Low plasma concentrations of diet-derived antioxidants in association with microalbuminuria in Indigenous Australian populations

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ABSTRACT

Microalbuminuria is a risk factor for renal and cardiovascular diseases. Oxidant stress may contribute to vascular disease risk by promoting damage to renal and vascular tissues. This study examined the associations of plasma levels of diet-derived antioxidants with albuminuria in Australian population groups at high risk of renal and cardiovascular disease. Data on microalbuminuria and diet-derived plasma antioxidants were drawn from results of cross-sectional community-based risk factor surveys of Aboriginal and Torres Strait Islander peoples (n = 698, 15 years and older). Prevalence of microalbuminuria ranged from 17–21%. After adjustment for age, gender, body mass index, diabetes, smoking status, plasma lipids and blood pressure, microalbuminuria was associated with significantly lower plasma concentrations of lycopene (−29%; \(P < 0.001\)), \(\beta\)-carotene (−22%; \(P < 0.001\)), \(\alpha\)-carotene (−22%; \(P < 0.001\)) and cryptoxanthin (−17%; \(P < 0.001\)) compared with normalbuminuric persons. Significant associations of microalbuminuria with plasma concentrations of \(\alpha\)-tocopherol, retinol, lutein plus zeaxanthin and homocysteine were absent. The data are consistent with a protective effect of diets rich in carotenoids on vascular endothelium and/or renal tissues, and support the need for interventions to address affordable food supplies and dietary quality among Indigenous Australians.

INTRODUCTION

Microalbuminuria is a risk factor for atherosclerotic vascular disease and predicts cardiovascular disease (CVD) mortality and renal failure in diabetic and non-diabetic populations [1–3]. Albuminuria arises from excess permeability of the renal vascular endothelium and may also represent a more generalized dysfunction of the vascular endothelium [4–6]. Studies from several populations have shown that major causes of microalbuminuria are diabetes, hypertension and glomerulonephritis [7–11].

The incidence of end-stage renal disease varies widely across Australian Indigenous populations, but overall is substantially higher than for Australia generally [12]. There is a direct correlation of the incidence of end-stage renal disease with social disadvantage in Australia [13]. In age-adjusted terms, Aboriginal and Torres Strait Islander people experience three times greater CVD mortality than do other Australians [14]. This excessive premature mortality is in parallel with certain social indicators, including dietary quality, as Indigenous populations screened to date have low fruit and vegetable intakes [15–17].

Key words: carotenoids, epidemiology, microalbuminuria, retinol, tocopherol.
Abbreviations: ACR, albumin/creatinine ratio; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease.
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Evidence is accumulating to suggest that oxidative damage to the vascular endothelium contributes to the initiation and progression of atherosclerosis and possibly microvascular disease [18]. Oxidant stress in renal tissues has been reported to mediate renal damage in several experimental models [19–23]. Sources of oxidative stress may include hyperglycaemia [24], smoking [25], products of catecholamine metabolism [26,27] and low antioxidant defences. Conversely, there is evidence that diets rich in antioxidant compounds are protective against CVD [28]. Although the compounds, or more likely the combinations of compounds, providing protection remain to be determined, a number of candidates have been proposed. These include polyphenolics, vitamins E and C and carotenoids. Experimentally, pharmacological doses of antioxidant compounds, including vitamins E and C, can prevent or ameliorate endothelial dysfunction [29–32]. Attempts to prevent coronary heart disease using antioxidant vitamin supplementation have proven ineffective, but studies to date have involved relatively short term supplementation of middle aged or elderly subjects who may already have significant (although clinically silent) vascular disease and who, as a group, were not vitamin deficient. Plasma carotenoids are, however, useful markers of dietary fruit and vegetable intake [33,34].

The relationship of diet to the development of microalbuminuria has not been extensively studied. The aim of the present study was to examine the relationships of circulating concentrations of diet-derived antioxidants to microalbuminuria in cross-sectional community-based survey samples drawn from several Indigenous population groups in Australia.

METHODS

Ethical approval

The study was approved by the Research Ethics Committees of Deakin University, Monash University, Queensland Health Service and Torres Strait Regional Health Authority and the Alice Springs Institutional Ethics Committee, and the work carried out in accordance with the Declaration of Helsinki. Participants gave written informed consent to screening procedures. This manuscript was submitted with the approval of the Indigenous communities involved in the surveys.

Participant recruitment

Participants (15 years and older) were volunteers to community-based risk-factor screening initiatives in remote areas of central and northern Australia as described previously [7,35]. Screening included measurement of height and weight, collection of early morning urine specimens, fasting blood samples and administration of a 2 h oral glucose tolerance test. Initial comparisons of risk factors between communities included all participants. For the remaining analyses, participants with macroalbuminuria ($n=95$) were excluded because (i) macroalbuminuria may be associated with overt renal dysfunction and thus altered clearance of circulating antioxidants, resulting in spuriously elevated concentrations, and (ii) dietary alteration may have been implemented subsequent to diagnosis of overt renal dysfunction. Complete data with respect to plasma lipid-soluble antioxidants, cholesterol, microalbuminuria, body mass index (BMI), current smoking status and glucose tolerance were available for 698 indigenous people from four communities in three geographical regions: Central ($n=408$) and North-western ($n=141$) Australia and Torres Strait ($n=149$).

Anthropometry, biochemical methods and diagnostic criteria

Body weight was measured to 0.1 kg using digital electronic scales (UC-300; A.N.D., Tokyo, Japan) and height to 0.1 cm using an anthropometer (Harpenden; Holtain Limited, Crymych, U.K.). Blood samples were collected by venepuncture early in the morning after an overnight fast and kept on ice until centrifugation ($<6$ h later). Plasma was then separated and frozen immediately at $−20^\circ C$ until transfer to storage at $−80^\circ C$ ($<2$ weeks). Plasma cholesterol, triacylglycerols (triglycerides) and glucose were assayed by standard enzymic techniques using commercial reagents (Boehringer-Mannheim, Sydney, NSW, Australia). Lipid-soluble antioxidants (α-tocopherol, retinol, lutein plus zeaxanthin, cryptoxanthin, lycopene, α-carotene and β-carotene) were assayed by HPLC as described previously [36]. Plasma total carotenoids was calculated as the sum of all quantified carotenoids. Homocysteine was measured by fluorescence polarization immunoassay [37]. Albumin and creatinine were assayed using immunonephelometry (Kallestadt QM300 or Beckman 360 Array nephelometers) and an alkaline picrate method (Olympus AU800) respectively. Microalbuminuria was defined as albumin/creatinine ratio (ACR) in the range 3.4 to less than 34 mg/mmol [38]. In those surveys conducted in central Australia, urine samples were screened for haematuria ($>80$ cells/µl) and urinary tract infection using reagent strips (N-Multistix SG; Bayer Diagnostics, Mulgrave VIC, Australia), the latter with confirmation by microbiological culture.

Diabetes status was determined using American Diabetes Association criteria, including results of a 75 g 2 h oral glucose tolerance test (fasting plasma glucose $\geq 7.0$ mmol/l and/or 2 h plasma glucose $\geq 11.1$ mmol/l [39]), or on the basis of a previously confirmed clinical diagnosis. Blood pressure was measured as the mean of three readings with the volunteer in the sitting position using a Dinamap vital signs monitor (Critikon, Tampa,
Table 1  Clinical and demographic characteristics of the four community-based survey samples
Values are means (SD), geometric means (95 % CI) or prevalence.

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<th>Aboriginal communities</th>
<th>Torres Strait Islander community</th>
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<td>Torres Strait</td>
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<td>n</td>
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<td>Age (years)</td>
<td>36 (16)</td>
<td>37 (15)</td>
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<td>Male (%)</td>
<td>47</td>
<td>32</td>
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<td>Obesity (%)</td>
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<td>Hypertension (%)</td>
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<td>33</td>
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<td>Current smoking (%)</td>
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<tr>
<td>Diabetes (%)</td>
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<td>Microalbuminuria (%)</td>
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<td>Macroalbuminuria (%)</td>
<td>10</td>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.2 (1.2)</td>
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<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>2.0 (1.9, 2.1)</td>
<td>2.1 (1.9, 2.4)</td>
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<tr>
<td>α-Tocopherol (µmol/l)</td>
<td>17.1 (15.0, 16.0)</td>
<td>16.8 (15.0, 16.0)</td>
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<td>Total carotenoids (µmol/l)</td>
<td>0.47 (0.45, 0.49)</td>
<td>0.55 (0.50, 0.61)</td>
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<td>Homocysteine (µmol/l)</td>
<td>13.7 (13.2, 14.2)</td>
<td>13.3 (12.0, 14.8)</td>
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Statistical analyses
Differences in concentrations of lipid-soluble antioxidants between categories of ACR were tested using ANOVA. Gender, diabetes status, current smoking and community of residence were entered as fixed factors in the model, and age, BMI, mean arterial pressure and plasma lipids (cholesterol and triacylglycerols) as covariates. Models also tested first-order interaction terms of ACR category with gender, diabetes, geographic region and smoking status. The General Linear Modelling function of SPSS version 10 (SPSS Inc., Chicago, IL, U.S.A.) was used for these analyses. Initial comparisons within gender categories were assessed by unpaired Student’s t test. Prevalence and exact mid-P 95 % confidence interval (CI) for categorical data were calculated using PEPI software [41]. Statistical significance was taken as $P < 0.05$.

RESULTS
Survey sample characteristics
The gender distribution and average age of Aboriginal and Torres Strait Islander survey samples did not vary significantly between the four communities surveyed (Table 1). There was significant variation in prevalences of obesity, hypertension, smoking, diabetes and albuminuria, and in mean cholesterol, triacylglycerols, α-tocopherol, carotenoids and homocysteine (Table 1).

Clinical and demographic characteristics of the participants (excluding those with macroalbuminuria) stratified by ACR category are shown in Table 2. Microalbuminuria was associated with significantly greater age, BMI, blood pressure, total cholesterol and triacylglycerols and prevalence of diabetes. Current smoking was significantly less prevalent among microalbuminuric persons. Antihypertensive medication was currently prescribed for 2.4 % of normalbuminuric and 7.2 % of microalbuminuric persons: for normalbuminuric persons,
Table 3  Plasma carotenoid concentrations (nmol/l) stratified by microalbuminuric status

Table 3 shows average concentrations of plasma carotenoids stratified by ACR category. In comparisons of crude (unadjusted) mean carotenoid concentrations in men and women, microalbuminuria was associated with lower levels of lycopene, β- and α-carotene and cryptoxanthin in men. In women, microalbuminuria was associated with significantly lower levels of lycopene and cryptoxanthin and the differences for α- and β-carotene approached significance. After adjustment for age, gender, BMI, mean arterial pressure, plasma lipids, community of residence, smoking and diabetes status, the presence of microalbuminuria was associated with significantly lower plasma concentrations of lycopene (29 % lower), β-carotene (22 % lower), α-carotene (22 % lower) and cryptoxanthin (17 % lower). The difference in lutein plus zeaxanthin was small in magnitude and did not reach statistical significance. Tests of interaction effects showed there was no significant variation in the relationship of carotenoids to microalbuminuria across categories of gender (although the P value approached significance in the case of β-carotene, due to a stronger relationship in men than in women), geographic region, diabetes (Table 3) or smoking (results not shown). In general, plasma carotenoids were higher in women.

Plasma carotenoids and microalbuminuria

Lipid-soluble antioxidant vitamins and microalbuminuria

There were no significant differences in retinol or α-tocopherol concentrations between normal and microalbuminuric persons: mean (95 % CI) for retinol in normal and microalbuminuric groups respectively, was 1.58 (1.51–1.64) and 1.61 (1.51–1.71) µmol/l (P = 0.336); and mean (95 % CI) for α-tocopherol in normal and microalbuminuric groups respectively, was 22.2 (21.5–23.0) and 22.2 (21.1–23.4) µmol/l (P = 0.324). Retinol varied significantly according to gender (higher in men; P < 0.001), age (P = 0.004), community (P = 0.007), cholesterol (P < 0.001) and triacylglycerols (P < 0.001). α-Tocopherol varied significantly according to all variables in the model (P < 0.01 or less), except smoking and microalbuminuria.
positively related to plasma cholesterol and inversely with blood pressure. In the subsample of central Australian participants, inclusion of haematuria or urinary tract infection in the model did not alter the association of carotenoids with microalbuminuria (results not shown). Exclusion of participants prescribed antihypertensive medication made no substantial difference to these relationships (results not shown).

**Homocysteine and microalbuminuria**

There was no significant difference in average plasma homocysteine concentration (adjusted for age, gender, diabetes and geographic region) between normal and microalbuminuric persons [mean (95% CI) homocysteine, 12.3 (11.5, 13.0) µmol/l and 13.0 (12.1, 14.0) µmol/l in normal and microalbuminuric persons respectively].

**DISCUSSION**

The present study has identified low circulating concentrations of certain diet-derived carotenoids in association with elevated urinary albumin excretion in Australian populations. Consistent with other studies, the presence of microalbuminuria was associated with greater BMI, blood pressure, plasma lipids and prevalence of diabetes in these populations. The associations of carotenoids with microalbuminuria were independent of these factors, and were found consistently across four Aboriginal and Torres Strait Islander communities in remote areas. With the possible exception of β-carotene, the associations also did not vary by gender. Although these data are observational, they are consistent with a protective effect of dietary antioxidant compounds (and/or other closely-associated phytochemicals) against damage to the vascular endothelium generally and perhaps specifically in renal tissues. They also highlight the problems associated with food supply and storage infrastructure in remote communities [42] and the consequentially low circulating levels of antioxidants derived from fruit and vegetables.

Carotenoids, which are a good marker of dietary vegetable and fruit intake [33,34], may be important in themselves or may merely represent a marker of a more generally healthy lifestyle, which includes a better quality diet. We [7] and others [11] have reported previously that, among Aboriginal people, haematuria, indicative of glomerulonephritis (usually post-streptococcal), is associated with substantially increased albuminuria. A healthy diet contributing to protection against streptococcal infection and subsequent glomerulonephritis is a potential mechanism by which this effect could be mediated. However, in the subset of Aboriginal people from central Australia among whom haematuria was assessed by dipstick analysis, haematuria levels indicative of glomerulonephritis were not associated with lower carotenoid concentrations independent of the presence of albuminuria (results not shown). We are not aware of any evidence for greater clearance rates of carotenoids from the circulation in association with microalbuminuria.

An alternative explanation may be that greater oxidative stress may simultaneously increase endothelial and/or renal damage and degrade circulating diet-derived antioxidant concentrations, with no interaction of antioxidants with the endothelium or renal tissues. However, the observed associations were independent of two major sources of oxidative stress: diabetes and smoking. Furthermore, another potential marker of oxidant stress, in the form of plasma homocysteine, did not differ significantly between normal and microalbuminuric persons. This is in contrast with some [43], but not all [44,45], other reports. We also note that plasma homocysteine concentrations were relatively high in all groups evaluated (Table 1). Levels above 12 µmol/l are associated with elevated risk of vascular disease [46]. Dietary folate is a major determinant of plasma homocysteine levels and the relatively high homocysteine we observed probably reflects low folate intake and thus poor dietary quality.

Of interest was the fact that the more lipophilic carotenoids (lycopene and β-carotene) showed stronger associations with microalbuminuria than did the more polar lutein and zeaxanthin. This difference may reflect antioxidant actions on circulating lipids, although a lack of association of α-tocopherol (largely carried on low-density lipoprotein particles) with microalbuminuria, together with the absence of independent associations of dyslipidaemia with albuminuria in these populations [7], does not support this proposition. If carotenoids (which in plasma are quantitatively minor antioxidants compared with α-tocopherol) do have a causal relationship with albuminuria, it may be exerted at the level of the endothelium rather than on circulating lipid particles, interactions of carotenoids with α-tocopherol in low-density lipoprotein particles notwithstanding [47–49]. Lycopene has been shown experimentally to modulate binding of monocytes to vascular endothelial cells [50]. Further investigation of potential specific roles for carotenoids at the tissue, cellular or subcellular level is warranted.

In several studies of Type I diabetic patients, no associations of vitamin E, retinol or β-carotene with albuminuria were found [51,52]. Average levels of tocopherol and retinol in those studies were similar to those seen in the present study (that is, within the normal range), but average β-carotene concentration was substantially higher than in the present study. Intervention studies have provided some evidence for a link between oxidant stress and renal damage. In one group of Type I and II diabetic patients, long-term treatment with ascorbate reduced albumin excretion rate [53], and in another similar group α-lipoic acid prevented progression...
of albuminuria [54]. None of these studies have looked specifically at the role of carotenoids or carotenoid-rich diets. One of few studies to examine dietary factors in renal disease identified a protective effect of dietary fish intake against albuminuria in Type I diabetic patients [55]. The renovascular protective effect of angiotensin II receptor antagonists may be partly due to modulation of oxidant stress [56,57].

In conclusion, microalbuminuria was associated with lower circulating concentrations of diet-derived antioxidants in people from remote communities with low average plasma carotenoid concentrations. Hence it is possible that oxidant stress arising from antioxidant-poor diets is contributing to excessive premature renal and vascular disease among Indigenous Australians. The present data provide support for policies and interventions to address food supply, food cost and diet in remote communities and Indigenous communities generally. Several community-directed programmes have had some success in improving dietary quality and circulating antioxidant levels among Aboriginal people [15,58].

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