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Reference Values for α-Tocopherol and β-Carotene in the Whitehall II Study

NICOLA C. ARMSTRONG[b], GEORGE PAGANGA[a], ERIC BRUNNER[b], NICHOLAS J. MILLER[a], KIRAN NANCHAHAL[†], MARTIN SHIPLEY[b], CATHERINE A. RICE-EVANS[a], MICHAEL G. MARMOT[b], and ANTHONY T. DIPLOCK[a,*]

[a]*International Antioxidant Research Centre, UMDS-Guy's Hospital, London SE1 9RT;* [b]*International Centre for Health and Society, Department of Epidemiology and Public Health, University College London Medical School, 1-19 Torrington Place, London WC1E 6BT*

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Plasma α-tocopherol, β-carotene, serum lipids and their derived ratios were determined in British Civil Servants (n = 7177) at the second medical examination of the Whitehall II Study, a longitudinal study of cardiovascular disease. For plasma α-tocopherol the non-parametric 95% reference interval (90% confidence limits) for the total population was: 11.1 (10.9–11.3) – 51.5 (50.6–52.7) μmol/l. For plasma β-carotene the non-parametric reference interval for the total population was: 0.05 (0.05–0.05) – 2.14 (2.08–2.21) μmol/l. The latter interval was wider than those previously published with a higher mean (0.61 μmol/l) and median (0.75 μmol/l). Plasma β-carotene concentrations were higher in women than men with age-adjusted means of 0.70 and 0.57 μmol/l respectively ($p < 0.001$). This may reflect differences in diet, lifestyle and metabolism between the sexes. The α-tocopherol/cholesterol ratio, as in other surveys, did not vary with age. Among men, current- and ex-smokers had a higher α-tocopherol/cholesterol ratio than never-smokers with age-adjusted means of 4.18, 4.19 μmol/mmol and 4.05 μmol/mmol respectively. This difference is as yet unexplained. Follow-up of these subjects will help to clarify the role of antioxidant nutrients as protective factors for cardiovascular disease and cancer.

Keywords: α-tocopherol, β-carotene, reference values

INTRODUCTION

Coronary heart disease (CHD) is a major cause of death in the Western world. Recent trends show that mortality rates are increasing in Eastern Europe[1] and its importance is growing in most developing countries.[2] Although epidemiological studies initially focused on the influence of dietary fat, recently emphasis has shifted towards the antioxidant hypothesis of atherosclerosis and CHD.[3–7] Simultaneously, large-scale research has expanded on the possible role of dietary antioxidants in cancer prevention.[8–11] Antioxidants such as vitamin C, carotenoids, and vitamin E may protect against cancer by several mechanisms[12] and against atherosclerotic disease by impeding

*Corresponding author. Tel.: (+44)-171 955 4521. Fax: (+44)-171 403 7195.
[†]Present address: Medical Statistics Unit, Department of Epidemiology and Population Sciences, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

the oxidative modification of low-density lipoprotein (LDL), and its incorporation into the arterial wall.[12,13] *In vitro* susceptibility of LDL particles to oxidation is directly associated with the severity of coronary atherosclerosis[14] and inversely associated with vitamin E dose in supplementation *trials*.[15] α-Tocopherol in plasma is considered to be a good indicator of protection offered by dietary vitamin E against oxidative damage at the tissue and membrane level.[16] Associations between dietary intake, plasma and tissue levels of β-carotene, are similar to those for α-tocopherol.[16] There is, however, uncertainty about the efficacy of β-carotene in primary prevention of CHD, and neoplasms of the lung and other sites.[8–10]

To date the largest published studies of reference values for the UK for these antioxidant nutrients are those of Gregory *et al.*[17] (n = 1889) and Duly *et al.*[18] (n = 1600). This report presents baseline measurements for 7177 Whitehall II study participants (4943 men and 2234 women) who are being followed up for incident CHD and cancer.[19] Dietary intake data, collected at the same time (1991–93), are reported elsewhere.[20] Reference values for plasma α-tocopherol, β-carotene, serum lipids and derived ratios analyzed by sex, age and self-reported smoking status are presented here, together with the proportion of study participants with biochemical deficiency of vitamin E[21,22] and those potentially at risk of CHD.[23]

MATERIALS AND METHODS

Subjects

The Whitehall II study cohort, set up primarily to investigate the causes of social inequalities in cardiovascular disease, comprises 10,308 persons (6895 men and 3413 women) aged 35–55 years who worked in the London offices of 20 government (Civil Service) departments in 1985–1988 (Phase 1).[19] The response rate was 73% (74% among men and 71% among women). The true response rates are likely to be higher, however, as around 4% of those on the provided list of employees had moved before the study began and thus were ineligible for inclusion. Subjects were re-examined in 1991–93 (Phase 3: 83% participation rate) when a blood sample was collected. Most subjects had fasted overnight for at least 9 hours; others attending the medical examination in the afternoon were instructed to have no more than a light, fat-free breakfast before 8 am on the day of venepuncture. This formed the reference sample for the present report.

Age was categorised into 5-year age-groups of 39–44 years, 45–49 years, 50–54 years and 55–63 years. A self-reported measure of smoking was used to categorise subjects into 3 groups: never smoker, ex-smoker and current smoker. Since the number of pipe and cigar smokers was small (men: 10.4%; women: 0.7%), they were combined with cigarette smokers. Women who answered 'yes' to the question: 'Have you ceased having your periods?' were identified as postmenopausal.

Blood samples were obtained by venepuncture of the left antecubital vein using a tourniquet. Blood was collected into lithium-heparin tubes and plasma stored at –80 °C until assay. Serum was prepared and stored at 4°C for total cholesterol determination within 72 hours. A random subsample of approximately 6% of participants returned for re-screening within 8 weeks of the first visit and laboratory measures were repeated in order to estimate the reliability of the measurements.

Laboratory Methods

α-Tocopherol and β-carotene were measured concurrently by normal phase high performance liquid chromatography (HPLC) using a modification of the methods of Buttriss and Diplock (1984)[24] and Thurnham et al (1988).[25] A mobile phase of 92% hexane/8% tert-butyl methyl ether and fluorescence detection was used.

δ-Tocopherol, added to the sample prior to extraction with hexane, was used as an internal standard for both α-tocopherol and β-carotene determination. HPLC equipment (Waters Chromatography Division, Watford) using a silica column (Novapak® 3.9 × 150mm, 4μm) was operated at a temperature of 30°C with a flow rate of 1 ml/min at a pressure of 450 psi. Calibration standards in hexane were prepared using the molar absorptivity of α-tocopherol at 292 nm (E_{mM} = 3.26), δ-tocopherol at 298 nm (E_{mM} = 3.67) and β-carotene at 455 nm (E_{mM} = 134.1). All reagents were of HPLC grade or better (Rathburn Chemicals Ltd., Walkerburn, Scotland). Detection limits of the HPLC system were estimated to be 1.10 μmol/l for α-tocopherol and 0.10 μmol/l for β-carotene.

Total cholesterol, HDL and triglycerides were determined with a Cobas Fara centrifugal analyser (Roche Diagnostic Systems, Welwyn Garden City) using commercially available kits. Total cholesterol was determined in the samples by the cholesterol oxidase/peroxidase colorimetric method (Boehringer Mannheim GmbH Diagnostica). HDL cholesterol was measured after LDL precipitation with dextran sulphate/magnesium chloride solution.[26] LDL cholesterol was calculated using the Friedwald formula.[27] External comparison and examination of age differences at Phases 1 and 3 suggested that serum total cholesterol levels were over-estimated by approximately 0.33 mmol/l at Phase 3. No correction was applied to the data.

Statistical Methods

Biochemical variables were skewed and the logarithmic transformation was used to achieve approximate normality. The 95% reference intervals were calculated using the non-parametric method.[28] The 90% confidence interval for each percentile was determined using a normal distribution approximation to the binomial distribution.[29] Regression methods were used to compare age-adjusted means between men and women and between smoking categories. Tests for trend across age-groups were performed by including a linear term for age-group in the regression model. Statistical analyses were performed using SAS version 6.04 (Cary, USA).

Estimates of laboratory imprecision were obtained from the 10% of samples with duplicate measurements. These were obtained by re-analysing every tenth sample after each HPLC run was complete. The technical error (TE), based on this subset, was calculated using the formula:

$$TE = \sqrt{\Sigma d_i^2 / 2n},$$

where di^2 is the difference between a duplicate pair of measurements for the ith sample (i = 1, . . ., n) and n is the number of pairs. The coefficient of variation was calculated using the formula:

$$CV = TE / m_n \times 100\%$$

where m_n, is the mean of the n pairs.[30]

The reliability of the measurements (between-person variation as a proportion of between-person + within-person variation), was estimated using intraclass correlation coefficients with data from 322 subjects who were re-screened.[31]

The coefficients of variation based on duplicate measurements were: α-tocopherol 10.5%, β-carotene 22.4%, serum cholesterol 3.4% and HDL cholesterol 3.5%. The intra-class correlations based on re-screened subjects were: α-tocopherol men 0.68, women 0.67; β-carotene men 0.46, women 0.49; total cholesterol men 0.85, women 0.86 and HDL cholesterol men 0.81, women 0.88. A substantial proportion of our study participants had undetectable β-carotene levels (men 10.7%, women 7.3%). This group was assigned a value of 0.05 μmol/l (i.e. half way between the detection limit and zero as the best estimate of the true β-carotene concentration). The national survey[17] did not report undetectable values.

RESULTS

The distributions of α-tocopherol and β-carotene in the total study sample are shown in Figure 1. The medians (2.5–97.5 percentile) for plasma α-tocopherol and β-carotene were 27.7 (11.1–51.5) μmol/l and 0.75 (0.05–2.14) μmol/l respectively. The distributions in the total study sample corrected for total cholesterol concentrations are shown in Figure 2. Medians (2.5–97.5 percentile) for serum α-tocopherol/cholesterol and β-carotene/cholesterol were 4.40 (1.85–7.30) μmol/mmol and 0.12 (0.01–0.34) μmol/mmol respectively.

Plasma α-tocopherol levels increased with age ($p < 0.0001$ both sexes). In the youngest age-group (ages 39–44) the medians (2.5–97.5 percentile) were for men 25.9 (10.9–49.1) μmol/l and for women 26.3 (10.1–47.3) μmol/l. In the oldest age-group (ages 55–63) the α-tocopherol levels were for men 29.1 (11.9–51.5) μmol/l and for women 29.6 (11.6–58.2) μmol/l. There were no age-related differences in the α-tocopherol/cholesterol ratio. β-Carotene levels in μmol/l for men in the youngest age-group were 0.70 (0.05–1.71) and 0.74 (0.05–2.12) in the oldest age-group ($p < 0.0001$). This trend with age remained after correction for total cholesterol concentrations ($p = 0.003$) in men. There was an inverse association with age in women after correction for total cholesterol ($p = 0.003$). The β-carotene/cholesterol ratios in μmol/mmol for women in the youngest and oldest age-groups were: 0.14 (0.01–0.38) and 0.12 (0.01–0.36) respectively. Total and LDL cholesterol concentrations were higher in older subjects ($p < 0.0001$) in both sexes. In the youngest men and women LDL cholesterol levels in mmol/l were respectively 4.16 (2.52–6.33) and 3.75 (2.19–6.12). In the oldest men and women the LDL cholesterol levels were respectively 4.57 (2.75–6.62) and 4.69 (2.90–7.45).

The medians, 95% reference intervals with 90% confidence limits and geometric means for plasma α-tocopherol, β-carotene, lipids, α-tocopherol/cholesterol and β-carotene/cholesterol for men and women are shown in Table I. Among women, levels of total and LDL cholesterol, but no other variables, were associated with menopausal status (see footnote to Table I). No significant differences were found between men and women for either the age-adjusted α-tocopherol levels or the cholesterol corrected α-tocopherol levels. Women had significantly higher ($p < 0.0001$) levels of β-carotene and β-carotene/cholesterol compared to men after age-adjustment. Compared to men, premenopausal women had significantly lower ($p = 0.0006$) total cholesterol whereas postmenopausal women had significantly higher ($p < 0.0001$) total cholesterol levels. Premenopausal women had significantly lower ($p < 0.0001$) LDL cholesterol levels compared to men and postmenopausal women after age-adjustment. Men had lower ($p < 0.0001$) HDL cholesterol levels than women.

Age-adjusted means by smoking status, and tests of heterogeneity, are presented in Table II. Plasma α-tocopherol levels differed significantly ($p < 0.0001$) by smoking status in men but not women. Median (2.5–97.5 percentile) α-tocopherol levels for men in the never-, ex- and current-smoker categories were: 26.4 (10.9–50.8), 28.4 (11.5–53.6) and 27.9 (11.9–50.4) μmol/l respectively. After α-tocopherol levels were corrected for total cholesterol, the group differences were again statistically significant only in men ($p = 0.008$): 4.31 (1.89–7.19), 4.47 (1.84–7.34) and 4.39 (1.93–7.36) μmol/mmol respectively. β-carotene levels in μmol/l for men in the never-, ex- and current-smoker categories were: 0.75 (0.05–2.03), 0.74 (0.05–1.96) and 0.63 (0.05–1.78) and for the women were: 0.91 (0.05–2.62), 0.85 (0.05–2.21) and 0.67 (0.05–1.92). Heterogeneity tests were significant ($p < 0.0001$) in both men and women. In both sexes self-reported current smokers had statistically significantly lower β-carotene/cholesterol ratios than non-smokers. The mean total cholesterol level in never-smokers was lower than in smokers among men, but among women this difference

(*text continues on p. 215*)

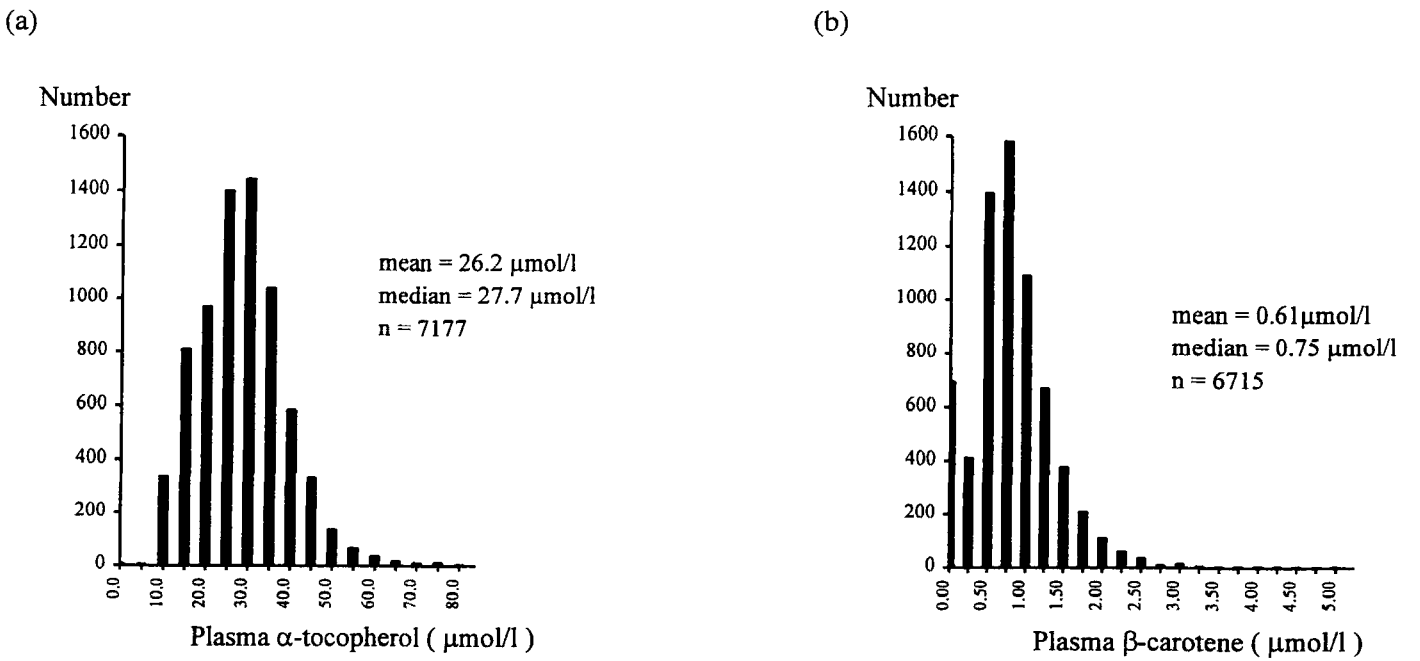

FIGURE 1 Distribution of α-tocopherol (**a**) and β-carotene (**b**) in plasma in the Whitehall II population. α–tocopherol 95% reference interval 11.1–51.5 µmol/l. 12 individuals (0.17% of 7177 assayed) had plasma α-tocopherol levels of greater than 80.0 µmol/l and are not displayed in the figure. β-carotene 95% reference interval 0.05–2.14 µmol/l. 16 individuals (0.24% of 6715 assayed) had plasma β-carotene levels greater than 5.00 µmol/l.

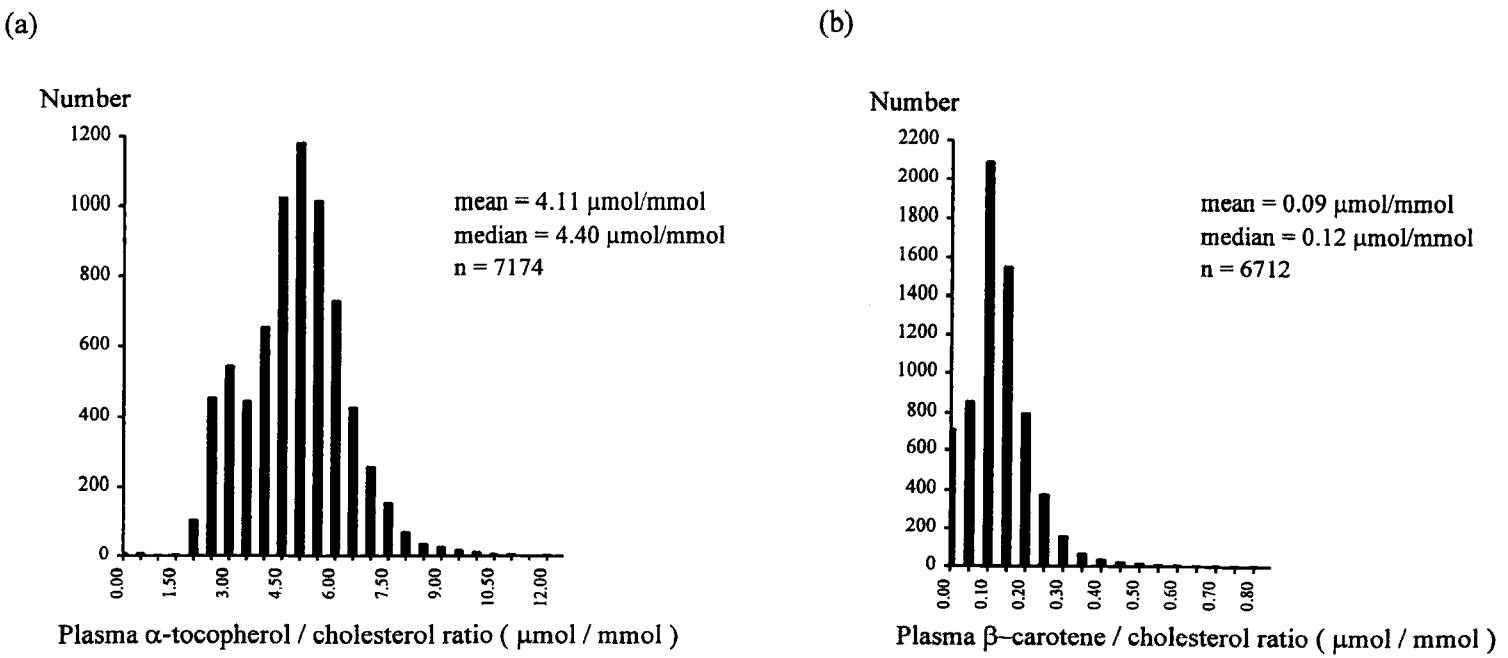

FIGURE 2 Distribution of α-tocopherol/cholesterol ratio (**a**) and β-carotene/cholesterol ratio (**b**) in plasma in the Whitehall II population. α-Tocopherol/cholesterol ratio 95% reference interval 1.85–7.30 μmol/l. 8 individuals (0.11% of 7174 derived) had α-tocopherol/cholesterol ratios greater than 12.00 μmol/mmol and are not displayed in the figure. β-carotene/cholesterol ratio 95% reference interval 0.01–0.34 μmol/l. 14 individuals (0.21% of 6712 derived) had plasma β-carotene/cholesterol ratios greater than 0.80 μmol/mmol.

TABLE I Geometric means, median and reference intervals (95% with 90% confidence limits) for plasma α-tocopherol, β-carotene, lipids and derived variables in the Whitehall II study population, by sex

VARIABLE	MEN					WOMEN				
	n	Mean	Median	Reference Interval 2.5%	Reference Interval 97.5%	n	Mean	Median	Reference Interval 2.5%	Reference Interval 97.5%
α-tocopherol (μmol/l)	4943	26.2	27.6	11.3 (11.0–11.5)	51.6 (50.6–52.9)	2234	26.3	28.0	10.6 (10.3–11.1)	51.40 (49.8–53.6)
β-carotene*** (μmol/l)	4609	0.57	0.72	0.05 (0.05–0.05)	1.98 (1.88–2.06)	2106	0.71	0.84	0.05 (0.05–0.05)	2.45 (2.33–2.57)
[1]Total cholesterol (mmol/l)	5566	6.38	6.42	4.46 (4.42–4.50)	8.85 (8.75–8.95)	2456	6.40	6.42	4.44 (4.33–4.49)	9.31 (9.17–9.51)
[1]LDL cholesterol*** (mmol/l)	5427	4.33	4.39	2.65 (2.61–2.71)	6.54 (6.47–6.62)	2437	4.15	4.20	2.44 (2.31–2.51)	6.78 (6.63–7.02)
HDL cholesterol*** (mmol/l)	5544	1.27	1.28	0.76 (0.74–0.78)	2.14 (2.11–2.18)	2452	1.63	1.64	0.96 (0.92–0.98)	2.60 (2.56–2.66)
α-tocopherol/cholesterol ratio (μmol/mmol)	4940	4.12	4.39	1.87 (1.83–1.90)	7.28 (7.20–7.39)	2234	4.10	4.40	1.77 (1.71–1.84)	7.35 (7.16–7.65)
β-carotene/cholesterol ratio*** (μmol/mmol)	4606	0.09	0.11	0.01 (0.01–0.01)	0.30 (0.29–0.31)	2106	0.11	0.13	0.01 (0.01–0.01)	0.38 (0.36–0.40)

Significance of sex difference * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
[1] The values for pre- and post-menopausal women are statistically significantly different ($p < 0.001$):
LDL cholesterol pre-menopausal n = 1151, median = 3.83 mmol/l, reference interval = 2.27 – 6.11 mmol/l
post-menopausal n = 1234, median = 4.50 mmol/l, reference interval = 2.60 – 7.25 mmol/l
Total cholesterol pre-menopausal n = 1154, median = 6.03 mmol/l, reference interval = 4.26 – 8.39 mmol/l
post-menopausal n = 1250, median = 6.77 mmol/l, reference interval = 4.88 – 9.97 mmol/l

TABLE II Age-adjusted geometric means and heterogeneity test for plasma α-tocopherol, β-carotene, lipids and derived variables in the Whitehall II study population, by self-reported smoking status

VARIABLE	MEN (Age-Adjusted Means)			p value†	WOMEN (Age-Adjusted Means)			p value†
	Never-Smoker	Ex-Smoker	Current Smoker		Never-Smoker	Ex-Smoker	Current Smoker	
α-tocopherol (μmol/l)	25.4	27.0	27.1	0.0001	25.9	26.0	27.2	0.16
β-carotene (μmol/l)	0.63	0.59	0.44	0.0001	0.78	0.73	0.53	0.0001
Total cholesterol (mmol/l)	6.28	6.46	6.48	0.0001	6.35	6.35	6.49	0.08
LDL cholesterol (mmol/l)	4.28	4.39	4.40	0.0003	4.11	4.06	4.27	0.005
HDL cholesterol (mmol/l)	1.29	1.29	1.23	0.0001	1.64	1.68	1.51	0.0001
α-tocopherol/cholesterol ratio (μmol/mmol)	4.05	4.19	4.18	0.008	4.08	4.10	4.18	0.60
β-carotene/cholesterol ratio (μmol/lmmol)	0.10	0.10	0.07	0.0001	0.12	0.11	0.08	0.0001

†p value for test of heterogeneity between smoking categories.

was not statistically significant. In both sexes never-smokers had lower LDL and higher HDL cholesterol levels than smokers.

DISCUSSION

This report describes plasma levels of α-tocopherol, β-carotene, serum lipids and the derived ratios at the second medical examination of the Whitehall II study. With a study sample of 4943 men and 2234 women we have established the largest single-centre prospective study to date of antioxidants and cardiovascular disease in the UK.

Our study population is an office-based group of Civil Servants, and there may be concerns that it is not representative of adults in the UK. We obtained a similar distribution of α-tocopherol levels as in other recent large community surveys in Britain and Northern Ireland.[17,18] Each of these cross-sectional surveys showed, as the present study has done, α-tocopherol levels to be higher among older participants, and that the age-related difference in α-tocopherol appeared to be explained by the tendency of total cholesterol to increase with age and, in women, after the menopause. Compared to the Dietary and Nutritional Survey of British Adults,[17] however, approximately three-fold higher mean β-carotene levels were observed in our population. This possibly reflects higher than average intakes of foods containing β-carotene in our study population. The poor precision of the β-carotene estimations (22.4%) may alter the range of values but does not influence our estimate of population means.

We have replicated the finding of higher levels of plasma β-carotene in women compared to men.[17] Plasma concentrations reflect carotene intake[16,32,33] and a likely explanation for the sex difference in β-carotene levels is that there are parallel differences in consumption of fruit and vegetables, particularly carrots and tomatoes which are major sources of β-carotene.[34] Reported intake of dietary β-carotene tends however, if anything, to be higher in men than women.[17] Differences in the bioavailability of β-carotene in raw and cooked foods may therefore be important. Absorption reportedly varies between 3 and 97%[35] according to preparation method and it may be that men and women tend to consume carotene-containing foods prepared in different ways. The finding of substantially higher plasma and adipose tissue levels in women despite similar β-carotene intakes in each sex[36] supports the suggestion that there may be sex differences in metabolism of β-carotene, including its distribution in body compartments.

Tobacco smoking is a major preventable cause of CHD and cancer.[37] Some heavy smoking populations, however, have unexpectedly low rates of coronary disease and it has been suggested that high dietary antioxidant intake may be responsible for this paradox.[38] In Britain smokers tend to have lower intakes of antioxidant micronutrients and have lower plasma antioxidant levels than non-smokers[17] and it may be that these two factors operate together to promote atherosclerosis. In our study non-smokers had, as expected,[39,40] higher plasma β-carotene than smokers, but male never-smokers had a lower mean plasma α-tocopherol and α-tocopherol/cholesterol ratio than smokers. Among women there was a similar finding only for the cholesterol corrected ratio. Previous studies in British populations found no association between vitamin E and smoking status.[18,39] Our finding suggests that the relationship between smoking and dietary nutrient intake may vary for different dietary antioxidants.

Plasma β-carotene is lower in smokers[32,39,40] and this is one way that it resembles vitamin C.[41] Lower levels of carotenoids in smokers may reflect differences in dietary intake. Smokers have been found to eat smaller amounts of β-carotene-containing foodstuffs,[42–44] however, differences in intake do not appear to explain all of the effect of smoking.[32,45] The additional difference has been ascribed to the pro-oxidant

stress effects of tobacco smoke[46] and other dietary differences such as the greater alcohol consumption of smokers.[32,47–49] This has led to the suggestion that the daily requirement of smokers for β-carotene, as well as for vitamin C, may be higher than that of non-smokers.[50,51] This argument was advanced[7] to explain the results of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study[8] which found an excess of CHD and lung cancer among those supplemented with β-carotene at a dose of 20 mg/day. Two further reports of trials of β-carotene, one involving American physicians[9] (50mg on alternate days) and the other current and former smokers, and workers exposed to asbestos[10] (30 mg/day, plus retinol 25,000 IU/day) suggest that, regardless of smoking history, doses of up to 30 mg/day do not produce benefit.

Observational[5–7] and metabolic[15] studies of the vitamin E—CHD association provide better support for the antioxidant hypothesis than the studies relating to β-carotene. A trial of vitamin E supplementation (400 or 800 IU/day) in secondary prevention produced partial support for this treatment.[52] Supplementation was associated with fewer non-fatal myocardial infarctions, but cardiovascular deaths and total mortality were not reduced. Experimental and epidemiological evidence of health benefit is probably strongest for vitamin E among the lipid-soluble antioxidant nutrients[12] and consequently it may be valuable to examine the distribution of plasma α-tocopherol levels in the Whitehall II population.

Horwitt *et al.* (1972)[21] defined a threshold for biochemical, as opposed to clinical,[53] vitamin E deficiency based on the sensitivity of erythrocytes to oxidant stress. This threshold of 0.8 mg tocopherol/g total lipid,[21] conventionally expressed as a plasma α-tocopherol concentration of 11.6 μmol/l,[22] is equivalent to an α-tocopherol/total cholesterol ratio of 2.25 μmol/mmol. The latter has been proposed by the Department of Health as the 'lowest satisfactory value' for plasma α-tocopherol.[54] 7.8% of men and 8.4% of women in the present study, distributed uniformly across age-groups, fell below this level. There was a tendency for fewer current smokers (men 6.3%, women 6.1%) to be classified below the lowest satisfactory value compared to never- and ex-smokers (men 8.3% and 8.2% respectively, women 9.5% and 7.9% respectively).

Gey *et al.* (1993)[23] have proposed plasma concentration ranges for several dietary antioxidants which reflect the concept of optimal health, rather than avoidance of deficiency.[21] 'Truly safe' levels for vitamins A, C, E and carotenes derived from epidemiological studies of cardiovascular disease, cancer and antioxidant status, have been proposed on the basis that disease risk may be reduced if intakes are sufficient to obtain the given plasma concentrations.[23] These are for vitamin E: >28.0 μmol/l, for α- plus β-carotene: >0.5 μmol/l and for vitamin C: >41 μmol/l.[23] An optimal range of β-carotene alone was estimated[23] to be approximately 0.3–0.4 μmol/l on the basis that an eightfold increase of β-carotene above the baseline value of 0.35 μmol/l produced no additional protection against basal-cell nonmelanoma skin cancer.[55] The proposed values for vitamins A, C, E and carotenes are consistent with those previously reported[4] to be associated with a reduced risk of angina pectoris. 51.3% of the present sample (men 51.9%, women 50.0%) fell below the 'truly safe' level of 28.0 μmol/l for α-tocopherol.[23] This proportion is somewhat less than that recently observed in Northern Ireland[18] (63%), perhaps reflecting the differing social class composition of the two study populations.[19]

Bearing in mind the uncertain role of β-carotene in degenerative disease prevention[8–10] we have nevertheless estimated the proportion in the Whitehall II population considered to be in the suboptimal range of plasma levels. Taking the suggested lower limit of 0.3 μmol/l[23] 14.6% of men and 10.9% of women had plasma β-carotene concentrations below this level. The proportion of participants with low β-carotene concentrations, as defined, tended to be smaller

in the older age-group (39–49 years: 14.2%, 50–63 years: 12.5%).

Firm dietary recommendations for vitamin E and β-carotene, and other antioxidants, for optimal health await further evidence from studies including this and the EPIC study (European Prospective Investigation of Cancer and Nutrition[11]). A number of important questions remain with regard to optimal intakes, possible multiplicative, or synergistic, effects on risk, and the unexpected results of the recent β-carotene trials,[8–10] which cast doubt on the advisability of reliance on supplements for antioxidant protection.[7] The available evidence suggests that dietary antioxidant intakes may be significant in explaining socioeconomic differences in cardiovascular risk.[56] The data presented here will, in later analyses and when incident disease has accumulated, provide further evidence on this question.

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