MICRONUTRIENTS IN THE PREVENTION AND TREATMENT OF CARDIOVASCULAR DISEASE

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PREVALENCE OF VASCULAR DISEASE IN SOUTH AFRICANS

The prevalence of coronary heart disease (CAD) reported in the late seventies was high in whites by world standards in contrast to blacks, who had a very low prevalence.[1,2] The difference in prevalence was ascribed to differences in lifestyle and diet, as blacks were reported to take in a high-fibre and low-fat diet.[3,4] Due to increased urbanisation, a trend towards a Westernised lifestyle was observed in the black population with a concomitant increase in hypertension,[5,6] obesity[6] smoking[7] and diabetes,[8] which are all recognised risk factors for vascular disease. Despite the high prevalence of CAD risk factors, the incidence of the disease is still remarkably low in this population group[9-11] in contrast to whites.[10] The low prevalence of CAD in black subjects despite a high prevalence of major risk factors shows that our understanding of atherogenesis and its clinical presentation(s) is still incomplete. Even more puzzling is the observation that atherosclerosis in black subjects appears to affect the cerebral circulation rather than extracranial and peripheral arteries.[12-15] Cerebrovascular disease (CVD) is regarded as a major cause of morbidity and mortality in this ethnic group[16-18] and approximately 32 - 50% of strokes are related to atherothrombosis.[16-19] Against this background the study on homocysteine as a CVD/CAD risk factor in both black and white South African subjects was performed in order better to understand the underlying differences in these vascular disease expressions. Elevated plasma total homocysteine (tHcy) concentrations have repeatedly been associated with increased vascular disease risk,[20,21] and explaining ethnic differences in CVD/CAD in terms of prevalence by differences in homocysteine metabolism is possible.

HOMOCYST(E)INE AND DETERMINANTS OF PLASMA TOTAL HOMOCYST(E)INE (THCY) CONCENTRATIONS

Homocysteine is an intermediate sulfhydryl α-amino acid formed during conversion of methionine to cysteine and is formed when transmethylation occurs between S-adenosyl methionine and a methyl group acceptor. Plasma levels of tHcy

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represent the sum of concentrations of free homocysteine (the reduced form), protein-bound homocysteine, the disulfide homocystine (the oxidised form) and homocysteine-cysteine mixed disulfide.[22] Homocysteine can be metabolised through a remethylation pathway to methionine where cobalamin/vitamin B_{12} acts as cofactor, and 5-methyltetrahydrofolate is the methyl donor. Alternatively, betaine acts as the methyl donor and the reaction is catalysed by betaine-homocysteine methyltransferase. Homocysteine may be condensed via the transsulfuration pathway with serine by a vitamin B_6-dependent enzyme, cystathionine B-synthase (CBS; E.C. 4.2.1.22) to form cystathionine, in an irreversible reaction. Cystathionine is then converted to cysteine and α-ketobutyrate in a reaction catalysed by α-cystathionase (E.C. 4.4.1.1), another vitamin B_6-dependent enzyme.[23,24] An inverse relationship has been observed between plasma folate and vitamin B_{12} and plasma homocysteine levels.[25-27] Supplementation with these two vitamins leads to a significant decrease in circulating homocysteine levels.[26,28-30] Vitamin B_6 status does not appear to affect circulating homocysteine levels,[31,32] but vitamin B_6 deficiency does cause post-load homocysteine levels to increase;[33] this effect may be reversed by pyridoxine supplementation.[34,36] Furthermore, an inadequate intake of the B vitamins has been reported to induce hyperhomocyst(e)-inaemia.[37,38]

Plasma homocysteine concentrations vary considerably between individuals[25,38] and gender.[39-42] Renal clearance of homocysteine also affects circulating levels.[43] Homozygosity for CBS deficiency or 5,10-methylenetetrahydrofolate reductase (MTHFR; E.C. 1.7.99.5) deficiency are associated with severe hyperhomocysteinaemia and premature vascular disease, such as atherosclerosis of major arteries and/or vascular thrombosis.[44-47] Milder forms of hyperhomocysteinaemia associated with heterozygous CBS deficiency, the labile form of methyl tetrahydrofolate reductase, or a suboptimal vitamin status, are also associated with premature vascular disease.[48-52]

General aim of the study

Homocysteine research is currently focused on its role as pro- or anti-oxidant at different plasma concentrations, and its effect on oxidative modification of LDL. Ueland *et al.*[53] recently suggested that the imbalance between thiols may affect redox status, and therefore anti-oxidant status, and/or vice versa. Since anti-oxidant vitamin deficiencies and hyperhomocyst(e) inaemia are associated with increased LDL peroxidation, vitamin B and anti-oxidant vitamin concentrations were also determined in this study performed in white subjects.

Anti-oxidant nutrients within the LDL particle and in the circulation presumably protect the LDL particle against oxidative damage and thereby inhibit the process of atherosclerosis. Vitamin E is the most effective chain-breaking lipid-soluble anti-oxidant found in biological membranes and it is particularly effective in protecting LDL from oxidation.[54] Cross-cultural and prospective studies that investigated cardiovascular disease incidence and/or mortality in populations with differing levels of dietary vitamin E intake, suggest that a higher dietary vitamin E intake or higher plasma vitamin E concentrations have a protective effect against CAD.[55-63] These findings are however not consistent.[64-66] Vitamin E supplementation in the Cambridge Heart Study was observed to reduce the risk of cardiovascular death and non-fatal myocardial infarction in subjects with angiographically proven coronary arteriosclerosis.[67] Vitamin C, an aqueous anti-oxidant, is the first line of defence against free radicals of aqueous origin[68] and helps regenerate α--tocopherol.[69-71] Cross-cultural studies in European populations have reported a geographical correlation between plasma vitamin C concentration and high rate of cardiovascular disease.[55,63,72] A higher dietary intake of vitamin C was associated with a trend towards decreasing risk of coronary heart disease, or risk of death due to cardiovascular disease,[73-75] although these findings are not consistent.[76,77]

Vitamin A may play a significant role in the protection of LDL against peroxidation as it can function as a lipoperoxyl radical scavenger[78] and as an anti-oxidant[79,80] Although an inverse relationship between vitamin A and mortality from ischaemic heart disease has been reported previously,[56,63,81] recent studies have not confirmed such an association.[77,82,83]

Results

Homocyst(e)ine as a risk factor for occlusive vascular disease in black subjects

The mean plasma tHcy concentrations were higher (10.91; range 4.95-23.05 μmol/l) in the stroke patients than in controls (8.73; 3.95 - 15.10 μmol/l) ($P = 0.031$). This difference, however, could not be explained by differences in vitamin B_{12}, vitamin B_6 and folate status. Hyperhomocyst(e)inaemia in black stroke patients may be partially caused by renal insufficiency, as a subgroup of 9 stroke patients with hypercreatininaemia (> 90 μmol/l, 75% of control concentrations) had significantly higher plasma tHcy concentrations ($P = 0.002$), while plasma tHcy concentrations of stroke patients with normal serum creatinine concentrations were not significantly different to those of controls. Hyperhomocyst(e)inaemia was also associated with significant risk of CVD (crude odds ratio of 5.0 (95% CI, 1.5 - 17.3)). The multivariate odds ratio adjusted for blood pressure and serum total cholesterol was 3.6 (95% CI, 1.0 - 12.7), and further inclusion of the vitamins in the logistic regression resulted in an odds ratio estimate of 4.3 (CI, 1.1. - 17.5). Addition of serum creatinine (entered as bivariate data: normal or elevated above 75th percentile of 90.0 μmol/l) to the logistic regression, resulted in an odds ratio estimate of 3.73 (CI, 0.83 - 16.70), which was no longer significant.

Hyperhomocyst(e)inaemia may therefore increase the risk of stroke, but it is unlikely to be a primary initiating factor in black subjects.

HOMOCYST(E)INE AS A RISK FACTOR FOR OCCLUSIVE VASCULAR DISEASE IN WHITES

Plasma tHcy concentration was significantly elevated and plasma vitamin B_6 concentration significantly decreased in 138 white male patients with angiographically proven CAD compared with 204 healthy control subjects ($P < 0.001$). Odds ratios determined for the B vitamins with inclusion of age and smoking status in the logistic regression showed that decreases in plasma vitamin B_6 concentrations were also associated with increased risk of CAD (odds ratio 1.26; CI, 1.03 - 1.55), while no significant CAD risk was associated with folate and vitamin B_{12} status. The significant and strong correlation between plasma tHcy and serum creatinine concentrations ($r = 0.803$; $P = 0.001$) that was observed in the black patients with vascular disease was much weaker, but still significant, in white vascular disease patients ($r = 0.184$; $P = 0.041$).The crude odds ratio of stroke, comparing subjects with elevated plasma tHcy concentrations (above the 75th percentile: > 9.66 µmol/l) with subjects with normal plasma tHcy concentrations, was 4.0 (95% CI, 2.5 - 6.4). The multivariate odds ratio adjusted for blood pressure and serum total cholesterol was 2.1 (95% CI, 1.4 - 3.0), and further inclusion of the vitamins in the logistic regression resulted in an odds ratio estimate of 2.0 (CI, 1.3 - 2.9). Addition of serum creatinine to the logistic regression resulted in an odds ratio estimate of 2.3 (CI, 1.5 - 3.5), which was still significant. Renal insufficiency, therefore, does not appear to play as large a role in hyperhomocyst(e)inaemia in white subjects as in black subjects, and hyperhomocyst(e)- inaemia appears to be independently associated with an increased CAD risk in white South African CAD patients.

Additional findings of anti-oxidant vitamin nutritional status in white subjects.

Plasma vitamin E, C and A concentrations were significantly decreased in CAD patients compared with controls ($P < 0.001$) after correcting for significant covariates. Vitamin deficiencies were related to increased CAD risk, when subjects within the highest anti-oxidant vitamin quartiles were compared with those in the lowest quartiles, after adjusting for other CAD risk factors. Risk of CAD associated with vitamin A was notably higher compared with the other highest of the anti-oxidant vitamins. Calculation of the odds ratio showed that risk of CAD increased 2.35-fold with decreased vitamin A plasma concentrations (lowest quartile), compared with vitamin A concentrations in the highest quartile (CI, 1.25 - 2.31). Interquartile risk assessment relating to vitamin status resulted in an odds ratio estimate for CAD of 1.49 (95% CI, 1.03 - 2.16) for vitamin E and 1.71 (95% CI, 1.04 - 2.81) for vitamin C.

Respective cutoff values used for estimate of CAD risk relating to vitamin C, A and E concentrations were: <5.6 versus >11.59 µmol/l, <3.53 versus >4.53 µmol/l, <6.02 versus > 8.01 µmol/l per mmol/l serum total cholesterol.

DISCUSSION

Decreased vitamin B_6 concentrations have been reported previously in CAD,[84-86] although a recent study reported slightly higher vitamin B_6 levels in patients compared with controls.[87] We found that decreased vitamin B_6 concentrations were associated with a significant increase in CAD risk, compared with normal vitamin B_6 levels after adjusting for age and smoking status. This finding is in accordance with other reports that low vitamin B_6 concentrations confer an independent risk of CAD.[86,87] An inverse association between plasma folate and risk of CAD has also been reported.[87,88] Folate concentrations were not significantly different in our population and in the study of Verhoef *et al.*[87] folate concentrations were significantly increased in CAD patients compared with controls. A possible explanation for the higher vitamin B levels observed in recent studies could be an increased awareness of the importance of vitamins in CAD and a concomitant change in lifestyle. Higher intake of folate and vitamin B_6 was associated with a decreased risk of CAD, and supplementation with folate and vitamin B_6 or multiple vitamin usage reduced risk of CAD in women.[89] In this regard, a significant decrease in the rate of progression of carotid plaque in CAD patients was recently reported with a supplement of 2.5 mg folate, 25 mg vitamin B_6 and 150 µg vitamin B12 daily.[90] Vitamin B supplementation could therefore play a role in the primary and secondary prevention of CAD. In our study performed in white subjects, hyperhomocyst(e)inaemia was evident in CAD patients, in spite of the plasma folate concentration that did not differ significantly between patients and controls. As Verhoef *et al.*[87] reported the same findings, it would appear that pharmacological doses, and not only dietary supplementation with folate and B vitamins, may be necessary in order to achieve a reduction in plasma tHcy. Furthermore, case-control studies suggest a significantly lower index of CAD risk in subjects in the highest percentiles of plasma vitamin concentrations compared with those in the lowest percentiles.[57,91] Nested case-control studies, however, did not confirm these findings,[64,66] possibly owing to prolonged serum storage. In our study, all the three major anti-oxidant vitamins were significantly decreased in CAD patients compared with controls and related to CAD risk, as observed in other studies.[57,91-93] Several studies found that dietary intake of vitamins A, C and E and beta-carotene was significantly lower in subjects with CAD compared with controls.[93-95] Dietary intake studies to assess anti-oxidant vitamin intake in South Africans are therefore clearly indicated.

To summarise, CAD in white South African males is

characterised by significantly decreased plasma vitamin C, E and A as well as decreased plasma vitamin B_6 concentration. A significantly increased plasma tHcy concentration was observed in these patients. Higher intake of anti-oxidant vitamins, as well as folate and B vitamins, are advocated to reduce primary and secondary CAD risk in South African males.

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