Cocoa Flavanol Cardiovascular Effects Beyond Blood Pressure Reduction

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The protective cardiovascular (CV) effect of cocoa flavanol has been a target of many recent clinical prospective and retrospective investigations. Epidemiological data in different patient cohorts revealed an association between higher intake of flavanol-rich foods and decreased incidence of CV events, especially stroke and myocardial infarction. Cocoa flavanol has been shown to reduce systolic (2.8 mm Hg) and diastolic (2.2 mm Hg) office blood pressure (BP). Greater BP reduction has been found in hypertensive patients and people younger than 50 years. Cocoa flavanol intake exerts beneficial effects on pathophysiologic mechanisms of hypertension-related organ damage, such as improved endothelial function, anti-inflammatory potency, inhibition of platelet activation, and increased vasodilatory capacity. Recent clinical trials have focused on establishing a potential link between epidemiology and pathophysiology of flavanol and identified possible mechanisms for prevention of end-organ damage in patients at CV risk. This review summarizes the available data on the antihypertensive effects of cocoa flavanol beyond BP lowering effects, accentuates subgroup-specific protective actions of cocoa according to patients' different CV risk profile, and outlines potential cocoa flavanol–associated clinical implications. *J Clin Hypertens (Greenwich).* 2016;18:352–358. © 2015 Wiley Periodicals, Inc.

The beneficial effect of cocoa consumption has been observed in the population of Kuna Indians who belong to one of the few cultures that are protected against the age-dependent increase in blood pressure (BP), the development of arterial hypertension, and the age-dependent decline in kidney function.[1] In this population that consumes large amounts of cocoa daily,[2] cardiovascular (CV) mortality is lower compared with other Pan-American citizens.[1,3] This protection is thought to depend on the daily amount of cocoa consumption and is widely attributed to the cocoa content of flavanols.[4,5] Flavanols occur as the monomers epicatechin and catechin.[1] Various flavonoid classes[6] have been shown to be associated with lower risk of fatal CV disease.[7]

In the past decade, several prospective clinical trials investigated the effect of chocolate and cocoa products on BP and CV disease. A PubMed search in March 2015 confined to "flavanol," "blood pressure," "cardiovascular," "cocoa," and "end-organ damage" indicated four reviews highlighting the effect of cocoa on BP (2012),[8] CV disease (2012),[9] development of type 2 diabetes mellitus (2013),[10] and endothelial function (2009).[1] In addition, this paper is based on 31 randomized controlled clinical trials (RCTs) and 28 further investigations. The aim of this comprehensive review is to include the most recent animal studies, epidemiological reports, randomized trials, and meta-analyses that investigated the effect of cocoa flavanols on BP and hypertensive end-organ damage, to accentuate subgroup-specific protective effects according to patients' different CV risk profile, and to specify potential cocoa flavanol–associated pathogenetic mechanisms.

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COCOA AND BP

The impact of flavanol on BP has been evaluated in a systematic review including 42 acute or short-term chronic RCTs (1297 participants). Normotensive and mildly hypertensive patients with 50 mg epicatechin intake daily revealed a significant reduction in mean arterial and diastolic office BP compared with placebo (Figure 1).[9,11] These results match well with the conclusion of a Cochrane Review from 2012 integrating 20 RCTs (n=856)[11–18] with 2- to 18-week duration of daily epicatechin and catechin intake (dose 7–236 mg daily).[8,19–27] There was a significant reduction in office systolic ($\bar{X}$: −2.77; 95% confidence interval [CI], −4.72 to −0.82 mm Hg; P=.005 [n=20 clinical trials, 856 participants]) and diastolic ($\bar{X}$: −2.20; 95% CI, −3.46 to −0.93 mm Hg; P=.006 [n=19] clinical trials, 824 participants) BP compared with placebo (Figure 1).[8] Two clinical trials using ambulatory 24-hour BP monitoring found a reduction of BP after cocoa intake (systolic: −4.52±3.94 mm Hg; diastolic: −4.17±3.29 mm Hg [n=19][18] and systolic −5.3±5.1 mm Hg; P=.001 and diastolic −3±3.2 mm Hg; P=.002 [n=52]).[24] On a population basis, a BP decrease even of only 3 mm Hg could still have important effects on reduction of end-organ damage. Incidentally, the Food and Drug Administration has granted some antihypertensive drugs superiority claims over others, which are based on BP differences of this range.

While there was a significant BP-lowering effect in the hypertensive (systolic BP ≥140 mm Hg at baseline) subgroup ($\bar{X}$: −3.99; 95% CI, −7.02 to −0.97 mm Hg; P=.01), normotensive patients did not show a significant

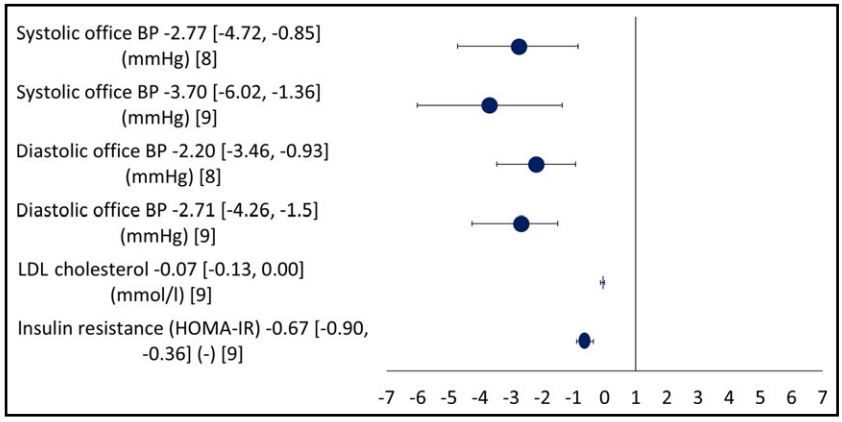

FIGURE 1. Results from systemic reviews: effect of cocoa flavanol on cardiovascular risk factors (×[95% confidence interval]).[8] BP indicates blood pressure; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment insulin resistance index.

BP reduction ($\bar{X}$: −2.04; 95% CI, −4.64 to 0.57 mm Hg; *P*=.13) (Table II).[8] BP reduction was found to be dose-dependent (Table I).[8,9,28] The BP-lowering effect of cocoa has been shown to attenuate with increasing age.[8] In trials with younger participants (mean age <50 years), systolic ($\bar{X}$: −4.57; 95% CI, −7.41 to −1.73 mm Hg; *P*=.002 [n=10]) and diastolic ($\bar{X}$: −3.85; 95% CI, −5.45 to −2.26 mm Hg; *P*<.001 [n=9]) BP was lower in comparison with placebo.[8]

COCOA AND CENTRAL HEMODYNAMICS

Cocoa intake has been shown to effect central hemodynamics. van den Bogaard and colleagues[25] described a reduction in central systolic and diastolic BP, augmentation index, and wave reflection in patients 2 hours after intake of a cocoa drink coinciding with the peak plasma levels of flavanols. These findings are in accordance with increased pulse wave amplitude (indicative of reduced pulse wave velocity) 90 minutes and 4 days after ingestion of a flavanol-rich cocoa drink (821 mg/d flavanol, n=27 healthy people, *P*=.01).[29] Of note, a recent clinical trial demonstrated a dose-dependent decrease (*P*<.001) of pulse wave velocity with increasing epicatechin concentrations in 20 healthy individuals after 1 week of epicatechin ingestion.[28] Furthermore there was an improvement in arterial function after 6 weeks of daily 446 mg flavanol intake.[30] These studies have all been conducted in healthy individuals and to our knowledge there are so far no clinical trials investigating central hemodynamics in hypertensive patients.

These findings that cocoa flavanol lowers office, ambulatory, and central BP raise questions about the underlying pathophysiological mechanisms.

COCOA AND PATHOGENETIC MECHANISMS

Endothelial Dysfunction

Endothelial dysfunction, especially impaired flow-mediated vasodilation (FMD), plays a key role in the development of arterial hypertension, correlates with coronary vascular dysfunction, and is predictive of future coronary events.[1,31–34] Cocoa flavanol consumption has been found to improve vasodilatory capacity (Figure 2). Several in vitro and in vivo studies proposed that these vasodilatory effects of flavanol occur via reduction of reactive oxygen species (ROS),[4,23] angiotensin-converting enzyme inhibition, endothelin 1 antagonism,[28] and endothelial nitric oxide (NO) synthetase (eNOS) activation.[1,14,16,35] Improved vasodilatory capacity of small and large arteries due to eNOS activation has been demonstrated in aortic rings from rabbits, and consistent administration of epicatechins resulted in endothelium-derived relaxation by activation of NO synthetase.[36]

It has been shown in different patient cohorts that cocoa flavanol augments endothelial NO synthetase via eNOS stimulation in the short (2 hours after ingestion) and long (after 2 weeks of cocoa intake) term. In addition, cocoa flavanol has been shown to augment the plasma circulating NO pool 2 hours after ingestion compared with baseline in healthy people[37] and smokers.[35] This was associated with an improvement in FMD[35] and supported by another clinical trial showing improved antioxidant status 2 hours after 40 g of chocolate ingestion compared with baseline (*P*=.03).[38] Urinary excretion of NO metabolites increased in the same population after ingestion of 2 mg/kg epicatechin daily for 2 weeks compared with baseline.[37] Moreover, serum asymmetric dimethylarginine, an NO-inhibiting factor, significantly decreased after 2 weeks of flavanol intake.[11,14] Other clinical trials have further observed that there is a cocoa flavanol dose-dependent release of NO.[4] Finally, in healthy individuals, NG-nitro-L-arginine methyl ester (L-NAME) infusion, which inhibits the NO synthases, has been shown to reduce cocoa-induced peripheral vasodilatory response to ischemia from 6.3% to 3.4% (*P*=.004).[4,29] Based on these data, there is little doubt that cocoa flavanol administration increases NO availability in humans.

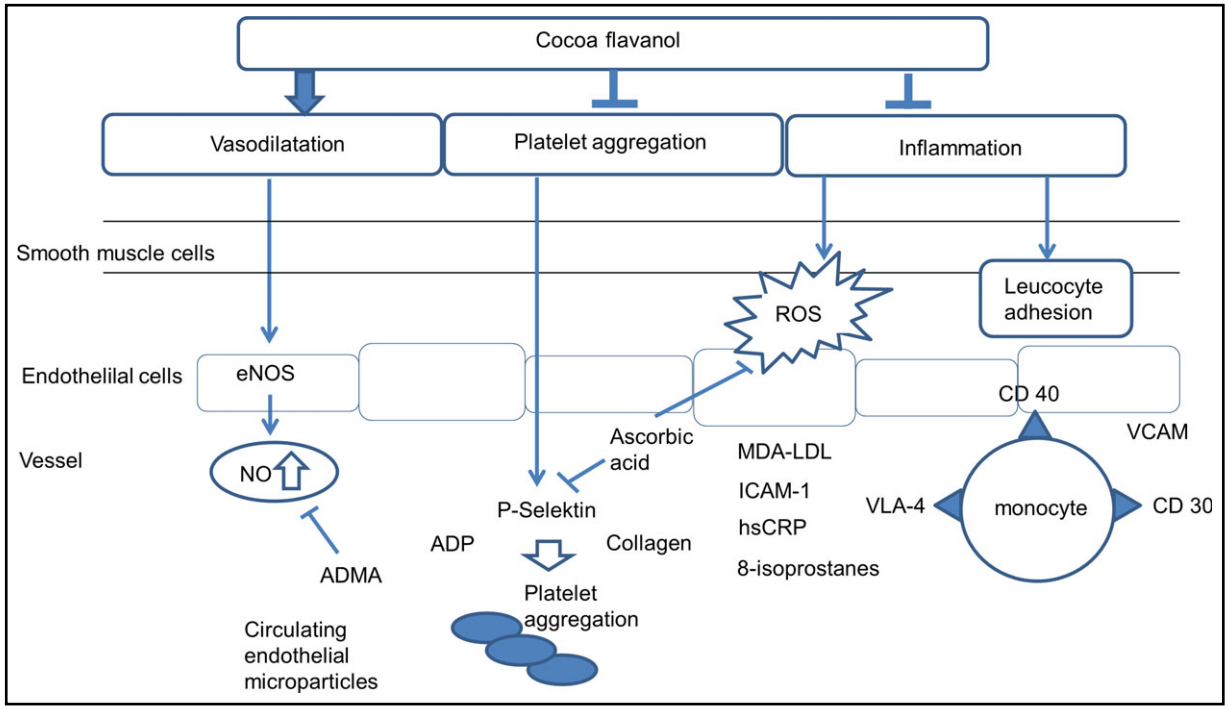

FIGURE 2. Endothelium-dependent vasodilatory effect of cocoa flavanol with the messenger molecules of vasodilatation, platelet aggregation, and inflammation. eNOS indicates, endothelial nitric oxide synthase; ROS, reactive oxygen species; NO, nitric oxide; ADMA, asymmetric dimethylarginine; ADP, adenosine diphosphate; MDA-LDL, malondialdehyde-modified low-density lipoprotein; ICAM-1, intracellular adhesion molecule; hsCRP, high-sensitivity C-reactive protein; VCAM, vascular cell adhesion molecule.

What data do we have in patients at CV risk? Grassi and colleagues[16–18] have shown a significantly improved FMD in untreated hypertensive patients and patients with hypertension and impaired glucose tolerance. Improvement in FMD caused by cocoa flavanol administration has also been shown in smokers,[35,38] overoverweight patients,[20] and patients with other CV risk factors.[4,23,29] Likewise, in patients with type 2 diabetes mellitus, flavanol intake leads to increased FMD.[39] In contrast, no improvement in forearm FMD was observed in patients with coronary artery disease (CAD) (>50% angiographically investigated stenosis).[40] These discrepant results between patients at CV risk as opposed to those with CAD may be related to differences in the mean age of the patients in the studies, since FMD decreases with increasing age. In addition, FMD has been shown to depend on the amount of flavonoid ingestion (Table I).[9] The flavanol dose used in the clinical trial, examining patients with CAD was lower than in the other trials. Thus, no definite conclusion can be drawn about whether cocoa flavanol intake improves FMD in patients with CV disease because of a lack of conducted studies.

Inflammation

Reduction of ROS[41] and anti-inflammatory properties[22] has been proposed as a pathogenetic mechanism of the vasoprotective flavanol effect and seems to contribute to the potential benefits of cocoa consumption against atherosclerosis (Figure 2). Data on the influence of cocoa flavanol on markers of inflammation are inconsistent and difficult to compare because of variations of CV risk profile and preexisting end-organ damage in the patient cohorts investigated. In patients at high risk for CV disease, leukocyte adhesion is decreased by cocoa flavanol via lower expression of VLA-4, CD40, and CD36 on the monocyte surface, in addition to lower serum concentrations of intercellular adhesion molecule 1, a marker molecule for ROS-induced inflammation.[22] On the contrary, there was no change in the inflammatory markers 8-isoprostanes[11,14] and malondialdehyde-modified low-density lipoprotein (LDL), both indicative of oxidative stress.[23] Moreover, three clinical trials conducted in hypertensive patients revealed no change in parameters of oxidative stress and antioxidant capacity after 730 mg quercetin/900 mg flavanol/100 mg dark flavanol-rich chocolate intake.[16,19,42]

Platelet Aggregation

It has been demonstrated that cocoa flavanol inhibits platelet aggregation in the short term (2 hours[38,43] and 6 hours[13] after intake) via a decrease in P-selectin, adenosine diphosphate (ADP)–induced aggregation, and collagen-induced aggregation in different patient cohorts (Figure 2).[13] Platelet adhesion was shown to decrease (P=.04) in 22 heart transplant recipients after

TABLE I. Dose-Dependent Flavanol Effect[9]

	Epicatechin Dose, mg/d	No. of Studies (No. of Participants)	Mean Effect (95% CI)	*P* Value for Difference Between Subgroups
Systolic BP, mm Hg	<50	6 (299)	0.10 (−2.20 to 2.41)	.002
	50–100	5 (161)	−4.48 (−6.32 to −2.63)	
	>100	3 (110)	−4.58 (−5.95 to −3.21)	
Diastolic BP, mm Hg	<50	6 (299)	−0.38 (−1.97 to 1.20)	.001
	50–100	5 (161)	−4.25 (−5.66 to −2.85)	
	>100	2 (78)	−3.62 (−5.50 to −1.74)	
FMD, 2 hours (%)	<50	0 (0)	No data	.005
	50–100	3 (89)	1.62 (1.33–1.92)	
	>100	4 (110)	3.53 (2.22–4.83)	
FMD, chronic (%)	<50	2 (95)	2.18 (0.93–3.43)	.13
	50–100	2 (111)	1.04 (0.59–1.49)	
	>100	3 (108)	1.60 (0.95–2.24)	
Fasting glucose, mmol/L	<50	3 (142)	−0.01 (−0.24 to 0.22)	.22
	50–100	3 (106)	−0.17 (−0.33 to −0.02)	
	>100	2 (78)	0.09 (−0.20 to 0.37)	
LDL cholesterol, mmol/L	<50	5 (217)	−0.00 (−0.09 to 0.08)	.27
	50–100	6 (296)	−0.02 (−0.08 to 0.04)	
	>100	4 (204)	−0.14 (−0.28 to 0.01)	

Abbreviations: BP, blood pressure; CI, confidence interval; FMD, flow-mediated dilatation; LDL, low-density lipoprotein.

intake of 40 g of dark chocolate[43] and in patients with congestive heart failure after intake of dark chocolate containing catechin 0.27 mg/g and epicatechin 0.9 mg/g.[44] Another clinical trial demonstrated that plasma total 8-iso-PGF2α decreased after 8 weeks of daily 990 mg flavanol consumption.[45]

Overall, the underlying pathogenetic mechanisms of the vasoprotective flavanol-associated effects are under discussion. Clinical trials have focused on markers of three key players of atherosclerosis, such as inflammation, platelet aggregation, and NO-induced vasodilatation (Figure 2). These in vivo and in vitro studies in various study populations indicate that cocoa flavanol intake improves endothelial function, mediated by increased FMD, by increasing NO activity and reducing oxidative stress as well as inflammatory markers. There are clinical trials indicating that platelet aggregation is reduced 2 to 6 hours after cocoa flavanol ingestion. This beneficial effect might be limited or absent in older patients and/or in patients with advanced CAD.

EFFECTS OF COCOA ON BLOOD LIPIDS

Clinical trials investigating the influence of cocoa flavanol on blood lipids have shown conflicting results. A systematic review including 42 acute or short-term chronic RCTs (1297 participants) and published in 2012 demonstrated an overall marginally significant decrease in LDL ($\bar{X}$: −0.07 mmol/L; 95% CI, −0.13 to 0.00 mmol/L, *P*=.05) (Figure 1) and increase in high-density lipoprotein (HDL) cholesterol ($\bar{X}$: 0.03 mmol/L; 95% CI, 0.00–0.06 mmol/L; *P*=.05).[9] In two further clinical trials not included in the review there were no significant changes in cholesterin, LDL, or HDL after daily ingestion of flavanol-rich chocolate for 2 or 6 weeks.[11,12,17] The conflicting results can be explained by the different number of patients integrated in the clinical trials. Although the total cohort in the systemic review comprised 1297 patients,[9] there was just a marginally significant effect on HDL and LDL cholesterol.

Patients with untreated essential hypertension revealed plasma total and LDL cholesterol reduction.[16] Hypertensive patients with impaired glucose tolerance showed a decrease in total cholesterol (−6.5%, *P*<.001) and LDL cholesterol (−7.5%, *P*=.001) after flavanol consumption.[18] In patients with type 2 diabetes mellitus, flavan-3-ols and isoflavones significantly lowered total and LDL cholesterol (*P*=.04).[46]

The existing data indicate that patients with untreated hypertension, impaired glucose tolerance, and type 2 diabetes mellitus might benefit from the improved lipid metabolism according to cocoa flavanol consumption (Table II). Further clinical studies are needed to confirm this hypothesis.

EFFECTS OF COCOA ON DIABETIC METABOLISM

Impaired glucose metabolism is an established risk factor for CV diseases and often coexists with hypertension. Insulin sensitivity is known to partly depend on insulin-mediated NO release.[1,47] This leads to the hypothesis that flavanols and dietary antioxidants may decrease insulin resistance by ameliorating NO bioavailability.[1] Existing data indicate that insulin resistance in patients at risk for CV disease might be positively influenced by cocoa flavanol, even though markers of glucose metabolism have just been analyzed in small patient cohorts and therefore remain difficult to

TABLE II. Effect of Cocoa Flavanol on CV Risk Factors

	Nonhypertensive	Hypertensive
Office blood pressure	0	↓
Ambulatory blood pressure	0	↓
Pulse wave velocity	↓	NA
Marker of endothelial function	↑	↑
Marker of inflammation	NA	0
Platelet aggregation	↓	NA
Lipids	↓ marginally	↓
Insulin resistance	↓[a]	NA
CV disease	↓	NA
Cerebrovascular disease	↓	NA

Abbreviations: CV, cardiovascular; NA, not applicable; 0, no effect; ↑, increase; ↓, decrease. [a]Decreased insulin resistance especially in elderly, overweight patients and patients with type 2 diabetes mellitus.

evaluate. In the existing clinical trials, the homeostatic model assessment insulin resistance index (HOMA-IR) was used as a marker for insulin resistance.

A systematic review including 42 acute or short-term chronic RCTs with 1297 individuals[9] and additional clinical trials in elderly[45] and overweight patients[20] and patients with type 2 DM[46] showed consistent improvement in insulin resistance after cocoa flavanol consumption (HOMA-IR $\bar{X}$: −0.67; 95% CI, −0.98 to −0.36) (Figure 1).[9] Insulin sensitivity (r=0.51, P=.001) and beta-cell function (r=0.40, P=.012) were found to be directly related to increased FMD and decrease in BP.[18]

Data on HOMA-IR reduction as a result of flavanol consumption in hypertensive patients are inconsistent. Two clinical trials found that there was no change in fasting plasma glucose and insulin resistance in untreated hypertensive patients after ingestion of 100 mg of dark chocolate daily for 2 weeks[12] and 900 mg flavanol daily for 2 weeks.[19] Grassi and colleagues described a significant decrease in HOMA-IR (P<.0001) and improved insulin sensitivity (P<.0001) after daily intake of 100 mg of dark chocolate for 15 days in untreated hypertensive patients.[16] In treated hypertensive patients with impaired glucose tolerance, insulin resistance decreased (P<.001) and beta-cell-function increased (P=.035).[18] Further studies with larger cohorts of hypertensive patients are needed to evaluate a potentially beneficial effect of cocoa flavanol on insulin resistance in hypertensive patients.

LONG-TERM EFFECTS OF COCOA FLAVANOL ON CV HEALTH

Population-based meta-analyses in different patient cohorts suggest an association between higher intake of flavanol-rich foods and decreased incidence of CV events,[7,48,49] especially stroke[50–52] and myocardial infarction (Table II).[53,54]

Epidemiological investigations have consistently revealed an inverse relationship between high dietary intake of flavanol-containing foods and reduced risk of coronary heart disease (CHD) mortality,[54] between high chocolate consumption (n=19,357) and reduced CV disease risk (myocardial infarction and stroke over 8 years),[46,53] and between higher intake of flavanol-rich foods and lower incidence of CV events.[48,49] However, a large meta-analysis of cohort studies comparing relative risk estimates of CHD for high compared with low flavanol intake found no effect.[55] Likely, there was a large difference in flavanol intake in the compared groups to find a difference in risk reduction of CHD. Patients with previous myocardial infarction who ate chocolate twice a week compared with patients who did not eat chocolate showed a 66% reduction in 8-year cardiac mortality.[56]

Interestingly, in one study, after oral ingestion of 100 mg/kg epicatechin daily, metabolites of flavanol (such as epicatechin glucuronide and 3′-*O*-methyl epicatechin) were detected in brain tissue.[57] In elderly individuals (n=90) with mild cognitive impairment, high flavanol intake was associated with better cognitive test results in the Trail Making Test A and B and verbal fluency test in a dose-dependent fashion.[45] Other studies have confirmed that observation. An improvement in the cognitive demand battery after consumption of 520 mg and 994 mg flavanol daily for 3 days was observed, again in a dose-dependent fashion.[58] A greater intake of chocolate (7.5 g/d compared with 1.7 g/d) was found to be associated with a lower 8-year risk of stroke.[53] Meta-analysis of six independent prospective cohort studies involving 111,067 persons revealed that intake of 760 mg flavonol daily was inversely associated with nonfatal and fatal stroke (pooled relative risk, 0.80; 95% CI, 0.65–0.98; P=.05)[50] and reduced the risk of ischemic stroke.[51] Moreover, flavonoid intake was associated with lower stroke mortality (RR $\bar{X}$, 0.63; 95% CI, 0.44–0.89; P trend=.04).[7]

Limitations

The protective effect of cocoa flavanol has been shown to be dependent on several side effects, which are at least partly difficult to control.[1] Variation in manufacture processes,[59] dose, agriculture origin,[60] additional supplements,[61,62] and further ingredients such as milk[63–65] influence the bioavailability and therefore therapeutic effectiveness of cocoa flavanol. Clinical trials using different cocoa flavanol supplements or chocolate types are difficult to compare. Flavanol content varies between 46 mg and 61 mg in 100 g of chocolate.[1] Precaution in all products containing chocolate is mandatory because of high caloric load of commercially available chocolate (about 500 kcal/100 g) and associated weight gain, a risk factor for hypertension, diabetes, and dyslipidemia.[1]

CONCLUSIONS

Population-based meta-analyses in different patient cohorts revealed an association between higher intake

of flavanol-rich foods and decreased incidence of CV events, especially stroke and myocardial infarction. Cocoa flavanol has been shown to lower systolic and diastolic office and ambulatory BP. Even though there is just a modest BP reduction of 3 mm Hg in an average mixed population, patients with hypertension and people younger than 50 years experienced larger effects. Shortly after application, cocoa flavanol dose-dependently improved pulse wave reflection, reduced ROS, inhibited platelet activation, and improved FMD via eNOS activation. In patients at risk for CV diseases such as hypertension, impaired glucose control, and type 2 diabetes, LDL and total cholesterol were reduced and insulin resistance improved after cocoa flavanol consumption in hypertensive patients, although not all studies showed concordant results. Healthy people did not display changes in lipid and glucose metabolism after flavanol intake. Further investigations are needed to evaluate the concept that improves CV progress and to specify the organ-protective effect in comparison to well-established pharmacologic options.

Disclosures: None.

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