Basic science for the clinician

Flavanols and cardiovascular disease prevention

Christian Heiss1, Carl L. Keen2, and Malte Kelm1*

1Department of Cardiology, Vascular Medicine and Pulmonology, Heinrich-Heine-University, Duesseldorf, Germany; and 2Nutrition Department, University of California, Davis, CA, USA

Received 26 February 2010; revised 8 July 2010; accepted 16 August 2010; online publish-ahead-of-print 18 September 2010

Introduction

Diet is a lifestyle factor that plays a major role in the primary and secondary prevention of numerous chronic diseases, including myocardial infarction, stroke, and diabetes. Epidemiological studies suggest that the beneficial cardiovascular health effects of diets rich in fruits and vegetables are in part mediated by their flavonoid content, with particular benefits provided by one member of this family, the flavanols. This concept is supported by findings from small-scale intervention studies with surrogate endpoints including endothelium-dependent vasodilation, blood pressure, platelet function, and glucose tolerance. Mechanistically, short-term effects on endothelium-dependent vasodilation following the consumption of flavanol-rich foods, as well as purified flavanols, have been linked to an increased nitric oxide bioactivity in healthy humans, and those with increased cardiovascular risk. The critical biological target(s) for flavanols have yet to be identified and the extent to which these acute results are important in the context of long-term human health is unknown. While flavanols represent a promising class of food components with respect to their ability to lower cardiovascular risk the flavanol-rich foods used in many trials have been poorly defined with respect to their flavanol content and flavanol-isomer profile; several studies have lacked appropriate controls, and the long-term randomized controlled intervention trials with flavanol-rich foods are missing. Thus, while the literature regarding flavanols and vascular health is encouraging, more in-depth and well-controlled clinical and experimental studies are needed to better define the potential protective vascular effects of these nutrients and their therapeutic value in cardiovascular medicine.

Keywords
Flavanols • Endothelial function • Nitric oxide • Flow-mediated dilation
subgroup of the flavonoid family (Figure 1), are a focus of attention as epidemiological investigations have shown an independent inverse correlation between the dietary intake of flavanol-rich foods and CAD mortality. While the epidemiological findings are provocative, no randomized controlled trials with hard clinical endpoints have been published that corroborate a cause and effect relationship between the intake of flavanols and vascular health.

However, several controlled human dietary intervention studies with flavanol-rich foods and beverages have demonstrated positive effects, including the recovery of endothelial function, improvements in insulin sensitivity, decreased blood pressure, and reductions in platelet aggregation. The pharmacological mechanisms of action of flavanols have yet to be identified, but they likely include an enhancement in nitric oxide (NO) bioactivity, modulation of the immune system, and enhanced endothelial homeostatic vascular repair. While it has been speculated that the intrinsic antioxidant capacity of flavanols, and flavonoids in general, underlies their positive vascular effects (refs 22,23 and references therein), this is as unlikely, although select flavonoids may influence the overall level of oxidative stress through secondary mechanisms.

Quantitatively, flavanols represent a major group of flavonoids in the western diet. Major sources include chocolate and cocoa (up to 920–1220 mg/100 g), apples (up to 120 mg/200 g), and tea (up to 300 mg/infusion), however, it must be noted that the profile of flavanols (e.g. (−)-epicatechin, (+)-epicatechin, (−)-catechin, (+)-catechin) in these foods can vary considerably, and it can be changed as a consequence of food processing. Importantly, the methodologies that are typically used to measure flavanols in foods, as well as in biological fluids, do not provide information on the profile of flavanol stereoisomers. The average daily flavanol intake of an adult has been approximated to be in the range of 50–100 mg. However, there is considerable confusion in the literature, as many authors when reporting dietary intakes do not make a distinction between flavanols per se (which are by definition monomers) and procyanidins, which are oligomers of flavanols. Generally, when viewed as a composite, the flavanol monomers (−) and (+)-epicatechin, (−)- and (+)-catechin are approximately 10% of the combined monomer and oligomer total. We suggest that the pooling of monomers and procyanidins when presenting dietary intake data is inappropriate, given that while monomers and dimers are absorbed in the small intestine, the longer oligomers are not absorbed. Thus, the longer oligomers are unlikely to have direct effects on the vascular endothelium, although we note they may have biological effects within the intestinal track (e.g. they might influence the microbiota, or act as immune modulators). Depending on many factors, peak monomer and

Figure 1 (A) Basic structures and examples of the main subclasses of dietary flavonoids. (B) Whereas the majority of flavanols are present as oligomers in food (i.e. cocoa), metabolized flavanol monomers are the dominant flavanols in blood and may be partly responsible for the observed vascular effects.
dimer plasma concentrations in the nanomolar and low micromolar range are reached at 1–2 h after the ingestion of a flavanol/dimer-rich meal.\textsuperscript{30} As a result of phases I and II metabolism during absorption and liver passage, the majority of circulating flavanol monomers are methylated, glucuronidated, and/or sulfated metabolites. Importantly, the metabolite profile that occurs in blood is influenced by the profile of the flavanol isomers that is present in the consumed food/beverage (Figures 1B and 2).\textsuperscript{31} In contrast to the monomers, dimers are not thought to be extensively modified subsequent to their absorption.\textsuperscript{32} The biological activities of dimers and the extent to which these activities are influenced by dimer type (e.g. A type vs. B type) are a subject of active research.

**Epidemiological evidence**

Epidemiological studies suggest that the chronic consumption of a diet rich in plant-based foods is associated with a reduced incidence of CAD, stroke, and myocardial infarction.\textsuperscript{3,4,33} The positive health effects of fruits and vegetables are often attributed to their macronutrient profile: they are typically low in fat, high in fiber, have low sodium/potassium ratios, and low-energy densities. Fruits and vegetables can also be important sources of a number of health-promoting micronutrients. These include essential nutrients (e.g. vitamin C, vitamin E, potassium, magnesium), as well as numerous phytochemicals whose bioactivities are poorly defined. Multiple investigators have reported an inverse correlation between the intake of total flavonoids, as well as specific flavonoid classes, and the incidence of cardiovascular mortality.\textsuperscript{10} A review of 15 cohort studies examined the relationship between dietary flavonoid intakes and the risk for developing CAD.\textsuperscript{10} Thirteen of these prospective studies demonstrated a protective effect of flavanols, procyanidins, flavones, and flavanones in the context of deadly and non-deadly CAD with a reduction of mortality of up to 65%.\textsuperscript{10}

Three prospective cohort studies (Zutphen Elderly Study\textsuperscript{34–36} and Iowa Women’s Health Study,\textsuperscript{7,37} European Prospective investigation into Cancer and Nutrition\textsuperscript{38}) and one retrospective anthropological study\textsuperscript{39} provide evidence for a primary protective effect of flavanols and procyanidins. In the Zutphen Elderly Study, 806 Dutch elderly men were followed over 15 years. The calculated dietary flavonoid intake was inversely associated with a reduction in ischaemic heart disease mortality with a risk ratio of 0.49 in the highest tertile of daily flavanol intake (average intake 124 ± 15 mg/d) as compared with the lowest tertile (average intake of 25 ± 40 mg/d).\textsuperscript{36} While the above paper is widely, and appropriately, accepted as a landmark study, a limited number of foods were included in the analysis, thus the flavonoid intakes that were reported in this study are lower than what was actually consumed. The prospective Iowa Women’s Health Study followed 34 489 postmenopausal women who were free of cardiovascular disease at inclusion. The results published after 13\textsuperscript{37} and 16\textsuperscript{7} years of follow-up differed slightly in their food classification and analysis, and as a result provide interesting insights into the types of foods that may be driving the epidemiological results. An inverse association between coronary heart disease mortality and catechin and epicatechin intake, as defined by intake of tea, apples and pears, and chocolate, was initially demonstrated.\textsuperscript{37} The analysis
at 16 years of follow-up continued to show reduced cardiovascular risk with increased intakes of foods the authors defined as procyanidin-rich (as noted above procyanidins are oligomers of flavanols; foods in this category included chocolate, apples and seeded grapes), however, as a separate class, the dietary intake of flavanols was not associated with a reduced risk. The flavanol-rich foods in their analysis were identified as apples, red wine, and green tea. The seeming discrepancy of the findings between Arts et al. and Mink et al. with respect to flavanols per se is likely due in part to differences in how the data from chocolate and seeded grapes were treated. In the paper by Mink et al. the flavanol intake data from these foods were pooled with the procyanidin intake data from these foods. Importantly, the analysis of the 16 years data continued to show an inverse association between cardiovascular mortality and high chocolate and fruit intake. Recently, Buijsse et al. extended these findings to middle-aged Germans of both sexes in the Potsdam Arm of the European Prospective Investigation into Cancer and Nutrition. The authors reported an inverse relationship between chocolate consumption and cardiovascular disease risk (myocardial infarction, stroke, 8 years follow-up) in a large cohort (n = 19 357) of middle-aged German participants of both sexes, without cardiovascular disease at inclusion. They observed that in the quartile characterized by the lowest chocolate consumption (1.7 g/day) 106 myocardial infarctions and strokes occurred, whereas 61 events occurred (combined relative risk of 0.61) in the quartile with the highest chocolate consumption (7.5 g/day). In the latter group, both systolic and diastolic blood pressures were significantly lower (1 mmHg) than the referent low-chocolate consumption quartile. Baseline blood pressure explained 10–12% of the risk reduction. Counter intuitive to the idea that a high vegetable intake is beneficial, the subgroup with the lowest risk was the group with the lowest vegetable intake while also having the highest chocolate intake. A limitation of this study is that chocolate consumption was only estimated as one item on the food-frequency questionnaire, making a more qualified evaluation of the associated intake of potential bioactive compounds, including flavanols, impossible. In addition, the flavanol/procyanidin content of cocoa/chocolate (as well as other flavanol/procyanidin-rich foods) products can be markedly influenced by food processing, as well as agricultural practices. This is an important issue that must be considered when evaluating data from epidemiological studies.

Evidence in support of the concept that a high intake of dietary flavanols and procyanidins is associated with positive vascular health effects is provided by studies investigating the mortality resulting from cardiovascular events in the Kuna Indians, who live on the San Blas Islands off the coast of Panama. Traditionally, the Kuna, who reside on the islands, consume large volumes of cocoa on a daily basis, and as a group they are characterized by a low incidence of age-related hypertension. When Kuna migrate from the islands to the mainland, they tend to decrease their cocoa intake, and adopt a more westernized diet that is relatively low in flavonoids. Following their movement to the mainland, they typically develop age-related hypertension. That the high cocoa consumption of the Kuna living on the islands can be linked to their reduced risk for hypertension is supported by the observation that urinary nitrite and nitrate excretion, potential NO markers, are higher in Kuna living on the islands than for those living on the mainland. It has been reported that cardiovascular mortality of the Kuna is considerably lower than that of other Pan-American populations (9 vs. 83 age-adjusted deaths/100 000). The determinants of this effect seem to be predominantly environmental rather than genetic, given that this protection is lost on migration of Kuna Indians to Panama City.

Recently, a prospective study (Stockholm Heart Epidemiology Program) was published that reported a secondary preventive effect of chocolate intake in a group of 1169 non-diabetic patients after first acute myocardial infarction with a mean follow-up of 8 years. In this study, a high chocolate intake was associated with a reduction in cardiac mortality in patients that consumed 50 g of chocolate twice or more per week, with a hazard ratio of 0.34. While the above epidemiological studies are promising, epidemiological studies inherently deliver associations. Thus, they are limited with respect to proving cause and effect relationships, or in giving mechanistic insights as the observed associations may be due to strong confounders. An important potential confounder in this context is reporting bias. Subjects with an unhealthy lifestyle may report lower chocolate intake as they may feel guilty about it. In several cases, nutrition intervention studies based on hypotheses generated from epidemiological studies have been disappointing, as exemplified by the report that vitamin E and C supplementation did not reduce major cardiovascular events the Physicians’ Health Study II.

Clinical intervention studies

While the data from epidemiological studies can be directional, randomized controlled clinical intervention studies are the preferred method for generating the evidence that is needed to causally link increased dietary flavanol consumption with effective cardiovascular disease prevention. Key questions that should be considered when conducting such trials include, what are the meaningful endpoints that need to be studied and what populations should be used? Ideally, flavanol-rich foods should be given to large groups of subjects in a randomized placebo-controlled study. Study populations should include subjects with (secondary prevention) or without (primary prevention) cardiovascular disease, and clinical hard endpoints including death, myocardial infarction, stroke, but also hospitalization or clinical symptoms, should be studied over a long-time frame. The study populations should be large enough to address age and sex considerations and to ensure that the results are applicable to the public. To our knowledge, no such trials have been conducted with any member of the flavonoid family. However, several small-scale and short-term clinical studies have been performed with cardiovascular surrogate endpoints thought to reflect key pathophysiological relevant entities. These studies are useful as they can help to elucidate the mechanisms by which flavanols mediate their pleiotropic effects, as these surrogate parameters reflect key pathophysiological entities implied in cardiovascular disease development and progression. These endpoints include, but are limited to, endothelial vasomotor function, blood pressure, blood lipids, glucose tolerance, and activity of platelets, inflammatory and circulating progenitor cells.
Independent of the endpoint studied, certain limitations apply to many of the flavonoid feeding studies that have been conducted to date. For example, many of the papers in this area describe studies that lack proper food/beverage controls; they often provide minimal compositional analysis of the foods that are being tested; the studies are often of limited duration; and in many cases there is a lack of relevant study populations. Ideally, the control food/beverage should be indistinguishable from the intervention by means of taste and appearance to assure proper blinding and exclude bias. The controls should be matched for micro- and macronutrient composition, including other potential bioactive compounds, such as theobromine and caffeine in the case of cocoa or chocolate. Illustrative of the above, in several studies where the putative positive health effects of a flavonoid-rich chocolate was being studied, a white or milk chocolate was used as a control for a ‘high flavanol’ dark chocolate. The use of this type of ‘control’ clearly prohibits blinding, and these food products, in addition to having differences in their flavonoid content, vary in their content of numerous other nutrients and bioactive compounds such as methylxanthines. Similar to the above example, in many tea studies, water has been used as a ‘control’. The lack of appropriate food/beverage controls in studies evaluating the putative vascular effects of specific flavonoids is a significant issue that needs to be addressed in a systematic manner. Until this is done, progress in the field will be hampered. Another difficulty with the interpretation of the collection of studies done to date is that when evaluating the information on the flavanol content of the test foods used, it is evident that the amounts vary considerably across studies, a fact that complicates the comparison of the results from the investigations. Another impediment to a comprehensive interpretation of the studies reported to date is that in several cases, plasma concentrations of flavanols and flavanol metabolites are not reported. In the cases where values are reported, there is minimal standardization as to when the blood samples were collected. This is an issue in the case of flavanols, as the metabolic turnover of these nutrients is very fast with a half-life of approximately 1–2 h. We suggest that one reason the importance of dietary flavanols with respect to vascular health has received minimal attention is the common use of fasted samples in clinical settings, as well as in many epidemiological studies. With the caveats noted above, below we review some of surrogate endpoints that have been studied in short-term flavonol studies. We primarily cite literature from the cocoa/chocolate field, as the test foods used in many of these studies have been better characterized than what is typically reported in studies with tea or grape products.

### Endothelial function

The endothelium maintains vascular homeostasis through multiple complex interactions with cells in the vessel wall and lumen. It regulates vascular tone by balancing the production of vasodilators, most importantly NO, prostaglandins, and endothelium-derived hyperpolarizing factor, and vasoconstrictors including endothelin-1 (Figure 3). The severity of endothelial dysfunction relates to a patient’s risk for experiencing an initial or recurrent cardiovascular event.43 A number of interventions known to decrease cardiovascular risk, including diets rich in plant foods, exercise, smoking cessation, weight reduction, medication with angiotensin-converting enzyme (ACE) inhibitors, and statin administration are thought to mediate their vascular protective effect in part by restoring endothelial function.43,44

Several clinical intervention studies have tested the effect of flavanol-rich foods including cocoa, dark chocolate, tea, grape

---

**Figure 3** Pharmacodynamics—summary of potential targets for flavanol-mediated vascular effects. (A and B) Cardiovascular risk factors induce endothelial dysfunction and lead to arteriosclerosis and facilitate cardiovascular events by inducing endothelial damage and interaction with platelets, WBCs, and EPCs. (C) Key signaling cascades by which the endothelium modulates its physiological functions and by which flavanols may modulate vascular function and structure. *Clinical human studies, †human ex vivo, #animal studies, ‡in vitro studies (ADMA, asymmetrical dimethylarginine, A I, angiotensin I; A II, angiotensin II, ACE, angiotensin-converting enzyme; AT1, angiotensin receptor 1, CACs, circulating angiogenic cells; COX, cyclooxygenase; EC, endothelial cells; ECE, endothelin-converting enzyme; EDHF, endothelium derived hyperpolarizing factor; EMPs, endothelial microparticles; EPCs, endothelial progenitor cells; ET-1, endothelin 1; GC, guanylyl cyclase; NO, nitric oxide; NOX, NADPH oxidases; PGL, prostaglandin; SMC, smooth muscle cells; O2–, superoxide anion; WBCs, white blood cells).
Regenerative processes: impact on circulating angiogenic cells

Bone marrow-derived circulating angiogenic cells (CACs; also referred to as early endothelial progenitor cells) may contribute to the recovery and maintenance of endothelial function. Primary and secondary preventive lifestyle interventions and statin therapy can enhance endogenous endothelial repair mechanisms by stimulating CAC mobilization. It has been reported that 2–4 weeks of flavanol-rich foods can mobilize EPCs in CAD patients, healthy smokers and non-smokers. In a randomized double-blind study in patients with coronary artery disease a cocoa–flavanol intervention (30 days)-related increase in FMD and decrease in blood pressure was paralleled by a significant increase in CD34+/KDR and CD133/KDR-CACs. Similarly, in healthy smokers, green tea (8 g/day) ingestion (one armed study) over 2 weeks led to a significant increase in FMD and EPCs. Interestingly, a smaller amount of this green tea (5 g/day) over 4 weeks failed to increase EPCs in haemodialysis patients despite significantly increased FMD. Huang et al. showed that 3 weeks of 300 ml red wine consumption in healthy subjects led to a significantly higher number of EPCs that was paralleled by increased FMD response, NOX levels, and decreased asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of NOS, however, other alcohol-containing drinks (beer and vodka) were used as controls, prohibiting proper blinding.

Blood pressure, lipids, glucose tolerance, and inflammation

Aside from flavanol-associated effects on conduit artery vasodilation, several studies have shown that there can be effects on blood pressure. Studies by Taubert, Grassi, and others suggest that in healthy subjects, patients with untreated essential hypertension, and pre-hypertension, blood pressure can be reduced following the consumption of dark chocolate compared with white chocolate. In the study by Taubert et al., participants with untreated essential hypertension received 6.3 g of dark chocolate per day over 18 weeks, and a significant drop in systolic and diastolic blood pressure was observed. While the results that are reported are provocative, a limitation of this study is that white chocolate was used as a control food product, thus the study was not blinded. Furthermore, the different compositions of white and dark chocolate make it difficult to identify specific bioactives, such as flavanols, in the context of blood pressure lowering. We suggest that at present we do not have conclusive data from sufficiently controlled interventional trials to support the notion that flavanols possess antihypertensive properties.

Similar limitations apply to several studies that have reported that the consumption of flavanol-rich foods can result in reductions in LDL cholesterol, and improvements in glucose tolerance. While these observations are provocative, they need to be confirmed in well-controlled studies. Multiple studies suggest that the acute consumption of flavanol-rich foods/beverages can also result in an inhibition of platelet activity and aggregation, and inhibit monocyte and neutrophil activation in vitro and ex vivo. It has also been reported that the chronic consumption of a high flavanol diet can result in a reduction in select pro-inflammatory markers. While space constraints do not allow for an in-depth review of this literature, these observations support the concept that dietary flavanols have positive vascular effects through a multiplicity of mechanisms. We note that the seemingly diverse actions of flavanols are likely interrelated. Illustrative of this are the observations that an increase in NO can result in a reduction in platelet activation, as can improvements in glucose tolerance.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study group</th>
<th>Study period</th>
<th>Study design</th>
<th>Intervention vehicle (dosing paradigm)</th>
<th>Flavanol (total) dosing</th>
<th>Vascular effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agewall et al.</td>
<td>2000</td>
<td>Healthy volunteers (n = 12)</td>
<td>1 h</td>
<td>Cross-over</td>
<td>Red wine vs. dealcoholized red wine (250 ml)</td>
<td>1950 vs. 1110 mg total polyphenols</td>
<td>FMD+ (dealcoholized red wine)</td>
</tr>
<tr>
<td>Alexopoulus et al.</td>
<td>2008</td>
<td>Healthy volunteers (n = 14, 50% smokers)</td>
<td>2 h</td>
<td>Cross-over</td>
<td>Green tea (6 g) vs. caffeine vs. water</td>
<td>nd</td>
<td>FMD+ (only green tea)</td>
</tr>
<tr>
<td>Engler et al.</td>
<td>2004</td>
<td>Healthy volunteers (n = 21)</td>
<td>14 days</td>
<td>Parallel groups</td>
<td>High flavanol vs. low flavanol dark chocolate (46 g/day)</td>
<td>213 vs. 0 mg</td>
<td>FMD+, LDL nc, ORAC nc, 8-isoprostanes nc</td>
</tr>
<tr>
<td>Fisher et al.</td>
<td>2003</td>
<td>Healthy volunteers (n = 27)</td>
<td>4 days</td>
<td>1 arm</td>
<td>High flavanol vs. matched low flavanol cocoa (4 x 230 ml/day)</td>
<td>821 mg/day</td>
<td>PAT+, BP nc</td>
</tr>
<tr>
<td>Grassi et al.</td>
<td>2009</td>
<td>Healthy males (n = 19)</td>
<td>7 days</td>
<td>Cross-over</td>
<td>Black tea (3 times/day, 5 doses)</td>
<td>0, 100, 200, 400 and 800 mg tea flavonoids/days</td>
<td>FMD+ (dose-dependent), BP − (not dose-dep.)</td>
</tr>
<tr>
<td>Hampton et al.</td>
<td>2010</td>
<td>Healthy volunteers (n = 10)</td>
<td>1 h</td>
<td>Cross-over</td>
<td>Red grape juice vs. red grape juice plus vodka vs. water, postprandial</td>
<td>nd</td>
<td>FMD+ (juices similar), Gluc. nc, Trigl. nc</td>
</tr>
<tr>
<td>Berry et al.</td>
<td>2010</td>
<td>Obese healthy volunteers (n = 21)</td>
<td>2 h</td>
<td>Cross-over</td>
<td>High flavanol vs. matched low flavanol cocoa</td>
<td>701 vs. 22 mg</td>
<td>FMD+, Exercise BP −</td>
</tr>
<tr>
<td>Heiss et al.</td>
<td>2005</td>
<td>Healthy smokers (n = 4)</td>
<td>2 h</td>
<td>Cross-over</td>
<td>High flavanol cocoa (50, 100, 200 ml) vs. water (100 ml)</td>
<td>88, 176, 352 vs. 0 mg (dose response)</td>
<td>FMD+, GTN nc, RXNO+</td>
</tr>
<tr>
<td>Heiss et al.</td>
<td>2007</td>
<td>Healthy smokers (n = 6)</td>
<td>6 h</td>
<td>Cross-over</td>
<td>High flavanol vs. matched low flavanol cocoa (powder in 300 ml water)</td>
<td>36–987 mg (dose response)</td>
<td>FMD+, RXNO+, nitrite-, MDA nc, ascorbate nc, urate nc, TEAC nc</td>
</tr>
<tr>
<td>Heiss et al.</td>
<td>2006</td>
<td>Healthy smokers (n = 5)</td>
<td>24 h</td>
<td>1 arm</td>
<td>Dark chocolate (40 g)</td>
<td>nd</td>
<td>FMD+</td>
</tr>
<tr>
<td>Hermann et al.</td>
<td>2008</td>
<td>Healthy smokers (n = 20)</td>
<td>2 h</td>
<td>Parallel groups</td>
<td>Dark vs. white chocolate (40 g)</td>
<td>nd</td>
<td>FMD+ (black and green equal), GTN nc</td>
</tr>
<tr>
<td>Jochmann et al.</td>
<td>2006</td>
<td>Healthy postmenopausal women (n = 21)</td>
<td>14 days</td>
<td>1 arm</td>
<td>Green tea (8 g/day)</td>
<td>744 mg</td>
<td>FMD+, EPIC+</td>
</tr>
<tr>
<td>Schroeter et al.</td>
<td>2006</td>
<td>Healthy volunteers (n = 10)</td>
<td>4 h</td>
<td>Cross-over</td>
<td>High flavanol vs. matched low flavanol cocoa (powder in 300 ml water)</td>
<td>917 vs. 35 mg</td>
<td>FMD+, PAT+, GTN nc, RXNO+</td>
</tr>
<tr>
<td>Loke et al.</td>
<td>2008</td>
<td>Healthy volunteers (n = 6)</td>
<td>6 h</td>
<td>Cross-over</td>
<td>(−)-Epicatechin (in 3 ml/kg water)</td>
<td>0, 1, 2 mg/kg</td>
<td>FMD+, PAT+</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>2010</td>
<td>Healthy volunteers (n = 80)</td>
<td>21 days</td>
<td>Parallel groups</td>
<td>Red wine (100 ml/day), beers 250 ml/day, vodka (80 ml/day), water (100 ml/day)</td>
<td>nd</td>
<td>FMD+, EPIC+, EPC fct+, NOX+, ADMA −</td>
</tr>
<tr>
<td>Vlachopoulos et al.</td>
<td>2005</td>
<td>Healthy volunteers (n = 17)</td>
<td>3 h</td>
<td>Cross-over</td>
<td>Dark chocolate vs. water (100 g)</td>
<td>2620 mg</td>
<td>FMD+, BP nc, HR−, Aix−, PWV nc, MDA nc, TEAC nc</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Author et al.</th>
<th>Year</th>
<th>Study group</th>
<th>Study period</th>
<th>Study design</th>
<th>Intervention vehicle (dosing paradigm)</th>
<th>Flavanol (total) dosing</th>
<th>Vascular effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgson et al.</td>
<td>2002</td>
<td>Mild serum cholesterol or triglycerides elevation (n = 21)</td>
<td>28 days</td>
<td>Parallel groups</td>
<td>Black tea vs. water (5x250 ml)</td>
<td>nd</td>
<td>FMD+, GTN+, BP nc, F2-Isoprostane excretion nc</td>
</tr>
<tr>
<td>Wang-Polagruto et al.</td>
<td>2006</td>
<td>Hypercholesterolemic postmenopausal women (n = 32)</td>
<td>42 days</td>
<td>Parallel groups</td>
<td>High flavanol vs. matched low flavanol cocoa</td>
<td>446 vs. 43 mg/day</td>
<td>FMD+, sVCAM-1</td>
</tr>
<tr>
<td>Grassi et al.</td>
<td>2005</td>
<td>Untreated essential hypertension (n = 20)</td>
<td>15 days</td>
<td>Cross-over</td>
<td>Dark (100 g/day) vs. white chocolate (90 g/day)</td>
<td>88 (epicatechin plus catechin) vs. 0 mg</td>
<td>FMD+, BP−, HOMA-IR−, QUICKI+, ISI+, LDL−</td>
</tr>
<tr>
<td>Grassi et al.</td>
<td>2008</td>
<td>Hypertensives with impaired glucose tolerance (n = 19)</td>
<td>15 days</td>
<td>Cross-over</td>
<td>Dark (100 g/day) vs. white chocolate (90 g/day)</td>
<td>88 (epicatechin plus catechin) vs. 0 mg</td>
<td>FMD+, BP−, HOMA-IR−, QUICKI+, ISI+, CIR−, LDL−</td>
</tr>
<tr>
<td>Taubert et al.</td>
<td>2003</td>
<td>Untreated mild systolic hypertension (n = 13)</td>
<td>14 days</td>
<td>Cross-over</td>
<td>Dark (100 g/day) vs. white chocolate (90 g/day)</td>
<td>500 vs. 0 mg polyphenols</td>
<td>BP−</td>
</tr>
<tr>
<td>Taubert et al.</td>
<td>2007</td>
<td>Untreated essential hypertension (n = 44)</td>
<td>126 days</td>
<td>Parallel groups</td>
<td>Dark vs. white chocolate (6.3 g/day)</td>
<td>30 vs. 0 mg polyphenols</td>
<td>BP−, GSNO+, 8-Isoprostanes nc</td>
</tr>
<tr>
<td>Davison et al.</td>
<td>2010</td>
<td>Untreated mild hypertension (n = 52)</td>
<td>42 days</td>
<td>Parallel groups</td>
<td>High flavanol cocoa drink</td>
<td>33, 372, 712, 1052 mg/day</td>
<td>BP− (not dose-dep.)</td>
</tr>
<tr>
<td>Flammer et al.</td>
<td>2007</td>
<td>Heart transplant recipients (n = 22)</td>
<td>2 h</td>
<td>Parallel groups</td>
<td>Dark vs. ‘flavonoid free’ chocolate (40 g)</td>
<td>47 mg (epicatechin plus catechin) vs. 0 mg</td>
<td>CA diameter+, CA vasodilation−, platelet adhesion−, 8-isoprostane−, TRAPnc, FRAP−</td>
</tr>
<tr>
<td>Heiss et al.</td>
<td>2003</td>
<td>CAD or CVRF (n = 6)</td>
<td>6 h</td>
<td>Cross-over</td>
<td>High flavanol vs. matched low flavanol cocoa (100 ml)</td>
<td>176 vs. &lt;10 mg</td>
<td>FMD+</td>
</tr>
<tr>
<td>Heiss et al.</td>
<td>2010</td>
<td>CAD (n = 16)</td>
<td>28 days</td>
<td>Cross-over</td>
<td>High flavanol vs. matched low flavanol cocoa (100 ml)</td>
<td>176 vs. &lt;10 mg</td>
<td>FMD+, GTN nc, RXNO+, Nitrite nc, Nitrate nc, BP nc</td>
</tr>
<tr>
<td>Widlansky et al.</td>
<td>2007</td>
<td>CAD (n = 44)</td>
<td>14 days</td>
<td>Cross-over</td>
<td>EGCG vs. placebo capsules</td>
<td>750 vs. 18 mg/day</td>
<td>FMD+, CAC+, BP−, Nitrite−</td>
</tr>
<tr>
<td>Stein et al.</td>
<td>1999</td>
<td>CAD (n = 15)</td>
<td>14 days</td>
<td>1 arm</td>
<td>Purple grape juice (7.8 ml/kg/day)</td>
<td>300 vs. 0 mg/day</td>
<td>FMD+ (acute), nc (chronic)</td>
</tr>
<tr>
<td>Duffy et al.</td>
<td>2001</td>
<td>CAD (n = 66)</td>
<td>28 days</td>
<td>Cross-over</td>
<td>Black tea vs. water (900 ml/day)</td>
<td>118 vs. 0 mg/day</td>
<td>FMD+, GTN+, LDL oxidation−</td>
</tr>
<tr>
<td>Balzer et al.</td>
<td>2008</td>
<td>Diabetics (n = 10, 100% CAD)</td>
<td>6 h</td>
<td>Cross-over</td>
<td>High flavanol vs. matched low flavanol cocoa (powder in water)</td>
<td>963, 371, 75 mg (dose response)</td>
<td>FMD+, GTN nc, BP nc</td>
</tr>
<tr>
<td>Balzer et al.</td>
<td>2008</td>
<td>Diabetics (n = 41, 71–80% CAD)</td>
<td>30 days</td>
<td>Parallel groups</td>
<td>High flavanol vs. matched low flavanol cocoa (powder in water three times/day)</td>
<td>963 vs. 75 mg/day</td>
<td>FMD+, GTN nc, BP nc</td>
</tr>
<tr>
<td>Farouque et al.</td>
<td>2006</td>
<td>CAD (n = 40)</td>
<td>42 days</td>
<td>Parallel groups</td>
<td>Flavanol rich chocolate (48 g) and cocoa drink (100 ml) vs. low flavanol products</td>
<td>440 vs. 20 mg/day</td>
<td>FMD nc, GTN nc, FBF nc, BP nc</td>
</tr>
<tr>
<td>Park et al.</td>
<td>2009</td>
<td>Chronic renal failure, dialysis (n = 40)</td>
<td>28 day</td>
<td>Parallel groups</td>
<td>Green tea (5 g/day) vs. water</td>
<td>465 vs. 0 mg</td>
<td>FMD+, EPC uc, HOMA nc, QUICKI nc, hsCRP nc, Fibrinogen nc (C. Heiss et al.)</td>
</tr>
</tbody>
</table>

(AIx, augmentation index; BP, blood pressure; CA, coronary artery; CAC, circulating angiogenic cells; EPC, CAD, coronary artery disease; CIR, corrected insulin response; CVRF, cardiovascular risk factor; EPC, endothelial progenitor cells = CAC; FBF, forearm blood flow; FMD, flow-mediated dilation; FRAP, ferric-reducing ability of plasma; GTN, glycerol trinitrate-mediated dilation; HR, heart rate; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; LDL, low-density lipoproteins; MDA, malondialdehyde; nc, not changed; nd, not determined; ORAC, oxygen radical absorbance assay; PAT, peripheral artery tonometry; QUICKI, quantitative insulin sensitivity check index; RXNO, nitroso NO adducts in plasma; TEAC, Trolox equivalent antioxidant capacity; TBARS, 2-thiobarbituric acid reactive substance; PWV, pulse wave velocity; ÷, increase; −, decrease).
Potential mechanisms contributing to flavanol-induced changes in endothelial vascular health: experimental evidence

The mechanisms by which flavanols mediate their vascular effects are not fully understood. As supported by clinical data, animal, and in vitro studies, short-term effects may be related to an increase in NOS activity. The precise mechanism and biological target structure(s) or receptor(s) have not been identified in vivo. Besides direct effects on eNOS, inhibitory effects on pathways that may negatively affect NOS activity including NADPH oxidase, ACE, ADMA, and endothelin-1 have been proposed to be affected by flavanols.

The oral administration of flavanol-rich food extracts (red wine polyphenol extract over 7–10 days) or the flavanol monomer catechin to rats led to increased acetylcholine NOS-dependent vasodilation, NO production, and decreased superoxide (O2•−) production of aortic rings isolated from these animals along with a significant decrease in blood pressure. These results were corroborated by Ramirez-Sanchez et al. showing that nano-micromolar concentrations of (−)-epicatechin acutely stimulate eNOS activity in human coronary endothelial cells and a membrane bound acceptor was proposed. Schnorr et al. reported that (−)-epicatechin, as well as select metabolites of cocoa flavanols, inhibits the expression of arginase-2 in cultured endothelial cells. An effect of flavanols on arginase was also demonstrated in vivo, providing evidence that a flavanol-rich intervention can result in an increase of arginase activity in human red blood cells and rat kidney ex vivo. These data suggest flavanols have the potential to increase NOS activity by increasing the availability of the substrate L-arginine. Notably, mono methylated flavanol metabolites were shown to enhance NO bioactivity by attenuating its degradation via O2•−. In a cell culture model, Steffen et al. reported that 3′-O-methyl-epicatechin is a bioactive metabolite of epicatechin that can inhibit NADPH oxidase, reducing O2•− generation through this system. The authors reported that, under these conditions, there was an increase in steady-state NO levels as indicated by DAF-2DA fluorescence. The authors reported that the methyl-ation of epicatechin in endothelial cells is required to inhibit angiotensin II mediated increases in NADPH oxidase dependent O2•− formation. However, this mechanism has yet to be demonstrated in vivo. Endothelins (ETs), a family of vasoactive peptides, are rapidly produced by endothelial cells in response to tissue injury and play a major role in vascular dysfunction and vascular disease. Whereas (−)-epicatechin did not inhibit ET-1 synthesis in vitro, oral application of epicatechin lowered ET-1 levels along with increasing NO species in healthy human subjects. Together with the results from studies showing that aortic rings do not dilate in the presence of flavanol monomers, the partly discrepant results from in vitro and in vivo systems imply that flavanol metabolites, rather than the parent molecule, trigger many of the critical biological activities that are pertinent with respect to vascular health.

In the context of long-term effects, general patterns of cellular adaptive or regenerative responses have to be considered on the overall tissue/organ level as well as on an individual cell level with the eNOS axis likely being only one of the pathways involved. Based on the results from murine models, it has been suggested that the regular ingestion of flavanols and flavanol extracts can inhibit aortic plaque size by up to 40%, inhibit the expression of inflammatory cytokines, and reduce I/R injury with a 30% reduction in infarct size.

While the above mechanistic work is of interest, several limitations have to be kept in mind when evaluating experimental studies aiming at understanding the mechanisms by which flavanols mediate their vascular effects. With respect to in vitro models, including cell culture systems and isolated vessel systems such as aortic rings, limitations include the relevance of flavanol compounds that are used (e.g. parent compounds vs. metabolites), and the concentrations of the flavanol/flavanol metabolite used in the experimental system. Regarding the relevance of flavanol compounds used in experimental set-ups, flavanol absorption and metabolism need to be considered and accounted for. As noted above, the majority of flavanols’ present in most foods, such as cocoa are present as oligomers (procyanidins), rather than monomers, and it is now well estab-

Conclusions

While flavanols represent a promising class of food components that are associated with lower cardiovascular risk and can positively affect cardiovascular surrogate parameters in the short term, epidemiological studies are prone to confounders and no long-term randomized controlled dietary intervention trials with hard clinical endpoints are available to date. More clinical and
experiments are needed before flavonols can be considered as a feasible therapeutic option in cardiovascular medicine.

Funding
M.K. is supported by the Deutsche Forschungsgemeinschaft (Ke405/5-1). C.H. receives research funding from the Forschungskommission of the Heinrich-Heine University, Duesseldorf, C.H., M.K., and C.L.K. have received unrestricted research funding from Mars Inc and are senior investigators in the FLAVIOA research consortium of the European Union (FP7-RIBBE-2008-2B, Targeted delivery of dietary flavanol for optimal human cell function: effects on cardiovascular health).

Conflict of interest: none declared.

References


