Inflammation and vascular endothelial activation in an Aboriginal population: relationships to coronary disease risk factors and nutritional markers

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ABSTRACT

Objective: To describe the levels of inflammation and vascular endothelial activation in an Aboriginal community, and the relationship of these factors to coronary heart disease (CHD) risk factors and markers of nutritional quality.

Design and participants: A cross-sectional survey of 95 women and 76 men participating in a chronic-disease prevention program.

Setting: A remote Aboriginal community in Western Australia in 1996.

Main outcome measures: Concentrations of markers of inflammation (C-reactive protein [CRP]) and vascular endothelial activation (soluble E-selectin [sE-selectin]); presence of metabolic syndrome; concentrations of diet-derived antioxidants.

Results: Participants exhibited very high plasma concentrations of CRP (mean, 5.4 mg/L; 95% CI, 4.6–6.3 mg/L) and sE-selectin (mean, 119 ng/mL; 95% CI, 111–128 ng/mL). Both CRP and sE-selectin concentrations were significantly higher in the presence of the metabolic syndrome. There were significant inverse linear relationships between concentrations of CRP and plasma concentrations of the antioxidants lycopene, β-carotene, cryptoxanthin and retinol. Even stronger inverse associations were evident between concentrations of sE-selectin and lycopene, β-carotene, cryptoxanthin and lutein.

Conclusions: Vascular inflammation and endothelial activation may be important mediators of elevated CHD risk in Aboriginal people. Inadequate nutrition and physical inactivity may contribute to this process.

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ABORIGINAL PEOPLE in Australia suffer very high mortality from coronary heart disease (CHD).[1] Nevertheless, screening in a number of Aboriginal communities has shown that the prevalence of two conventional coronary disease risk factors, hypercholesterolaemia and hypertension, is not excessive.[2,3] In contrast, pro-inflammatory and pro-oxidant stimuli — such as infection, obesity, smoking[4] and low intake of dietary antioxidant compounds[5] — are often prevalent. These, in turn, reflect social environmental conditions conducive to ill health. Hence, inflammation may be an important mediator of elevated CHD risk in Aboriginal populations.

Atherosclerosis is a chronic inflammatory state that is initiated and/or exacerbated by dyslipidaemia, cigarette smoking, hypertension, elevated plasma homocysteine levels, infection, obesity, insulin resistance and diabetes.[6] The early stages of this process involve binding of monocytes to the endothelial surface, initially through selectins expressed on the endothelium. This is followed by firmer binding through other cell adhesion molecules, penetration into the vessel intima, and perpetuation of a local inflammatory response that may lead to the initiation of atheroma. Oxidative damage to the endothelium may be important in initiation and progression of atherosclerosis. Elevated homocysteine levels potentially promote oxidative damage and are a CHD risk factor. Conversely, protection from endothelial damage can be provided by diets high in plant foods, antioxidant compounds (eg, vitamin C, vitamin E and carotenoids), n-3 polyunsaturated fatty acids, folate and L-arginine.[7]

Certain biomarkers of chronic inflammation are associated with and predictive of future cardiovascular events. These include proinflammatory cytokines (soluble cell-adhesion molecules shed from endothelial cells, the first of which to be upregulated is soluble E-selectin [sE-selectin]), and acute-phase reactants produced in the liver, such as C-reactive protein (CRP), which is now recognised as an independent predictor of CHD.[8,9,10]

Our study reports on levels of two biomarkers of inflammation and endothelial-cell activation (CRP and sE-selectin) in a cross-sectional survey of Aboriginal people. It also examines relationships between these biomarkers and indicators of dietary quality and other CHD risk factors.

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1: Clinical characteristics of participants (data given as mean [or geometric mean*] and 95% CI, except for prevalences, which are given as percentages)

	Desirable range	Men (*n*=76)	Women (*n*=95)	All (*n*=171)
Age (years)		38 (34–42)	37 (34–41)	38 (35–40)
Body mass index (kg/m^2)	< 25	23.7 (22.8–24.7)	26.7 (25.6–27.8)	25.4 (24.6–26.2)
Current smoking (%)		57%	38%	46%
Hypertension (%)		12%	18%	15%
Diabetes (%)		25%	28%	27%
Impaired glucose tolerance (%)		20%	22%	21%
Fasting insulin (μU/mL)*	5–15	15 (13–18)	19 (17–21)	17 (16–19)
Insulin resistance (HOMA)*	< 4	4.1 (3.4–4.9)	5.3 (4.6–6.0)	4.7 (4.2–5.3)
Total cholesterol (mmol/L)	< 5.5	4.8 (4.6–5.1)	4.5 (4.3–4.8)	4.7 (4.5–4.8)
HDL-cholesterol (mmol/L)	> 1.0	0.86 (0.81–0.91)	0.88 (0.84–0.92)	0.87 (0.84–0.90)
Triglycerides (mmol/L)*	< 2.0	1.9 (1.6–2.2)	1.6 (1.5–1.8)	1.7 (1.6–1.9)
Homocysteine (μmol/L)*	< 15	13.6 (12.4–15.0)	11.3 (10.4–12.2)	12.3 (11.6–13.1)
Carotenoids (μg/dL)*	†	26 (23–30)	28 (26–31)	27 (25–29)
C-reactive protein (mg/L)*	< 2.9	4.1 (3.2–5.2)	6.6 (5.4–8.1)	5.4 (4.6–6.3)
Soluble E-selectin (ng/mL)*	12–81	119 (107–132)	120 (108–132)	119 (111–128)

† Median for an Australian reference population was 88 μg/dL. HDL = high-density lipoprotein. HOMA = homoeostasis model assessment.

METHODS

Recruitment and screening

Participants were volunteers undergoing CHD risk-factor screening in 1996 as part of an evaluation of a community-directed intervention program. Screening was offered to all residents aged 15 years or over in a remote Aboriginal community in Western Australia. Our analyses are based on 95 women and 76 men, representing 67% of the eligible adult population present in the community at the time of screening. The survey sample did not differ significantly from the target population with respect to age and sex distribution. Non-responders were younger on average (mean age, 29 years) and included a higher proportion of men (71%).

Age was determined by self-report in the majority of cases and from clinical records for the remainder. Anthropometric measurements (weight, height, waist and hip circumferences) were performed using standard techniques. Blood pressure, recorded using an automated Dinamap Vital Signs Monitor (Critikon Inc., Tampa, Fla), was expressed as the mean of three measurements taken after the participant had been seated quietly for five minutes. Fasting blood samples were collected by venepuncture. Participants were then given a 75 g oral glucose load (except those with a clearly elevated fasting glucose level as indicated by a blood-glucose meter reading), and a second blood sample was collected 2 h after the start of glucose ingestion. Samples were kept cold until centrifugation (within 6 h), and plasma samples were frozen immediately thereafter. Samples were held at –20°C until transfer to –80°C storage (within two weeks).

Biochemical analyses

The biochemical analyses carried out and the methods used were as follows:

- Plasma glucose, cholesterol and triglyceride levels: standard, automated, colorimetric methods using commercial reagents (Hitachi 704 autoanalyser; Boehringer Mannheim, Sydney, NSW);
- Plasma insulin concentration: radioimmunoassay using commercial reagents (Linco Research Inc, St Louis, Mo);
- Plasma homocysteine concentration: automated fluorescence polarisation immunoassay (IMx autoanalyser; Abbott Laboratories, Abbott Park, Ill);
- Plasma carotenoid, α-tocopherol and retinol concentrations: high-pressure liquid chromatography;[11]
- Plasma CRP concentration: high-sensitivity commercial assay (BN-A nephelometer; Dade Behring Diagnostics, Lane Cove, NSW);
- Plasma sE-selectin concentration: enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn);
- Insulin resistance: homoeostasis model assessment (HOMA).[12]

Diagnostic criteria

The diagnostic criteria for the CHD-related conditions we assessed were as follows:

- Hypertension: systolic blood pressure ⩾ 140 mm Hg and/or diastolic blood pressure ⩾ 90 mm Hg and/or current antihypertensive medication;
- Diabetes: fasting plasma glucose concentration ⩾ 7.0 mmol/L and/or 2 h plasma glucose concentration ⩾ 11.1 mmol/L;
- Impaired glucose tolerance: 2 h plasma glucose concentration 7.8–11.0 mmol/L;
- Metabolic syndrome: having three or more of abdominal obesity (waist circumference > 102 cm for men or > 88 cm for women), plasma triglyceride concentration ⩾ 1.7 mmol/L, plasma HDL-cholesterol concentration < 1.1 mmol/L for men or < 1.3 mmol/L for women, blood pressure ⩾ 130/85, and fasting plasma glucose concentration ⩾ 6.1 mmol/L.[13]

Statistical analyses

Statistical analyses were performed using an SPSS statistical software package.[14] Continuous data are presented as the mean and 95% CI. Variables that had skewed distributions (ie, concentrations of insulin, triglycerides, homocysteine, carotenoids, α-tocopherol, CRP and sE-selectin) were log-transformed before analysis. Average levels of these variables are expressed as the geometric mean and 95% CI.

Relationships between CRP or sE-selectin levels and CHD risk factors were examined using Pearson product-moment correlation coefficients. Gen-

2: Bivariate correlations of C-reactive protein and soluble E-selectin with coronary heart disease risk factors and nutritional markers (data given as Pearson product-moment correlation coefficients [*r)**

	C-reactive protein†			Soluble E-selectin†		
	Men	Women	All	Men	Women	All
Age	0.271‡	0.294§	0.265§	0.042	0.206‡	0.133
Body mass index	0.056	0.369¶	0.299¶	0.276‡	0.291§	0.272¶
Waist : hip circumference ratio	0.288‡	0.337§	0.315¶	0.330§	0.334§	0.330¶
Systolic BP	0.098	0.310§	0.176‡	0.060	0.261‡	0.176‡
Diastolic BP	0.200	0.302§	0.228§	0.149	0.244‡	0.202‡
Fasting glucose	0.315§	0.141	0.218§	0.237‡	0.238‡	0.237§
2-hour glucose	0.291‡	0.230‡	0.266§	0.180	0.261‡	0.227§
Fasting insulin†	0.178	0.151	0.197‡	0.234‡	0.194	0.209§
Insulin resistance (HOMA)†	0.286‡	0.238‡	0.287¶	0.291‡	0.325§	0.300¶
Total cholesterol	0.129	0.023	0.029	0.084	0.106	0.095
HDL-cholesterol	–0.265‡	–0.121	–0.161‡	–0.099	–0.216‡	–0.163‡
Triglycerides†	0.083	0.185	0.093	0.301‡	0.289§	0.288¶
Homocysteine†	0.020	0.044	–0.022	0.013	0.139	0.078
Carotenoids†	–0.271‡	–0.293§	–0.256§	–0.279‡	–0.226‡	–0.248§
Retinol	–0.089	–0.178	–0.187‡	0.107	0.155	0.128
α-Tocopherol†	0.059	0.056	0.042	-0.054	0.114	0.045

* *r* describes degree of linear association between two variables (1 indicates a perfect positive correlation, –1 a perfect inverse correlation, 0 no association at all). † Log-transformed. ‡ $P < 0.05$. § $P < 0.01$. ¶ $P < 0.001$. BP = blood pressure. HDL = high-density lipoprotein. HOMA = homeostasis model assessment.

eral linear modelling was used to compare concentrations of CRP and sE-selectin between metabolic syndrome categories, with age, cholesterol concentrations and carotenoid concentrations as covariates, and sex, smoking status and the interaction term between sex and metabolic syndrome included in the model. Antioxidant concentrations across tertiles of CRP and sE-selectin were examined using general linear modelling, with adjustment for age and cholesterol levels, and sex, smoking status, metabolic syndrome and the interaction of sex with tertile included as factors in the model. Tests for linear trend in antioxidant concentrations across tertiles were also examined in these models. Statistical significance was inferred at a P value of < 0.05.

Ethics approval

The study was approved by the University of Melbourne Human Research Ethics Committee, the Monash University Human Research Ethics Committee and the Joint Ethics Committee of the Menzies School of Health Research and the Royal Darwin Hospital. Approval was also obtained from the Council of the community involved. Participants gave written, informed consent before screening procedures.

RESULTS

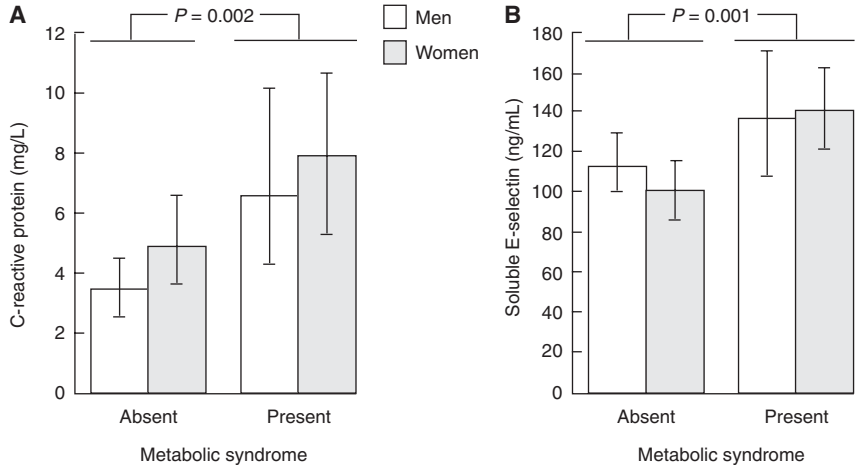
Smoking, diabetes and impaired glucose tolerance were common in the survey sample (Box 1). The population showed a high degree of insulin resistance, as indicated by high fasting insulin levels and HOMA insulin resistance values. Low mean levels of carotenoids and high levels of homocysteine indicated that dietary quality was poor. Fourteen participants were currently taking antihypertensive medication, eight were taking antidiabetic medication and none were taking lipid-lowering drugs. Mean levels of CRP and sE-selectin were very high, with the majority of both men and women having levels above the upper limit of the reference ranges for CRP (2.9 mg/L) and sE-selectin (81 ng/mL). After excluding participants with CRP values indicative of clinically significant inflammation (> 10 mg/L),[15] the mean CRP level was 3.7 mg/L (95% CI, 3.2–4.2 mg/L) ($n = 122$).

Pearson correlation coefficients (r) for CRP and sE-selectin levels with other CHD risk factors are shown in Box 2. CRP and sE-selectin levels correlated significantly with each other ($r = 0.202$; $P = 0.013$). In men, CRP level correlated significantly with age, waist:hip circumference ratio, glucose concentration and insulin resistance, and showed a significant inverse correlation with HDL-cholesterol and carotenoid concentrations. Similar associations were apparent in women (except for fasting glucose and HDL-cholesterol concentrations), and CRP concentration was also significantly associated with body mass index and blood pressure in women. Excluding participants with CRP levels > 10 mg/L from the analysis gave largely similar results, except that correlations with lipid and α-tocopherol concentrations were stronger (total cholesterol, $r = 0.205$, $P = 0.024$; HDL-cholesterol, $r = –0.169$, $P = 0.063$; triglycerides, $r = 0.216$, $P = 0.017$; and α-tocopherol, $r = 0.189$, $P = 0.042$), while the inverse correlations with concentrations of carotenoids ($r = –0.169$, $P = 0.063$) and retinol ($r = –0.101$, $P = 0.268$) were weaker.

In men, sE-selectin concentration correlated significantly with body mass index, waist:hip circumference ratio, concentrations of glucose, fasting insulin and triglycerides, and insulin resistance, and correlated inversely with carotenoid concentrations (Box 2). Similar associations were apparent in women, in whom there were also significant correlations of sE-selectin concentration with blood pressure and HDL-cholesterol concentration.

Twenty-seven per cent of men and 51% of women exhibited the metabolic syndrome as defined by the criteria of an expert panel.[13] After adjustment for age, sex, smoking status and carotenoid concentration, mean CRP concentration was significantly higher in the presence of the metabolic syndrome (Box 3). The effect was similar in both men and women, as indicated by the non-

3: Levels of C-reactive protein and soluble E-selectin in men and women with or without the metabolic syndrome



Plasma concentrations of C-reactive protein (A) and soluble E-selectin (B) according to presence or absence of the metabolic syndrome in men and women. Data are expressed as geometric mean (95% CI), adjusted for age and smoking status. P-values are for the difference in means between strata of the metabolic syndrome.

significance of the interaction term of sex with the metabolic syndrome ($P = 0.596$). Exclusion of individuals with CRP level > 10 mg/L from the analyses made no substantial difference to the results (data not shown). Inclusion of sE-selectin as a covariate made no substantial difference to the results (data not shown).

The mean concentration of sE-selectin was also significantly higher in the presence of the metabolic syndrome (Box 3). The interaction between sex and the metabolic syndrome was not significant ($P = 0.311$). Inclusion of CRP concentration as a covariate made no substantial difference to the results (data not shown). In these models, neither CRP concentration ($P = 0.315$) nor sE-selectin concentration ($P = 0.755$) varied by smoking status.

Data for CRP and sE-selectin concentrations were stratified into tertiles to examine the relationship with individual plasma antioxidant concentrations (Box 4). After adjustment for age, sex, smoking status, plasma cholesterol concentration and the metabolic syndrome, there were significant linear decreases in lycopene, β-carotene, cryptoxanthin and retinol levels with increasing CRP (Box 4). Excluding participants with CRP level > 10 mg/L made little difference to the results (other than to strengthen the association with lycopene; data not shown). Inclusion of sE-selectin concentration as a covariate in these models made little difference to the results (data not shown).

After adjustment for age, sex, smoking status, plasma cholesterol concentration and the metabolic syndrome, there were significant linear decreases in concentrations of lycopene, β-carotene, cryptoxanthin and lutein + zeaxanthin with increasing sE-selectin concentration (Box 4). For the relationship of cryptoxanthin and lutein + zeaxanthin with sE-selectin, there were significant interactions with sex, such that their concentrations decreased linearly with increasing sE-selectin in men but not in women (data not shown). Inclusion of CRP as a covariate in these models made no substantial difference to the results (data not shown).

Homocysteine concentration did not vary across tertiles of either CRP or sE-selectin.

DISCUSSION

Our study shows very high levels of circulating markers of vascular endothelial damage and subclinical inflammation in a remote population of Aboriginal people. Average CRP concentration (5.4 mg/L) was within the range associated with the highest risk of CHD mortality in men and women.[8,9] Average sE-selectin concentration (119 ng/mL) was well above the mean for people with incident CHD or carotid atherosclerosis in the United States (40–50 ng/mL)[10] and that for Europeans with cardiovascular disease (80 ng/mL).[16] Average levels of both markers were higher in the presence of the metabolic syndrome, as reported in other populations.[17,18] Moreover, these markers were inversely and independently associated with diet-derived antioxidants from fruit and vegetables. Our results are consistent with the very high mortality rate from cardiovascular disease in the fourth and fifth decades of life among Aboriginal people.[1] The data suggest that vascular inflammation is a major factor mediating the elevated CHD risk associated with adverse social environmental circumstances and the high prevalence of the metabolic syndrome in this population.

Our study did not exclude prevalent cases of CHD, and this may have resulted in higher average CRP and sE-selectin levels than are seen in other populations. However, the fact that both markers of vascular damage were elevated, even in the absence of risk factors such as the metabolic syndrome, suggests that endothelial activation and inflammation are preceding, and mediating, overt CHD in this population. Acute infection also raises CRP levels dramatically, but exclusion of people with CRP levels > 10 mg/L from statistical analyses did not substantially alter our conclusions.

Some major studies of CRP level as a CHD risk factor have not applied an upper limit of CRP level as an exclusion criterion,[8,9] and to apply such a limit assumes that elevated circulating (and tissue) CRP levels *per se* are less important than the underlying source of inflammation. As this assumption is yet to be definitively tested, it is difficult to interpret CRP levels unequivocally. In addition to being a marker of inflammation, CRP may have direct detrimental effects on vascular tissues,[19] which may partly explain the association of insulin resistance with inflammation and endothelial dysfunction.

Cross-sectional studies in other populations have shown relationships

between chronic inflammation and body mass index, insulin resistance, diabetes, blood pressure and other metabolic syndrome components, as well as an inverse relationship with physical activity.[17,20,21] The association of CRP (and sE-selectin) concentration with blood pressure was stronger in women in our study, as it was in the Mexico City Diabetes Study.[22] Furthermore, CRP level predicted six-year incidence of metabolic syndrome components among women in that study.[22] Like CRP, sE-selectin concentration was correlated with markers of insulin resistance and other metabolic syndrome components in the Aboriginal population we studied.

Concentrations of CRP and, to an even greater extent, sE-selectin were inversely related to plasma levels of antioxidants derived from vegetables and fruit. Such evidence for dietary antioxidant protection against endothelial dysfunction and inflammation is consistent with previous studies. Endothelial dysfunction, induced in healthy subjects by instigating hyperinsulinaemia, can be ameliorated by the administration of vitamin C.[23] In people with peripheral arterial disease, vitamin C levels have been found to be inversely associated with CRP levels.[24] Lycopene has also been shown to reduce the expression of sE-selectin and other cell-adhesion molecules on human aortic endothelial cells in culture, and also to modulate leukocyte binding.[25] Together, these results suggest that protection from oxidative stress provided by carotenoids and other dietary components of fruit and vegetables is important for the vasculature and thus prevention of CHD. However, our cross-sectional study cannot identify cause and effect relationships, and it may be that plasma carotenoid levels (related to fruit and vegetable consumption) are markers of a generally more healthy lifestyle rather than being of specific importance in themselves. Furthermore, if inflammation and its associated metabolic abnormalities are associated with greater degrees of oxidative stress, it may be that carotenoids are being removed from circulation at a greater rate when inflammation and metabolic abnormalities are present.

Poor dietary quality has been reported in other remote Aboriginal communities[5] and, coupled with high oxidant stress in the form of high prevalences of diabetes and smoking, suggests that diet and food supply may be important contributors to excessive CHD risk. Endothelial dysfunction and CHD risk have also been linked to psychological stress,[26] catecholamine metabolism[27] and chronic conditions such as periodontal disease and *Helicobacter* and *Chlamydia* infections.[28] We believe that the inverse associations of vascular inflammatory markers with dietary antioxidants, and positive relationships to metabolic syndrome components, provide further evidence of the need for broad-based interventions, including nutrition and physical activity, such as those community-directed interventions shown to be effective.[29,30] Optimal nutrition and physical activity are, in turn, determined by issues such as food supply, education, infrastructure, social inequalities and psychosocial factors.

In conclusion, high levels of markers of inflammation and vascular endothelial dysfunction suggest these processes may be important mediators of CHD risk in Aboriginal people.

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COMPETING INTERESTS

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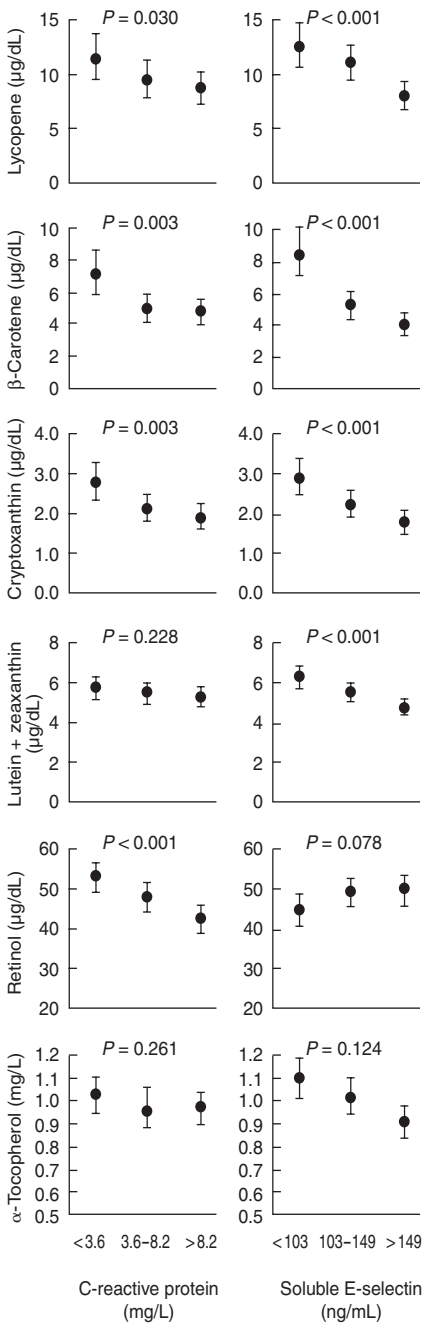
4: Association between plasma levels of dietary antioxidants and the inflammatory markers C-reactive protein and soluble E-selectin



Plasma antioxidant concentrations were stratified by tertiles of inflammatory marker levels. Data are expressed as mean or geometric mean (95% CI), adjusted for age, sex, plasma cholesterol concentration, metabolic syndrome status and smoking. P-values indicate significance of tests for linear trend in antioxidant concentrations across tertiles.

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