The Journal of Nutrition
Nutrition and Disease

Supplementation of the Pure Flavonoids Epicatechin and Quercetin Affects Some Biomarkers of Endothelial Dysfunction and Inflammation in (Pre)Hypertensive Adults: A Randomized Double-Blind, Placebo-Controlled, Crossover Trial[1,2]

James I Dower,[3,4] Johanna M Geleijnse,[3,4] Lieke Gijsbers,[3,4] Casper Schalkwijk,[3,5] Daan Kromhout,[4] and Peter C Hollman[3,4]*

[3]Top Institute Food and Nutrition, Wageningen, The Netherlands; [4]Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands; and [5]Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands

Abstract

Background: Consumption of flavonoid-rich foods such as cocoa and tea may reduce cardiovascular disease risk. The flavonoids epicatechin (in cocoa and tea) and quercetin (in tea) probably play a role by reducing endothelial dysfunction and inflammation, 2 main determinants of atherosclerosis.

Objective: We studied the effects of supplementation of pure epicatechin and quercetin on biomarkers of endothelial dysfunction and inflammation.

Methods: Thirty-seven apparently healthy (pre)hypertensive men and women (40–80 y) participated in a randomized, double-blind, placebo-controlled crossover trial. Participants ingested (-)-epicatechin (100 mg/d), quercetin-3-glucoside (160 mg/d), or placebo capsules for a period of 4 wk, in random order. Plasma biomarkers of endothelial dysfunction and inflammation were measured at the start and end of each 4-wk intervention period. The differences in changes over time between the intervention and placebo periods ($\Delta_{intervention} - \Delta_{placebo}$) were calculated and tested with a linear mixed model for repeated measures.

Results: Epicatechin changed $\Delta_{epicatechin} - \Delta_{placebo}$ for soluble endothelial selectin (sE-selectin) by −7.7 ng/mL (95% CI: −14.5, −0.83; $P = 0.03$) but did not significantly change this difference (−0.30; 95% CI: −0.61, 0.01; $P = 0.06$) for the z score for endothelial dysfunction. Quercetin changed $\Delta_{quercetin} - \Delta_{placebo}$ for sE-selectin by −7.4 ng/mL (95% CI: −14.3, −0.56; $P = 0.03$), that for IL-1β by −0.23 pg/mL (95% CI: −0.40, −0.06; $P = 0.009$), and that for the z score for inflammation by −0.33 (95% CI: −0.60, −0.05; $P = 0.02$).

Conclusions: In (pre)hypertensive men and women, epicatechin may contribute to the cardioprotective effects of cocoa and tea through improvements in endothelial function. Quercetin may contribute to the cardioprotective effects of tea possibly by improving endothelial function and reducing inflammation. This trial was registered at clinicaltrials.gov as NCT01691404. *J Nutr* 2015;145:1459–63.

Keywords: atherosclerosis, CVD, endothelial dysfunction, inflammation, epicatechin, quercetin, flavonoids, clinical trial

Introduction

Flavonoids (a subclass of polyphenols) are structurally related secondary metabolites that are ubiquitous in plant foods. Consumption of flavonoid-rich foods such as cocoa and tea are associated with a lower risk of cardiovascular disease (CVD)[6]. Data from 7 studies ($n = 114{,}009$) showed that the risk of CVD was 37% lower in subjects with the highest amount of

[1] Supported by Top Institute Food and Nutrition (TIFN) (JID, JMG, and PCH), a public-private partnership on pre-competitive research in food and nutrition. TIFN had no role in the study design, data acquisition and analysis or drafting of the manuscript, or decision to publish.
[2] Author disclosures: JI Dower, JM Geleijnse, L Gijsbers, C Schalkwijk, D Kromhout, and PC Hollman, no conflicts of interest.
* To whom correspondence should be addressed. E-mail: peter.hollman@wur.nl.

[6] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; FMD, flow-mediated dilation; MCP-1, monocyte chemotactic protein-1; SAA, serum amyloid A; sE-selectin, soluble endothelial selectin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule; vWf, von Willebrand factor.

Manuscript received February 6, 2015. Initial review completed February 25, 2015. Revision accepted April 10, 2015.
First published online May 13, 2015; doi:10.3945/jn.115.211888.

chocolate consumption (1). Likewise, data from 9 studies (n = 194,965) showed a 21% lower risk of stroke for subjects who consumed 3 cups/d of tea than for subjects who consumed <1 cup/d (2). It is suggested that the beneficial effects of flavonoid-rich foods are mediated through reduction of endothelial dysfunction and inflammation which are important early steps in the pathogenesis of atherosclerosis (3–5).

Atherosclerosis is characterized by the accumulation of lipids and fibrous elements in large arteries and is one of the main contributors to CVD (6). At sites of inflammation, activated endothelial cells release cytokines such as TNF-α, C-reactive protein (CRP), and IL-1β, IL-6, and IL-8. In response to these cytokines, adhesion molecules such as soluble endothelial selectin (sE-selectin), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), and von Willebrand factor (vWf) are synthesized by endothelial cells and induce adhesion of leukocytes to the endothelium. After adhesion, monocyte chemotactic protein-1 (MCP-1) induces the transendothelial migration of monocytes into the intima (7). Once in the intima, monocytes take on the role and properties of macrophages as they scavenge oxidized LDL, form foam cells, and cause fatty streaks and fibrous plaques, leading to atherosclerosis. Indeed, studies have shown that biomarkers of inflammation (e.g., CRP, TNF-α, and IL-6) and endothelial dysfunction (e.g., sVCAM-1, sICAM-1, and sE-selectin) are predictors of CVD risk (8–10).

A limited number of human intervention studies with a duration of 2–6 wk investigated the effects of flavonoid-rich foods, such as cocoa and tea, on biomarkers of endothelial dysfunction and inflammation. Consuming a high flavan-3-ol (the flavonoid subclass of epicatechin) cocoa beverage decreased plasma concentrations of sVCAM-1 by 11% (11) but did not change sICAM-1. In contrast, consuming 40 g of cocoa decreased serum concentrations of sICAM-1 by 10% but did not change sVCAM-1 or markers of inflammation, including IL-6 and high-sensitivity CRP (12). Similarly, consumption of dark chocolate and a cocoa drink had no effect on IL-1β, IL-6, high-sensitivity CRP, and TNF-α (13). For black tea, Hodgson et al. (14) found that consumption of 5 cups/d (1 cup = 250 mL) had no effect on the adhesion molecules E-selectin, P-selectin, sICAM-1, and sVCAM-1, which was confirmed by Steptoe et al. (15) for P-selectin. Only 1 tea intervention studied the effect on MCP-1 and found a decrease of 19% (16). A reduction in CRP of 27%–47% was found in 2 tea interventions (15, 16), whereas another did not find an effect on CRP, IL-1β, IL-6, or TNF-α (17).

Until now as shown in the above paragraph, human intervention studies have only investigated the effects of flavonoid-rich foods such as cocoa and tea on a limited number of biomarkers of inflammation and endothelial dysfunction. These foods are complex mixtures of flavonoids and other substances. To unravel the role of individual flavonoids, pure flavonoids should be studied. Cocoa and tea are rich dietary sources of epicatechin, whereas tea is the main dietary source of quercetin (18, 19). For this reason, we hypothesized that epicatechin and quercetin are responsible for the cardioprotective effects of cocoa and tea. The aim of the present study was to investigate whether pure (-)-epicatechin and quercetin could account for the beneficial effects of cocoa/chocolate and tea on inflammation and endothelial function. This was done for the first time, by measuring a comprehensive set of biomarkers of these 2 functions in a double-blind, placebo-controlled crossover study in 37 healthy (pre)hypertensive adults who ingested supplements with pure isolated (-)-epicatechin, quercetin-3-glucoside, or placebo daily for periods of 4 wk.

Methods

Study design. The study was conducted as a 3-armed, randomized, double-blind, placebo-controlled, crossover study as described previously (20). In brief, 37 apparently healthy nonsmoking men and women between the ages of 40 and 80 y with a BMI (in kg/m^2) between 20 and 40 and systolic blood pressure between 125 and 160 mm Hg took part in the study. Excluded from participation were subjects with chronic diseases, users of medication, users of prescribed diet, and pregnant or lactating women (20).

Participants ingested equimolecular amounts of (-)-epicatechin (100 mg/d = 345 μmol/d) and quercetin-3-glucoside (160 mg/d = 345 μmol/d) and placebo capsules for periods of 4 wk, in random order, separated by 4-wk washout periods. The dosage of epicatechin chosen was in line with the amount of epicatechin present in previous cocoa/chocolate intervention studies (46–107 mg/d) (21–24). For quercetin an equimolecular dose was chosen. In The Netherlands, the habitual intake of epicatechin is 11 mg/d and of quercetin is 16 mg/d (20). Subjects were asked to avoid consumption of flavonoid-rich foods (cocoa, tea, apples, onion, and red wine) during the study. They consumed a standardized low-flavonoid meal (boiled potato with a meatball and spinach) the evening before each measurement day. Fasted blood samples were collected at the research center at the start and end of each intervention period. All participants provided written consent before the start of the study. The study was approved by the Medical Ethics Committee of Wageningen University (NL 40772.081.12), and informed consent of all participants was obtained. The trial was registered at clinicaltrials.gov as NCT01691404.

Biomarkers of endothelial dysfunction and inflammation. Serum biomarkers of endothelial dysfunction (sICAM-1, sVCAM-1, sE-selectin, and MCP-1) and inflammation [CRP, serum amyloid A (SAA), TNF-α, IL-1β, IL-6, IL-8, and sICAM-1] were assessed by an electrochemiluminescence detection system and vWf by ELISA, as described previously (25). The interassay CVs ranged from 1.8% to 11.1% for all biomarkers.

Individual z scores were calculated for each biomarker by using the following formula: individual value − population mean)/population SD. Overall z scores were calculated by averaging the individual z scores according to clusters of biomarkers for endothelial dysfunction and inflammation (26, 27). The endothelial dysfunction cluster consisted of sICAM-1, sVCAM-1, sE-selectin, vWf, and MCP-1. The inflammation cluster consisted of TNF-α, CRP, SAA, IL-1β, IL-6, IL-8, and sICAM-1.

Statistical analysis. Statistical analyses were performed according to a predefined analysis plan by using SAS 9.2 (SAS Institute). Baseline variables were checked for normality of distribution and were expressed as mean ± SD for normally distributed variables and median with IQRs for skewed variables. The difference between the change during each 4-wk treatment period and the change during the 4-wk placebo period was calculated. This difference, $\Delta_{treatment} - \Delta_{placebo}$, was defined as the treatment effect of epicatechin and quercetin and was statistically tested. Treatment effects were checked for normality of distribution, and all variables were considered to be normally distributed. A linear mixed model for repeated measures (SAS, PROC MIXED) was used to test the treatment effects. Treatment and period were set as fixed effects and subject was set as random effect. Compound symmetry was used as covariant structure because this resulted in the best fit according to a likelihood ratio test. No clear carryover effect was apparent; therefore, previous treatment was not included in the model. Treatment effects are expressed as least squares mean with 95% CIs. Statistical significance was set at a 2-sided α level of 0.05. Effects of outliers were checked by performing additional analyses after removal of outliers >5 times the SD from the mean. This resulted in the removal of 1 outlier for MCP-1, vWf, IL-1β, IL-6, IL-8, TNF-α, and CRP and 2 outliers for SAA, distributed over 7 subjects.

Results

Of the 37 subjects who participated in the study, 4 subjects did not complete all 3 interventions (20). These dropouts occurred at different points in the study, meaning that 35 completed the epicatechin intervention, 35 the quercetin intervention, and 35

the placebo intervention. At the start of the study, the mean age of the study population was 66.4 ± 7.9 y. The average blood pressure (systolic blood pressure/diastolic blood pressure) was 129/75 mm Hg, and 23 subjects (62%) had a BMI > 25 (**Table 1**). Body weight remained stable throughout the study period (mean change body weight: 0.2 ± 1.3 kg).

The treatment effect of epicatechin supplementation, $\Delta_{epicatechin} - \Delta_{placebo}$, was a significant decrease of plasma sE-selectin by 7.7 ng/mL (95% CI: 14.5, 0.83; $P = 0.03$) (**Table 2**). The magnitude of this effect equaled 10% of the baseline value (Table 1). All other markers of endothelial dysfunction did not change significantly. Epicatechin supplementation did not significantly change the z score for endothelial dysfunction biomarkers ($\Delta = -0.30$; 95% CI: −0.61, 0.01; $P = 0.06$). Epicatechin had no significant effect on markers of inflammation or the z score for inflammation.

The treatment effect of quercetin supplementation, $\Delta_{quercetin} - \Delta_{placebo}$, was a significant decrease of plasma sE-selectin by 7.4 ng/mL (95% CI: 14.3, 0.56; $P = 0.03$) (Table 2). The magnitude of this effect equaled 10% of the baseline value (Table 1). No other changes in markers of endothelial dysfunction or the z score for endothelial dysfunction were seen. Quercetin significantly decreased $\Delta_{quercetin} - \Delta_{placebo}$ for IL-1β by 0.23 pg/mL (95% CI: 0.40, 0.06; $P = 0.009$) (Table 2). The magnitude of this effect equaled 29% of the baseline value (Table 1). All other markers of inflammation did not change significantly. However, quercetin supplementation significantly lowered $\Delta_{quercetin} - \Delta_{placebo}$ for the z score for biomarkers of inflammation ($\Delta = 0.33$; 95% CI: 0.60, 0.05; $P = 0.02$).

Removal of outliers did not change these effects, with the exception of vWf that decreased by 22.6% absolute (95% CI: 40.1, 5.0; $P = 0.01$) after epicatechin supplementation once the outlier was removed.

Discussion

In this randomized crossover study of 37 healthy (pre)hypertensive adults, supplementation of pure epicatechin decreased sE-selectin. All other markers for endothelial dysfunction, including the z score, did not change significantly. Supplementation of pure quercetin significantly decreased sE-selectin and IL-1β and the z score for inflammation. These data suggest that the cardiometabolic effects of quercetin and epicatechin only partly overlap, which may distinguish the effects of cocoa and tea.

When assessing markers of inflammation and endothelial dysfunction, it is important to consider within-subject biological variation (28). To minimize within-subject variation, we designed a crossover study that used a rather strict study protocol. Subjects avoided consumption of flavonoid-rich foods throughout the study and also consumed a standardized low-flavonoid meal the evening before each measurement day. To reduce diurnal variation (29), blood samples were taken at the same time of day. Despite these precautions, a large biological variation in markers of inflammation and endothelial dysfunction is evident from wide confidence intervals. Baseline values and variation of markers of inflammation and endothelial dysfunction were, however, similar to values reported in a study with younger Dutch adults ($n = 293$) that used the same analytical methods (30). Another strength of our study was the high compliance, because >98% of capsules distributed were consumed. In addition, increases in plasma epicatechin and quercetin concentrations due to supplementation also showed that the flavonoids in the capsules were absorbed (20).

Because of the number of biomarkers ($n = 11$) measured, we cannot exclude the possibility of a false-positive finding. Nevertheless, we did not correct for multiple testing because these markers are mutually dependent, and correction may hide a true effect. With the use of a Bonferroni correction for multiple testing, P values would need to be <0.005 (0.05/11) to be considered statistically significant. After this correction, the changes found would no longer be statistically significant. The z scores for biomarkers of endothelial dysfunction and inflammation were calculated because the pathogenesis of atherosclerosis is a complex process, involving numerous cytokines and adhesion molecules. As mentioned by van Bussel et al. (31), the calculation of a z score does hold some limitations because its calculation is based on the assumption that each biomarker carries a similar weight. This may not be the case because some biomarkers may outweigh others. As such, the z scores calculated here do provide pooled measures of endothelial dysfunction and inflammation biomarkers but may not optimally reflect the pathophysiology of endothelial dysfunction and inflammation.

To our knowledge, the present study is the first to assess the effects of pure flavonoids on a comprehensive set ($n = 11$) of biomarkers of endothelial dysfunction and inflammation. Only a limited number of interventions with cocoa and tea have been published, which all addressed only a few of these biomarkers at the same time.

Interventions with cocoa and tea did not find effects on E-selectin (11, 12, 14). The magnitude of the effect on E-selectin of ~7% found in the present study is similar to the effect of ~10% found for other adhesion molecules in some of the cocoa and tea interventions (11, 12). E-selectin is involved in the adhesion of leukocytes to the endothelium (32). A previous study showed that E-selectin concentrations in patients with carotid artery atherosclerosis were 15% higher than in control subjects (33). Furthermore, E-selectin was inversely associated to flow-mediated dilation (FMD), a functional marker of endothelial function (28). This suggests that the reductions in sE-selectin of 7% found after both epicatechin and quercetin

TABLE 1 Baseline characteristics of study population[1]

Characteristic	Value
Male/female, *n*/*n*	25/12
Age, y	66.4 ± 7.9
Body mass index, kg/m^2	26.7 ± 3.3
Systolic blood pressure, mm Hg	129.3 ± 14.1
Diastolic blood pressure, mm Hg	74.8 ± 9.8
IL-1β, pg/mL	0.78 (0.50–1.26)
IL-6, pg/mL	2.5 (1.8–4.7)
IL-8, pg/mL	5.3 (4.7–6.8)
TNF-α, pg/mL	7.1 (6.5–9.1)
CRP, μg/mL	0.97 (0.52–2.32)
SAA, μg/mL	2.4 (1.6–5.4)
sVCAM-1, ng/mL	427 (359–477)
sICAM-1, ng/mL	264 (242–291)
sE-selectin, ng/mL	73.6 (60.3–119)
vWf, %	144 (105–181)
MCP-1, pg/mL	284 (247–327)

[1] Data are means ± SDs or medians (IQRs), $n = 37$. Biomarkers of endothelial dysfunction and inflammation were determined in fasting plasma samples. CRP, C-reactive protein; MCP-1, monocyte chemotactic protein-1; SAA, serum amyloid A; sE-selectin, soluble endothelial selectin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; vWf, von Willebrand factor.

TABLE 2 Effects of (-)-epicatechin (100 mg, 345 μmol) and quercetin-3-glucoside (160 mg, 345 μmol) supplementation on markers of endothelial dysfunction and inflammation in (pre)hypertensive subjects[1]

	(-)-Epicatechin		Quercetin-3-glucoside	
	Treatment effect	P^2	Treatment effect	P^2
Markers of endothelial dysfunction				
sVCAM-1, ng/mL	−10 (−58, 37)	0.66	5 (−42, 52)	0.84
sICAM-1, ng/mL	−11 (−28, 4.7)	0.16	−6 (−22, 10)	0.47
sE-selectin, ng/mL	−7.7 (−14.5, −0.83)	0.03	−7.4 (−14.3, −0.56)	0.03
vWf, %	−15 (−37, 8)	0.19	−2 (−25, 20)	0.84
MCP-1, pg/mL	−28 (−77, 22)	0.27	−39 (−88, 11)	0.12
z score	−0.30 (−0.61, 0.01)	0.06	−0.20 (−0.52, 0.11)	0.20
Markers of inflammation				
IL-1β, pg/mL	−0.07 (−0.24, 0.10)	0.42	−0.23 (−0.40, −0.06)	0.009
IL-6, pg/mL	0.13 (−0.92, 1.17)	0.81	−0.31 (−1.35, 0.74)	0.56
IL-8, pg/mL	−1.1 (−3.4, 1.2)	0.35	−1.5 (−3.8, 0.8)	0.19
TNF-α, pg/mL	−0.09 (−0.84, 0.66)	0.81	−0.48 (−1.23, 0.27)	0.20
CRP, μg/mL	−1.3 (−6.0, 3.3)	0.57	−0.90 (−5.5, 3.7)	0.70
SAA, μg/mL	−6.4 (−21.1, 8.2)	0.38	−8.7 (−23.4, 6.0)	0.24
z score	−0.12 (−0.39, 0.16)	0.40	−0.33 (−0.60, −0.05)	0.02

[1] Data are least squares means; 95% CIs in parentheses (all such values) from linear mixed model for repeated measures with compound symmetry as covariant structure, $n = 35$. All biomarkers were determined in fasting plasma samples. Treatment effect, $\Delta_{treatment} - \Delta_{placebo}$, = (value biomarker at end treatment − value at start treatment) − (value biomarker at end placebo − value at start placebo). CRP, C-reactive protein; MCP-1, monocyte chemotactic protein-1; SAA, serum amyloid A; sE-selectin, soluble endothelial selectin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; vWf, von Willebrand factor.
[2] *P* for treatment effect.

supplementation could beneficially affect endothelial function and the development of atherosclerosis. However, we previously published that in this study FMD did not change significantly after epicatechin or quercetin supplementation (20). Endothelial function is a complex process that involved numerous molecules and pathways and also pathologic conditions. The lack of an effect on FMD of our supplementations, despite a decrease in sE-selectin, suggests that other molecules besides sE-selectin and other factors may play a role.

The lack of an effect of epicatechin on markers of inflammation is in line with previous chocolate and cocoa intervention studies that showed no effects on markers of inflammation (12, 13). In contrast to epicatechin, we found that quercetin supplementation decreased IL-1β and the *z* score for inflammation. To our knowledge, no studies have investigated the effects of pure quercetin on inflammation. Only a limited number of studies with tea, the main dietary source of quercetin, were reported. Two of 3 of these tea interventions found that tea suppressed CRP (15, 16). However, only 1 tea intervention measured IL-1β, IL-6, and TNF-α, which did not change (17). These results should be compared cautiously, because the dosage of pure quercetin used in the present study (100 mg/d) is notably higher than the amount of quercetin present in 4 cups (1 cup = 250 mL) of black tea (19 mg) (34).

In this intervention study, we previously reported improvements in insulin resistance after epicatechin supplementation only (20). It was suggested that endothelial dysfunction is closely related to the pathogenesis of insulin resistance and impaired glucose metabolism (5). Higher concentrations of sE-selectin and, to a lesser extent, sICAM-1 were associated with an increased risk of developing diabetes (35). Similarly, studies have shown higher concentrations of sE-selectin and sICAM-1 in subjects with insulin resistance (36–38). In the present study, the decrease in sE-selectin after epicatechin supplementation may have contributed to the improvements in insulin resistance reported previously. However, quercetin also decreased sE-selectin but did not affect insulin resistance, suggesting that other markers are also involved.

In conclusion, in (pre)hypertensive men and women, epicatechin supplementation reduced sE-selectin, a marker of endothelial dysfunction, but did not significantly change other markers of endothelial dysfunction. This suggests that epicatechin may contribute to the cardioprotective effects of epicatechin-rich foods such as cocoa and tea. Similarly quercetin supplementation reduced sE-selectin but also reduced IL-1β and the *z* score for inflammation. This suggests that quercetin may contribute to the cardioprotective effects of quercetin-rich foods such as tea by improving endothelial function and reducing inflammation. Further long-term studies are needed to confirm these results and to gain more insight into the potential mechanisms behind the proposed effects of both epicatechin and quercetin.

Acknowledgments

We thank Harrie Robins for practical assistance in performing the study, Marjo van de Waarenburg for performing biochemical analyses, and Prof. dr. Hendriek Boshuizen for statistical advice. JID, JMG, DK, and PCH designed the research; JID and LG conducted the research; JID, JMG, DK, and PCH analyzed the data; JMG, DK, and PCH supervised the study; JID, JMG, DK, and PCH wrote the paper; PCH had primary responsibility for the final content; JID, JMG, LG, CS, DK, and PCH provided critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

References

1. Buitrago-Lopez A, Sanderson J, Johnson L, Warnakula S, Wood A, Di Angelantonio E, Franco OH. Chocolate consumption and cardiometabolic disorders: systematic review and meta-analysis. BMJ 2011;343;d4488.
2. Arab L, Liu W, Elashoff D. Green and black tea consumption and risk of stroke: a meta-analysis. Stroke 2009;40:1786–92.

3. González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martínez-Augustin O, Sánchez de Medina F. Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci Nutr 2011;51:331–62.
4. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, Esposito K, Jönsson LS, Kolb H, Lansink M, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr 2011;106:S5–78.
5. Esper RJ, Nordaby RA, Vilariño JO, Paragano A, Cacharrón JL, Machado RA. Endothelial dysfunction: a comprehensive appraisal. Cardiovasc Diabetol 2006;5:4.
6. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
7. Libby P. Vascular biology of atherosclerosis: overview and state of the art. Am J Cardiol 2003;91:3A–6A.
8. Mulvihill NT, Foley JB, Murphy RT, Curtin R, Crean PA, Walsh M. Risk stratification in unstable angina and non-Q wave myocardial infarction using soluble cell adhesion molecules. Heart 2001;85:623–7.
9. Tuomisto K, Jousilahti P, Sundvall J, Pajunen P, Salomaa V. C-reactive protein, interleukin-6 and tumor necrosis factor alpha as predictors of incident coronary and cardiovascular events and total mortality. A population-based, prospective study. Thromb Haemost 2006;95:511–8.
10. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GDO, Fowkes FGR. Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh artery study. Circulation 2007;115:2119–27.
11. Wang-Polagruto JF, Villablanca AC, Polagruto JA, Lee L, Holt RR, Schrader HR, Ensunsa JL, Steinberg FM, Schmitz HH, Keen CL. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. J Cardiovasc Pharmacol 2006;47:S177–86.
12. Monagas M, Khan N, Andres-Lacueva C, Casas R, Urpí-Sardà M, Llorach R, Lamuela-Raventós RM, Estruch R. Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. Am J Clin Nutr 2009;90:1144–50.
13. Mathur S, Devaraj S, Grundy SM, Jialal I. Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. J Nutr 2002;132:3663–7.
14. Hodgson JM, Puddey IB, Mori TA, Burke V, Baker RI, Beilin LJ. Effects of regular ingestion of black tea on haemostasis and cell adhesion molecules in humans. Eur J Clin Nutr 2001;55:881–6.
15. Steptoe A, Gibson EL, Vuononvirta R, Hamer M, Wardle J, Rycroft JA, Martin JF, Erusalimsky JD. The effects of chronic tea intake on platelet activation and inflammation: a double-blind placebo controlled trial. Atherosclerosis 2007;193:277–82.
16. Oyama J, Maeda T, Kouzuma K, Ochiai R, Tokimitsu I, Higuchi Y, Sugano M, Makino N. Green tea catechins improve human forearm endothelial dysfunction and have antiatherosclerotic effects in smokers. Circ J 2010;74:578–88.
17. de Maat MP, Pijl H, Kluft C, Princen HM. Consumption of black and green tea has no effect on inflammation, haemostasis and endothelial markers in smoking healthy individuals. Eur J Clin Nutr 2000;54: 757–63.
18. Arts ICW, Hollman PCH, Feskens EJM, Bueno De Mesquita HB, Kromhout D. Catechin intake and associated dietary and lifestyle factors in a representative sample of Dutch men and women. Eur J Clin Nutr 2001;55:76–81.
19. Hertog MGL, Hollman PCH, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutr Cancer 1993;20:21–9.
20. Dower JI, Geleijnse JM, Gijsbers L, Zock PL, Kromhout D, Hollman PCH. Effects of the pure flavonoids epicatechin and quercetin on vascular function and cardiometabolic health: a randomised double-blind, placebo-controlled, crossover trial. Am J Clin Nutr 2015;101: 10.3945/ajcn.114.098590.
21. Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, Kwak HK, Milbury P, Paul SM, Blumberg J, et al. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr 2004;23:197–204.
22. Faridi Z, Njike VY, Dutta S, Ali A, Katz DL. Acute dark chocolate and cocoa ingestion and endothelial function: A randomized controlled crossover trial. Am J Clin Nutr 2008;88:58–63.
23. Farouque HMO, Leung M, Hope SA, Baldi M, Schechter C, Cameron JD, Meredith IT. Acute and chronic effects of flavanol-rich cocoa on vascular function in subjects with coronary artery disease: A randomized double-blind placebo-controlled study. Clin Sci 2006;111:71–80.
24. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. J Nutr 2008;138:1671–6.
25. van Bussel BCT, Henry RMA, Schalkwijk CG, Ferreira IF, Feskens EJM, Streppel MT, Smulders YM, Twisk JWR, Stehouwer CDA. Fish consumption in healthy adults is associated with decreased circulating biomarkers of endothelial dysfunction and inflammation during a 6-year follow-Up. J Nutr 2011;141:1719–25.
26. de Jager J, Dekker JM, Kooy A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CDA. Endothelial dysfunction and low-grade inflammation explain much of the excess cardiovascular mortality in individuals with type 2 diabetes: the Hoorn Study. Arterioscler Thromb Vasc Biol 2006;26:1086–93.
27. Yudkin JS, Stehouwer CDA, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: Associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972–8.
28. Eschen O, Christensen JH, Dethlefsen C, Schmidt EB. Cellular adhesion molecules in healthy subjects: short term variations and relations to flow mediated dilation. Biomark Insights 2008;3:57–62.
29. Osmancik P, Kvasnicka J, Widimsky P, Tarnok A. Diurnal variation of soluble E- and P-selectin, and intercellular adhesion molecule-1 in patients with and without coronary artery disease. Cardiology 2004;102:194–9.
30. van Bussel BC, Schouten F, Henry RM, Schalkwijk CG, de Boer MR, Ferreira I, Smulders YM, Twisk JW, Stehouwer CD. Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period. Hypertension 2011;58:588–95.
31. van Bussel BCT, Soedamah-Muthu SS, Henry RMA, Schalkwijk CG, Ferreira I, Chaturvedi N, Toeller M, Fuller JH, Stehouwer CDA. Unhealthy dietary patterns associated with inflammation and endothelial dysfunction in type 1 diabetes: the EURODIAB study. Nutr Metab Cardiovasc Dis 2013;23:758–64.
32. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, Holgate ST, Jönsson LS, Latulippe ME, Marcos A, et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br J Nutr 2013;109:S1–34.
33. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr., Boerwinkle E. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997;96:4219–25.
34. Hertog MGL, Hollman PCH, Van de Putte B. Content of potentially anticareinogenic flavonoids of tea infusions, wines, and fruit juices. J Agric Food Chem 1993;41:1242–6.
35. Song Y, Manson JE, Tinker L, Rifai N, Cook NR, Hu FB, Hotamisligil GS, Ridker PM, Rodriguez BL, Margolis KL, et al. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. Diabetes 2007;56:1898–904.
36. Hak AE, Pols HAP, Stehouwer CDA, Meijer J, Kiliaan AJ, Hofman A, Breteler MMB, Witteman JCM. Markers of inflammation and cellular adhesion molecules in relation to insulin resistance in nondiabetic elderly: the Rotterdam Study. J Clin Endocrinol Metab 2001;86:4398–405.
37. Matsumoto K, Miyake S, Yano M, Ueki Y, Tominaga Y. High serum concentrations of soluble E-selectin in patients with impaired glucose tolerance with hyperinsulinemia. Atherosclerosis 2000;152:415–20.
38. Weyer C, Yudkin JS, Stehouwer CDA, Schalkwijk CG, Pratley RE, Tataranni PA. Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. Atherosclerosis 2002;161:233–42.