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Cocoa, Blood Pressure, and Cardiovascular Health

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ABSTRACT: High blood pressure is an important risk factor for cardiovascular disease and cardiovascular events worldwide. Clinical and epidemiological studies suggest that cocoa-rich products reduce the risk of cardiovascular disease. According to this, cocoa has a high content in polyphenols, especially flavanols. Flavanols have been described to exert favorable effects on endothelium-derived vasodilation via the stimulation of nitric oxide-synthase, the increased availability of L-arginine, and the decreased degradation of NO. Cocoa may also have a beneficial effect by protecting against oxidative stress alterations and via decreased platelet aggregation, decreased lipid oxidation, and insulin resistance. These effects are associated with a decrease of blood pressure and a favorable trend toward a reduction in cardiovascular events and strokes. Previous meta-analyses have shown that cocoa-rich foods may reduce blood pressure. Long-term trials investigating the effect of cocoa products are needed to determine whether or not blood pressure is reduced on a chronic basis by daily ingestion of cocoa. Furthermore, long-term trials investigating the effect of cocoa on clinical outcomes are also needed to assess whether cocoa has an effect on cardiovascular events. A 3 mmHg systolic blood pressure reduction has been estimated to decrease the risk of cardiovascular and all-cause mortality. This paper summarizes new findings concerning cocoa effects on blood pressure and cardiovascular health, focusing on putative mechanisms of action and "nutraceutical " viewpoints.

KEYWORDS: *cocoa, blood pressure, cardiovascular health, polyphenols, endothelium*

INTRODUCTION

Cardiovascular disease represents a continuum that starts with risk factors such as hypertension and progresses to endothelial dysfunction, atherosclerosis, target organ damage, and ultimately to myocardial infarction, heart failure, stroke, or death.[1−3] Because risk of cardiovascular disease is linear throughout the entire range of blood pressure (BP), a large segment of the population, although not classically defined as "hypertensive", may still be at risk.[2] Therefore, lowering BP, even in the normal range, through dietary means may decrease the rate of end-organ damage caused by hypertension.[1,2] Lifestyle modifications, including dietary habits, have substantial effects on risk factors for cardiovascular disease such as hypertension.[2] Increased consumption of fruitsand vegetables has been recommended as a key component of a healthy diet for the prevention of cardiovascular diseases.[4−6] The beneficial effects of fruits and vegetables have been largely ascribed to their content in flavonoids.[4−6] In fact, a large body of evidence supports the dietary intake of polyphenols,particularly of flavonoids and the specific class of flavonoids named flavanols that are largely contained in cocoa beans, might be able to exert some beneficial vascular effects, reduce the risk for cardiovascular morbidity and mortality, and contribute to the prevention of other chronic diseases.[6−9] Among phytochemicals, polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom, with more than 8000 phenolic structures.[6−10] They occur in a variety of fruits, vegetables, seeds, flowers, beverages, and even some manufactured foods as a component of the natural ingredients used. Cocoa is specifically rich in polyphenols, and the total polyphenol content of the cocoa bean has been evaluated to be 6−8% by weight of the dry bean.[6−11]

There exist several mechanisms of how cocoa flavonoids may be protective against cardiovascular disease; these include antioxidant, antiplatelet, and anti-inflammatory effects, as well as possibly increasing high-density lipoprotein (HDL), decreasing BP, and improving endothelial function.[6−10] These effects might be responsible for the reported substantial reduction in cardiovascular risk.

In this brief review we aim to discuss the clinically relevant cardiovascular effects of cocoa, particularly focusing on the BP responses to cocoa and the potential clinical implications associated with its consumption.

EVIDENCE FROM EPIDEMIOLOGICAL STUDIES

With regard to the effects of cocoa on BP, a key study is represented by the Amerinds living on the San Blas Islands off the coast of Panama. Of note, authors have shown that these native Kuna Indians, daily drinking several servings of unprocessed cocoa, may represent the only known society with regular daily sodium intake that is free from age-related increments in BP and presents a very low incidence of hypertension [prevalence of hypertension was 2.2% and reflected minimal criteria with a mean systolic blood pressure (SBP) = 140 mmHg and diastolic blood pressure (DBP) < 85 mmHg] and BP did not rise with age. Conversely, all of these features disappeared in Kuna Indians who moved to the

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mainland of Panama (the overall prevalence of hypertension was 10.7% and was an especially striking, 45.1%, in subjects older than 60 years of age, and BP rose significantly with age; $p < 0.001$) and consumed cocoa from commercial stores and consequent drank either lower amounts of flavanol-rich cocoa beverages or no flavanols at all.[12] Furthermore, in the Kuna residents of the suburban community of Kuna Nega, all findings were intermediate but resembled those of urban Panama City more than the isolated islands in overall hypertensive prevalence (l0%), prevalence in those over 60 years of age (35 9%), and rise of average BP with age ($p < 0.001$). The absent age-related rise in BP and the low prevalence of hypertension might be attributed to environmental rather than familial or genetic factors, as BP rose with age and hypertension prevalence rose strikingly among Kuna who had moved to Panama City.

This suggested that migration favored cultural changes with a decrease in cocoa consumption, making cocoa potentially responsible for the observed changes in BP.[12]

A BP-lowering effect of chocolate was also suggested by the Zutphen Elderly Study.[13] In this cohort of 470 elderly men, free of chronic diseases, BP was measured at baseline and 5 years later, and causes of death were evaluated during 15 years of follow-up. Even after multivariate adjustment (also for food intake), the mean SBP in the highest tertile of cocoa intake (>2.30 g/day) was 3.7 mmHg lower [95% confidence interval (CI), −7.1 to −0.3 mmHg; $p = 0.03$ for trend] and the mean DBP was 2.1 mmHg lower (95% CI, −4.0 to −0.2 mmHg; $p = 0.03$ for trend) compared with the lowest tertile (<0.36 g/day). After adjustment for age, BMI, lifestyle factors, drug use, and food and calorie intake, the relative risk for cardiovascular mortality for men in the highest tertile of cocoa intake was 0.50 (95% CI, 0.32−0.78; $p = 0.004$ for trend) and 0.53 (95% CI, 0.39−0.72; $p < 0.001$) for all-cause mortality.[13] Of further note, the authors reported that in the studied population cocoa intake was positively associated with calorie intake. However, they did not observe a positive association of cocoa intake with BMI or physical activity. Therefore, data from this study suggested for the first time that cocoa intake was inversely associated with BP levels and cardiovascular and all-cause mortality.[13] Accordingly, the same research group[14] evaluated a large cohort of 19357 middle-aged German participants of both sexes, without cardiovascular disease at inclusion. After a mean follow-up of 8 years the authors observed the relative risk of the combined outcome (myocardial infarction and stroke) for the quartile with the highest chocolate consumption (7.5 g/day) versus the quartile with the lowest chocolate consumption (1.7 g/day) was 0.61 (95% CI 0.44−0.87; p linear trend = 0.014). Furthermore, chocolate consumption was related to lower SBP and DBP in a linear manner. The difference between top and bottom quartiles was 1.0 mmHg for SBP (95% CI, 21.6 to 20.4 mmHg; p linear trend = 0.0008) and 0.9 mmHg for DBP (95% CI, 21.3 to 20.5 mmHg; p linear trend < 0.0001), also after adjustment for age and sex, lifestyle variables, indicators of socioeconomic status, dietary factors, and the prevalence of type 2 diabetes.[14] Results were substantially similar after excluding participants with diabetes at baseline. Baseline BP explained 12% of the inverse relationship between chocolate and the combined outcome of myocardial infarction and stroke. These estimates were 16% for myocardial infarction and 10% for stroke. Surprisingly, despite the well-known association between high vegetable intake and cardiovascular benefits, the subgroup with the lowest risk was also the group with the lowest vegetable intake, while also having the highest chocolate intake.[14] Concordantly, Cassidy et al.[15] evaluated the association between habitual flavonoid intake and incident hypertension in men and women, involving a total of 87242 women from the Nurses' Health Study (NHS) II, 46672 women from the NHS I, and 23043 men from the Health Professionals Follow-Up Study (HPFS). Mean amounts of flavan-3-ols ranged from 50.1 to 61.7 mg of flavan-3-ols/day [interquartile range (IQR), 12.0−72.0 mg of flavan-3-ols/day] across cohorts, whereas mean anthocyanin intakes ranged from 12.5 to 15.2 mg of anthocyanin/day (IQR, 4.6−19.3 mg of anthocyanin/day). During 14 years of follow-up were reported 29018 cases of hypertension in women and 5629 cases of hypertension in men. A high anthocyanin intake was associated with an 8% decreased risk of hypertension (quintile 5 compared with quintile 1, relative risk, 0.92; 95% CI 0.86, 0.98; p for trend < 0.03). The magnitude of the association was greater (12%) in participants <60 years of age (quintile 5 compared with quintile 1, relative risk 0.88; 95% CI 0.84, 0.93; p for trend < 0.001; p for age interaction = 0.02). With regard to the flavan-3-ol subclass, in analyses restricted to participants <60 years of age, lower rates of hypertension were observed in participants in the highest versus lowest quintiles of catechin (7%; 95% CI 3%, 12%; $p = 0.002$) and epicatechin (5%; 95% CI 0%, 9%; $p = 0.05$) intakes. In all participants, there was evidence of an effect modification of the epicatechin and hypertension association (p for sex interaction = 0.03); in women, the relative risk was 0.95 (95% CI 0.92, 0.99; $p = 0.015$).

Contrasting with the above, in the Seguimiento Universidad de Navarra Study[16] chocolate intake was not associated with the incidence of hypertension in a cohort of healthy university graduates. The authors indicated that differences with respect to previous studies could be the result of cocoa- and flavanol-poor chocolate intake by the general population. They also reported that chocolate consumption was significantly associated ($p < 0.001$, adjusted for age and sex) with snacking (individuals did not consume chocolate in isolation but in an indulgent dietary pattern with high-energy food intake) in the studied population.[16]

EXPERIMENTAL AND CLINICAL EVIDENCE: COCOA EFFECTS ON BP

Besides epidemiological evidence, in vitro as well as randomized intervention studies indicate flavonoid-rich cocoa products such as dark chocolate and cocoa beverages have BP-lowering properties. A recent study by Galleano et al.[17] investigated the possible antihypertensive effect of dietary (−)-epicatechin on spontaneously hypertensive rats (SHRs).

Consumption of a (−)-epicatechin-supplemented diet [3 g (−)-epicatechin/kg diet] decreased BP in SHR by 27 and 23 mmHg on days 2 and 6, respectively. Compared with nonsupplemented SHRs, on day 6, a 173% increase of nitric oxide synthase (NOS) activity was observed in the aorta of SHR supplemented with (−)-epicatechin ($p < 0.05$). Furthermore, evaluating the responses to acetylcholine (ACh) in femoral arteries in the absence and presence of L-NAME, a nonselective eNOS inhibitor, authors reported ACh-induced endothelium-dependent relaxation in the femoral artery was significantly higher in (−)-epicatechin-supplemented SHRs with respect to nonsupplemented SHRs, with a predominance of the NO-dependent component of this relaxation. The endothelium-independent relaxation, assayed by using the NO donor sodium nitroprusside, resulted in a nonsignificant

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difference in the three experimental groups, demonstrating an unaffected function of vascular smooth muscle cells. These results give further support to the concept that (−)-epicatechin might modulate BP in hypertension by increasing NO levels in the vasculature.

Moreover, the same group of authors[18] showed that (−)-epicatechin administration prevented the 42 mmHg increase in BP associated with the inhibition of NO production in a dose-dependent manner (0.2−4.0 g/kg diet). This BP effect was associated with a reduction in L-nitro-L-arginine-methyl ester (L-NAME)-mediated increase in the indices of oxidative stress (plasma TBARS and GSSG/GSH^2 ratio) and with a restoration of the NO concentration. At the vascular level, none of the treatments modified NOS expression, but (−)-epicatechin administration avoided the L-NAME-mediated decrease in eNOS activity and increase in both superoxide anion production and NOX subunit p47(phox) expression. Thus, (−)-epicatechin resulted in the prevention of an increase in BP and oxidative stress and restored NO bioavailability.[18]

An additional experimental study by Cienfuegos-Jovellanos et al.[19] showed that a single oral administration of natural flavonoid-enriched cocoa powder (procyanidins = 128.9 mg/g, especially monomers, dimers, and trimers = 54.1 mg/g, and mainly (−)-epicatechin = 19.36 mg/g) at different doses (50, 100, 300, and 600 mg/kg) decreased BP in SHRs but not in normotensive Wistar−Kyoto rats. The maximum effect in decreasing the SBP was caused by 300 mg/kg of cocoa powder. Interestingly, the antihypertensive effect was similar to that caused by 50 mg/kg captopril, a recognized antihypertensive agent inhibiting the angiotensin converting enzyme (ACE), thus supporting the hypothesis that, although the limitation of experimental data, flavanol-enriched cocoa powder might be used as a functional ingredient with antihypertensive effects.[19] Accordingly, Sánchez et al.[20] studied the effect of long-term intake of a soluble cocoa fiber product on the development of hypertension in SHRs. The active treatment reduced the development of hypertension, whereas the withdrawal of the cocoa intake promoted a BP increase.[20]

With regard to intervention trials, a number of clinical studies with cocoa and chocolate have involved different groups of subjects: normotensive (young, old, overweight, hypercholesterolemic), pre-hypertensive, and hypertensive with and without impaired glucose tolerance. Most of these trials reported an anti-hypertensive effect after cocoa/chocolate consumption.[21] Of note, we observed that a 15-day consumption of flavanol-rich but not flavanol-free chocolate was able to significantly lower both SBP and DBP in healthy subjects[22] in hypertensive patients without (after dark chocolate, 24-h SBP −11.9 ± 7.7 mmHg, $p < 0.0001$; 24-h DBP −8.5 ± 5.0 mmHg, $p < 0.0001$)[23] and with[24] glucose intolerance. In the latter study,[24] we observed that monitored as well as clinical SBP and DBP decreased ($p < 0.0001$) after flavanol-rich (SBP, −3.82 ± 2.40 mmHg; DBP, −3.92 ± 1.98 mmHg; 24-h SBP, −4.52 ± 3.94 mmHg; 24-h DBP, −4.17± 3.29 mmHg) but not after flavanol-free chocolate intake.[24] Furthermore, the flavanol-rich chocolate administration significantly improved NO-dependent flow-mediated dilation of the brachial artery ($p < 0.0001$). Of clinical relevance, the decrement in BP was inversely correlated with the increase in flow-mediated dilation (SBP $r = -0.547$, $p = 0.0004$; DBP $r = -0.488$, $p = 0.001$; 24-h SBP $r = -0.460$, $p = 0.003$; 24-h DBP $r = -0.457$, $p = 0.003$). In agreement with the experimental findings, these results suggested a possible pathophysiological relationship between the observed vascular and BP changes. According to this, Taubert and colleagues[25] showed additional evidence on potential BP-lowering effects of cocoa ingestion by comparing the long-term effect of dark versus white chocolate consumption in patients with pre-hypertension or stage I hypertension.[25] In this intervention study, the daily administration for 18 weeks of 6.3 g (30 kcal) of dark chocolate was able to decrease mean SBP by 2.9 ± 1.6 mmHg and DBP by 1.9 ± 1.0 mmHg. In addition, the prevalence of hypertension declined from 86 to 68%.[25] These changes were accompanied by a sustained increase in plasma markers of NO, suggesting an improved formation of this vasodilative agent as a potential mechanism of the reduction in BP following dark chocolate consumption.[25] Confirming this, Faridi et al.[26] reported that, compared with placebo, consumption of dark chocolate and sugar-free cocoa was able to significantly decrease BP levels (dark chocolate, SBP −3.2 ± 5.8 mmHg versus 2.7 ± 6.6 mmHg, $p < 0.001$; and DBP −1.4 ± 3.9 mmHg versus 2.7 ± 6.4 mmHg, $p = 0.01$; sugar-free cocoa, SBP −2.1 ± 7.0 mmHg versus 3.2 ± 5.6 mmHg, $p < 0.001$; and DBP −1.2 ± 8.7 mmHg versus 2.8 ± 5.6 mmHg, $p = 0.014$).

Furthermore, Saftlas et al.[27] determined whether regular chocolate intake during pregnancy was associated with reduced risks of pre-eclampsia and gestational hypertension. The authors reported that chocolate intake was more frequent among normotensive (80.7%) than pre-eclamptic (62.5%) or gestational hypertensive women (75.8%). Additionally, chocolate consumption was associated with reduced odds ratio (OR) of pre-eclampsia [first trimester, OR 0.55; 95% confidence interval (95% CI), 0.32−0.95; third trimester, OR, 0.56; 95% CI, 0.32−0.97] and only in first trimester was associated with reduced odds of gestational hypertension (OR, 0.65; 95% CI, 0.45−0.87).

The study of Davison and colleagues,[28] aiming at evaluating the effect of the intake of different doses of cocoa flavanols (33, 372, 712, or 1052 mg/day during 6 weeks) on 24-h mean arterial BP in untreated mild hypertensive patients, reported significant reductions in 24-h SBP (−5.3 ± 5.1 mmHg; $p = 0.001$), DBP (−3.0 ± 3.2 mmHg; $p = 0.002$), and mean arterial BP (−3.8 ± 3.2 mmHg; $p = 0.0004$) only at the highest dose studied. The same research group[29] observed that high-flavanol but not low-flavanol cocoa beverage administration was able to attenuate the BP response to exercise (DBP increase was 68% lower; $p = 0.03$ and mean BP was 14% lower; $p = 0.05$).

Of clinical relevance, we also recently reported that cocoa dose-dependently increased the NO-dependent flow-mediated dilation (FMD) ($p < 0.0001$).[30] Compared with the control, even 80 mg of cocoa flavonoids per day increased FMD ($p < 0.0001$). Moreover, with respect to control, office SBP and DBP significantly decreased during each of the 4 weeks of active treatment (SBP, mean difference −4.8 ± 1.03 mmHg, $p < 0.001$; DBP, mean difference −3.03 ± 1.07 mmHg, $p < 0.001$); SBP, but not DBP, decreased dose-dependently [significant effects for higher doses of flavonoids (>200 mg flavonoids and >42 mg of epicatechin), with a trend for dose-dependent effects for SBP]. With respect to control, cocoa flavonoids decreased SBP ($p < 0.0001$ for treatment) and DBP ($p = 0.0011$ for treatment). Accordingly, with respect to control, 24-h (mean difference −2.28 ± 1.22 mmHg) and daytime (mean difference −2.45 ± 1.57 mmHg) ambulatory SBP significantly decreased after active treatments, whereas no significant differences between treatments were observed for the monitored DBP levels. In addition, compared with control,

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Table 1. Mean Changes in SBP and DBP in Hypertensives, Pre-hypertensives, and Normotensives after Active Treatment or Placebo[a]

	treatment/control groups	difference in SBP/DBP at the end of treatment (active)	difference in SBP/DBP at the end of treatment (control)
Studies in Hypertensives			
Taubert et al. (2003)[38]	dark/white chocolate	−4.8/−1.6 mmHg	+0.4/+0.3 mmHg
Grassi et al. (2005)[23]	dark/white chocolate	−12/−7.8 mmHg	−0.7/−0.6 mmHg
Grassi et al. (2008)[24]	dark/white chocolate	−3.8/−3.9 mmHg	−0.1/−0.2 mmHg
Muniyappa et al. (2008)[39]	high-flavanol cocoa/low-flavanol drink	−2.0/−3.0 mmHg	−1.0/−4.0 mmHg
Davison et al. (2010)[28]	high/low-flavanol cocoa drinks	−4.1/−2 mmHg	−2.1/−0.1 mmHg
Studies in Pre-hypertensives			
Taubert et al. (2007)[25]	dark/white chocolate	−2.9/−1.9 mmHg	+0.1/0.0 mmHg
Heiss et al. (2010)[40]	high/low-flavanol drink	−7/?	−2/?
Studies in Normotensives			
Murphy et al. (2003)[41]	high/low-flavanol cocoa tablets	+2.0/−1.0 mmHg	+3.0/0.0 mmHg
Engler et al. (2004)[42]	high/low-flavanol chocolate	−1.0/+0.9 mmHg	−2.8/−0.1 mmHg
Fraga et al.(2005)[43]	dark/white chocolate	−6.0/−5.0 mmHg	−2.0/−1.0 mmHg
Grassi et al. (2005)[22]	dark/white chocolate	−7.0/−4.2 mmHg	−0.5/−0.3 mmHg
Crews et al. (2008)[44]	dark chocolate + cocoa drink/low-flavanol chocolate + drink	−3.5/−0.5 mmHg	−3.1/−0.6 mmHg
Davison et al. (2008)[45]	high-flavanol cocoa/low-flavanol drink	−1.9/−1.8 mmHg	+4.2/+2.8 mmHg
Al-Faris (2008)[46]	dark/white chocolate	−8.9/−5.3 mmHg	−1.3/−0.8 mmHg
Shiina et al. (2009)[47]	dark/white chocolate	+4.6/+6.6 mmHg	+4.0/+5.2 mmHg
Monagas et al. (2009)[48]	cocoa powder in milk/milk	0.0/−2.0 mmHg	−3.0/−3.0 mmHg
Njike et al. (2011)[49]	high/low-flavanol chocolate	+2.2/−0.5 mmHg	−0.1/+0.8 mmHg

[a]Studies without control and without office blood pressure measures were excluded.

24-h daytime and night-time pulse pressure (PP) and night-time heart rate significantly decreased after active treatments, but the effects did not seem to be dose-dependent.[30] Consistent with our data, Shrime et al.,[31] in a meta-analysis evaluating 24 short-term studies, showed that in response to flavonoid-rich chocolate consumption, SBP, but not DBP, decreased by −1.63 mmHg ($p = 0.033$). Finally, reviewing all of the findings regarding BP effects of cocoa and chocolate intake, an early meta-analysis[32] reported that chocolate and cocoa intake was able to significantly reduce SBP (−5.88 mmHg; −9.55, −2.21; five studies) and DBP (−3.30 mmHg; −5.77, −0.83; four studies) (effects appeared greater in studies with higher doses and shorter duration), whereas chronic intake of other flavanols (with ≥3 included studies) did not show any effect on BP. In agreement with our findings on BP, Hooper et al.[33] systematically reviewed 42 acute or short-term chronic (≤18 weeks) studies involving 1297 participants and recently reported chocolate and cocoa are able to reduce DBP and mean arterial pressure. In particular, we observed a decrease in office DBP (mean difference − 3.03 ± 1.07 mmHg; $p < 0.001$), with doses of cocoa flavonoids >500 mg and epicatechins >105 mg. Consistent with our results on DBP, the above cited meta-analysis showed significant reductions in DBP after cocoa and/or chocolate administration (−1.60 mmHg; 95% CI −2.77, −0.43 mmHg) considering 22 trials with 918 participants.

Furthermore, in line with our study on dose−responses in heathy subjects (significant effects in SBP for epicatechins >42 mg and DBP >105 mg), the meta-analysis showed (with different kinds of treatments: cocoa, chocolate, or both) that doses >50 mg of epicatechin/day reduced SBP and DBP, whereas doses <50 mg/day did not.[33]

However, Ried et al.[34] in a pooled meta-analysis of all trials (13 assessed studies) revealed a significant BP-reducing effect of cocoa−chocolate compared with control (mean BP change ± standard error, SBP −3.2 ± 1.9 mmHg, $p = 0.001$; DBP, −2.0 ± 1.3 mmHg, $p = 0.003$). However, meta-analysis of subgroups suggested a significant anti-hypertensive effect only for the hypertensive or pre-hypertensive subgroups (SBP −5.0 ± 3.0 mmHg, $p = 0.0009$; DBP −2.7 ± 2.2 mmHg, $p = 0.01$), whereas BP was not significantly reduced in the normotensive subgroups (SBP −1.6 ± 2.3 mmHg, $p = 0.17$; DBP −1.3 ± 1.6 mmHg, $p = 0.12$). Of interest, nine trials used chocolate containing 50−70% cocoa compared with white chocolate or other cocoa-free controls, whereas six trials compared high- with low-flavanol cocoa products. Daily flavanol dosages ranged from 30 to 1000 mg in the active treatment groups, and interventions ran for 2−18 weeks. In addition, meta-regression analysis showed study design and type of control to be borderline significant but possibly indirect predictors for BP outcome.[34] In contrast to previous meta-analyses, subgroup analyses by Ried et al.[34] suggested that there was a difference in outcome dependent on baseline BP (hypertensive versus normotensive). Moreover, it seems important to remark that the relatively modest but significant BP-lowering effect of cocoa observed in the hypertensive subgroup should be considered of clinical relevance. A reduction of 5 mmHg in SBP has been shown to reduce the risk of cardiovascular events by about 20% over 5 years.[1,2,35] Therefore, the effect of cocoa in hypertensive patients is comparable to other lifestyle modifications, such as moderate physical activity (30 min/day), which has been reported to decrease SBP by 4−9 mmHg.[1,2,35]

Thus, all of the above data confirmed that the ingestion of cocoa-rich and therefore flavonoid-rich chocolate might promote BP-lowering effects. However, not all of the results are univocal and in some case are even conflicting. Significant statistical heterogeneity across studies was observed, and considering the small number of subjects studied, the different quality assessment of trials and BP measurement method-

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ologies (number, accuracy, devices, etc.) and the variable dose of flavanols and/or chocolate used, a large, well-controlled, interventional study should be warranted.[30−36] Furthermore, meta-regression analyses (40) suggested study design (parallel versus crossover) and type of control (flavanol-free versus low-flavanol) could be significant predictors of BP outcome. Results of trials using flavanol-free controls, including white or milk chocolates, could be considered a potential bias for unblinded participants and might overestimate the effect of the active treatment.[36] Nevertheless, it has been recently stated that the placebo effect is an unlikely explanation for BP effects of flavanol-rich dark chocolate administered in randomized, open-label crossover studies.[36] Finally, a Cochrane review[37] combined data from 20 trials involving 856 patients. Patients using medications or other interventions to treat hypertension were included. Cocoa was consumed in several forms, including dark and milk chocolate and cocoa powder. Overall, cocoa had a statistically significant effect of lowering SBP by −2.8 mmHg and DBP by −2.2 mmHg (Table 1). Results were more significant in shorter trials, which tended to use flavonol-free control products and were not dose-dependent. In the eight trials in which the control group used a low-flavonol product, BP reduction was similar between the treatment and control groups. However, in the 12 trials in which the control group used a flavonol-free product, those in the treatment group had reductions in SBP of −3.7 and −2.7 mmHg compared with the control group. None of the studies measured health outcomes (e.g., cardiovascular events, mortality).

Treated participants received a mean of 545.5 mg of flavonols or about 50 g of cocoa per day (range = 3.6−105 g of cocoa per day). One serving of typical cocoa powder for drinking contains 5−10 g of cocoa. The BP-lowering effect was greater in persons younger than 45 years, in those who consumed <10 g of sugar per serving of cocoa product, and in those with an initial SBP ≥140 mmHg. Five percent of treated patients had adverse effects; the most common were gastrointestinal irritation and laxative effects.

COCOA AND BP: PUTATIVE MECHANISMS OF ACTION AND PERSPECTIVES

Beyond the flavanol-dependent increase in NO bioavailability likely representing the main mechanism underlying the BP reduction, NO is known to play a pivotal role in the regulation of BP and endothelial function,[3] after flavanol-rich chocolate ingestion,[21−25] the reported evidence suggested flavonoids from food might also act to reduce BP levels by modulating the renin−angiotensin−aldosterone system.[21] Supporting this, in a randomized, controlled, double-blind, crossover study, we demonstrated for the first time that only 10 g of cocoa with a very low caloric (38 kcal) and flavanol (80 mg) intake per day is already significantly ameliorating vascular function. Furthermore, we also reported that cocoa dose-dependently increased NO-mediated FMD from 6.2% (control) to 7.3, 7.6, 8.1, and 8.2% after the different flavonoid doses, respectively ($p < 0.0001$).[30] As suggested in the above meta-analysis,[33] our study also provided evidence on dose-dependent effects with respect to the epicatechin content. Regardless of the flavonoid content, we administered cocoa presented with similar color, taste, flavor, and possible vasoactive compounds. Starting from this evidence, the European Food Safety Authority (EFSA) for the first time concluded that a cause-and-effect relationship has been established between the consumption of cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.[50] With regard to the possible involvement of the renin−angiotensin−aldosterone system, Actis-Goretta et al.[51] demonstrated that incubation of purified angiotensin converting enzyme (ACE) in the presence of flavanol-rich foods resulted in a volume-dependent inhibition of the enzyme activity and high-procyanidins chocolate and chocolate presented within the lowest IV_{50} values. The IV_{50} values for each food were correlated with both the phenolic content ($R^2 = 0.73$, $p < 0.003$) and the flavanol content ($R^2 = 0.85$, $p < 0.001$). Moreover, to evaluate ACE activity inhibition closer to physiological conditions, membrane suspensions isolated from rat kidney were incubated in the presence of captopril (positive control) or (−)-epicatechin, dimer, hexamer, high-procyanidin chocolate, and low-procyanidin chocolate.[51] In this specific context, the ACE activity in kidney membrane suspensions was inhibited by 100 μM of dimer ($p < 0.001$) or hexamer ($p < 0.001$) but not by (−)-epicatechin. The use of equal volumes of high-procyanidin chocolate (634 μM (+)-catechin equivalents) and low-procyanidin chocolate (314 μM (+)-catechin equivalents) inhibited ACE activity by 70 and 45% ($p < 0.001$), respectively.

According to this, an experimental study suggested[52] a significant and dose-dependent inhibition of ACE activity in cultured human umbilical vein endothelial cells (HUVEC) after incubation with (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate. This effect was combined to yield a significant dose-dependent increment in NO production.[40] Moreover, Persson et al.[52] also recently observed a significant inhibition of ACE activity (mean 18%) 3 h after the intake of dark chocolate in healthy subjects. According to ACE genotype, significant inhibition of ACE activity was seen after 3 h in individuals with genotype insertion/insertion and deletion/deletion (mean 21 and 28%, respectively).[53] Considering the above, also confirming the hypothesis suggesting a putative involvement of endothelium and renin−angiotensin−aldosterone system in regulating BP responses after high-flavonoid cocoa intake, it has been indicated that exposure of human endothelial cells to (−)-epicatechin resulted in elevation of cellular levels of NO and cyclic GMP and in protection against oxidative stress elicited by pro-inflammatory agonists.[54] In keeping with this, the authors suggested that endothelial NO bioavailability and metabolism rather than general antioxidant activity may be a major target of dietary flavanols and that NADPH oxidase activity may represent a crucial site of action.[54] Nevertheless, Schewe et al.[54] also concluded that besides NADPH oxidase, the observed results on ACE inhibition[50−52] should be absolutely considered in the cocoa-dependent cardiovascular protection, thus supporting the hypothesis that flavanols could counteract angiotensin II in a dual way, by both inhibiting formation at the level of ACE and then decreasing the well-known pro-oxidant action of this octapeptide.[54] Accordingly, as it is known, ACE is implicated in the regulation of BP by transforming angiotensin I to angiotensin II, a potent vasopressor peptide, and pharmacological inhibition of ACE is currently considered to be a relevant therapeutic approach in treating hypertensive patients.[2] ACE inhibition decreases angiotensin II levels and the consequent activation of NAD(P)H oxidase. This could favor a lower production of oxidants, the production of which is associated with the NAD(P)H oxidase-dependent formation of superoxide anion.[3,8] Interestingly, in vivo ACE inhibition has been also associated with both increased levels of NO and decreased

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oxidative stress.[3,8] In this regard, cardiovascular risk factors significantly cause oxidative stress, which contributes to a disruption in the balance between NO and reactive oxygen species, with a resulting relative decrease in NO bioavailability. The resulting endothelial dysfunction has been supposed to be the first step of atherosclerosis also early influencing BP regulation. Furthermore, the majority of cardiovascular diseases follow from complications of cardiovascular risk factors and atherosclerosis. In addition, an important initiating event for atherosclerosis may well be the transport of oxidized low-density lipoprotein across the endothelium into the artery wall.[8] Reactive oxygen species are produced by various oxidase enzymes, including nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, uncoupled endothelial NO synthase (eNOS), cyclooxygenase, glucose oxidase, lipooxygenase, and mitochondrial electron transport.[8,55,56] An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, has been defined as "oxidative stress".[8] The term describes a metabolic condition of cells, organs, or the entire organism characterized by an oxidative overload.[8,56−58] Therefore, oxidative stress has been implicated in a number of human diseases as well as in the aging process. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms. Consistent with this, a putative controversial effect exerted by flavanols may be the antioxidant effect. According to this, metabolic modifications of flavonoids may alter their described antioxidant nature, which is defined mainly by the presence of a B-ring catechol group (dihydroxylated B-ring) capable of readily donating hydrogen (electron) to stabilize a radical species.[8,56−58] Spencer et al. showed that circulating metabolites of flavonoids, such as glucuronides and O-methylated forms, and intracellular metabolites, for example, flavonoid−GSH adducts, have a reduced ability to donate hydrogen[57] and are less effective scavengers of reactive oxygen and nitrogen species relative to their parent aglycone forms.[56−58] Different studies have suggested that although such conjugates and metabolites may participate directly in plasma antioxidant reactions and scavenge reactive oxygen and nitrogen species in the circulation, their effectiveness is reduced relative to that of their parent aglycone. In addition, it has also been described that concentrations of flavonoids and their metabolite forms accumulated in vivo in plasma and organs may be lower than those recorded for other antioxidant nutrients such as ascorbic acid and α-tocopherol.[8,59,60] Therefore, flavonoids are unlikely to express beneficial action in vivo through outcompeting antioxidants such as ascorbate.

Specifically, accumulating findings suggest that the cellular effects of flavonoids may be mediated by their interactions with specific proteins central to intracellular signaling cascades.[8,58,61]

Schroeter et al.[61] indicated that flavonoids may interact selectively within the mitogen-activated protein kinase (MAP kinase) signaling pathway. Suggesting and supporting a non-antioxidant activity, experimental evidence showed that flavonoids are able to protect neurons against oxidative stress more effectively than ascorbate, even when the latter was used at 10-fold higher concentrations.[62] Thus, flavonoids might exert modulatory effects in cells independent of classical antioxidant capacity through selective actions at different components of a number of protein kinase and lipid kinase signaling cascades such as phosphoinositide 3-kinase (PI 3-kinase), Akt/PKB, tyrosine kinases, protein kinase C (PKC), and MAP kinases.[8,58−62] Inhibitory or stimulatory actions at these pathways are likely to profoundly affect cellular function by altering the phosphorylation state of target molecules and/or by modulating gene expression. Although selective inhibitory actions at these kinase cascades may be beneficial in cancer, proliferative diseases, inflammation, and neurodegeneration, flavonoid interactions with these pathways could have unpredictable outcomes and will be dependent on both the cell type and the disease studied. The cellular effects of flavonoids will ultimately depend on the extent to which they associate with cells, either by interactions at the membrane or by uptake into the cytosol. Information regarding uptake of flavonoids and their metabolites from the circulation into various cell types and whether they are modified further by cell interactions has become increasingly important as attention focuses on the new concept of flavonoids as potential modulators of intracellular signaling cascades vital to cellular function. Relatively few studies have examined the ability of these metabolites to exert modulatory effects on signaling pathways. The ability of flavonoids and flavonoid-rich foods and beverages to reduce NO oxidation and increase NO bioavailability appears to contribute significantly to their vascular benefits[3,8,9] and thus, finally, protection against atherosclerosis.[3,8,9] Moreover, the polyphenol-induced NO formation is due to the redox-sensitive activation of the phosphatidylinositol 3-kinase/Akt pathway leading to direct eNOS activation subsequent to its phosphorylation on Ser1177. Besides the phosphatidylinositol 3-kinase/Akt pathway, polyphenols have also been shown to activate eNOS by increasing the intracellular free calcium concentration and by activating estrogen receptors in endothelial cells.[63] In addition, Schini-Kerth et al.[63] also showed that to cause a rapid and sustained activation of eNOS by phosphorylation, polyphenols might increase the expression level of eNOS in endothelial cells, leading to an increased formation of NO. Considering the putative biological and pathophysiological mechanisms involved in the flavonoid benefits on cardiovascular health, the emerging evidence is that flavonoids are likely to exert eventual beneficial effects on cells not through their potential to act as antioxidants but rather through their modulation of signaling cascades. Flavonoids have been demonstrated as potent bioactive molecules, and a clear understanding of their mechanisms of action as either antioxidants or modulators of cell signaling is crucial to the evaluation of their specific potential in cardiovascular protection.

DISCUSSION AND CONCLUSIONS

It has been reported that the polyphenol-rich Mediterranean diet and flavonoid-rich foods (cocoa, red wine, tea, etc.) are able to reduce cardiovascular morbidity and mortality.[4,5,64,65] According to this, the Mediterranean diet has been considered a correlated food model to the style of life. Furthermore, flavonoids seem to play a pivotal role in "healthy diets". The Mediterranean diet is progressively abandoned, and industrial manufacturers progressively change healthy ingredients to unhealthy foods. In particular, cocoa beans have been considered the richest known source of flavonoids. Even so, during chocolate processing up to 85% of the original flavanol content vanishes. Losing the healthy active constituents of a diet could promote the development of a variety of diseases collectively considered significant causes of disability and premature death, also imposing a substantial economic burden.[64,65]

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The medicinal and nutritional properties of the cocoa bean have been known and exploited by traditional cultures for centuries.[66] However, it is only recently that scientific studies have begun to define the healthy effects of this ancient medicinal source. Of note, a large body of evidence has been focused on the role of polyphenols found in abundance in the cocoa bean.[6−9] Research on the effects of cocoa polyphenols is daily suggesting that flavonoids from cocoa present all of the biological potential to significantly decrease cardiovascular risk and disease.[6−9] Nevertheless, despite the claims of several chocolate manufacturers, the vast majority of the polyphenols found in the cocoa bean are in fact destroyed during the conventional chocolate-making process, thus converting certain healthy ingredients into "undefinable" healthy foods. Consistent with this, the wide variation in cocoa processing and in the content and profile of flavonoids makes it difficult to determine to what extent the findings about positive effects expressed in different studies might be translated into tangible clinical benefits.[6−11] Epidemiological studies reported an inverse relationship between flavonoid-rich cocoa, BP, and the risk of cardiovascular disease. Experimental data from both in vivo and in vitro studies suggested that flavonoids and flavanol-rich cocoa products present all of the biological potential to reduce BP in humans. Clinical intervention studies suggest consumption of flavanol-rich cocoa and chocolate may reduce the cardiovascular risk by improving endothelial function and decreasing BP. It has been estimated that a 3 mmHg reduction in SBP would reduce the relative risk of stroke mortality by 8%, of coronary artery disease mortality by 5%, and of all-cause mortality by 4%.[55] Thus, the BP changes in response to the consumption of flavanol-rich cocoa in healthy subjects as well as in pre-hypertensive and hypertensive patients might interestingly suggest the inclusion of moderate amounts of flavanol-rich cocoa or chocolate in the daily diet to potentially delay the onset of hypertension. Interest in the biological activities of cocoa flavonoids is steadily increasing; nevertheless, the practicality of chocolate or cocoa products as long-term treatment for hypertension should be completely clarified. Critical attention must be paid to the flavanol content of the finished cocoa products (manufacturing processes significantly reduce its concentration) and to the high fat and sugar contents of many cocoa snacks and confectionaries. According to this, further research should be addressed to the identification of the active constituents of diet in the formulation of appropriate dietary guidelines. Research into the pharmacological properties of original healthy ingredients and, therefore, of final flavonoid-rich products might promote natural functional foods and "nutraceuticals". Investigation on intervention controlled long-term effects of cocoa products should clarify these critical points. Furthermore, future research should focus on structure−activity relationships regarding the complex effects of flavonoids and metabolites on cardiovascular risk factors and cardiovascular protection.

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Notes

The authors declare no competing financial interest.

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