Serum Tocopherol (Vitamin E) Status of Malaysians.

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INTRODUCTION

Recently there has been a renewal of interest in vitamin E. This is partly due to reports in the popular press and magazines extolling the benefit of vitamin E supplements in the prophylaxis of cardiovascular disorders, skin conditions and athletic performance. Although none of these claims have been proven on firm scientific foundations, it cannot be denied that such reports stemmed primarily from the proceedings of scientific forums or conferences dealing with vitamin E which are yet to make their appearance in print.

Until the exact roles of vitamin E are ascertained, human daily vitamin E requirements are put at 5 – 30 mg. Recently, the Food and Nutrition Board, National Academy of Sciences U.S.A. recommended a daily dietary allowance of 10 mg for a healthy adult (*Am. J. Publ. Hlth.*, Vol. 63, 1973). However, when relatively large amounts of polyunsaturates are consumed as in the treatment of primary hyperlipoproteinaemia, opinions are divided as to whether there is an increased need for vitamin E (Horwitt, 1962; Jager, 1972; Briggs and Briggs, 1974).

Owing to the complete lack of information on vitamin E nutriture in Malaysians, we thought it worthwhile to undertake some fundamental studies in this connection.

The objectives in this study are therefore as follows:–

(i) to obtain some baseline data on serum tocopherol status of Malaysians in apparent good health.

(ii) to compare the vitamin E status of the above subjects with patients treated for primary hyperlipoproteinaemia with a low-cholesterol, polyunsaturates-rich diet.

(iii) to study the effect of a daily supplementation of alpha-tocopherol on serum tocopherol status and the levels of serum beta-lipoproteins, total serum lipids, serum cholesterol and serum triglycerides.

MATERIALS AND METHODS

Subjects

a. **Subjects in apparent good health:** These consisted of 63 Malaysian blood donors and subjects undergoing routine medical examination. Their ages ranged from 7 to 55 years with a mean age of 34 years. The majority were males and consisted of an approximately equal number of Malays, Chinese and Indians.

b. **Hyperlipoproteinaemic patients on a modified diet:** These consisted of 63 patients of Malay, Chinese and Indian origin, aged 7 to 55 years with a mean age of 39 years. The majority were outpatients found to have primary hyperlipoproteinaemia (mostly Type II) and had been instructed to avoid cholesterol-rich foods such as eggs and organ foods and to use corn oil for cooking purposes instead of other edible oils. Hyperlipoproteinaemia was diagnosed when beta-lipoproteins exceeded 700 mg/100 ml serum, cholesterol exceeded 260 mg/100 ml serum and triglycerides (fasting) over 200 mg/100 ml serum, occurring singly or in combination.

c. **Hyperlipoproteinaemic subjects on a normal diet:** These consisted of 56 subjects of various races, aged 11 to 69 years with a mean age of 43 years. They were not known to be on drug or diet therapy at the time of investigation.

d. **Subjects on vitamin E supplementation:** These comprised 3 healthy, non-obese laboratory personnel, aged 24, 26 and 28 years. They were given 100 mg dl-alpha-tocopherol acetate daily (except Sundays) after meals for a total of 5 weeks.

During the period of the study, they were instructed to adhere to their normal eating habits.

Methods

Single venous samples were obtained usually after an over-night fast and for the vitamin E supplementation experiment, weekly fasting venous samples were obtained before, during and after the 5-week supplementation period. The sera were analysed for total tocopherol, beta-lipoproteins, total lipids, total cholesterol and triglycerides.

Serum total tocopherol was estimated by the Emmerie and Engel reaction which involved the reduction by vitamin E of ferric ions to ferrous ions, the latter forming a red colour with dipyridyl (Baker and Frank, 1968). n-Heptane was used as the serum extractant (Hashim and Scuttringer, 1968). Tocopherol levels were expressed as mg per 100 ml serum and also as mg per gram total serum lipids, referred to henceforth as Horwitt's ratio (Horwitt et al, 1972).

Serum beta-lipoproteins were determined by a turbidimetric method involving the specific precipitation of beta-lipoproteins by dextran sulphate in the presence of calcium ions at pH 9.0 (B.D.H. beta-lipoproteins kit).

Serum total lipids were estimated using a turbidimetric method (Huerga et al, 1953). The concentration of lipids was read from a standard curve obtained by plotting the total serum lipid levels determined gravimetrically (Friedman, 1968) against turbidimetric readings of corresponding samples. Aliqouts from the same chloroform-methanol extract of serum were used to obtain the turbidimetric and gravimetric values.

Serum total cholesterol was determined by the method of Abell et al (1952) which involved a preliminary saponification, extraction with petroleum ether (B.P. 40-60°C) and colorimetry by the Liebermann-Burchard reaction.

Serum triglycerides were determined essentially by the method of Van Hendel et al (1957) as modified by Harding et al (1967); phospholipid-free sera extracts were obtained by adsorption onto 'Florisil'. The samples were saponified and the glycerol liberated was oxidised to formaldehyde which reacted with chromotropic acid forming a purple colour. Triglycerides were expressed as mg tripalmitin per 100 ml serum.

All readings were read on a Coleman 44 spectrophotometer.

RESULTS AND DISCUSSION

The serum tocopherol levels (expressed as mg/100 ml serum) for the various categories of subjects are summarised in Table I.

Table I
Mean ± S.D. and range of serum tocopherol in the various categories of subjects

Subjects	No. of samples	Mean ± S.D. (mg/100ml serum)	Range (mg/100ml serum)
Subjects in apparent good health	63		0.30-2.07
Malays		0.83 ± 0.39	
Chinese		0.92 ± 0.36	
Indians		0.98 ± 0.32	
Means of all races		0.92 ± 0.36	
Hyperlipoproteinaemic patients on a modified diet	63	1.20 ± 0.38	0.34-2.10
Hyperlipoproteinaemic subjects on a normal diet	56	1.21 ± 0.30	0.69-2.12

No significant difference was observed between the mean values for Malays, Chinese and Indians; the overall mean of all races was 0.92 mg/100 ml. This is slightly lower than the mean of serum tocopherol obtained by other investigators (Harris et al, 1961; Bieri et al, 1964) but higher than values reported by others using chromatographic procedures for the estimation of serum alpha-tocopherol (Dayton et al, 1965; Christiansen and Wilcox, 1973). However, higher tocopherol values were apparent in the patients with hyperlipoproteinaemia.

We observed that the levels of serum tocopherol correlated positively with serum beta-lipoproteins, cholesterol and triglycerides (Figs. 1-3). This corroborates the findings of others who reported similar associationships (Davies et al, 1969; Rubenstein et

al, 1969). Consequently, the expression of tocopherol in terms of mg per 100 ml serum is of little value particularly when there is concurrent hyperlipoproteinaemia or hypolipoproteinaemia. Thus it can be argued that a high level of serum tocopherol may be secondary to lipid disorders and need not reflect vitamin E nutriture.

To overcome this, Horwitt et al (1972) suggested the use of the expression serum tocopherol : total serum lipid ratio (i.e. mg tocopherol per gram total serum lipids). These values for our subjects are now shown in Table II.

Table II
Serum tocopherol : total serum lipid ratios and incidence of vitamin E deficiency in the 3 groups of subjects

Subjects	No. of samples	(Horwitt's ratio) mg tocopherol per g total lipids (Mean ± S. D.)	% subjects <0.8 mg tocopherol per g total lipids
Subjects in apparent good health	63	1.32 ± 0.34	5
Hyperlipoproteinaemic patients on a modified diet	63	1.29 ± 0.35	8
Hyperlipoproteinaemic subjects on a normal diet	56	1.26 ± 0.29	2

It is evident from Table II that when expressed in this fashion, there was little difference in the serum tocopherol status of the 3 groups of subjects which differed when the levels were expressed in mg tocopherol per 100 ml serum.

Thus while none of our subjects had serum tocopherol levels below 0.3 mg/100 ml, a level below which Leonard and Losowsky (1969) considered indicative of serious vitamin E deficiency, when expressed as Horwitt's ratio it may be seen that 5% of the normal healthy subjects and 8% of the primary hyperlipoproteinaemic patients on the modified diet had ratios below 0.8, a ratio suggested as representing minimal vitamin E adequacy (Horwitt et al, 1972).

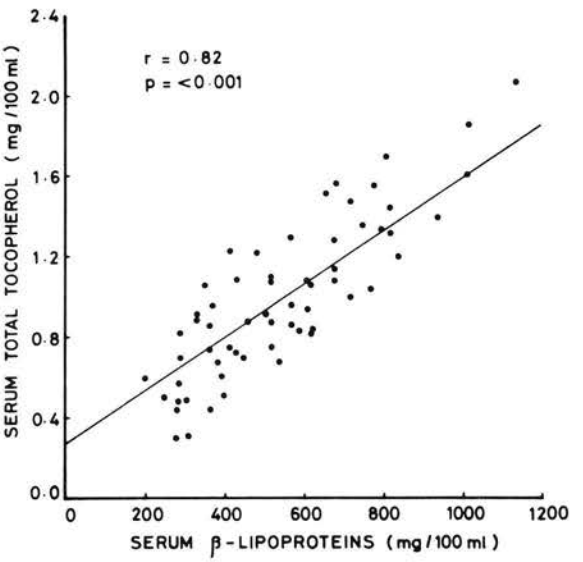

Fig 1
Correlation between serum tocopherol and serum beta-lipoproteins.

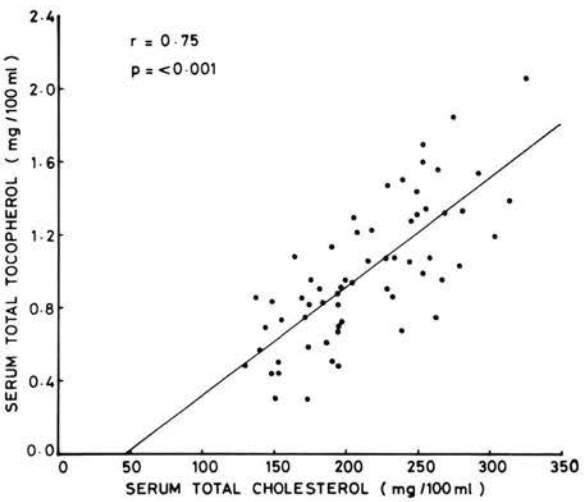

Fig. 2.
Correlation between serum tocopherol and serum total cholesterol.

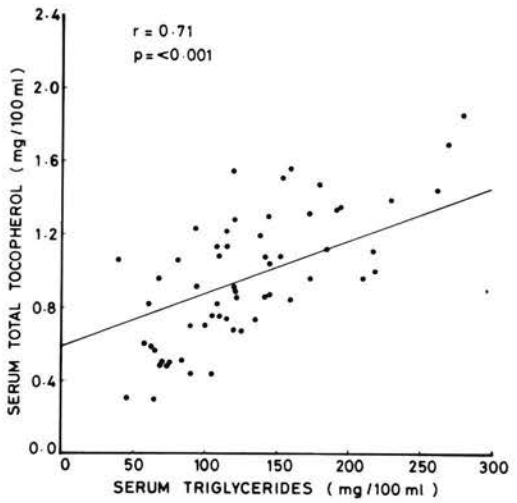

Fig. 3
Correlation between serum tocopherol and serum triglycerides.

This slight difference in the incidence of vitamin E inadequacy however, is of no statistical significance. But it would appear that expression of serum tocopherol in terms of the tocopherol : serum lipid ratio not only gives a truer picture of vitamin E status but it also possesses the added advantage of a greater sensitivity in predicting vitamin E insufficiency.

The highly significant correlation between the levels of serum tocopherol expressed as mg/100 ml and the various serum parameters e.g. beta-lipoproteins, cholesterol and triglycerides (see Figs. 1-3) prompted us to investigate the levels of these substances with daily supplementation of vitamin E.

The effect of a daily supplementation of 100 mg dl-alpha-tocopherol acetate for a period of 5 weeks on the levels of serum tocopherol, serum beta-lipoproteins, total serum lipids and serum lipid fractions in the 3 human volunteers is shown in Tables III-VI.

Table VI shows that only serum tocopherol levels, expressed either as mg/100 ml or mg per g total serum lipids, were altered significantly during the period of vitamin E supplementation. No significant changes could be observed in the levels of serum beta-lipoproteins, total serum lipids and serum lipid fractions. This is in agreement with the observations

Table III
The effect of alpha-tocopherol supplementation on the level of serum constituents in subject A.

Serum constituents (mg/100ml unless otherwise stated)	Before supplementation (Mean of 3 consecutives samples)	During supplementation (Mean of 5 consecutive samples)	After supplementation (Mean of 3 consecutive samples)
Tocopherol	0.70	1.45	0.99
Tocopherol (mg per g total lipids)	1.10	2.15	1.36
Beta-lipoproteins	422	427	533
Total lipids	613	653	708
Total cholesterol	196	218	224
Triglycerides (fasting)	117	92	131

Table IV
The effect of alpha-tocopherol supplementation on the level of serum constituents in subject B

Serum constituents (mg/100ml unless otherwise stated)	Before supplementation (Mean of 3 consecutive samples)	During supplementation (Mean of 5 consecutive samples)	After supplementation (Mean of 3 consecutive samples)
Tocopherol	0.61	1.19	0.68
Tocopherol (mg per g total lipids)	1.06	1.97	1.18
Beta-lipoproteins	217	271	258
Total lipids	575	583	563
Total cholesterol	171	180	168
Triglycerides (fasting)	72	95	125

Table V
The effect of alpha-tocopherol supplementation on the level of serum constituents in subject C.

Serum constituents (mg/100ml unless otherwise stated).	Before supplementation (Mean of 3 consecutive samples)	During supplementation (Mean of 5 consecutive samples)	After supplementation (Mean of 3 consecutive samples)
Tocopherol	1.45	1.79	1.34
Tocopherol (mg/g total lipids)	1.45	1.85	1.50
Beta-lipoproteins	920	905	840
Total lipids	948	932	862
Total cholesterol	324	307	279
Triglycerides (fasting)	219	234	231

Table VI
Statistical Analysis of effect of Vitamin E supplementation on the levels of serum tocopherol, serum beta-lipoproteins and serum lipids

Serum constituents	During supplementation vs before		
	subject A	subject B	subject C
Tocopherol (mg/100ml)	S $p=<0.001$	S $p=<0.001$	raised but N.S.
Tocopherol (mg per g total lipids)	S $p=<0.001$	S $p=<0.001$	S $p=<0.001$
Beta-lipoproteins	N.S.	N.S.	N.S.
Total lipids	N.S.	N.S.	N.S.
Total cholesterol	N.S.	N.S.	N.S.
Triglycerides	N.S.	N.S.	N.S.

S = significant; N.S. = not significant

of Harman (1960) and suggests that supplementation with vitamin E has no direct effect on the levels of serum lipoproteins and lipids.

SUMMARY

1. Normal, healthy Malaysians had a mean serum tocopherol level of 0.92 ± 0.36 mg/100 ml with a range of 0.30 - 2.07 mg/100 ml.
2. The mean serum tocopherol levels of healthy subjects appeared significantly lower than those of hyperlipoproteinaemic subjects either on a normal or modified diet. However, when expressed as mg tocopherol per gram total serum lipids, no difference was observed between healthy subjects and patients suffering from hyperlipoproteinaemia.
3. Serum tocopherol when expressed as mg per g total serum lipids (Horwitt's ratio) appeared to be a better indicator of vitamin E nutritional status than the expression in terms of mg per 100 ml serum. The mean Horwitt's ratio for normal, healthy Malaysians was 1.32 ± 0.34.
4. In 3 human volunteers, alpha-tocopherol supplementation at a daily level of 100 mg for a period of 5 weeks significantly raised the serum tocopherol levels but had no significant effect on the levels of serum beta-lipoproteins, total serum lipids, serum total cholesterol and serum triglycerides.

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