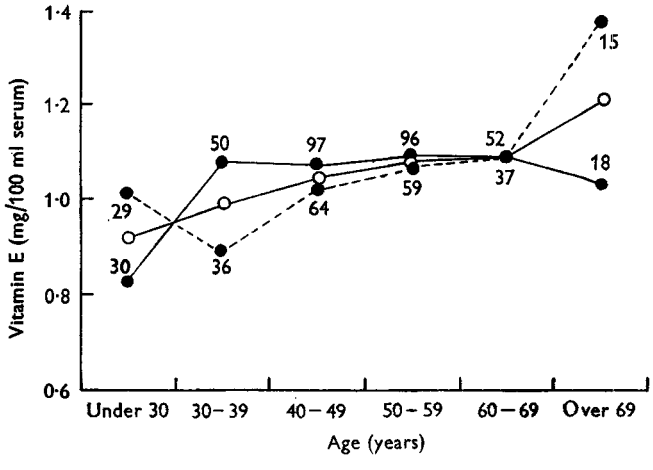

Fig. 4. Relationship between the vitamin E content of the blood of British men and women and their age. Numbers of subjects are shown beside each point; upper numbers, males; lower numbers, females. ●——●, males; ● - - - ●, females; ○——○, mid-point of range, males and females.

Vitamin E levels in different years. Comparison of the means, for both sexes combined, showed that the changes from year to year were slight and inconsistent. Thus the mean was 1·06 mg/100 ml for 1952, 1·06 for 1953, 0·95 for 1954, 1·02 for 1955, 1·01 for 1956, and 1·17 for 1957.

DISCUSSION

Blood levels of vitamin E in various investigations. In Table 1 the results described here are compared with those obtained in previous investigations. It will be seen that the means found fell within the range 0·89–1·20 mg/100 ml. Variations between the results of different workers were therefore much smaller than for vitamin A or carotenoids (Leitner *et al.* 1960). For both sexes together there was no disparity between our findings and those from the United States.

Seasonal and annual variations. It will be remembered that for vitamin A and carotenoids considerable variations were found according to season or to the biennial period during which the specimens were collected. For vitamin E, with both sexes together, seasonal variation was negligible, and changes from year to year were small and inconsistent in direction.

Table 1. *Mean values for vitamin E in the blood of 'normal' human subjects, found in various investigations*

Reference	Country	Subjects		Vitamin E (mg/100 ml serum)
		No.	Details	
Harris, Hickman, Jensen & Spies (1946)	U.S.A.	70	Both sexes	1·04
Popper, Dubin, Steigmann & Hesser (1949)	U.S.A.	9	—	0·94
Lemley, Gale, Furman, Cherrington, Darby & Meneely (1949)	U.S.A.	21	Healthy young adults	1·09
Klatskin & Krehl (1950)	U.S.A.	23	Normal adults	1·12
Chieffi & Kirk (1951)	U.S.A.	78	Men, including old	0·92
		110	Women, including old	1·01
Ferguson, Bridgforth, Quaife, Martin, Cannon, McGanity, Newbill & Darby (1955)	U.S.A.	1575	Pregnant women	0·89–1·40*
Kramer (1955–6)	Hungary	86	Non-pregnant women	0·99
Postel (1956)	U.S.A.	30	Normal	1·20
Leitner, Moore & Sharman (this work)	U.K.	343	Men	1·05
		240	Women	1·06

* According to stage of pregnancy.

Sex and age. Chieffi & Kirk (1951) reported sex differences in vitamin E levels at various ages, with a mean for all ages of 0·92 mg/100 ml for men and 1·01 mg for women. The sex differences found in our work appeared only when the subjects were divided into age groups. Otherwise the superiority in vitamin E levels for women over men under the age of 30, and over 69 years, was counterbalanced by the superiority of levels in men between those ages. When the means for each sex were averaged for each age group the vitamin E level increased steadily with age.

Differences between vitamins A, E and other lipids in sex and age relationships. The sex differences for vitamin E, which vary in their direction according to age, may be

contrasted with the much more consistent sex differences found for vitamin A and carotenoids. Thus we have seen that vitamin A means are consistently about 20% higher in men than in women, except in extreme old age, and that carotenoids are 8% higher in women than in men. Obviously there must be important differences in the mechanisms by which vitamins A and E and carotenoids are carried in the blood, and in the response of the mechanisms to the influence of hormones.

Evidence that such sex differences extend to other lipids may be found in the results of Adlersberg, Schaefer, Steinberg & Wang (1956) on the influence of age and sex on the serum levels of total cholesterol and of phospholipids. For both these lipid fractions we have the same reversal of the sex relationship as for vitamin E, with higher levels for females than for males up to about the age of 27 years, followed by a change to higher mean values for men. The finding by the same workers of a further reversal, occurring as the age of 50 years is approached, lends plausibility to our finding of higher vitamin E levels for women than for men in old age, over 69 years. Although the difference between our means for men and women, in this age group, was not statistically significant it may well have been genuine.

A further indication of the change in the influence of sex according to age may perhaps be seen in the carotenoid levels which we have found for young females (Leitner *et al.* 1960). Although the graphs for carotenoids in males and females did not cross, there was the same tendency as was found for vitamin E, cholesterol, and phospholipids, for values to be unexpectedly high in females under the age of 30 years.

Further evidence of the different response of vitamin A and vitamin E to the influence of hormones may be derived from changes in their values during pregnancy. Thus, it is well known that in pregnancy the content of vitamin A falls (Lund & Kimble, 1943; Bodansky, Lewis & Lillienfeld, 1943), whereas that of vitamin E is raised (Straumfjord & Quaife, 1946; Kramer, 1955–6; Ferguson *et al.* 1955).

On the evidence available it appears that the levels in the blood of vitamin E, cholesterol, phospholipids and probably carotenoids are controlled by similar types of mechanism but that the level of vitamin A is controlled by a different mechanism. As we have already suggested (Leitner *et al.* 1960) the mechanism for maintaining the level of vitamin A in the blood may be influenced by a sex difference in the efficiency of converting carotene.

SUMMARY

1. Vitamin E was estimated over the period 1952–7 in the blood of over 300 men and more than 200 women, in whom no serious disease could be detected.

2. Considerable individual variations were found for both sexes. The mean value, for equal numbers of each sex, expressed as α-tocopherol was 1·05 mg/100 ml serum. Variations in different seasons and in different years were small.

3. Sex differences were less consistent than for vitamin A or carotenoids. When all ages were combined for each sex no difference between the means was found.

4. When results for both sexes were combined the values for vitamin E rose with increasing age.

5. Simultaneous analysis of the results according to both age and sex revealed sex differences between certain age groups. Thus, levels tended to be higher in women than in men at ages under 30 and over 69, and higher in men between these ages.

6. The variations in vitamin E according to age and sex were similar to those known to hold for total cholesterol and phospholipids, but differed from those shown by vitamin A.

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REFERENCES

Adlersberg, D., Schaefer, L. E., Steinberg, A. G. & Wang, C. (1956). *J. Amer. med. Ass.* **162**, 619.
Bodansky, O., Lewis, J. M. & Lillienfeld, M. C. C. (1943). *J. clin. Invest.* **22**, 643.
Chieffi, M. & Kirk, J. E. (1951). *J. Geront.* **6**, 17.
Emmerie, A. & Engel, C. (1938). *Rec. Trav. chim. Pays-Bas*, **57**, 1351.
Ferguson, M. E., Bridgforth, E., Quaife, M. L., Martin, M. P., Cannon, R. O., McGanity, W. J., Newbill, J. & Darby, W. J. (1955). *J. Nutr.* **55**, 305.
Harris, P. L., Hickman, K. C. D., Jensen, J. L. & Spies, T. D. (1946). *Amer. J. Publ. Hlth*, **36**, 155.
Klatskin, G. & Krehl, W. A. (1950). *J. clin. Invest.* **29**, 1528.
Kramer, M. (1955–6). *Int. Z. Vitaminforsch.* **26**, 58.
Leitner, Z. A., Moore, T. & Sharman, I. M. (1952). *Brit. J. Nutr.* **6**, x.
Leitner, Z. A., Moore, T. & Sharman, I. M. (1960). *Brit. J. Nutr.* **14**, 157.
Lemley, J. M., Gale, R. G., Furman, R. H., Cherrington, M. E., Darby, W. J. & Meneely, G. R. (1949). *Amer. Heart J.* **37**, 1029.
Lund, C. J. & Kimble, M. S. (1943). *Amer. J. Obstet. Gynec.* **46**, 486.
Popper, H., Dubin, A., Steigmann, F. & Hesser, F. P. (1949). *J. Lab. clin. Med.* **34**, 648.
Postel, S. (1956). *J. clin. Invest.* **35**, 1345.
Quaife, M. L. & Harris, P. L. (1944). *J. biol. Chem.* **156**, 499.
Straumfjord, J. V. & Quaife, M. L. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 369.

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