



Plasma ascorbate and vitamin E levels in Hong Kong Chinese

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Objective: To describe fasting plasma total- and lipid standardised-vitamin E and ascorbate concentrations in Hong Kong Chinese subjects, and to explore age-, diet- and sex-related differences.

Design: Observational study.

Setting: Hong Kong.

Subjects: One hundred and fifty randomly selected Chinese subjects.

Interventions: Fasting plasma concentrations of ascorbate, total vitamin E and lipid standardised vitamin E (Vit E_{LS}, expressed as μmol vitamin E/ mmol total cholesterol plus triglycerides) were measured.

Results: Total vitamin E concentrations ranged from 6–53 $\mu\text{mol}/\text{l}$ (mean 24; s.d. 8.5), and Vit E_{LS} from 0.85–7.09 $\mu\text{mol}/\text{mmol}$ (mean 3.61; s.d. 1.19). Plasma Vit E_{LS} concentrations in women were higher ($P = 0.02$) than in men: mean (median) concentrations 3.82 (3.88) and 3.37 (3.28) $\mu\text{mol}/\text{mmol}$, respectively. Vit E_{LS} concentrations in men decreased with age. Mean (s.d.) plasma ascorbate concentrations in men and women were 49.7 (14.0) and 51.6 (21.6) $\mu\text{mol}/\text{l}$ respectively, and did not show the significant age- and sex- differences reportedly found in Western studies.

Conclusions: The hypothesis that Hong Kong Chinese, with their low risk of CHD, might have high Vit E_{LS} levels in fasting plasma is not supported by the results obtained. Moreover, results showed male–female differences and, in men, a previously unreported age-related decrease in plasma Vit E_{LS} concentration which cannot be accounted for by concomitant differences in dietary intake of vitamin E alone. Results also suggest that the age- and sex- differences in plasma ascorbate seen in Western populations can be avoided by increased vitamin C intake.

Sponsorship: This work was financially supported by The Hong Kong Polytechnic University and the UK/HK Joint Research Scheme.

Descriptors: vitamin E; alpha tocopherol; ascorbate; vitamin C; Chinese; diet; CHD risk; age

Introduction

Vitamin E is the generic name given to a group of biologically active, lipid soluble antioxidant compounds, the tocopherols and tocotrienols (Traber & Packer, 1995; Cohn, 1997). Owing to the very small amount of trienols and 'non-alpha' tocopherols in fasting plasma, the concentration of 'vitamin E' is regarded as practically equivalent to that of alpha tocopherol in fasting plasma, and, although not entirely correct, these terms are often used synonymously. Ascorbate is a water soluble antioxidant of major importance (Frei *et al*, 1989). A redox interaction between these vitamins has been proposed, the lipid bound vitamin E being recycled from its oxidised tocopheroxyl radical form by ascorbate in the aqueous surroundings (Thomas *et al*, 1995).

Ascorbate and vitamin E levels in plasma do not usually directly reflect simple dietary intake (Bates, 1997; Benzie & Strain, 1997; Cohn 1997). Plasma levels of vitamin E are, however, directly increased by supplemental intake (Weber *et al*, 1997), and this is, in turn, associated with an increase in the resistance of low density lipoprotein (LDL) to oxidation (Esterbauer & Jurgens, 1993; Jialal & Devaraj, 1996). Vitamin E might, therefore, help lower risk of coronary heart disease (CHD) by decreasing formation of

highly atherogenic oxidised LDL, as well as by virtue of other anti-atherogenic properties (Ferns *et al*, 1993; Halliwell, 1995; Jialal *et al*, 1995; Benzie, 1996a; Berliner & Heinecke, 1996; Maxwell & Lip, 1997). The strong inverse correlation found between plasma vitamin E concentrations, or intake, and CHD mortality is consistent with this (Riemersma *et al*, 1990; Rimm *et al*, 1993; Stampfer *et al*, 1993; Bellizzi *et al*, 1994; Parfitt *et al* 1994; Gey, 1995). There are, however, conflicting reports of age-related changes, and there is a reported lack of male–female differences in plasma vitamin E concentrations (Vandewoude & Vandewoude, 1987; Succari *et al*, 1991; Frei, 1995; Machlin, 1995), although subjects with higher plasma lipid levels exhibit higher vitamin E levels (Duthie *et al*, 1993). These observations are not consistent with the proposed CHD protective effect of vitamin E. Diets rich in ascorbate are beneficial, but whether this is cause or coincidence is not yet clear (Block *et al*, 1992; Frei, 1995; Machlin, 1995). Furthermore, results of intervention studies involving dietary supplementation with antioxidant vitamins have not so far shown convincing evidence of a subsequent significant decrease in CHD risk (Rapola *et al*, 1996; Stephens *et al*, 1996; Maxwell & Lip, 1997; Weber *et al*, 1997). The clear and marked age, sex and geographical differences in CHD prevalence cannot be adequately accounted for by corresponding differences in established risk factors. Further exploration of other putative risk factors, such as micronutrient

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Received 4 January 1998; revised 11 March 1998; accepted 14 March 1998

antioxidant status, remains important. This is particularly true if such factors can be readily modified by simple dietary means.

The male all-age CHD mortality rate in Hong Kong is < 60 per 100 000 (WHO, 1995; Hong Kong Department of Health, 1997), low when compared to European populations. Cholesterol and triglycerides concentrations are not, however, concomitantly low. In a population wide study of 2900 individuals aged 25–74 y, 46% of men and 41% of women had plasma cholesterol levels of ≥ 5.2 mmol/l; 12% of men and 13% of women had plasma cholesterol levels ≥ 6.2 mmol/l. These proportions increased with age (Janus *et al*, 1997). Plasma ascorbate and vitamin E concentrations in Hong Kong Chinese have not been previously reported. Based on European studies (Gey *et al*, 1991; Gey, 1995; Winkelhofer-Roob *et al*, 1997), however, our hypothesis was that plasma lipid standardised vitamin E (Vit E_{LS}) would be high in this population. The aims of this study, therefore, were to determine plasma vitamin E concentrations in Hong Kong Chinese and to test for age-related and male–female differences in vitamin E levels. Plasma ascorbate concentrations were also investigated, and plasma levels of both ascorbate and vitamin E were related to recently published dietary data (Leung *et al*, 1997).

Materials and methods

Samples

Samples used were fasting, non-haemolysed, EDTA plasma samples from 150 (78 women, 72 men) apparently healthy, consenting, free-living normal Chinese aged 21–70 y from a range of socio-economic backgrounds and geographical areas in Hong Kong. This was an unselected subsample from a study (Janus *et al*, 1997) of coronary heart disease risk factors in 2900 subjects recruited by telephone interview using randomly generated telephone numbers. This method of subject recruitment is well validated in Hong Kong, where telephone accessibility is not linked with socio-economic status. Blood was kept chilled and in the dark until separation of plasma, which was within three hours of sampling. Plasma was then stored at -70°C until assayed for lipids and vitamin E, with no intermediate thawing/refreezing. Ascorbate measurements on whole plasma were performed immediately after separation. Ethical approval for this study was obtained from the Ethics Committees of both the Hong Kong Polytechnic University and University of Hong Kong; all procedures involving human subjects complied with the Declaration of Helsinki as revised in 1989.

Test procedures

Vitamin E was measured by a fluorimetric method (Hansen & Warwick, 1969), using pure (> 98%) DL-alpha-tocopherol (Merck, Darmstadt, Germany) as calibrator. In-run CVs on plasma samples at 24 and 44 $\mu\text{mol/l}$ were $\leq 2.0\%$ ($n = 10$ in each case). Between-run CV on plasma samples was 7.1% (mean 33 $\mu\text{mol/l}$, $n = 13$). Recovery of alpha-tocopherol added to plasma was 83–110% (mean 93%; CV 9.0%, $n = 18$). Lipid standardised vitamin E was expressed as μmol vitamin E per mmol of total cholesterol plus triglycerides (Thurnham *et al*, 1986). Total cholesterol and triglycerides concentrations in plasma were measured using enzymatic methods (Roche Diagnostic Systems, Switzerland) on a Cobas Fara centrifugal analyser (Roche). In 139 of the 150 freshly separated whole plasma samples ascorbate was measured using a specific enzyme assisted spectrophotometric method known as EFTSA (Benzie, 1996b), on a Cobas Fara centrifugal analyser; freshly prepared aqueous solutions of ascorbate (from extra pure ascorbic acid, Merck) were used as calibrators; within- and between-run CVs were < 5% at 25, 50, 100 and 440 $\mu\text{mol/l}$, $n \geq 10$ in each case. Statistical analysis of results for age- and sex- related differences used the Mann–Whitney non-parametric test. Comparison of population dietary data was performed using Welch's approximate *t*-test.

Results

Plasma Vit E_{LS} concentrations were lower than expected, and showed a male–female difference not previously reported. Concentrations ranged from 6–53 $\mu\text{mol/l}$ (mean 24; s.d. 8.5) for total vitamin E; 0.85–7.09 $\mu\text{mol/mmol}$ total cholesterol plus triglycerides (mean 3.61; s.d. 1.19) when lipid standardised (Table 1). As expected there was a significant, direct correlation between plasma total vitamin E and lipid concentrations (results not shown). However, while total vitamin E increased with lipid concentrations, hyperlipidaemic subjects tended to have lower concentrations of Vit E_{LS} (Figure 1; $r = -0.259$; $P = 0.0013$).

A significant, direct correlation was found between age and total vitamin E (results not shown), most probably owing to the age-associated increase in lipid levels. Women had similar total vitamin E concentrations to men. Vit E_{LS} concentrations of women, however, were significantly ($P = 0.02$) higher than those of men (Table 1). Figure 2 shows mean + s.e.m. plasma Vit E_{LS} concentrations in men (diagonal lines) and women (horizontal lines) across 10 y age groups. Results showed that, while Vit E_{LS} concentrations in men and women were very similar in the youngest age group (21–30 y), men aged > 30 y had slightly lower plasma Vit E_{LS} than age-matched women.

Table 1 Total vitamin E, lipid standardised vitamin E, and ascorbate concentrations in fasting plasma of apparently healthy Chinese subjects, aged 21–70 y

	All Mean (median; s.d.; n)	Men mean (median; s.d.; n)	Women mean (median; s.d.; n)
Total Vitamin E ($\mu\text{mol/l}$)	24 (25; 8.5; 150)	23 (23; 9.2; 72)	25 (25; 7.9; 78)
Vitamin E _{LS} ($\mu\text{mol/mmol}$ cholesterol + triglycerides)	3.61 (3.64; 1.19; 150)	3.37 (3.28; 1.20; 72)	3.82 ^a (3.88; 1.16; 78)
Ascorbate ($\mu\text{mol/l}$)	50.6 (49.0; 18.2; 139)	49.7 (48; 14.0; 69)	51.6 (49.5; 21.6; 70)

^a Significantly different from male value; $P = 0.02$.

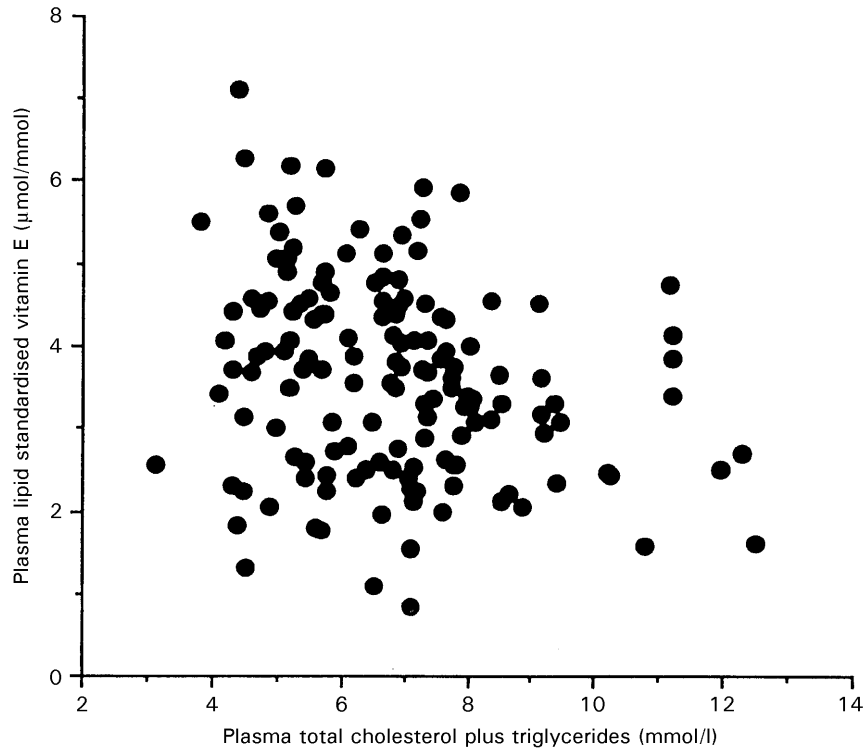


Figure 1 Relationship between plasma cholesterol plus triglycerides concentration and lipid standardised vitamin E concentration ($r = -0.259$; $P = 0.0013$).

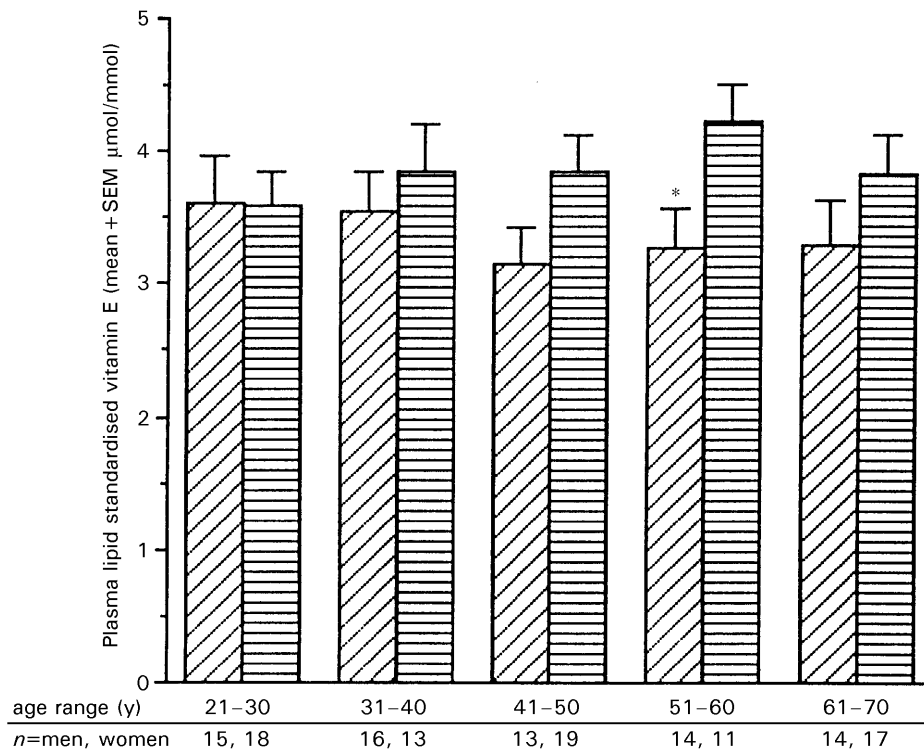


Figure 2 Comparison of the mean (+ s.e.m.) plasma lipid standardised vitamin E concentration in men (diagonal lines) and women (horizontal lines) across 10 y age groups. Men aged > 30 y had consistently lower levels than women of similar age, although statistical significance was reached only in the 51-60 y age group ($P = 0.046$).

Comparing 21–40 and 41–60 y age groups, older men had significantly ($P=0.001$) lower plasma Vit E_{LS} concentrations.

To explore possible methodological effects in relation to the lower than expected vitamin E levels found, a small ($n=10$; 4 females, 6 males) sample of Caucasians living in Hong Kong was studied. Results of this pilot study revealed mean (median) total and lipid standardised vitamin E concentrations in the fasting plasma of Caucasians of 33.2 (31.0) $\mu\text{mol/l}$ and 4.50 (4.48) $\mu\text{mol/mmol}$ respectively, significantly ($P<0.01$) higher than those obtained, using the same analytical methods and conditions, in the fasting plasma of Chinese subjects. These data imply that the lower Vit E_{LS} concentration obtained in our Chinese subjects, compared to those previously published for European subjects, was not owing to analytical differences between the current study and the European studies.

Fasting plasma ascorbate concentrations of the Chinese men and women studied were similar (Table 1), and showed no age-related effects.

Discussion

The novel finding that women have higher plasma Vit E_{LS} concentrations than men deserves further study, but is consistent with observations of dietary intake of vitamin E in Hong Kong adults (Leung *et al*, 1997). While intake of vitamin E is generally higher in men than women (Weber *et al*, 1997), when expressed as mg/kcal nutrient intake, average vitamin E intake of Hong Kong Chinese women (5.8 mg/kcal) is significantly ($P<0.01$) higher than that of men (4.7 mg/kcal). This may help explain the male–female difference in plasma levels reported here.

Age-related decreases in plasma Vit E_{LS} concentrations seen in men also warrant further study, particularly as these are not associated with parallel age-related differences in vitamin E intake. Indeed, in Hong Kong men (but not women), dietary intake of vitamin E increases with age (Leung *et al*, 1997); mean (s.d.) vitamin E intake of men aged ≥ 55 y is reported to be 5.22 (2.09) mg/kcal, significantly ($P<0.0001$) higher than that of men aged ≤ 34 y (4.05 (1.14) mg/kcal).

With reference to ascorbate concentrations in Hong Kong Chinese, no male–female difference was found, and older subjects did not show lower concentrations. This contrasts with the reportedly well established finding in Western populations that women have generally higher plasma ascorbate concentrations than men, and that elderly people have generally lower levels than younger people (Bates, 1997). However, there is no clear physiological mechanism to account for sex- and age-related differences, and these are likely to be caused, at least in part, by different dietary habits (Bates, 1997). There may, however, be higher rates of ascorbate utilisation in men and in older subjects, possibly related to increased urate levels (Benzie & Strain, 1996), and to subclinical age-related disease. The absence of detectable sex- and age-related effects on plasma ascorbate concentrations in this current study could be owing to relatively high vitamin C dietary intakes in the local Hong Kong population: mean (s.d.) intakes are 60 (35) and 94 (54) mg per 1000 kcal per day for men and women respectively (Leung *et al*, 1997). These figures are significantly ($P<0.001$) higher than those reported in UK men and women (28 (17) and 38 (26) respectively) (Gregory *et al*, 1990). Furthermore, vitamin C intake in Hong Kong increases with age, rising from a mean (s.d.) intake,

in men, of 48 (26) mg per 1000 kcal at age ≤ 34 to 73 (43) ($P<0.0001$) at age ≥ 55 y, and from 88 (53) to 104 (61) mg per 1000 kcal for women ($P<0.05$) in the same age groups (Leung *et al*, 1997). It is possible that putative age- and sex-related differences in ascorbate demand are more than matched by the dietary supply, preventing the sex- and age-related differences in plasma ascorbate seen in populations whose dietary intake of vitamin C is relatively poor.

It must be noted that, while the dietary data are assumed to be representative of the subjects in this current study, the data on dietary intake of ascorbate and vitamin E were collected from a representative 1010 subjects from a study total of 2900 participants (Leung *et al*, 1997), while the plasma vitamin levels were measured on 150 subjects randomly selected from the 2900 study participants. There may have been some overlap between the sub-samples, but results must be interpreted with this reservation in mind. Having clarified this, however, it must also be noted that plasma ascorbate and vitamin E concentrations are not simply a function of dietary intake. Ascorbate status of plasma is affected by the form in which the vitamin is ingested, stability, size of dose, renal loss and rate of utilisation (Bates, 1997; Benzie & Strain, 1997). Various factors, including the isomeric form of dietary vitamin E (Traber, 1994), the amount of PUFA in the diet (Weber *et al*, 1997), the rate of irreversible oxidation of the vitamin (Schultz *et al*, 1995), the rate of release from adipose tissue stores and, possibly, *in vivo* redox recycling of the alpha tocopheroxyl radical (Niki, 1987; Bowry *et al*, 1995), help define vitamin E status of plasma.

In this context, it may be significant that Hong Kong Chinese women of all age groups have a higher daily intake of vitamin C and a lower intake of PUFA (approximately 20% lower) than men (Leung *et al*, 1997). Moreover, women generally have greater adipose tissue mass than men, and are likely, therefore, to have concomitantly greater vitamin E stores. These factors may be important contributors to the higher plasma vitamin E levels seen in women in this study and, possibly, to their lower CHD risk.

This study has also shown that fasting plasma Vit E_{LS} concentrations of apparently healthy Hong Kong Chinese are similar to those of European populations with a considerably higher CHD mortality rate (Gey *et al*, 1991). The very strong inverse relationship between plasma vitamin E and CHD risk found in European studies (Gey *et al*, 1991; Gey, 1995), therefore, does not appear to apply to this population. Average daily vitamin E intake in Hong Kong Chinese is 11.22 mg and 10.47 mg for men and women respectively (Leung *et al*, 1997). This is similar to intakes of men in UK (11.7 mg/d) and in the US (10.0 mg/d), and slightly higher than those reported for women in UK (8.6 mg/d) and US (7.6 mg/d) (Gregory *et al*, 1990; Alaimo *et al*, 1994). Relationships between dietary intake and plasma levels of vitamin E, and between vitamin E levels and CHD risk in Hong Kong Chinese, therefore, may be different from those in European populations. This may reflect other important, as yet unexplored, dietary and other factors which may modulate CHD risk.

Conclusions

The hypothesis that Hong Kong Chinese, with their low risk of CHD, might have high lipid standardised vitamin E levels in fasting plasma is not supported by the results obtained. Moreover, results showed male–female

differences and, in men, an age-related decrease in plasma Vit E_{LS} concentrations not previously reported. Furthermore, the absence of sex- and age-related differences in plasma ascorbate concentrations suggests that such differences, reportedly commonly found in Western populations, may be prevented by increased vitamin C intake. The results also highlight the importance of the various factors which determine vitamin E status, and the overall complexity of CHD risk assessment.

Acknowledgements—The authors wish to thank Mrs M Janus for her expert phlebotomy skills and Miss I Wu for her technical assistance. Thanks go also to The Hong Kong Polytechnic University and to the UK/HK Joint Research Scheme for financially supporting this work.

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