Serum α-tocopherol, retinol and carotenoid profiles of coronary artery disease patients versus healthy controls

Ng TKW[1], Khor SC[1], Tee ES[1], Thong ML[2] and Seow TY[2] *[1]Institute for Medical Research, 50588 Kuala Lumpur, and [2]National Heart Institute, 50400 Kuala Lumpur, Malaysia*

Abstract

The present study was conducted to test the hypothesis that Malaysian patients with coronary atherosclerotic lesions which impede blood flow to the heart, have a poorer antioxidant nutrient status compared to healthy controls. Fasting serum samples from 316 coronary angioplasty (PTCA)/coronary artery bypass graft (CABG) patients (271 males, 45 females) and 162 apparently healthy individuals (102 males, 60 females), both groups aged 37-75 years, were analysed simultaneously for α-tocopherol (α-T), retinol and carotenoid profile (β-carotene, α-carotene, lycopene, and total carotenoids) using a new HPLC technique that involved a ternary mobile phase of acetonitrile, methanol and ethyl acetate (88:10:2, v/v) and a μBondapak C_{18} column. The results showed no evidence that serum α-T_{std} (standardized for total cholesterol plus triglycerides), retinol or lycopene status were poorer in patients versus healthy controls. However, serum α-carotene and β-carotene were 60-69% and 42-58% higher respectively, in healthy controls compared to the PTCA/CABG patients (all subgroups $p<0.05$ except for β-carotene in females). The present findings for α-tocopherol conflict with the reports on the vitamin's role as the principal antioxidant *in vivo*.

Key words: Serum α-tocopherol; retinol; carotenoids; coronary artery disease

Introduction

There is overwhelming evidence from epidemiological and case-control studies that habitual diets which are low in animal fat and protein but high in cereals or bread, vegetables and fruits, eg. the vegetarian-type diet (Register & Sonnenberg, 1973) and the "Mediterranean diet" (Ferro-Luzzi *et al.*, 1984; Spiller, 1991), are protective against coronary heart disease (CHD) and certain types of cancer. Thus, international agencies such as the World Health Organisation (WHO, 1990) and the professional bodies in several countries have formulated dietary guidelines or population nutrient goals that provide for generous amounts of these food items which are rich in vitamins and fibre.

Several reports in the literature suggest that the antioxidant nutrients in vegetables and fruits protect the human body from the potentially harmful effects of free radicals-reactive by-products of aerobic metabolism which can damage membrane lipids, cellular enzymes and the genetic material, deoxyribonucleic acid (DNA). Free radical damage has been implicated in the causal pathway of lipid peroxidation- a major factor in atherogeneisis and CHD (Witztum & Steinberg, 1991; Ballmer *et al.*, 1994; Gaziano, 1994), as well as in the initiation of cancer development (Halliwell & Gutteridge, 1989). Antioxidants such as glutathione, ubiquinol and uric acid are produced by normal metabolism, while others are found in the diet, the best known being vitamin E (tocopherols and tocotrienols), ascorbic acid and certain carotenoids, i.e. β-carotene, α-carotene, and lycopene (Bendich & Butterworth, 1991; Agarwal & Rao, 1997). However, beneficial effects of these antioxidants are only apparent at relatively high doses-much more than can be obtained from a normal diet (Langseth, 1995).

The evidence for a protective role of antioxidant vitamins in chronic diseases is particularly strong for vitamin E, and recent updates in this area are continually being provided by the Vitamin E Research and Information Service (VERIS) in several countries. Of the plasma concentrations of vitamins A, C, and E and carotene, vitamin E has been reported to have the strongest inverse relationship with risk of angina pectoris (Riemersma et al., 1991) and mortality due to ischaemic heart disease (IHD) (Gey *et al.*, 1991). Subsequently, Gey (1995) postulated that there are optimal plasma levels for these antioxidants in the protection against CHD. Recently, Tomeo *et al.* (1995) demonstrated that *palmvitee*, a vitamin E concentrate from palm oil, could regress carotid stenosis in patients who have been receiving daily supplements of the vitamin for only six months. Similarly, Stephens *et al.* (1996) demonstrated in the Cambridge Heart Antioxidant Study (CHAOS) that α-tocopherol treatment substantially reduced the rate of non-fatal myocardial infarction, with beneficial effects apparent after one year of treatment.

The present study was conducted to test the hypothesis that Malaysians who are inflicted with clogged arteries in coronary artery disease (CAD), eg. PTCA or CABG patients, have significantly poorer status of these antioxidant nutrients, as measured by serum levels of these antioxidants, compared to healthy controls.

Materials and Methods

Blood samples

Fasting serum samples were obtained from a total of 316 PTCA and CABG patients, aged 37-75 years (271 males, 45 females) of the National Heart Institute (IJN), Kuala Lumpur. The 162 controls (102 males, 60 females) consisted of apparently healthy subjects with no past history of CHD and who underwent routine screening for serum lipid profile at the IJN, as well as at the Institute for Medical Research (IMR), Kuala Lumpur.

Serum α-T, retinol and carotenoids (α-catotene, β-carotene, lycopene, and total carotenoids) were measured simultaneously by isocratic HPLC, with detection at 295 nm, 325 nm and 450 nm, respectively (Fig. 1). A 200 µl aliquot of serum extract was injected into a C_{18} reverse-phase column and separated by a mobile phase comprising a mixture of 88% acetonitrile:10% methanol:2% ethyl acetate (v/v) (Tee & Khor, 1995). Serum (-tocopherol was standardized for serum total cholesterol (TC) plus triglycerides (TG), i.e. $\alpha\text{-}T_{std}$.

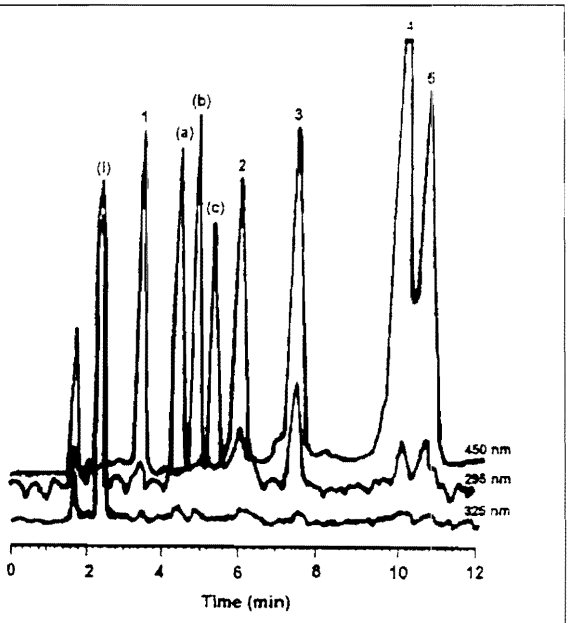

Fig. 1. Sample chromatograms for tocopherols, carotenoids and retinol: (a) = δ-tocopherol; (b) = γ-tocopherol; (c) = α-tocopherol; (1) = lutein; (2) = β-cryptoxanthin; (3) = lycopene; (4) = α-carotene; (5) = β-carotene; (i) = retinol.

Statistical analysis

Serum levels of the antioxidant nutrients measured were expressed as mean ± 1SD. Between group comparisons were analysed with the Student's t test, using $p<0.05$ to indicate significance. Effects of age on the parameters measured were investigated by analysing the data according to quartiles by age in 235 apparently healthy individuals (133 males, 102 females, aged 30-75 years).

Results

The results of the various serum antioxidant nutrient levels measured are shown in Table 1 below. From Table 1, it is evident that serum levels of $\alpha\text{-}T_{std}$, retinol and lycopene in CAD patients and healthy controls are comparable. However, β-carotene, α-carotene and total carotenoid levels are lower in CAD patients versus controls ($p<0.05$), both for combined sexes and in the males. These findings for carotenoids, retinol and α-tocopherol in the present study agree to a large extent with that of a similar study in Korean CAD patients (Kim *et al.*, 1996), i.e. serum β-carotene, but not α-tocopherol, was lower in CAD patients compared to healthy controls. The result obtained for serum α-tocopherol lever in both these studies are not in agreement with the report that α-tocopherol is the primary antioxidant in biological systems (Serbinova *et al.*, 1991), and plays a major role in protecting low density lipoproteins (LDL) against oxidative modification (Fuller *et al.*, 1996). If the "antioxidant hypothesis" of IHD is indeed true, one would expect the CAD patients in the study to possess a sub-optimal vitamin E status and thus exhibit lower serum α-tocopherol levels compared to in the healthy controls. Similarly, the in vivo antioxidant role of dietary lycopene reported elsewhere (Agarwal & Rao, 1997) was not apparent in the present study based on the serum levels of the parameter.

In this study, the healthy controls were only marginally younger than the CHD subjects (50.1±7.5 vs 53.9±9.4 years) and any confounding influence of age on serum α-tocopherol levels would at most be small, as the values of this serum parameter have been corrected for serum TC plus TG in line with the correction for serum total lipids recommended by Horwitt *et al.* (1972). Moreover, the analysis of the data on vitamin E, retinol and the carotenoids by quartiles of age in a larger healthy group (n=235; 133 males, 102 females) indicated that age had no influence on the serum levels of these antioxidants except for the case of vitamin E where subjects in the 4th quartile by age (56.5 ± 6.5 yr) had significantly higher serum α-tocopherol levels compared to the subjects in the "younger" quartiles ($p<0.05$) (Table 2).

Table 1. Serum α -Tocopherol, retinol and carotenoid profile in PTCA/CABG patients versus healthy controls

Group	N	Age (yrs)	α-T (mg/dl)	α-T_{std} (μg/mg TC+TG)	Retinol (μg/dl)	Lycopene (μg/dl)	β-carotene (μg/dl)	α-carotene (μg/dl)	Total carotenoids (μg/dl)
PATIENTS									
Males	271	53.5 ± 9.4	1.77 ± 0.61	4.28 ± 1.47	91 ± 33	11.2 ± 9.4	^{a}21.0 ± 18.2	^{a}5.8 ± 6.9	^{a}141 ± 66
Females	45	56.3 ± 8.5	1.93 ± 0.73	4.65 ± 1.68	89 ± 36	11.2 ± 8.9	^{bc}28.0 ± 24.0	^{a}6.2 ± 7.3	^{ab}156 ±75
Combined	316	53.9 ± 9.4	1.79 ± 0.63	4.34 ± 1.51	91 ± 34	11.2 ± 9.3	^{ab}22.0 ± 19.3	^{a}5.9 ± 7.0	^{a}143 ±63
CONTROLS									
Males	102	49.3 ± 7.4	1.61± 0.72	4.22 ± 1.46	82 ± 22	11.4 ± 8.5	^{c}33.7 ± 41.6	^{b}9.0 ± 12.8	^{b}171 ± 90
Females	60	51.9 ± 7.4	1.75 ± 0.69	4.99 ± 1.93	71 ± 17	12.8 ± 7.9	^{c}39.7 ± 23.3	^{b}10.5 ± 11.6	^{b}170 ± 69
Combined	162	50.1 ± 7.5	1.65 ± 0.71	4.44 ± 1.84	78 ±21	11.8 ± 8.3	^{c}34.7 ± 36.0	^{b}9.6 ± 12.0	^{b}171 ±78

Values with different superscripts, within the same column, are significantly different (p<0.05)

Table 2. Means of the serum parameters by quartiles of age in 235 healthy individuals (133 males, 102 females)

Quartile by age	α-tocopherol (mg/dl)	α-$tocopherol_{std}$ (μg/mg TC+TG)	Retinol (μg/dl)	β-carotene (μg/dl)	α-carotene (μg/dl)	Lycopene (μg/dl)	Total carotenoids (μg/dl)
1st Quartile (35.9 ± 2.4 yr)	1.35 ± 0.39	4.07 ± 1.47	73.8 ± 27.4	34.9 ± 25.8	9.2 ± 14.9	11.4 ± 7.3	163 ± 72
2nd Quartile (41.9 ± 1.9 yr)	1.44 ± 0.57	3.97 ± 1.68	72.2 ± 21.2	30.1 ± 28.7	7.5 ± 7.1	12.1 ± 8.3	162 ± 68
3rd Quartile (46.7 ± 1.1 yr)	1.46 ± 0.54	4.05 ± 1.46	74.7 ± 21.9	38.2 ± 45.0	7.5 ± 7.5	11.7 ± 7.5	176 ± 86
4th Quartile (56.5 ± 6.5 yr)	*1.80 ± 0.81	*4.86 ± 1.88	78.2 ± 18.4	36.3 ± 26.8	10.2 ± 13.2	10.7 ± 8.4	165 ± 83

**Significantly higher than the other means in the same column (p<0.05)*

Discussion

The basic assumption in this study is that the antioxidant nutrient status of the subjects is indicated by the serum levels of the respective antioxidant. This appears valid for the case of vitamin E which is readily absorbed, transported in the blood bound non-specifically to lipoproteins, taken up by the liver, and released in low-density lipoproteins (LDL). Also, tocopherol exchanges rapidly between the lipoproteins and the erythrocytes, and plasma tocopherol levels are inversely related to the susceptibility of erythrocytes to oxidative haemolysis (Combs, 1992).

Similarly, carotenoids are well absorbed and serum levels of carotenoids reflect current dietary intake of carotenoids (Olsen, 1984). Of interest, β-carotene and α-carotene (but not lycopene) are precursers of vitamin A, but serum levels of carotenoids correlate poorly with vitamin A status (Sauberlich *et al.*, 1984).

The use of serum retinol levels to reflect vitamin A status puts a "dent" in the basic assumption of the study as serum retinol levels only accurately reflects vitamin A status when liver vitamin A stores are either severely depleted or excessively high. When liver vitamin A concentrations are between these limits, serum retinol concentrations are homeostatically controlled. i.e.

levels remain relatively constant and do not reflect total body reserves of vitamin A (Olsen, 1984). As such, the use of the Relative Dose Response test recommended by Underwood & Olsen (1993) would have been a better index of vitamin A status. In addition, the inclusion of measurement of serum indices of lipid peroxidation [eg. malonyl dialdehyde (MDA) and conjugated dienes] or erythrocyte metalloenzymes such as glutathione peroxidase, superoxide dismutase or catalase, would have served as indirect indicators of antioxidant nutrient status, thus providing a clearer picture of the antioxidant nutrient status of the subjects.

Gey (1995) proposed that the threshold protective level for vitamin E against IHD is >30 μm/L (>1.29 mg/dl). In the present data set, 244 out of 316 (77%) CAD patients versus 70 out of 162 (57%) healthy controls ($p<0.05$) have serum α-tocopherol levels above this threshold level. This observation is in direct conflict with the results of many population case-control studies (Riemersma *et al.*, 1991; Stampfer *et al.*, 1993; Rimm *et al.*, 1993) and clinical trials (Tomeo *et al.*, 1995; Stephens et al., 1996) which suggest that vitamin E play a major role in reducing risk to CHD.

The CAD patients were not known to be on vitamin supplementation at the time of blood collection, and thus any confounding influence on serum vitamin levels arising from the intake of vitamin supplements is ruled out. The influence of smoking habits on the serum levels of the antioxidants measured is not clear as data on the smoking habits of the subjects was not available. However, it has been reported that smoking increased the susceptibility to erythrocyte peroxidation (Duthie *et al.*, 1991) but had no significant significant influence on plasma vitamin E and retinol levels (Duthie *et al.*, 1992).

The significantly higher serum levels of α-carotene, β-carotene and total carotenoids in all healthy subgroups (except β-carotene in females) compared to CAD patients would suggest that there was a protective role for these carotenoids in the pathogenesis of CHD in Malaysians. In contrast, recent reports of long-term supplementation with essentially *synthetic* β-carotene, either singly (Hennekens et al., 1996), or in combination with vitamin A (Omenn et al., 1996), have yielded no apparent beneficial effect on CHD or malignant neoplasms, especially lung cancer.

Conclusion

The findings in this study indicate that serum carotenoid levels, particularly for β-carotene and α-carotene, were lower in PTCA/CABG patients versus healthy controls which seem to support the antioxidant hypothesis for these nutrients in the pathogenesis of CAD in Malaysians. A similar but much weaker finding ($p>0.05$) was observed for serum lycopene levels.

However, the reason for the absence of a similar finding here for α-tocopherol, widely reported as the prinicipal antioxidant nutrient in biological systems, is unclear and remains unresolved.

Acknowledgements

The authors would like to thank the Director of the Institute for Medical Reseach and the Chief Executive Officer of the National Heart Institute, Kuala Lumpur for their support and permission to publish the current manuscript.

References

Agarwal S & Rao AV (1997). In vivo antioxidant proeties of dietary lycopene. *Abstracts 16th International Congress of Nutrition*, July 27-August 1 1997, Montreal, Canada: **PM 184**, p.117.

Ballmer PE, Reinhart WH & Gey KF (1994) Antioxidant vitamins and disease- risks of a subop timal supply. *Ther Umsch* **51,** 467-474.

Bendich A & Butterworth CE (1991). *Micronutrients in Health and in Disease Prevention*, Marcel Dekker, Inc., New York, 1991.

Combs Jr GF (ed) (1992). *The Vitamins: Fundamental Aspects in Nutrition and Health*, Academic Press, Inc., San Diego, California.

Duthie GG, Arthur JR & James WPT (1991). Effects of smoking and vitamin E on blood antioxidant status. *American Journal of Clinical Nutrition* **53(4),** 1061S-1063S.

Duthie GG, Shortt CT, Robertson JD, Walker KA & Authur JR (1992) Plasma antioxidants, indices of lipid peroxidation and coronary heart disease risk factors in a Scottish population. *Nutrition Research* **12,** Suppl 1, S61-S67.

Ferro-Luzzi A, Strazzullo P, Scaccini C, Siani A, Sette S, Mariani MA, Mastranzo P, Dougherty RM, Iacono JM & Mancini M (1984). Changing the Mediterranean diet: effects on blood lipids. *American Journal of Clinical Nutrition* **40,** 1027-1037.

Fuller CJ, Chandalia M, Garg A, Grundy SM & Jialal I (1996). RRR-alpha-tocopheryl acetate supplemention at pharmacologic doses decreases low-density lipoprotein oxidative susceptibility but not protein glycation in patients with diabetes mellitus. *American Journal of Clinical Nutrition* **63,** 753-759.

Gaziano JM (1994).Antioxidant vitamins and coronary artery disease risk. *American Journal of Medicine* **97,** 18S-28S.

Gey KF (1995). Ten-year retrospective on the antioxidant hypothesis of arteriosclerosis: Threshold plasma levels of antioxidant micronutrients related to minimum cardiovascular risk. *Nutritonal Biochemistry* **6,**

Gey KF, Puska P, Jordan P & Moser UK (1991). Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epi demiology. *American Journal of Clinical Nutrition* **53,** 326S-334S.

Halliwell B & Gutteridege (1989). *Free Radicals in Biology and Medicine*, 2nd Edition, Clarendon Press, Oxford.

Hennekens C, Buring J, Manson JE, Stampfer MJ, Rosner B, Cook NR, Belanger C, LaMutte F, JM, Ridker PM, Willet W & Peto R (1996). Lack of effect of long term supplementation with beta-carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine* **334,** 1145-1149.

Horwitt MK, Harvey CC, Dahm Jr GH & Searcy MT (1972). Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Annals of the New York Academy of Science* **203,** 223.

Kim SY, Lee-Kim YC & Kim MK (1996). Serum levels of antioxidant vitamins in relation to coronary artery disease: a case-control study of Koreans. *Biomedical Environmental Science* **9,** 229-235.

Langseth L (1995). Oxidants, antioxidants, and disease prevention. *ILSI Europe Concise Monographs*, Bracco U & Jardien NJ (eds). ISLI, Belgium, ISBN 0-944398 -52-9.

Olson JA (1984). Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *Journal of the National Cancer Institue* **73,** 1439-1444.

Omenn GS, Goodman GE, Thornquist M, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams Jr JH, Barnhart S & Mammar S (1996). Effects of combination of beta-carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine* **334,** 1150-1155.

Register UD & Sonnenberg LM (1973). The vegetarian diet. *Journal of the American Dietetic Association* **62,** 253-261.

Riemersma RA, Wood DA, Macintyre CCA, Elton RA, Gey KF & Oliver MF (1991). Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet* **337,** 1-5.

Rimm EB, Stampfer MJ, Ascherio A, Giovannuci E, Golditz GA & Willet WC (1993). Vitamin E consumption and the risk of coronary heart disease inmen. *New England Journal of Medicine* **328,** 1450-1456.

Sauberlich HE, Dowdy RP & Skala JH (1984). *Laboratory tests for the assessment of nutritional status, CRC Press*, Inc., Boca Raton, Florida, 1984; p.6.

Serbinova E, Kagan V, Han D & Packer L (1991). Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. *Free Radical Biology & Medicine* **10,** 263-275.

Spiller GA (1991). *The Mediterranean Diets in Health and Disease*. Van Nostrand Reinhold, New York, 1991.

Stampfer MJ, Hennekens CH, Manson JE, Golditz GA, Rosner B & Willet WC (1993). Vitamin E consumption and the risk of coronary disease in women. *New England Journal of Medicine* **328,** 1444-1449.

Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchison MJ & Brown MJ (1996). Randomised'controlled trial of vitamin E in patients with coronary disease:Cambridge Heart Antioxidant Study (CHAOS). *Lancet* **347,** 781-786.

Tee ES & Khor SC (1995). Simultaneous determination of retinol, carotenoids and tocopherols in human serum by high pressure liquid chromataography. *Malaysian Journal of Nutrition* **1(2),** 151-170.

Tomeo Ac, Geller M, Watkins TR, Gapor A & Bierenbaum ML (1995). Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis. *Lipids* **30(12),** 1179-1183.

Underwood BA & Olson JA (eds) (1993). *A Brief Guide to Current Methods of Assessing Vitamin A Status. A Report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation Inc.*, Washington D.C., USA, 1993; p. 14.

WHO (1990). *Diet, nutrition, and the prevention of chronic diseases*. Report of a WHO Study Group, *WHO Technical Report Series* **797,** WHO, Geneva.

Witztum Jl & Steinberg D (1991). Role of oxidized low density lipoprotein in atherogenesis. *Journal of Clinical Investigation* **88,** 1785-1792.

Received 4 June 1998; revised 25 August 1998; accepted for publication 1 September 1998.