

Effect of Grape Seed Extract and Quercetin on Cardiovascular and Endothelial Parameters in High-Risk Subjects

Peter M. Clifton*

CSIRO Health Sciences and Nutrition, CSIRO, PO Box 10041, Adelaide BC, SA 5000, Australia

Received 3 March 2004; revised 18 May 2004; accepted 15 June 2004

Grape seed extract (GSE) has *in vitro* antioxidant activity but whether or not it works *in vivo* is not clear. In a fully randomised, crossover trial with 4-week treatment periods on 36 men and women with above-average vascular risk, we aimed to demonstrate that 2 g/day of GSE (1 g of polyphenols) alone, or with 1 g/day of added quercetin in yoghurt, favourably alters vascular function, endothelial function, and degree of oxidative damage in comparison to a control yoghurt. GSE alone improved flow-mediated dilatation determined ultrasonically by an absolute 1.1% compared with control. There was no effect of the combination of GSE with quercetin. No other blood or urine measure was altered. Thus sufficient polyphenols from GSE appear to be absorbed to influence endothelial nitric oxide production, and GSE has the potential to favourably influence vascular function.

BACKGROUND

Wine polyphenols have been postulated to have many favourable effects [1, 2, 3, 4] but most of this data has been obtained *in vitro* [5, 6]. Grape seed extracts (GSEs) contain a high concentration of many of the polyphenols in grape skins, in particular, the proanthocyanidins, which are also found in red wine. Green tea also contains polyphenols, in particular, the catechins, which are believed to mediate many of the cancer chemopreventive effects [7]. Green tea has been epidemiologically associated with protection from both cancer and heart disease [8]. Although there is abundant *in vitro* evidence that polyphenols have antioxidant and anticancer effects, there is a dearth of animal and human experiments [9]. CSIRO data (unpublished) indicates that GSEs inhibit low-density lipoprotein (LDL) oxidation and reduce aortic ring constriction *in vitro*. Extract of oligomeric proanthocyanidins from other sources such as Pycnogenol from pine bark enhances nitric oxide (NO) production from vascular endothelium *in vitro* [10]. Grape seed proanthocyanidin extract (GSPE, 0.1% level) has been shown to reduce atherosclerosis in cholesterol-fed rabbits by 30%–50% probably by inhibiting LDL oxidation as lipid levels were not altered while malondialdehyde levels in the aorta (an index of lipid oxidation) were reduced by 25% [11]. Plasma proanthocyanidins were not detectable, suggesting that absorption is very low. Rats given a dose as a single bolus do have low (0.5 μ M) but detectable levels. The dose of GSPE was equivalent to a dose of 3.5 g for a 70 kg human [12]. Most over-the-counter forms of GPSE

are in doses of 50–100 mg with a recommendation to take 1 capsule/day. This material has been available for many years and is regarded as safe and nontoxic.

Quercetin, a flavanoid prominent in onions and apples, has been epidemiologically associated with protection from coronary artery disease and cancer [13, 14] and is now available over the counter in 300–500 mg dose forms, with daily doses of up to 1500 mg. No clinical trials in the cardiovascular area have been performed with quercetin although it has been shown to inhibit monocyte adhesion to endothelial cells [15]. This is believed to be the first step in the process of atherosclerosis. One trial using 4 g/day has shown no effects on lipids, blood pressure, or platelet activation in normal volunteers [16].

In this study we hypothesised that 2 g/day of GSE would improve flow-mediated dilatation (FMD) and this might be mediated by changes in the production of NO. We also hypothesised that GSE and quercetin would function as antioxidants in plasma, reduce the level of F2 isoprostane in urine, and possibly influence the level of oxidized LDL in plasma and, secondary to this, reduce the activation of the endothelium. This would be assessed by changes in adhesion, clotting, and fibrinolytic molecules.

METHODS

Subjects

Forty-three men and women with above-average vascular risk due to high cholesterol, smoking, or high blood pressure were recruited by public advertisement

and screened at the Clinical Research Unit, CSIRO Health Sciences and Nutrition, Adelaide. There were no exclusion criteria on the basis of medication or consumption of alcohol. Subjects were excluded if their body mass index (BMI) was greater than 35 or if they suffered from diabetes mellitus, untreated metabolic disorders such as thyroid or adrenal disease, liver or kidney disease, or unstable coronary artery disease. All subjects gave written, informed consent and the protocol was approved by the Human Ethics Committee of CSIRO.

The trial was 12 weeks long and consisted of 3 four-week periods of a double-blind randomised crossover with control and active ingredients (1 g GSE from Tarac +/- 0.5 g quercetin) in 240 g of yoghurt taken twice daily. Blood samples and vascular compliance measures were taken at baseline and at the end of each period. The background diet was a low-polyphenol, low-quercetin diet. This was achieved by restricting tea and coffee to a maximum of 2 cups per day, restricting apples to one per day, and forbidding red wine and onions throughout the 12 weeks. Measures included FMD using ultrasound, vascular compliance using radial pulse analysis (Hypertension Diagnostics Inc/PulseWave CR-2000), fasting lipids, oxidized LDL, nitrates (to assess the antioxidant effects), C reactive protein (CRP), von Willebrand factor (VWF), tissue-type plasminogen activator (tPA), plasminogen activator inhibitor 1 (PAI-1), vascular cell adhesion molecule (VCAM1), and intercellular adhesion molecule 1 (ICAM-1). Twenty-four-hour urine was collected to measure the oxidized lipid isoprostane $F_{2\alpha}$.

FMD was assessed in the brachial artery after blockage of blood flow in the forearm with a blood pressure cuff at 200 mmHg for 5 minutes. The response of the vessel 5 minutes after administration of 100 μ g of glyceryl trinitrate (GTN) sublingually was also assessed [17].

Serum lipids

Serum lipids (total cholesterol, triglyceride, HDL cholesterol) were measured on 2 consecutive days at baseline and at the end of each 3-week intervention period. Venous blood samples (20 mL) were taken into plain tubes after an overnight fast of 12 hour. Serum was separated by low-speed centrifugation at 600 g for 10 minutes at 5°C (GS-6R centrifuge; Beckman, Fullerton, Calif) and frozen at -20°C. At the end of the study, all samples from each subject were analysed within the same analytic run. Total cholesterol and triacylglycerol were measured on a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland) using enzymatic kits (Hofmann-La Roche Diagnostica, Basel, Switzerland) and standard control sera. Plasma HDL-cholesterol concentrations were measured after precipitation of apoB containing lipoproteins by PEG 6000. The coefficients of variation for the individual lipids were all less than 5%. The following modification of the Friedewald equation for molar concentrations was used to calculate LDL cholesterol in mmol/L: total cholesterol—(triacylglycerol/2.18)—HDL cholesterol.

All other tests were enzyme linked immunosorbent assays (ELISAs): VWF (Helena Laboratories, Melbourne Australia), Coaliza tPA (Chromogenix, Sweden), Coaset tPA (Chromogenix), Coaliza PAI-1 (Chromogenix), Coatest PAI-1 (Chromogenix), oxidised LDL: Mercodia oxidised LDL ELISA (Mercodia, Sweden), SVCAM1 (Immunokontakt, Sweden), ICAM1 (Immunokontakt), CRP (Alpha Diagnostic International, Texas), 8-isoprostane (8-iso PGF $_{2\alpha}$) (Cayman Chemical), nitrate/nitrite assay kit (Cayman Chemical).

Statistical analysis

Repeated measures analysis of variance was calculated with type of yoghurt as the within-subject factor and with sex and order as the between-subject factors. Where there was a significant treatment effect detected by repeated measures, paired Student *t* tests were used to locate differences. Bivariate correlation was conducted using Pearson's correlation coefficient. Analyses were performed with SPSS 10.0 for Windows (SPSS Inc, Chicago, Ill). Significance was set at $P < .05$.

RESULTS AND DISCUSSION

Twelve women and twenty-four men completed the study and one additional woman missed the last phase of treatment. Six subjects withdrew after commencement and 6 withdrew prior to commencement.

The risk profile of subjects was as follows: 6 subjects had high blood pressure (5 on medication), 3 were smokers, and 31 had high cholesterol (greater than 5 mmol/L on finger prick). Two volunteers on atorvastatin to lower cholesterol stopped the medication prior to beginning the trial. The average cholesterol was 6.5 mmol/L (range 4.68 to 8.63), average age 58 years (range 34–70), weight 83.1 Kg (63.1 kg to 118.7 kg), BMI 28.4 (19.8–37.5). Mean blood pressure was 127 mm Hg systolic and 74 mm Hg diastolic.

Blood pressure/vascular compliance

There was a weak ($P < .05$) trend to a lowering of systolic blood pressure over the duration of the trial with a fall from 127 mm Hg at baseline to 124 mm Hg at week 12. This is quite usual in clinical trials in which blood pressure is measured. There were no changes in any vascular parameter with treatment (see Table 1).

Flow-mediated dilatation after compression release and GTN dilatation

GSE alone produced an absolute 1.1% greater dilatation compared with control ($P < .05$) but the addition of quercetin apparently nullified this completely. GTN-induced dilatation was not influenced by GSE but quercetin again appeared to diminish the response compared with baseline ($P < .05$), but not compared with control.

TABLE 1. Cardiovascular measures produced by the HDI compliance instrument; mean of 35 complete measures \pm SD.

	Baseline	GSE	GSE/quercetin	Control
Systolic BP (mmHg)	127 \pm 15	124 \pm 14	125 \pm 11	124 \pm 13
Diastolic BP (mmHg)	74 \pm 9	73 \pm 8	73 \pm 10	73 \pm 9
Mean BP (mmHg)	94 \pm 13	91 \pm 18	94 \pm 12	91 \pm 12
Pulse pressure (mmHg)	53 \pm 9	51 \pm 8	51 \pm 7	51 \pm 8
Pulse rate (beats/min)	58 \pm 8	59 \pm 8	58 \pm 7	57 \pm 7
Estimated cardiac ejection time (ms)	335 \pm 25	337 \pm 24	336 \pm 23	333 \pm 36
Estimated stroke volume (mL)	93 \pm 13	92 \pm 12	93 \pm 12	94 \pm 12
Estimated stroke volume index (mL/m ²)	47 \pm 5	47 \pm 4	47 \pm 4	47 \pm 6
Estimated cardiac output (L/min)	5.4 \pm 0.7	5.5 \pm 0.7	5.5 \pm 0.7	5.4 \pm 0.7
Estimated cardiac output index (L/min/m ²)	2.8 \pm 0.2	2.7 \pm 0.3	2.8 \pm 0.3	2.7 \pm 0.2
Large artery elasticity index	17.5 \pm 4.6	18.4 \pm 4.8	18.7 \pm 6.1	18.2 \pm 4.7
Small artery elasticity index	7.4 \pm 3.8	7.4 \pm 3.4	7.9 \pm 3.9	7.4 \pm 3.2
Systemic vascular resistance	1364 \pm 275	1345 \pm 229	1364 \pm 221	1352 \pm 209
Total vascular impedance	131 \pm 32	124 \pm 33	125 \pm 35	127 \pm 35

TABLE 2. Flow-mediated dilatation as measured by ultrasound. $N = 35$, mean SD. Treatments with different superscripts are different at $P < .05$.

	Baseline	GSE	GSE/quercetin	Control
Precompression cm ⁻²	44.3 \pm 6.3	45.1 \pm 6.4	45.9 \pm 7.2	45.5 \pm 7.3
Postcompression	46.2 \pm 5.8	47.4 \pm 6.5	47.6 \pm 7.5	47.3 \pm 7.3
	($n = 30$)	($n = 35$)	($n = 32$)	($n = 36$)
Change	1.9 ^{1,2} \pm 1.3	2.3 ¹ \pm 1.4	1.7 ² \pm 1.0	1.8 ² \pm 1.3
	(4.3%)	(5.1%)	(3.7%)	(4.0%)
Pre-GTN	44.8 \pm 7.1	45.8 \pm 7.1	46.9 \pm 7.5	46.2 \pm 7.1
Post-GTN	52.1 \pm 6.8	52.7 \pm 6.9	52.9 \pm 7.3	52.8 \pm 7.0
	($n = 38$)	($n = 30$)	($n = 29$)	($n = 31$)
Change	7.3 ¹ \pm 2.4	6.9 ^{1,2} \pm 2.3	6.0 ² \pm 3.0	6.5 ^{1,2} \pm 1.8
	(16.3%)	(15.1%)	(12.8%)	(14.1%)

TABLE 3. Effect of GSE and GSE/quercetin on serum lipids mean (mmol/L) \pm SD.

	Period 1 baseline	Period 2	Period 3	Period 4	GSE	GSE/quercetin	Control
Total cholesterol	6.57 \pm 1.07	6.63 \pm 1.06	6.59 \pm 0.99	6.58 \pm 1.06	6.63 \pm 0.93	6.64 \pm 1.10	6.64 \pm 1.05
Triglyceride	1.80 \pm 0.80	1.98 \pm 1.06	1.90 \pm 0.83	1.73 \pm 0.88	1.88 \pm 0.92	1.88 \pm 0.85	1.92 \pm 1.03
HDL cholesterol	1.18 \pm 0.31	1.18 \pm 0.31	1.19 \pm 0.34	1.16 \pm 0.33	1.18 \pm 0.33	1.16 \pm 0.34	1.15 \pm 0.31
LDL cholesterol	4.59 \pm 0.98	4.56 \pm 0.93	4.55 \pm 0.93	4.65 \pm 0.98	4.61 \pm 0.83	4.62 \pm 1.00	4.63 \pm 0.99

This indicates that GSE favourably influences the endothelium enhancing NO production, release or slowing down oxidative destruction of it, but quercetin appears to interfere with this. It is known that quercetin inhibits LPS-induced NO release in RAW 264.7 macrophages [18] and can act as a prooxidant in other systems [19, 20, 21] at both low and high levels (see Table 2).

Serum lipids

No changes were noted but none were expected. Subjects were at high risk of cardiovascular disease by virtue of the high average cholesterol (see Table 3).

C reactive protein

CRP is an acute-phase protein produced by the liver in response to tissue damage or inflammation and may increase 500 fold acutely [22]. It is also elevated but to low levels by low-grade inflammatory conditions such as atherosclerosis and can be used to predict clinical events [23]. Statins which lower cholesterol by inhibiting synthesis in the liver also lower CRP and the mechanism appears to be unrelated to the degree of cholesterol lowering [24]. It may be related to their antioxidant activity or a direct anti-inflammatory activity. Thus in this study it was used to check both for potential toxic effects and to

TABLE 4. Effect of GSE and GSE/quercetin on plasma CRP, nitrate/nitrite, and adhesion molecules. *N* = 35, mean SD.

	Period 1 baseline	Period 2	Period 3	Period 4	GSE	GSE/quercetin	Control
CRP (mg/L)	—	3.63 ± 5.01	3.48 ± 4.19	3.69 ± 4.17	3.40 ± 3.53	3.63 ± 5.10	3.73 ± 4.64
Nitrate (μmol/L)	31.1 ± 12.7	28.0 ± 11.0	36.9 ± 36.7	30.0 ± 13.2	35.6 ± 36.7	30.5 ± 12.4	28.4 ± 12.7
ICAM1 (μg/mL)	—	0.49 ± 0.10	0.49 ± 0.11	0.48 ± 0.11	0.49 ± 0.11	0.48 ± 0.11	0.49 ± 0.11
VCAM1 (μg/mL)	—	0.99 ± 0.28	0.99 ± 0.22	1.00 ± 0.24	0.98 ± 0.20	0.98 ± 0.26	1.02 ± 0.27

TABLE 5. Effect of GSE and GSE/quercetin on clotting and fibrinolytic factors. *N* = 35, mean SD.

	Baseline	Period 2	Period 3	Period 4	GSE	GSE/quercetin	Control
VWF (%)	—	115.58 ± 39.12	101.66 ± 35.82	102.37 ± 35.55	109.11 ± 36.06	104.50 ± 39.58	106.00 ± 36.40
PAI-1 ng/mL	—	50.47 ± 33.51	45.22 ± 21.41	49.27 ± 31.72	50.47 ± 35.77	48.58 ± 29.24	45.90 ± 21.41
PAI-1 activity	16.45 ± 9.72	16.27 ± 9.08	16.28 ± 8.05	16.28 ± 10.37	15.39 ± 9.30	16.78 ± 10.05	16.76 ± 8.13
tPA (ng/mL)	—	7.235 ± 3.396	6.636 ± 2.350	6.857 ± 2.378	6.929 ± 2.686	6.908 ± 2.835	6.891 ± 2.766
tPA activity	1.432 ± 1.204	1.295 ± 0.865	1.140 ± 0.715	1.269 ± 1.011	1.261 ± 0.822	1.146 ± 0.764	1.297 ± 1.011
tPA/PAI-1 activity	20.37 ± 21.63	20.91 ± 21.15	24.46 ± 24.06	27.11 ± 32.45	24.12 ± 27.75	26.05 ± 29.90	23.04 ± 21.3
tPA/PAI-1 mass	—	0.205 ± 0.348	0.188 ± 0.483	0.194 ± 0.648	0.187 ± 0.119	0.206 ± 0.179	0.195 ± 0.138

demonstrate a potential of GSE to act like a statin in the vessel wall. No differences were found between periods or treatments. One person was excluded as he had a respiratory infection requiring antibiotics which caused a sharp rise in CRP levels (over a 100-fold rise) (see Table 4).

Nitrate/Nitrite

Plasma nitrate was measured as a surrogate index of NO production [25, 26]. NO is an endogenous vasodilator produced by endothelial cells. Red wine polyphenols enhance vasorelaxation and NO production in vitro [27, 28]. Ethanol itself also enhances NO production [29]. No differences were found either by period or by treatment with or without an outlier whose value rose 6-fold in the GSE period. However, dietary nitrites and nitrates can confound this measure quite easily so the absence of change does not mean that NO production did not rise (see Table 4).

Adhesion molecules

ICAM1 and VCAM1 are molecules which bind white cells to the endothelium and reflect the state of the health of the endothelium, particularly in relationship to atherosclerosis [30, 31]. If the endothelium is damaged by oxidized lipid, cigarette smoke, or high blood pressure, these markers increase [32]. An antioxidant might be expected to lower the level of these markers (vitamin E does in some studies [33]) as does a statin which lowers plasma lipid level [34]. Polyphenols from blue and red berries reduce adhesion molecules in endothelial cells in vitro [35]. GSE and GSE/quercetin had no effect, nor were there any time effects (see Table 4).

Clotting and fibrinolytic factors

VWF mediates the binding of platelets to injured vessels and protects coagulation factor VIII. It is produced in the endothelium and is released when the endothelium

is damaged by atherosclerosis, diabetes, insulin resistance, or hypertension [36, 37, 38]. Tissue-type plasminogen activator is released from endothelial cells to initiate the process of breaking down clots in the vessel by activating plasminogen to plasmin which then breaks down fibrin. Endothelial dysfunction impairs the release of active tPA [39] and is associated with an enhanced release of PAI-1 [40]. GSE and GSE/quercetin had no effect on VWF, tPA, or PAI-1 and there were no time effects. In [41] de Maat et al found no effect of black or green tea on any of the markers we measured, but wine polyphenolics have been shown in cultured human endothelial cells [42] to increase production of tPA (see Table 5).

Urine isoprostanes

8-Isoprostane (8-iso PGF₂α) is a stable end product formed from arachidonic acid by free radical action and is measurable in plasma and urine. The level is believed to represent the degree of oxidative stress in lipids [43, 44].

In this experiment, we saw no changes in urinary isoprostanes either absolute or expressed in relation to creatinine to adjust for incomplete urinary collections (see Table 6). The values measured in this study were in the range described for subjects with type 2 diabetes. Vitamin E has been shown to reduce plasma and urine isoprostanes in some studies [45, 46] but not in others [47]. Subjects with type 2 diabetes have elevated (double) level of urinary isoprostane compared with controls and it falls by 32% with treatment with vitamin E [48]. Ide et al [49] found that in healthy young men vitamin E could reduce urine isoprostane levels by 48%. Whole grains have been found to lower isoprostane by 28% in one study [50], as did fruit and vegetables in another study [51], although van den Berg found no effects of fruit and vegetables [52]. Tea polyphenols did not alter isoprostanes [53] while dealcoholised red wine reduced the levels in plasma with a trend in urine [54].

TABLE 6. Effect of GSE and GSE/quercetin on urine isoprostane (iso PGF₂α). N = 35, mean SD.

	Period 2	Period 3	Period 4	GSE	GSE/quercetin	Control
Isoprostane (pg/mL)	617 ± 348	713 ± 507	709 ± 578	668 ± 407	741 ± 644	630 ± 362
Creatinine (mmol/L)	11.59 ± 4.92	11.37 ± 5.51	12.72 ± 5.46	11.59 ± 4.92	11.69 ± 5.23	12.72 ± 5.46
Isoprostane/creatinine pg/mg	529 ± 392	561 ± 327	519 ± 328	534 ± 362	562 ± 416	513 ± 254
Isoprostane excretion ng/d	1178 ± 986	1243 ± 1220	1107 ± 743	1147 ± 407	1270 ± 1348	1112 ± 694

TABLE 7. Effect of GSE and GSE/quercetin on oxidised LDL levels U/L; N = 35, mean SD.

Period 2	Period 3	Period 4	GSE	GSE/quercetin	Control
94973±26906	94637±24862	94359±31705	95284 ± 24563	99142 ± 22728	98383 ± 22973

Although the results are negative except for the FMD changes, they are not incompatible with the current literature which is not uniform in its results. They are also compatible with the observed lack of change in the levels of oxidized LDL (see Table 7) in this study. There is no published data on measurement of oxidized LDL in plasma using this method.

There were no changes in urine chemistry, haematology, clotting, or biochemistry with GSE or GSE/quercetin. There were some time-related changes in urea and creatinine chloride and bicarbonate which may have been due to warmer weather (data not shown).

CONCLUSIONS

We have demonstrated that sufficient antioxidant polyphenols from GSE were absorbed to influence FMD but no other endothelial functions were affected. It is known from rat and rabbit studies that the absorption of proanthocyanidins from GSE is very limited. In one rat study [55], after feeding 0.25 g/kg of GSE (equivalent to feeding over 20 g to humans), a level of 18 µg/mL of dimer was achieved after 1 hour. In the rabbits, despite an equivalent dose spread over the day, no proanthocyanidins were detected, even though in this model lipid peroxidation and aortic atherosclerosis were reduced [11]. In vitro studies often use levels of 10–50 µg/mL [6] which is 10–20 times higher than what might be achieved in human studies. Quercetin is known to be absorbed and 1 g/day can produce levels 23 times higher than control capsules. Despite this level and its demonstrable in vitro antioxidant action, it has no effects on platelets or lipids [56]. Although red wine extract inhibits endothelin (ET-1) production, neither isolated red wine polyphenols (quercetin, resveratrol, D-,L-catechin, D-,L-epicatechin) nor the anthocyanins delphinidin, pelargonidin, cyanidin, peonidin, petunidin, or malvidin affect ET-1 production [6].

Although there is no data yet relating impaired FMD to cardiac events in subjects without coronary disease, those patients with coronary disease who have very impaired FMD have more events [57]. Subjects with impaired FMD are more likely to have coronary disease

on angiography [58]. Statins improve mortality and one mechanism may be via their improvement of FMD [59]. Subjects with low acetylcholine induced forearm vasodilatation are more likely to have acute events [60].

REFERENCES

- [1] St Leger AS, Cochrane AL, Moore F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet*. 1979;1(8124):1017–1020.
- [2] Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*. 1992;339(8808):1523–1526.
- [3] Aviram M, Fuhrman B. Polyphenolic flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis. *Atherosclerosis*. 1998;137 (suppl):S45–S50.
- [4] Das DK, Sato M, Ray PS, et al. Cardioprotection of red wine: role of polyphenolic antioxidants. *Drugs Exp Clin Res*. 1999;25(2-3):115–120.
- [5] Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*. 1993;341(8843):454–457.
- [6] Corder R, Douthwaite JA, Lees DM, et al. Endothelin-1 synthesis reduced by red wine. *Nature*. 2001;414(6866):863–864.
- [7] Chung FL, Schwartz J, Herzog CR, Yang YM. Tea and cancer prevention: studies in animals and humans. *J Nutr*. 2003;133(10):3268S–3274S.
- [8] Hollman PC, Feskens EJ, Katan MB. Tea flavonols in cardiovascular disease and cancer epidemiology. *Proc Soc Exp Biol Med*. 1999;220(4):198–202.
- [9] Higdon JV, Frei B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit Rev Food Sci Nutr*. 2003;43(1):89–143.
- [10] Fitzpatrick DF, Bing B, Rohdewald P. Endothelium-dependent vascular effects of Pycnogenol. *J Cardiovasc Pharmacol*. 1998;32(4):509–515.
- [11] Yamakoshi J, Kataoka S, Koga T, Ariga T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis

- in cholesterol-fed rabbits. *Atherosclerosis*. 1999;142(1):139–149.
- [12] Baba S, Osakabe N, Natsume M, Terao J. Absorption and urinary excretion of procyanidin B2 [epicatechin-(4beta-8)-epicatechin] in rats. *Free Radic Biol Med*. 2002;33(1):142–148.
- [13] Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet*. 1993;342(8878):1007–1011.
- [14] Knekt P, Kumpulainen J, Jarvinen R, et al. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr*. 2002;76(3):560–568.
- [15] Koga T, Meydani M. Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. *Am J Clin Nutr*. 2001;73(5):941–948.
- [16] Ferry DR, Smith A, Malkhandi J, et al. Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. *Clin Cancer Res*. 1996;2(4):659–668.
- [17] Faulx MD, Wright AT, Hoit BD. Detection of endothelial dysfunction with brachial artery ultrasound scanning. *Am Heart J*. 2003;145(6):943–951.
- [18] Wadsworth TL, Koop DR. Effects of the wine polyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages. *Biochem Pharmacol*. 1999;57(8):941–949.
- [19] Anderson D, Dhawan A, Yardley-Jones A, Ioannides C, Webb J. Effect of antioxidant flavonoids and a food mutagen on lymphocytes of a thalassemia patient without chelation therapy in the Comet assay. *Teratog Carcinog Mutagen*. 2001;21(2):165–174.
- [20] Sahu SC, Gray GC. Pro-oxidant activity of flavonoids: effects on glutathione and glutathione S-transferase in isolated rat liver nuclei. *Cancer Lett*. 1996;104(2):193–196.
- [21] Stadler RH, Markovic J, Turesky RJ. In vitro anti- and pro-oxidative effects of natural polyphenols. *Biol Trace Elem Res*. 1995;47(1–3):299–305.
- [22] Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today*. 1994;15(2):81–88.
- [23] Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA*. 1998;279(18):1477–1482.
- [24] Balk EM, Lau J, Goudas LC, et al. Effects of statins on nonlipid serum markers associated with cardiovascular disease: a systematic review. *Ann Intern Med*. 2003;139(8):670–682.
- [25] Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. *Clin Chem*. 1995;41(6 pt 1):892–896.
- [26] Baylis C, Vallance P. Measurement of nitrite and nitrate levels in plasma and urine—what does this measure tell us about the activity of the endogenous nitric oxide system? *Curr Opin Nephrol Hypertens*. 1998;7(1):59–62.
- [27] Stoclet JC, Kleschyov A, Andriambelosen E, Diebolt M, Andriantsitohaina R. Endothelial NO release caused by red wine polyphenols. *J Physiol Pharmacol*. 1999;50(4):535–540.
- [28] Martin S, Andriambelosen E, Takeda K, Andriantsitohaina R. Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. *Br J Pharmacol*. 2002;135(6):1579–1587.
- [29] Venkov CD, Myers PR, Tanner MA, Su M, Vaughan DE. Ethanol increases endothelial nitric oxide production through modulation of nitric oxide synthase expression. *Thromb Haemost*. 1999;81(4):638–642.
- [30] Hope SA, Meredith IT. Cellular adhesion molecules and cardiovascular disease. Part I. Their expression and role in atherogenesis. *Intern Med J*. 2003;33(8):380–386.
- [31] De Caterina R, Basta G, Lazzarini G, et al. Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 1997;17(11):2646–2654.
- [32] Mazzone A, Cusa C, Mazzucchelli I, et al. Cigarette smoking and hypertension influence nitric oxide release and plasma levels of adhesion molecules. *Clin Chem Lab Med*. 2001;39(9):822–826.
- [33] Theriault A, Chao JT, Gapor A. Tocotrienol is the most effective vitamin E for reducing endothelial expression of adhesion molecules and adhesion to monocytes. *Atherosclerosis*. 2002;160(1):21–30.
- [34] Mueck AO, Seeger H, Wallwiener D. Further evidence for direct vascular actions of statins: effect on endothelial nitric oxide synthase and adhesion molecules. *Exp Clin Endocrinol Diabetes*. 2001;109(3):181–183.
- [35] Youdim KA, McDonald J, Kalt W, Joseph JA. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *J Nutr Biochem*. 2002;13(5):282–288.
- [36] Preston RA, Ledford M, Materson BJ, Baltodano NM, Memon A, Alonso A. Effects of severe, uncontrolled hypertension on endothelial activation: soluble vascular cell adhesion molecule-1, soluble intercellular adhesion molecule-1 and von Willebrand factor. *J Hypertens*. 2002;20(5):871–877.
- [37] Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes*. 2002;51(4):1157–1165.
- [38] Weyer C, Yudkin JS, Stehouwer CD, Schalkwijk CG, Pratlley RE, Tataranni PA. Humoral markers of inflammation and endothelial dysfunction in relation

- to adiposity and in vivo insulin action in Pima Indians. *Atherosclerosis*. 2002;161(1):233–242.
- [39] Newby DE, McLeod AL, Uren NG, et al. Impaired coronary tissue plasminogen activator release is associated with coronary atherosclerosis and cigarette smoking: direct link between endothelial dysfunction and atherothrombosis. *Circulation*. 2001;103(15):1936–1941.
- [40] Huvers FC, De Leeuw PW, Houben AJ, et al. Endothelium-dependent vasodilatation, plasma markers of endothelial function, and adrenergic vasoconstrictor responses in type 1 diabetes under near-normoglycemic conditions. *Diabetes*. 1999;48(6):1300–1307.
- [41] de Maat MP, Pijl H, Kluit C, Princen HM. Consumption of black and green tea had no effect on inflammation, haemostasis and endothelial markers in smoking healthy individuals. *Eur J Clin Nutr*. 2000;54(10):757–763.
- [42] Abou-Agag LH, Aikens ML, Tabengwa EM, et al. Polyphenolics increase t-PA and u-PA gene transcription in cultured human endothelial cells. *Alcohol Clin Exp Res*. 2001;25(2):155–162.
- [43] Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ. A series of prostaglandin F₂-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci USA*. 1990;87(23):9383–9387.
- [44] Praticò D. In vivo measurement of the redox state. *Lipids*. 2001;36(suppl):S45–S47.
- [45] Huang HY, Appel LJ, Croft KD, Miller ER 3rd, Mori TA, Puddey IB. Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial. *Am J Clin Nutr*. 2002;76(3):549–555.
- [46] Dietrich M, Block G, Hudes M, et al. Antioxidant supplementation decreases lipid peroxidation biomarker F(2)-isoprostanes in plasma of smokers. *Cancer Epidemiol Biomarkers Prev*. 2002;11(1):7–13.
- [47] Meagher EA, Barry OP, Lawson JA, Rokach J, FitzGerald GA. Effects of vitamin E on lipid peroxidation in healthy persons. *JAMA*. 2001;285(9):1178–1182.
- [48] Davi G, Ciabattoni G, Consoli A, et al. In vivo formation of 8-iso-prostaglandin f₂alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation*. 1999;99(2):224–229.
- [49] Ide T, Tsutsui H, Ohashi N, et al. Greater oxidative stress in healthy young men compared with premenopausal women. *Arterioscler Thromb Vasc Biol*. 2002;22(3):438–442.
- [50] Jang Y, Lee JH, Kim OY, Park HY, Lee SY. Consumption of whole grain and legume powder reduces insulin demand, lipid peroxidation, and plasma homocysteine concentrations in patients with coronary artery disease: randomized controlled clinical trial. *Arterioscler Thromb Vasc Biol*. 2001;21(12):2065–2071.
- [51] Thompson HJ, Heimendinger J, Haegele A, et al. Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage. *Carcinogenesis*. 1999;20(12):2261–2266.
- [52] van den Berg R, van Vliet T, Broekmans WM, et al. A vegetable/fruit concentrate with high antioxidant capacity has no effect on biomarkers of antioxidant status in male smokers. *J Nutr*. 2001;131(6):1714–1722.
- [53] Hodgson JM, Croft KD, Mori TA, Burke V, Beilin LJ, Puddey IB. Regular ingestion of tea does not inhibit in vivo lipid peroxidation in humans. *J Nutr*. 2002;132(1):55–58.
- [54] Abu-Amsha Caccetta R, Burke V, Mori TA, Beilin LJ, Puddey IB, Croft KD. Red wine polyphenols, in the absence of alcohol, reduce lipid peroxidative stress in smoking subjects. *Free Radic Biol Med*. 2001;30(6):636–642.
- [55] Koga T, Moro K, Nakamori K, et al. Increase of antioxidative potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape seeds. *J Agric Food Chem*. 1999;47(5):1892–1897.
- [56] Conquer JA, Maiani G, Azzini E, Raguzzini A, Holub BJ. Supplementation with quercetin markedly increases plasma quercetin concentration without effect on selected risk factors for heart disease in healthy subjects. *J Nutr*. 1998;128(3):593–597.
- [57] Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation*. 2002;105(13):1567–1572.
- [58] Furumoto T, Fujii S, Saito N, Mikami T, Kitabatake A. Relationships between brachial artery flow mediated dilation and carotid artery intima-media thickness in patients with suspected coronary artery disease. *Jpn Heart J*. 2002;43(2):117–125.
- [59] Dupuis J, Tardif JC, Cernacek P, Theroux P. Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes. The RECIFE (reduction of cholesterol in ischemia and function of the endothelium) trial. *Circulation*. 1999;99(25):3227–3233.
- [60] Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation*. 2001;104(2):191–196.

* Corresponding author.

E-mail: peter.clifton@csiro.au

Fax: +61 8 8303 8899; Tel: +61 8 8303 8826