

Cocoa and Chocolate in Human Health and Disease

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Abstract

Cocoa contains more phenolic antioxidants than most foods. Flavonoids, including catechin, epicatechin, and procyanidins predominate in antioxidant activity. The tricyclic structure of the flavonoids determines antioxidant effects that scavenge reactive oxygen species, chelate Fe²⁺ and Cu⁺, inhibit enzymes, and upregulate antioxidant defenses. The epicatechin content of cocoa is primarily responsible for its favorable impact on vascular endothelium *via* its effect on both acute and chronic upregulation of nitric oxide production. Other cardiovascular effects are mediated through anti-inflammatory effects of cocoa polyphenols, and modulated through the activity of NF- κ B. Antioxidant effects of cocoa may directly influence insulin resistance and, in turn, reduce risk for diabetes. Further, cocoa consumption may stimulate changes in redox-sensitive signaling pathways involved in gene expression and the immune response. Cocoa can protect nerves from injury and inflammation, protect the skin from oxidative damage from UV radiation in topical preparations, and have beneficial effects on satiety, cognitive function, and mood. As cocoa is predominantly consumed as energy-dense chocolate, potential detrimental effects of overconsumption exist, including increased risk of weight gain. Overall, research to date suggests that the benefits of moderate cocoa or dark chocolate consumption likely outweigh the risks. *Antioxid. Redox Signal.* 15, 2779–2811.

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I. Introduction

A. History

CHOCOLATE IS BEST KNOWN as an indulgent confection, but historically it has also been consumed for its purported healing properties (60). Foods and beverages made from beans from the *Theobroma cacao* tree (cocoa, cacao) have been consumed by humans since at least as early as 460 AD (224). The medicinal uses of cacao or chocolate either as a primary remedy or as a vehicle to deliver other medicines originated in Mesoamerica, where it was consumed by indigenous peoples, and diffused to Europe in the mid-1500s. Between the 16th and 20th centuries, well over 100 uses for cacao or chocolate, as a medical treatment, have been documented (60). Among these, three applications are most common: (i) to induce weight gain in emaciated patients; (ii) to stimulate the nervous system; and (iii) to improve digestion and elimination (60).

The Kuna Indians of the San Blas islands of Panama consume an average of three 10-ounce cups of cocoa beverage daily, ingesting approximately 1880 mg of procyanidins (39, 162). The prevalence of hypertension among the Kuna islanders is very low (2.2%) and blood pressure (BP) does not increase with age (104). The population also experiences lower rates of diabetes mellitus, myocardial infarction, stroke, and cancer than mainland Panamanians (103). Among Kuna who have migrated to urban areas on mainland Panama, the prevalence of hypertension is higher (10.7%) and reaches 45% among those over age 60 (Fig. 1) (104). McCullough *et al.* have hypothesized that the high intake of a traditional cocoa beverage may be partly responsible for the low incidence of cardiovascular disease among the Kuna islanders (162). Compared to Kuna living in a suburb of Panama City, those still living on the remote islands consume twice as much fruit, four times as much fish, and 10 times as much cocoa (162).

The majority of research on chocolate and cocoa has taken place over the last decade (41) and has primarily focused on the relationship between cocoa consumption and cardiovascular risk. More recent research has provided insights to the possible benefits of cocoa consumption on other organ systems. This review will discuss the reported physiologic effects of cocoa consumption and possible mechanisms by which they might occur.

B. Economic data

Worldwide, chocolate consumption ranges from 0.12 kg per person per year in China to 11.85 kg in Ireland. The United States falls somewhere in the middle of this range, with per capita annual consumption of 5.18 kg (33). Developed countries

account for 64% of worldwide consumption (75a). The largest cocoa bean producing country in the world is Côte d'Ivoire, which produced 1.2 million tons of cocoa in 2006–2007 (113a).

C. Components of cocoa

Cocoa, or cacao, is the dried and fully fermented fatty seed of the fruit of the cocoa tree, *Theobroma cacao* (163). Cocoa liquor is the paste made from ground, roasted, shelled, and fermented cocoa beans, called nibs. It contains both nonfat cocoa solids and cocoa butter. Cocoa liquor is what is referred to as “percent cacao” on food packaging. Cocoa powder is made by removing some of the cocoa butter from the liquor. Chocolate is a solid food made by combining cocoa liquor with cocoa butter and sugar. The proportion of cocoa liquor in the final product determines how dark the chocolate is. Milk chocolate is made with the addition of condensed or powdered milk to the chocolate mixture (281). The type of chocolate consumed most in the United States is milk chocolate, which typically contains 10%–12% cocoa liquor (18). Semisweet or bittersweet chocolate is often referred to as dark chocolate and must contain no less than 35% by weight of cocoa liquor. White chocolate contains only cocoa butter (at least 20% by weight) combined with sweeteners and dairy ingredients (258).

Cocoa liquor is a complex food and contains many bioactive compounds. Cocoa butter contains significant amounts of fatty acids, whereas the nonfat cocoa solids contain vitamins, minerals, fiber, and polyphenols.

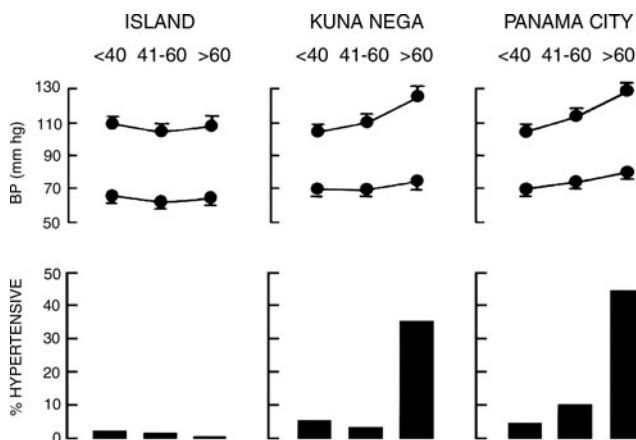


FIG. 1. Blood pressure and prevalence of hypertension among island-dwelling and mainland Kuna Indians. Reprinted with permission from Hollenberg (102).

1. **Lipids.** The oil in cacao, referred to as cocoa butter, is a mixture of monounsaturated and saturated fatty acids. In the monounsaturated fraction, oleic acid predominates, as it does in olive oil (26). The majority of the saturated fatty acids are palmitic acid and stearic acid. In general, saturated fatty acid consumption has been associated with an increased risk of coronary heart disease as saturated fatty acids elevate total cholesterol and LDL (10, 280). A single meal containing relatively high levels of saturated fat may destabilize coronary plaque and impair endothelial function (128, 198, 275). Stearic acid is unusual in that it does not elevate serum lipid levels to the same degree that other saturated fatty acids do (112). Thus, whereas shorter-chain saturated fatty acids such as myristic acid (14:0) and palmitic acid (16:0) are associated with increased LDL and atherogenesis, stearic acid is not (32, 124, 223). Although the lipid content of chocolate is relatively high, one-third of the lipids in cocoa butter is stearic acid (18:0), which is believed to be nonatherogenic and to exert a neutral cholesterolemic response in humans (26). The 2010 Dietary Guidelines Advisory Committee specifically acknowledged stearic acid's unusual nature and has recommended that it be considered separately from cholesterol-raising fats (262).

2. **Fiber.** Although the bran of the cocoa bean is high in fiber, and its consumption has been shown to improve the LDL:HDL ratio, much of the bran is lost in processing (117). Still, some fiber remains in commercial cocoa products, though there is a wide range of fiber content. A 100-kcal portion of dark chocolate (70%–85% cacao) contains 1.7 g of fiber, whereas semisweet chocolate and milk chocolate contain 1.2 g and 0.6 g per 100 kcal, respectively. Unsweetened cocoa powder contains almost 2 g of fiber per tablespoon, and provides only 12 kcal (1). The majority of fiber in cocoa is insoluble (139). Although soluble fiber is noted for reducing serum cholesterol (19, 71), total dietary fiber is recognized as important for weight maintenance, and insoluble fiber has been associated with reduced risk of type 2 diabetes (270).

3. **Minerals.** The cocoa bean contains several minerals necessary for vascular function. Dietary magnesium, copper, potassium, and calcium all reduce risk of hypertension and atherosclerosis (248).

Dark chocolate (70%–85% cacao) provides 36 mg of magnesium per 100 kcal serving, which is 9% of the U.S. recommended dietary allowance (RDA) for middle-aged men—more than three times the amount provided by milk chocolate (11 mg) (1, 184). Magnesium is a cofactor in protein synthesis, muscle relaxation, and energy production (249). Magnesium is an antiarrhythmic and hypotensive (4, 36, 53, 87, 237, 260).

Copper is a cofactor for a number of enzymes and is required for processes, including iron transport, glucose metabolism, infant growth, and brain development (190, 259). Copper deficiency can lead to anemia and pancytopenia, causing hypertension, inflammation, and myocardial hypertrophy (214). Copper deficiency has been linked to glucose intolerance, cardiac arrhythmia, and hypercholesterolemia in animals and humans (134); however, elevated copper status may also be harmful. High serum copper concentration is associated with an increased risk of cardiovascular death (206), all-cause, cancer, and cardiovascular mortality (142).

Chocolate is a significant source of copper for Americans; milk chocolate provides 10% of the U.S. RDA for copper per 100-kcal serving, whereas dark chocolate provides 31%, and cocoa powder 23% per tablespoon (1, 184). Despite the potential detrimental effects of excess copper, the prevention of copper deficiency is, nevertheless, important for the maintenance of cardiovascular health. Because a 1000-kcal serving of chocolate would need to be consumed to reach the RDA for copper, it is unlikely that chocolate consumption would elevate serum copper concentrations to harmful levels.

Dietary potassium may protect against hypertension caused by excess sodium intake (8). Low potassium intake has been associated with increased risk of cardiovascular mortality (261). Chocolate is relatively low in potassium; dark chocolate contains 114 mg potassium (2% RDA) per 100 kcal, whereas unsweetened cocoa powder contains 82 mg per tablespoon, and milk chocolate contains 67 mg (1% RDA) (1, 184).

Iron (Fe) deficiency is one of the most important nutritional problems in the world (50). Milk chocolate contains 5% of the RDA for iron for adult men and postmenopausal women (0.42 mg) per 100 kcal; dark chocolate provides 25% of the RDA (1.90 mg). A tablespoon of unsweetened cocoa powder contains more iron than milk chocolate, but less than solid dark chocolate (0.75 mg) (1, 184).

4. **Polyphenols and antioxidant activity.** Cocoa powder contains up to 50 mg of polyphenols per gram. Single servings of cocoa and cocoa products contain more phenolic antioxidants than most foods (Table 1), and more procyanidins than the average amount consumed by Americans per day (86).

Cocoa contains a number of polyphenolic compounds, but it is particularly rich in flavonoids—specifically, flavanols, also called flavan-3-ols. Flavanols form complexes with salivary proteins and are responsible for the bitterness of cocoa (13, 150). Although flavanols impart a bitter astringent flavor to foods, the flavor is frequently masked in chocolates by aggressive processing and the addition of other flavors. Estimates vary, but in one study, the average total flavanol content of commercially available dark chocolate was more than five times that of milk chocolate (168). Table 2 summarizes the polyphenol content and antioxidant capacity of selected commercially available cocoa products.

The main flavanols found in cocoa are epicatechin and catechin, and procyanidins (Table 2). Procyanidins provide the majority of antioxidant activity in cocoa products (200).

In addition to polyphenols, cocoa contains methylxanthine compounds—predominantly theobromine—about 2% to 3% by weight. Caffeine is also present in small amounts (0.2%). Theobromine has antioxidant activity similar to caffeine (232) and relatively little stimulating effect on the central nervous system (272).

Flavonoids consist of two aromatic carbon rings, benzopyran (A and C rings) and benzene (B ring) (Fig. 2), and are subdivided into 13 classes based on the degree of hydroxylation and oxidation of the rings; they are anthocyanidins, flavonols, isoflavones, flavones, flavanones, and flavanols (150). Flavanols in cocoa are present as either the monomers (–) epicatechin and (+) catechin or oligomers of epicatechin and/or catechin, called proanthocyanidins or condensed tannins. A full listing of flavonoids and other phenols in cocoa is provided in Table 3.

TABLE 1. FLAVAN-3-OL CONTENT AND ANTIOXIDANT CAPACITY OF VARIOUS FOODS AND BEVERAGES^a

	Flavanols+ procyanidins, mg	ORAC, mmol Trolox equivalents
Cocoa liquor		
Per 100 g	1400.0	40.0
Dark (semisweet) chocolate		
Per 100 g	170.0	13.1
Per 100 kcal	85.0	2.7
Milk chocolate		
Per 100 g	70.0	6.7
Per 100 kcal	14.0	1.3
Apples		
Per 100 g	106.0	0.2
Per 100 kcal	130.0	0.3
Cranberry juice cocktail		
Per 100 g	12.6	0.2
Per 100 kcal	20.0	0.4
Red wine		
Per 100 g	22.0	0.7
Per 100 kcal	25.0	0.9
Brewed black tea		
Per 2 g tea bag/200 ml water ^b	40.0	1.6

Reprinted with permission from Steinberg *et al.* (248).

^aAxioxidant activity is reported as oxygen radical absorbance capacity (ORAC) and expressed as mmol Trolox equivalents. Data are provided on a per-weight and per-kcal basis to facilitate comparison among foods. Data from refs. (10, 12, 87, 89, 90, 123).

^bData for tea are provided in an amount relevant to what might normally be consumed.

The tricyclic structure of the flavonoids determines their antioxidant (and possibly pro-oxidant) effects; phenolic-quinoid tautomerism and the delocalization of electrons over the aromatic system scavenge reactive oxygen species (Fig. 3). These aromatic rings directly neutralize free radicals, chelate metals (Fe²⁺ and Cu⁺) that enhance reactive oxygen species, inhibit enzymes, and upregulate antioxidant defenses (Fig. 3) (44, 216). Enzymes inhibited by cocoa flavonoids include xanthine oxidase, NADPH-oxidase, tyrosine kinases, and protein kinases (64). Cocoa intake increases serum antioxidant capacity, protecting the endothelium from oxidative stress and endogenous ROS (136).

Flavonoids, flavan-3-ols, and their oligomeric derivatives, procyanidins, have a variety of beneficial physiologic actions. Flavonoids have a number of properties that may contribute to their cardioprotective effects, including antioxidant and antiplatelet activity, immunoregulatory properties, and beneficial effects on the endothelium (42). The epicatechin content of cocoa is primarily responsible for its favorable impact on vascular endothelium, which is the result of both acute and chronic upregulation of nitric oxide production (222, 277). The combined catechin/epicatechin content in chocolate is 460–610 mg/kg (150). Epicatechins improve vascular function, reduce BP, improve insulin sensitivity, and reduce platelet activity (42). Epicatechin in cocoa quenches OH· 100 times more effectively than mannitol, a typical OH· scavenger (239).

Food products with significant antioxidant properties, such as green tea (79), degrade in antioxidant activity over time. Hurst *et al.* (113) assessed commercial preparations of chocolate and found that milk chocolate bars maintain oxygen radical absorbance capacity (ORAC), total polyphenols, and flavan-3-ol monomers for at least 50 weeks in commercial preparations, whereas cocoa powder and cocoa beans demonstrate stability in samples over 75 years old. Based on these results, Hurst posited that gallated flavan-3-ols (epigallocatechin-3-gallate [EGCG] and ECG; found in green tea) may be more susceptible to oxidation in relation to epicatechin (113).

a. Vascular effects mediated through NO. Independent of their antioxidant effects, plant polyphenols also promote the vasodilating factors nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor and inhibit pro-angiogenic factors endothelin-1 and vascular endothelial growth factor (VEGF) (217, 250). Cocoa is one of many food sources of substances that increase the production or bioavailability of endothelial nitric oxide. Other sources include grapes and berries, red wine, black and green tea, soy beans, pomegranates, olive oil, fish oil, and garlic (217). The vasodilatory response to cocoa flavanols is dependent on NO and can be reversed by blocking nitric oxide synthesis (74).

Endothelium-derived NO regulates vasodilation and the environment of the blood vessel wall (228), and is therefore critical for vascular function. Endothelial NO is produced by endothelial nitric oxide synthase (eNOS), which converts L-arginine to L-citrulline in the presence of necessary cofactors (143). Adequate production and bioavailability of eNOS-derived NO is necessary for the maintenance of healthy endothelium. A reduction in the bioavailability of eNOS-derived

TABLE 2. POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF SELECTED COMMERCIALY AVAILABLE COCOA PRODUCTS

Type of product	n	% NFCS	% fat	ORAC (μmol of TE)	Total polyphenols ^a	Epicatechin (mg/g)	Catechin (mg/g)
Cocoa powder	3	81.6 (8.2)	15.0 (5.8)	803.7 (78.2)	52.4 (7.5)	1.854 (0.849)	0.578 (0.285)
Baking chocolate	4	47.5 (2.2)	52.6 (0.7)	456.8 (50.7)	27.7 (1.3)	1.142 (0.103)	0.491 (0.222)
Dark chocolate	3	23.4 (5.3)	34.7 (5.5)	198.0 (47.0)	13.0 (1.7)	0.336 (0.031)	0.164 (0.064)
Semisweet chocolate chips	3	16.9 (1.7)	28.9 (1.0)	180.3 (8.5)	12.4 (0.6)	0.483 (0.085)	0.194 (0.071)
Milk chocolate	3	6.2 (1.2)	32.6 (4.0)	62.0 (17.6)	4.4 (1.1)	0.099 (0.067)	0.043 (0.038)
Chocolate syrup	3	6.2 (1.3)	0.9 (0.3)	63.4 (4.9)	4.2 (0.6)	0.074 (0.046)	0.042 (0.015)

Adapted from Miller *et al.*

Values are presented as means and standard deviations.

^aTotal polyphenols expressed as gallic acid equivalents.

NFCS, nonfat cocoa solids.

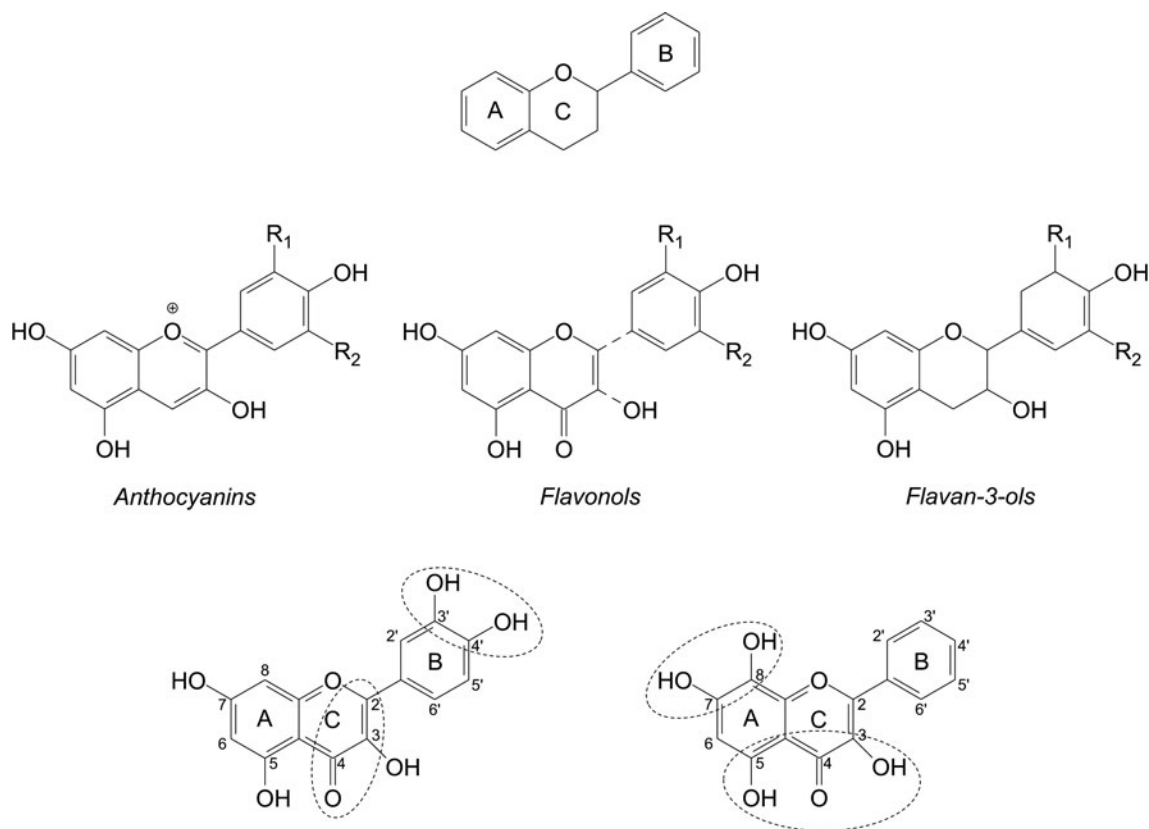


FIG. 2. C₁₅ (C₆-C₃-C₆) Flavonoid basic skeleton with high multifunctional activities including free radical scavenging, metal ion chelating, and enzyme inhibiting.

NO results in endothelial dysfunction, which is associated with all major risk factors for atherosclerosis. There is reason to believe that improving the function of the eNOS pathway may be effective in the prevention and treatment of atherosclerosis (Fig. 4) (143).

Endothelial NOS gene expression is regulated primarily by shear stress elicited by the circulating blood or endothelial cell proliferation, but its enzyme activity increases in response to receptor-operated substances such as acetylcholine, bradykinin, and serotonin (12).

The action of NO is mediated by the activation of the soluble guanylate cyclase in the smooth muscle cells and platelets, which increases the level of cyclic guanosine monophosphate (cGMP). The rise in cGMP inhibits calcium flux and decreases cytosolic calcium concentration, resulting in smooth muscle relaxation and platelet aggregation inhibition (173, 174). Prostacyclin is another vasodilator that works synergistically with NO to inhibit thrombosis. Increases in cGMP can increase cyclic adenosine monophosphate (cAMP) in the cell; cAMP is required for the activation of prostacyclin (171, 173, 174).

In addition to causing vasodilation, NO also prevents leukocyte adhesion and migration, smooth muscle cell proliferation, and platelet adhesion and aggregation (80). Nitric oxide and other endothelium-derived factors are important for the control of vascular biology, not only in the peripheral but also in the cerebral circulation (81). Deficiency of NO favors the development of atherosclerosis and is associated with increased cardiovascular risk in conditions such as type 2 dia-

betes, metabolic syndrome, hypertension, and atherosclerosis (31, 80). Pure epicatechin ingestion has been shown to acutely reduce the plasma levels of endothelin-1 in healthy men (144).

The specific mechanisms by which cocoa flavanols improve vascular function are the subject of ongoing research, but their effects on NO metabolism appear to be more substantial than their general antioxidant effects; NADPH oxidase may be the site of action.

NADPH oxidase is implicated in vascular dysfunction; NADPH oxidase isoforms generate O₂•⁻ that scavenge NO•. Cocoa polyphenols may help maintain NADPH oxidase activity at levels low enough to not harm vascular endothelium. Epicatechin increases bioavailability of NO by inhibiting NADPH oxidase (216, 247). Schewe and colleagues demonstrated that exposure to (-)-epicatechin elevates cellular levels of NO• and cGMP and protects against oxidative stress elicited by oxidized LDL (216); however, these effects tend to be transient. Longer-term effects of cocoa flavonoids on endothelial cells may be an adaptive mechanism to long-term exposure to high-flavonoid foods. Schewe *et al.* postulate that the effect on endothelial function may be mediated by changes in gene expression and protein synthesis or breakdown, including an elevated level of endothelial NO• synthase (eNOS) in the vascular endothelium (216, 245). Figure 5 illustrates structures of selected polyphenols and half maximal inhibitory concentration (IC₅₀) values for inhibition of NADPH oxidase activity. The 4-hydroxyphenyl group of apocynin is a determinant for NADPH oxidase inhibition by structurally similar flavonoids (216).

TABLE 3. COCOA FLAVONOID AND NONFLAVONOID PHENOLS

Class	Compounds
Cocoa nonflavonoid phenols	
Flavanols	(-)-Epicatechin (+)-Catechin (-)-Epicatechin-3-O-gallate (-)-Epigallocatechin Procyanidin B1 (epicatechin-(4 β (8)-catechin) Procyanidin B2 (epicatechin-(4 β (8)-epicatechin) Procyanidin B2-O-gallate (epicatechin-3-O-gallate-(4 β (8)-epicatechin) Procyanidin B2-3,3-di-O-gallate (epicatechin-3-O-gallate-(4 β (8)-epicatechin-3-O-gallate) Procyanidin B3 (catechin-(4 β (8)-catechin) Procyanidin B4 (catechin-(4 β (8)-epicatechin) Procyanidin B4-3-O-gallate (catechin-(4 β (8)-epicatechin-3-O-gallate) Procyanidin C1 (epicatechin-(4 β (8)-epicatechin-(4 β (8)-epicatechin)
Flavonols	Quercetin Isoquercetin (quercetin-3-O-glucoside) Quercetin-3-O-arbinoside Quercetin-3-O-galactoside
Anthocyanins	3-alpha-L-Arabinosidyl cyanidin 3-beta-D-Galactosidyl cyanidin
Flavones	Luteolin Luteolin-7-O-hyperoside Iso-orientin Vitexin
Flavanones	Naringenin Naringenin-7-O-glucoside
Cocoa nonflavonoid phenols	
Phenolic acids	Chlorogenic acid Vanillic acid Coumaric acid Phloretic acid Caffeic acid Ferulic acid Phenylacetic acid Syringic acid
Others	Clovamide Deoxyclovamide

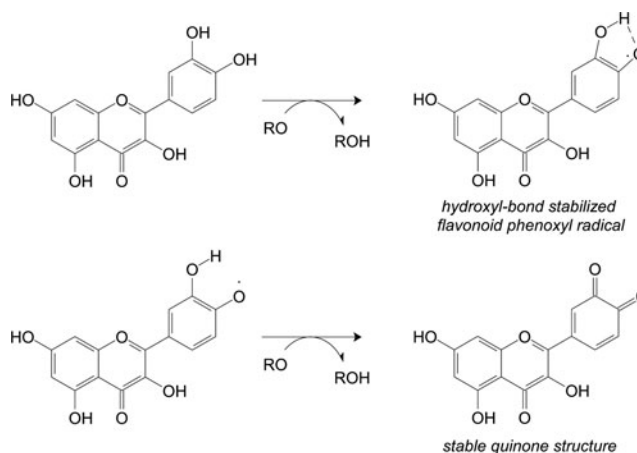


FIG. 3. Mechanism of antioxidant action of flavonoids (3', 4'-diOH polyphenols).

studies have mostly supported a protective association between cocoa or chocolate consumption and a variety of health indicators. These include total and cardiovascular mortality, serum C-reactive protein, psychological well-being, and risk of diabetes, myocardial infarction, and/or stroke (30, 59, 116, 189, 236).

The first cohort study of cocoa intake and cardiovascular outcomes was published in 2006 (29). The study population included 470 Dutch men free of cardiovascular disease and diabetes from the Zutphen Elderly Study cohort. Systolic and diastolic BPs were inversely associated with cocoa intake after adjustment for a wide range of possible confounders. This relationship was statistically significant for diastolic BP ($p=0.03$) and near significant for systolic BP ($p=0.06$). Cocoa consumption was associated with significantly lower cardiovascular and all-cause mortality. Those in the highest tertile of cocoa intake compared with those in the lowest tertile had 50% lower rates of all-cause mortality and cardiovascular disease. Individuals in the highest tertile consumed more than 2.30 g of cocoa daily, whereas those in the lowest tertile consumed less than 0.36 g/day. Consumption of other food groups, including confectionary, was not associated with mortality.

In 2009, a study in Stockholm, Sweden assessed cardiac mortality in a particularly high-risk group: nondiabetic patients hospitalized with a first myocardial infarction (116). After a mean 8.6 years of follow-up, patients who reported eating chocolate twice or more per week were 66% less likely to suffer a cardiac death compared to those who reported never eating chocolate. After adjustment for demographic and socioeconomic variables, coffee consumption, and intake of sweets, there was a significant, linear, inverse relationship between frequency of chocolate consumption and cardiac mortality ($p=0.01$). Total mortality, however, was not associated with chocolate consumption.

Other observational studies have identified a number of positive health outcomes associated with cocoa or chocolate consumption. In a Japanese study, risk of being found to have diabetes was reduced 35% among men who consumed "chocolate snack pieces" once per week or more compared with those who never or almost never ate chocolate (189). A similar but nonsignificant reduction in risk was observed for

II. Epidemiology: Chocolate and Health Outcomes

The majority of research on cocoa and health has been in the form of clinical trials on surrogate markers. However, a few prospective cohort studies are notable. An early ecological study investigated coronary mortality in relation to consumption of coffee, tea, cocoa, alcohol, and tobacco in 20 countries, but found no significant associations (28). Later

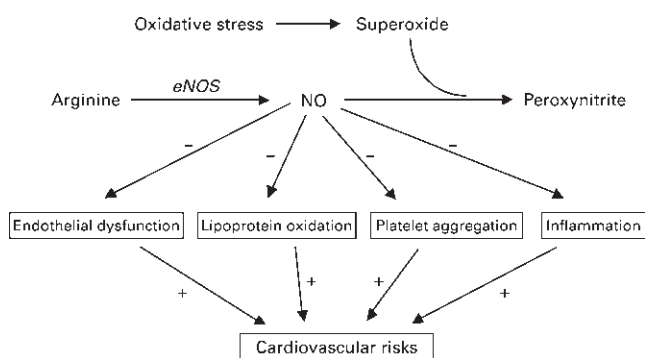


FIG. 4. Diagram to show how the downstream effects of cocoa polyphenols might affect the vascular system, with nitric oxide (NO) as the target. Endothelial dysfunction, lipoprotein oxidation, platelet aggregation, and inflammation all increase cardiovascular risk (+), whereas vascular effects of cocoa polyphenols reduce risk (-) from these intermediary factors. Reprinted with permission from Cooper *et al.* (41). eNOS, endothelial nitric oxide synthase.

women (189). In another study, a J-shaped relationship was observed between dark chocolate consumption and serum C-reactive protein (CRP) levels in Italian men and women (59). Individuals consuming a 20-g serving of dark chocolate daily had the lowest CRP concentrations. A 2009 study suggested a relationship between psychological health and chocolate preference in which elderly men preferring chocolate reported feeling less lonely and depressed and happier than men preferring other types of candy (251).

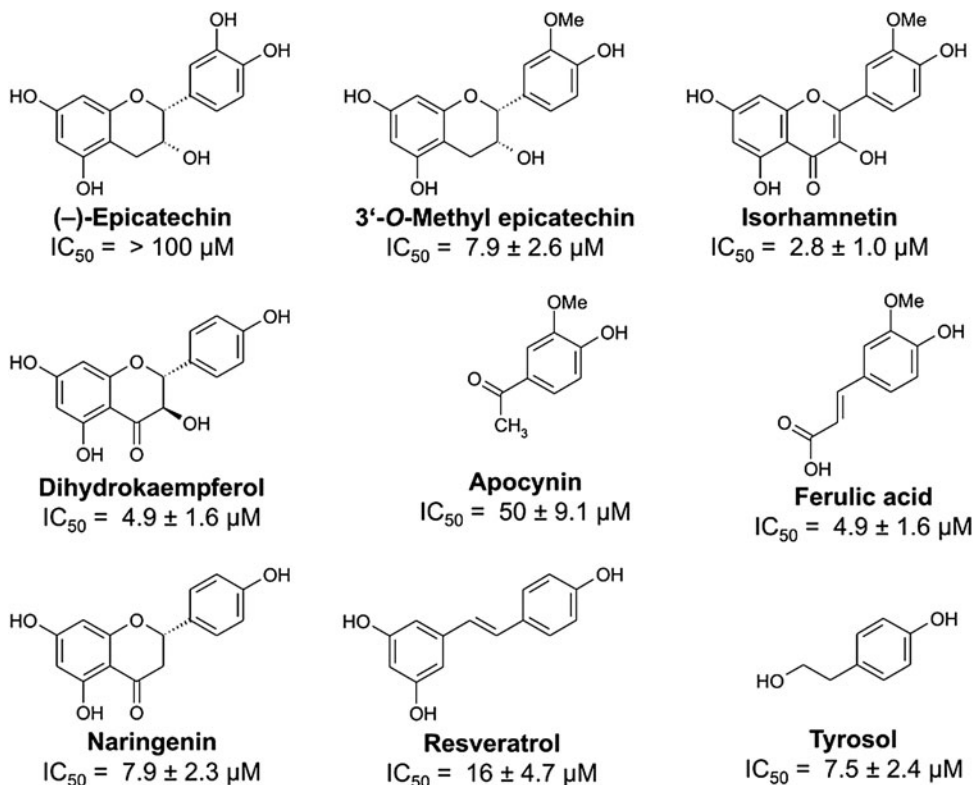
A cross-sectional analysis of data from 2217 participants in the NHLBI Family Heart Study identified an inverse rela-

tionship between chocolate consumption and calcified atherosclerotic plaque in the coronary arteries (61). Individuals who reported consuming (any type of) chocolate two or more times per week were 32% less likely to have prevalent coronary artery calcification compared with those who never ate chocolate. The odds ratios decreased with increasing frequency of chocolate consumption and were adjusted for age, sex, energy intake, waist-to-hip ratio, education, smoking, alcohol consumption, total cholesterol:HDL ratio, non-chocolate candy intake, and diabetes mellitus.

In 2010, Buijsse and colleagues released results from the first cohort study to demonstrate a reduced risk of myocardial infarction and stroke associated with chocolate consumption (30). The study followed 19,357 men and women in Germany over a mean of 8 years. Compared with the bottom quartile of chocolate consumption, those in the highest quartile had a 39% reduced risk of myocardial infarction or stroke ($p=0.014$). The highest quartile reported a mean chocolate intake of 7.5 g/day. The food frequency questionnaire used in this study did not distinguish between white, milk, and dark chocolate. However, a more detailed analysis of intake among a subset of participants found that milk chocolate was the type most often consumed (57%), followed by dark chocolate (24%) and white chocolate (2%).

Another 2010 study partially supports the findings of Buijsse *et al.* In this study, Mostofsky and colleagues assessed the relationship between chocolate consumption and heart failure in 31,823 middle-aged and elderly women over 9 years of follow-up (175). They found that women consuming 1–3 servings per month or 1–2 servings per week of chocolate had significantly lower rates of heart failure hospitalization or heart failure death compared with those consuming no chocolate (OR, 0.74; 0.68, respectively). However, the odds of

FIG. 5. Structures of selected polyphenols and IC₅₀ values for inhibition of NADPH oxidase activity. The noncatechol 4-hydroxyphenyl group of apocynin is a determinant for inhibition of NADPH oxidase by structurally related flavonoids and derivatives of cinnamic acid and silbenes. IC₅₀ is the half-maximal inhibitory concentration, a measure of a compound's effectiveness in inhibiting a biochemical function. IC₅₀ values are dependent on the specific conditions of the assay and cannot simply be translated to *in vivo* conditions. They permit, however, comparison of structurally related compounds. Reprinted with permission from Schewe *et al.* (216).



heart failure outcomes were slightly, but not significantly, higher among women who reported consuming 3–6 servings per week (OR, 1.09; 95% CI, 0.74–1.62) or one or more servings per day (OR, 1.23; 95% CI, 0.73–2.08).

III. Effects on Cardiovascular Disease

A. Pathogenesis

Cardiovascular disease is the leading cause of death and disability-adjusted life years globally and is associated with risk factors, including hypertension, smoking, hyperlipidemia, and diabetes mellitus (286). The vast majority (80%) of cardiovascular disease burden is now experienced in low- and middle-income countries (286). Atherosclerosis is the result of a series of inflammatory responses at the cellular and molecular level that leads to the development of lesions in medium-sized arteries. Chronic inflammation is responsible for the progression of atherosclerotic lesions through advancing stages. It has been proposed that endothelial dysfunction initiates the process of atherosclerosis. There are many possible etiological factors for endothelial dysfunction, including elevated LDL or homocysteine in the blood, infectious microorganisms, genetics, and oxidative stress. The injury caused by these varied agents initiates an inflammatory response that may continue indefinitely (209). The resulting chronic inflammatory state is characterized by increasing numbers of macrophages and lymphocytes and proliferation of smooth muscle cells. Unabated inflammation will further increase the concentration of macrophages and lymphocytes that promote the formation of fibrous tissue in the lesion through the release of hydrolytic enzymes, cytokines, chemokines, and growth factors. The resulting complicated lesion has the potential to narrow the lumen of the artery and block blood flow (209). Advanced plaques high in lipid content are particularly vulnerable to disruption, which can lead to the occlusive thrombosis that characterizes acute coronary syndromes (43).

Diet has been established as one of the most important lifestyle factors that can strongly influence the incidence of cardiovascular disease (51, 76, 125). Dietary flavonoids may decrease cardiovascular risk by protecting lipids, proteins, and nucleic acids from oxidative damage, as well as reducing inflammation and regulating vascular homeostasis (205). Flavonoid intake has been inversely associated with coronary artery disease (CAD) incidence and mortality (98).

A number of pro-inflammatory enzyme systems are involved in the pathogenesis of atherosclerosis, including xanthine oxidase and NADH/NADPH oxidase (159) that produce reactive oxygen species (ROS), which can cause endothelial dysfunction (265). Flavonols, particularly epicatechin (222), in cocoa have anti-inflammatory properties that inhibit the production of ROS, and have antihypertensive and vasoprotective effects (42).

Cocoa polyphenols modulate the activity of NF- κ B (141), a protein complex involved in DNA transcription that is a pivotal factor in a number of inflammatory processes (Fig. 6) (226). NF- κ B activation in leukocytes (as well as in endothelial cells and macrophages) results in leukocyte adhesion to the endothelium, tissue invasion, and the secretion of mediating factors leading to tissue injury. Cocoa polyphenols can reduce the activity of NF- κ B, downregulating leukocyte activation and attenuating the production of inflammatory mediators

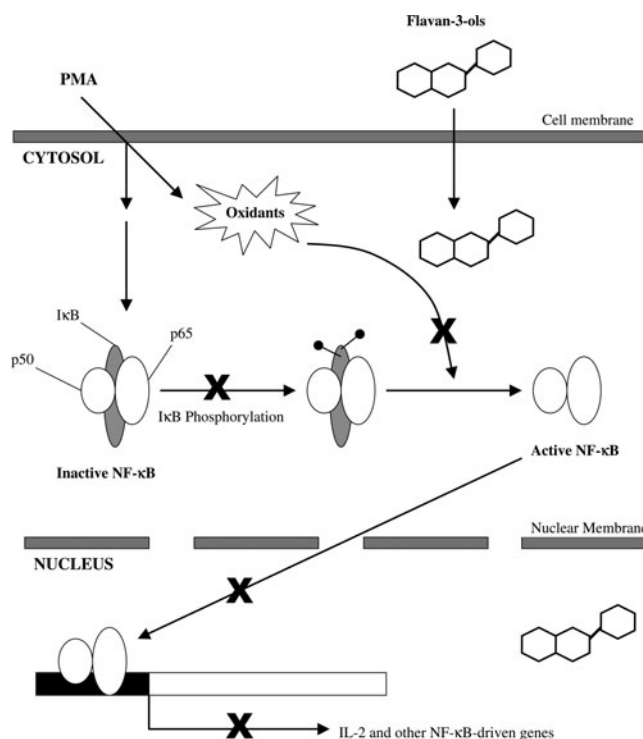


FIG. 6. Inhibition mechanisms of phorbol myristate acetate (PMA)-induced NF- κ B activation by cocoa flavanols, including a dimer. The specific steps in the NF- κ B activation cascade by which cocoa flavanols can interfere are indicated with an "x." Reprinted with permission from Selmi *et al.* (226).

and ROS (226, 229). Further, polyphenols act synergistically with other nutrients, such as vitamin C and selenium, to increase endogenous antioxidant capacity (229).

Cocoa flavanols can affect oxidant enzymes such as lipoxygenases, involved in arachidonic acid metabolism and the biosynthesis of leukotrienes. High-procyanidin chocolate was found to increase plasma prostacyclin and decrease plasma leukotrienes (220, 233), reflecting anti-inflammatory and vasoprotective properties (233).

B. Effects on endothelial function

Endothelial function is recognized as an important measure of cardiac risk, as impaired endothelial function has been found to predict both recurring and incident cardiovascular events (7, 34, 52, 57, 68, 209, 215, 253, 284, 285) (Fig. 7).

Endothelial function refers to arterial vasomotor responses mediated predominantly by the release of NO (vasodilating), and endothelin (vasoconstricting) from the vascular endothelium (145, 266), and plays an important role in the pathogenesis of atherosclerosis, hypertension, and cardiovascular disease (266). Endothelial dysfunction has been shown in the coronary and peripheral circulations before the development of atherosclerotic plaque (284, 285). Individuals with cardiac risk factors develop endothelial dysfunction *via* decreased NO production, abnormal signaling, and increased oxidative stress (266). Endothelial dysfunction has been shown to reverse in response to cardiac risk modification efforts (266). NO

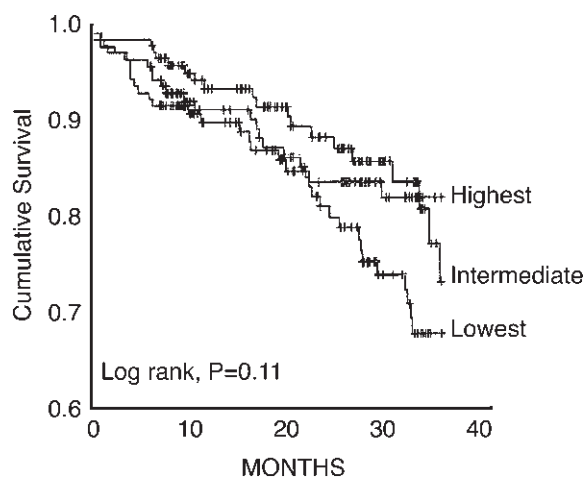


FIG. 7. Kaplan-Meier curves for cumulative event-free survival associated with endothelial function measured as flow-mediated dilatation (FMD) (lowest tertile <2%, highest tertile >6.3%). Log-rank analysis revealed a significant difference between the low and intermediate tertiles ($p=0.037$). Further analysis revealed a significant difference in event rate between those with the most severe FMD abnormality (tertile 1) and the combined group of second and third tertiles (log-rank $p=0.029$). When this analysis was restricted to all-cause mortality alone, it remained significant (log-rank $p=0.047$). Adapted from Fathi *et al.* (68).

also has an anti-inflammatory effect, counteracting leukocyte recruitment and platelet aggregation at the site of inflammation (225) though other substances play important roles in modulating vascular tone, fibrinolysis, coagulation, and inflammation (274).

Human trials have demonstrated vasoprotective effects mediated by NO (92, 225, 256). A 2008 meta-analysis of randomized, controlled trials of flavonoid-containing foods concluded that cocoa was the only food to show significant effects acutely and chronically on flow-mediated dilatation (FMD) of the brachial artery, a noninvasive method to measure endothelial function (107). Data from two long-term studies and six acute studies indicated that chocolate or cocoa significantly increased FMD by 1.45% and 3.99%, respectively (107). Corti *et al.* reviewed 11 human trials of cocoa and endothelial function and found that all demonstrated significant improvement in FMD after cocoa consumption (42). Acute studies were 2 h in duration, whereas longer-term studies were 2 weeks long, with the exception of one 4-week study (17) and a 5-day study (73). Four of the trials were conducted on healthy individuals (65, 73, 222, 231) and two on smokers (95, 97). Other study populations included diabetic patients (17); hypertensives (85); hypertensives with impaired glucose tolerance (84); patients with at least one cardiovascular risk factor (92); and heart transplant recipients (75). In five of the studies, a cocoa beverage was administered (17, 73, 92, 95, 222); the other six used dark chocolate (65, 75, 84, 85, 97, 231).

1. Cocoa beverage consumption in healthy subjects. Fisher and colleagues, in their 5-day trial in 27 healthy individuals, found that consumption of flavanol-rich cocoa (821 mg flavanols/day) induced vasodilation, as measured by pulse wave amplitude, which was reversible by the NO syn-

these inhibitor NG-nitro-L-arginine methyl ester, indicating that the vasodilating effect was attributable to activation of the NO system. The effects of low-flavanol cocoa were significantly smaller ($p=0.005$) (73).

Schroeter *et al.* conducted a series of studies to determine the acute vascular effects of cocoa and the role of cocoa flavanols in mediating those effects. In one of these studies, the group assessed the effects of a single dose of high-flavanol cocoa beverage (917 mg total flavanols) compared to a low-flavanol cocoa (37 mg flavanols) in 10 healthy men. In a subgroup of three participants, the dependence of endothelial function improvements on NO synthase was tested by inhibiting NOS before consumption of the high-flavanol cocoa beverage (222). To determine the effects of flavanols, isolated from other cocoa components, the researchers used a randomized, cross-over design to test two different doses of pure epicatechin (1 or 2 mg/kg of body weight) mixed with water, compared to water only in six individuals (three per treatment arm). Further, a cross-sectional study was completed to compare urinary flavanol metabolites in 18 mainland-dwelling Kuna Indians and 16 of those still living on the San Blas Islands, where cocoa intake is very high. Key findings from these studies include the following: (i) FMD was significantly increased at 1–4 h after ingestion paralleled by increases in plasma flavanols and metabolites; (ii) peripheral arterial tonometry (PAT, a measure of microvascular circulation) responses increased after high-flavanol cocoa consumption; (iii) FMD response was significantly attenuated by NO synthase inhibition; (iv) FMD and PAT increased significantly 2 h after ingestion of either 1 or 2 mg of pure epicatechin per kg of body weight, whereas no changes were associated with water alone—these changes were similar to those observed after high-flavanol cocoa ingestion; (v) urinary flavanol metabolites were more than six times higher in Kuna Islanders than in the mainland Kuna sample.

Fisher and colleagues also reported significantly greater improvements in endothelial function (FMD) after 4–6 days of flavanol-rich cocoa consumption in older (≥ 50 years) than in younger (< 50 years) individuals (72). Consistent with the findings of Heiss *et al.* (93) endothelial function was also further improved by acute ingestion of cocoa at the end of the period of sustained consumption.

Our lab has conducted two studies to investigate the effects of cocoa or chocolate on endothelial function. A randomized, placebo-controlled, single-blind crossover design was utilized to test the acute effects of dark chocolate, sugared cocoa and sugar-free cocoa, and the long-term effects of sugared and sugar-free cocoa. The placebos for both studies were white chocolate and white cocoa, as appropriate. The test products contained between 805 and 821 mg total flavanols, whereas the placebos contained no flavanols. The dark chocolate and white chocolate products were similar in energy, fat, and carbohydrate content, as were the sugared cocoa and white cocoa (66). The composition of test products used in this study is provided in Table 4.

The acute study had two phases. In the first, participants received single doses of 74 g dark chocolate and 74 g white chocolate, in random sequence and with a 7-day washout period between treatments (66). FMD and BP were measured after an 8-h fast and 2 h after ingestion of each treatment. Compared with placebo, dark chocolate improved FMD from baseline ($4.3 \pm 3.4\%$ compared with $-1.8 \pm 3.3\%$; $p < 0.001$) and

TABLE 4. COMPOSITION OF TEST PRODUCTS^a

Content	Placebo chocolate	Solid dark chocolate	Sugar- free cocoa	Sugared cocoa	Placebo cocoa
Weight (g) ^b	74	74	23.4	114.9	125.3
Cocoa powder (g)	0	22	22	22	0
Energy (kcal)	389	327	90	460	500
Total fat (g)	22	27	2	2	2
Carbohydrates (g)	44	39	12	104	110
Protein (g)	6	6	6	6	8
Sodium (mg)	97	4	110	110	410
Potassium (mg)	306	366	334	334	512
Calcium (mg)	215	33	33.4	33.4	314
Magnesium (mg)	19	119	133.6	133.6	38
Catechin (mg)	0	10.4	20.9	20.9	0
Epicatechin (mg)	0	21.5	48.4	48.4	0
Procyanidin dimer (mg)	0	81.4	92.0	92.0	0
Procyanidin trimer (mg)	0	67.3	98.1	98.1	3.3
Procyanidin tetramer (mg)	0	37.0	30.6	30.6	0
Procyanidin pentamer and hexamer (mg)	0	67.0	54.8	54.8	5.5
Total procyanidins (total flavanols) (mg)	0	821	805.2	805.2	8.8
Theobromine (mg)	1.5	525	436	436	0
Caffeine (mg)	3.7	44	28.1	28.1	0

Reprinted with permission from Faridi *et al.* (66).

^aEnergy and nutrient data of the tested products are provided by the Hershey Company.

^bRefers to total product weight.

reduced BP (systolic: -3.2 ± 5.8 mm Hg compared with 2.7 ± 6.6 mm Hg; $p < 0.001$; diastolic: -1.4 ± 3.9 mm Hg compared with 2.7 ± 6.4 mm Hg; $p < 0.001$).

In the second phase of the study, participants were randomized to one of six possible permutations of treatment sequences (188). All received each treatment (two cups of sugared, sugar-free, or placebo cocoa providing 805, 805, and 0 mg flavanols, respectively) with 7-day washout periods between each treatment assignment. Single doses of sugared and sugar-free cocoa improved FMD significantly from baseline compared with placebo ($5.7\% \pm 2.6\%$ and $2.0 \pm 1.8\%$ compared with $-1.5\% \pm 2.8\%$; $p < 0.001$), but the effect was significantly greater after sugar-free cocoa consumption compared with sugared cocoa ($p < .001$). Significant reductions in systolic and diastolic BPs were also observed after consumption of sugar-free, but not sugared, cocoa compared with placebo (systolic: -2.1 ± 7.0 mm Hg compared with 3.2 ± 5.6 mm Hg; $p < 0.001$; diastolic: -1.2 ± 8.7 mm Hg compared with 2.8 ± 5.6 ; $p = 0.014$).

In the long-term study (188) 44 subjects were enrolled and 37 completed the study. Using the same cocoa preparations from the acute study (sugared cocoa, sugar-free cocoa, and placebo cocoa), we assigned subjects to consume each of the cocoa preparations daily (two cups per day providing 805 mg flavanols or no flavanols) for 6 weeks, in random sequence. Both flavanol-containing cocoas improved FMD compared to placebo (2.4 and 1.5% for sugar-free and sugared cocoa, re-

spectively, compared with -0.8% ; $p < 0.01$). Although improvement was greater after sugar-free cocoa than sugared cocoa, the difference was not significant ($p = 0.15$). BP did not improve significantly in either intervention period compared with placebo.

2. Cocoa beverage consumption in subjects at risk for cardiovascular disease. Heiss and colleagues have contributed much to the study of cocoa. In a 2003 study, they assessed the effects of high- and low-flavanol cocoa drinks on FMD and nitrosylated and nitrosated species (RNO) in 20 participants with at least one cardiovascular risk factor (92). In the double-blind, crossover trial, participants received 100 ml of high-flavanol cocoa drink (176 mg) and a low-flavanol cocoa (< 10 mg) on 2 consecutive days, in random sequence. The high-flavanol and low-flavanol cocoas contained 73 and 66 kcal, respectively. In an initial pilot test, the high-flavanol cocoa increased FMD maximally 2 h after ingestion, whereas the low-flavanol drink had no effect. Based on these results, outcome measures were assessed 2 h after ingestion of the test products in the full study. Principal findings from this study include the following: (i) flavanol-rich cocoa, but not low-flavanol cocoa, significantly increased RNO and FMD (from 3.4% to 6.3%); (ii) changes in RNO and FMD were correlated ($r = 0.42$, $p = 0.02$); (iii) there were no significant changes in other vascular variables. These findings were replicated in a subgroup of 11 smokers with no other cardiovascular risk factors (95).

In a 2007 study series, Heiss *et al.* investigated the effects of acute (up to 6 h after ingestion) and chronic (daily ingestion for 7 days) high-flavanol cocoa consumption on FMD in healthy male smokers (93). In the 7-day study, high-flavanol cocoa contained 306 mg total flavanols and 80 kcal per dose and was consumed three times per day (93). Two acute studies were conducted using a randomized, double-blind crossover design to test the effects of three different doses of cocoa flavanols for 1–6 h. In the first, five subjects received cocoa drinks containing 36, 330, and 918 mg on different days in random order. In the second acute study, a separate group of six subjects received cocoa with 28, 179, and 483 mg of flavanols. The drinks were closely matched for other nutrients. The acute studies found that maximum increases in FMD occurred 2 h after ingestion of high-flavanol cocoa, regardless of dose, but the magnitude of FMD response and the time to return to baseline was dose dependent. For example, ingestion of cocoa containing 36 mg of flavanols did not significantly change FMD, whereas cocoa containing 330 mg increased FMD at 1, 2, and 3 h after ingestion and cocoa containing 918 mg increased FMD at 1, 2, 3, 4, and 6 h after ingestion. Peak FMD was also significantly different across all three groups. In the chronic study, fasting FMD increased progressively during 1 week of daily high-flavanol cocoa consumption. FMD responses on days 1, 3, 5, and 8 were 3.7%, 5.2%, 6.1%, and 6.6%, respectively. Further, the degree of acute FMD response after cocoa ingestion on each day was maintained, so that both fasting and post-cocoa ingestion measures were higher on successive days. FMD returned to baseline levels after a 1-week washout period. These findings suggest that although cocoa's effects on the vascular endothelium occur primarily in the short-term, chronic consumption may result in sustained benefits.

In a randomized, controlled, double-blind study by Balzer *et al.*, FMD increased by 30% among 21 medicated diabetics who consumed cocoa containing 321 mg of flavanols per dose three times per day for 30 days, but not in the 20 subjects who consumed a nutrient-matched low-flavanol cocoa (17). No significant differences in BP, heart rate, or glycemic control were observed between the groups. The test products provided between 160 and 170 kcal, approximately, per day. Like Heiss *et al.*, Balzer and colleagues also observed maximal increases in FMD 2 h after ingestion of cocoa and greater increases among participants ingesting cocoa containing higher amounts of flavanols. Consistent with Heiss *et al.*, baseline FMD improved after 8–30 days of sustained cocoa consumption compared with control, and acute FMD responses to cocoa ingestion also increased relative to baseline (Fig. 8).

3. Solid dark chocolate consumption in healthy subjects. Engler *et al.* investigated the effects of sustained dark chocolate consumption on endothelial function, as well as measures of oxidative stress, blood lipids, and BP in 21 healthy men and women using a randomized, double-blind, placebo-controlled design (65). The test products were 46 g of high-flavonoid (21 mg procyanidins, 46 mg epicatechin) or low-flavonoid (trace flavanols) dark chocolate, consumed once daily for 2 weeks. A nonsignificant decrease in FMD from baseline was observed in the low-flavonoid group ($p=0.17$), whereas a near-significant increase was observed in the high-flavonoid group ($p=0.05$). The change in FMD between the two groups was significantly different, however

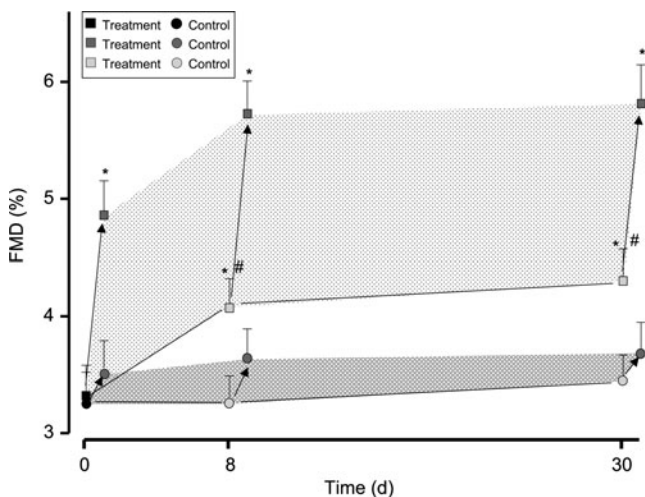


FIG. 8. Acute and sustained effects of flavanol-containing cocoa. At study entry, baseline values for flow-mediated dilation (FMD) were similar in both groups. In the treatment group (squares), FMD was significantly augmented over time. On top of sustained FMD increases, acute improvements were observed at 2 h after ingestion of flavanol-containing cocoa. No significant changes could be observed in the control group (circles). Data are given as mean \pm standard deviation. *Indicates significant differences in FMD compared with baseline differences within each group, $p < 0.001$; #Indicates significant differences in FMD between the control group and the treatment group, $p < 0.05$. Reprinted with permission from Balzer *et al.* (17).

($p=0.024$). No significant changes in any other outcome measures were observed. In a separate study with two individuals, consumption of a single 120 g dose of high-flavonoid chocolate did not increase plasma epicatechin levels further compared with the 46 g dose used in the larger study (65).

Hermann and colleagues also studied the effects of dark chocolate on endothelial function in smokers (97). They randomly assigned 20 men to receive a single dose of either 40 g of dark chocolate or 50 g of white chocolate. FMD and shear stress-dependent platelet function were assessed before and 2 h after chocolate ingestion. Consistent with other studies, dark chocolate improved FMD response significantly from 4.4% to 7.0% ($p=0.026$), whereas white chocolate had no effect. Platelet function was also reduced in the dark chocolate group (5.0%–3.2%, $p=0.03$) but not the white chocolate group. Accordingly, antioxidant status increased after dark chocolate ingestion only.

Two endothelial function studies involving dark chocolate were conducted by Grassi *et al.* (84, 85). In the first, 20 patients with essential hypertension received 100 g of dark chocolate (88 mg flavanols) and 90 g of white chocolate (no flavanols) daily for 15 days, in random sequence with a 7-day washout period between treatments (85). Outcome measures included FMD, 24-h ambulatory BP, 2-h glucose, serum cholesterol, and markers of vascular inflammation. Measures of insulin sensitivity were also calculated. Dark chocolate significantly improved FMD, systolic (–11.9 mm Hg) and diastolic (–8.5 mm Hg) BPs, insulin sensitivity, and serum LDL (–0.4 mM). All measures remained unchanged after white chocolate consumption.

In their second study, Grassi and colleagues used the same research design and chocolate dose to test the effects of regular dark chocolate consumption in 19 hypertensive subjects who were also glucose intolerant (84). In addition to the variables measured in the first study, serum CRP, plasma homocysteine, and β -cell function (corrected insulin response) (120) were also assessed. In this population, dark chocolate again significantly improved FMD, insulin sensitivity, 24-h ambulatory systolic and diastolic BP, and LDL. Dark chocolate also significantly decreased total cholesterol (–6.5%, $p < 0.0001$) and improved β -cell function ($p=0.035$).

4. Dark chocolate and coronary circulation. Another dark chocolate study by Flammer *et al.* provides support for the extension of cocoa's vascular effects to coronary arteries (75). In this double-blind, randomized trial, 22 heart transplant recipients received a single dose of either 40 g of dark chocolate (15.6 mg epicatechin equivalents per gram) or a flavonoid-free control chocolate. Coronary artery diameter and endothelium-dependent coronary vasomotion increased significantly and platelet adhesion decreased significantly 2 h after ingestion of the flavonoid-rich dark chocolate, but not flavonoid-free chocolate. These changes were paralleled by increases in serum epicatechin concentrations.

A 2009 study by Shiina *et al.* also found improvement in coronary circulation associated with dark chocolate ingestion (231). In this randomized, single-blind study, 39 healthy men were assigned to consume either 45 g of flavonoid-rich dark chocolate (550 mg polyphenols) or 35 g flavonoid-free white chocolate daily for 2 weeks. Coronary flow velocity reserve (CFVR) measured by echocardiography, BP, serum lipids, and markers of oxidative stress were assessed at baseline and

at the end of the study. Dark chocolate, but not white chocolate, significantly improved CFVR, independent of changes in other variables.

5. Cocoa and chocolate consumption in CAD patients. In contrast to the positive findings of studies in healthy individuals and those with cardiovascular risk factors, the results of a study in 40 subjects with CAD were null (67). In this randomized, double-blind, placebo-controlled study, the active treatment was a chocolate bar and cocoa beverage, containing a total of 444 mg of flavanols. Isocaloric placebo chocolate bars and cocoa drinks containing only 19.6 mg of flavanols but matched for macronutrient, caffeine, and theobromine content were provided to the control group. Each treatment was consumed daily for 6 weeks and provided approximately 275 kcal and 18 g fat per day. Endothelial function, high-sensitivity CRP, oxidized LDL, lipids, glucose, and insulin were assessed. No significant differences in any measure were observed between the treatment groups.

However, another study in CAD patients published in July 2010 had different results (94). This study used a randomized, double-blind, cross-over design to investigate whether cocoa flavanols improve endothelial function in this population and whether improvement involves an increase in number and function of circulating angiogenic cells (CACs). All 16 subjects consumed each of the test beverages—high-flavanol cocoa (375 mg) and low-flavanol cocoa (9 mg)—twice daily for 30 days, in random sequence. FMD improved significantly from baseline in both groups, but high-flavanol cocoa increased FMD significantly more than low-flavanol cocoa (3.8% *vs.* 1.3%, respectively, $p=0.001$). High-flavanol cocoa also significantly increased the mobilization of functional CACs and reduced BP compared with low-flavanol cocoa. CAC numbers more than doubled after the high-flavanol cocoa intervention, a finding the authors suggest is clinically relevant and comparable to effect sizes reported for other interventions including statins, estrogen, exercise, and smoking cessation.

The conflicting results of this study and the study by Farouque *et al.* are noteworthy. Flavanol intake and sample size per treatment arm or phase were similar in both studies, as were age, gender, and BMI. Some differences in baseline characteristics of the study populations include higher proportions of diabetics and hypertensives in the Heiss *et al.* study (38% *vs.* 7.5% and 88% *vs.* 55%, respectively). Fewer subjects in the Farouque *et al.* study were using beta blockers (45% *vs.* 88%). The Farouque *et al.* study was also longer in duration (6 weeks *vs.* 30 days). The form in which cocoa flavanols were delivered did differ between the studies, and may have affected the results. In the Heiss *et al.* trial, the active treatment was a cocoa beverage and provided 50 kcal and <1 g fat per day. In comparison, the cocoa beverage/solid chocolate combination used in the Farouque *et al.* study provided 275 kcal and 18 g fat. Additional research is needed in CAD patients to determine whether there is a clinical benefit of cocoa flavanol consumption and to identify the most beneficial way to deliver them.

C. Effects on platelets

When the endothelium is damaged and the underlying fibrous matrix exposed, as it is when atherosclerotic plaque ruptures, platelets can adhere to the matrix and become ac-

tivated (9). Once activated, platelets can recruit leukocytes and endothelial progenitor cells, and interact with these cells to induce inflammatory responses and promote thrombus formation (88, 158).

Dietary flavonoids, and flavanols in particular, have been associated with decreased platelet reactivity. Although the specific mechanisms responsible for this association have not yet been identified, it has been theorized that flavanols may affect the activity of platelets by inducing changes in membrane fluidity, ligand-receptor affinity, and intracellular signaling pathways (105). It has also been suggested that platelet-derived nitric oxide may be a target of flavanols (204). Although many plant foods contain flavanols, cocoa is an especially concentrated source (150), and has been recognized as having significant effects on platelets in humans. A systematic review of 25 intervention studies of polyphenol-rich diets and platelet function noted cocoa's platelet-inhibiting effects as the only consistent finding among the trials (194). In 2007, Bordeaux and colleagues found that, among healthy participants in a platelet function study, those who had consumed chocolate before testing ($n=141$) had reduced platelet activity compared to nonconsumers (23). A small study of 30 healthy subjects found that platelet activation decreased 2 and 6 h after cocoa consumption (205). In another trial, cocoa consumption inhibited platelets *in vivo*, whereas dealcoholized red wine consumption did not (204). Hamed and colleagues demonstrated that these short-term effects could also be seen after 1 week of solid dark chocolate consumption (containing 700 mg of flavonoids per day) in 28 healthy subjects (89). Flammer and colleagues found that dark chocolate consumption decreased platelet adhesion 2 h after consumption in 22 heart transplant patients (75). A study by Heptinstall *et al.* evaluated the effects of cocoa beverage consumption on platelets and leukocytes *in vitro* and *ex vivo* supporting previous findings of platelet inhibition, and identifying a potential role for cocoa flavanols in suppressing leukocyte activation (96).

Existing evidence strongly supports a beneficial effect of cocoa consumption on platelet activation, an important contributor to the inflammation and thrombosis that leads to advanced cardiovascular disease. The effects are likely due mostly to the actions of flavanols, though the minerals (potassium, magnesium, and calcium) in cocoa or stearic acid present in chocolate may also play a role (196).

D. Effects on lipids

Cocoa's effects on serum lipids are not as clear as its effects on endothelial function and platelet activity. Evidence for a lipid-lowering effect is limited and inconclusive.

Numerous studies have investigated the effects of cocoa consumption on the lipid profile, with conflicting results. In one trial in patients with hypertension, daily consumption of 100 g flavonoid-rich chocolate over 2 weeks significantly reduced serum total cholesterol by 7% and LDL by 12% (85). Consumption of cocoa and dark chocolate increased HDL by 4% in one study (267).

Mellor *et al.* found that HDL increased in subjects consuming a high-polyphenol chocolate, but not in persons consuming a low-polyphenol chocolate over the course of 16 weeks (166).

In contrast, Engler *et al.* found no difference in serum lipids between subjects consuming 46 g of high-flavonoid dark

chocolate and those consuming low-flavonoid chocolate daily for 2 weeks (65). Almoosawi *et al.* also did not observe significant changes in total cholesterol among overweight and obese subjects after 2 weeks of daily consumption of dark chocolate containing either 500 mg or 1000 mg polyphenols (6).

Feeding studies of cocoa beverages containing only cocoa powder have also had mixed results. Wang-Polagruto and colleagues studied the effects of consuming a high flavanol (446 mg total flavanols) cocoa beverage relative to a low flavanol (43 mg) cocoa beverage daily for 6 weeks in hypercholesterolemic postmenopausal women (269). In this study, HDL levels increased 6.6% in the high flavanol group, whereas HDL decreased 9.6% in the low flavanol group ($p < 0.05$). Another study investigated the effects of consuming a cocoa beverage containing four different levels of polyphenols (0 g, 13 g, 19.5 g, or 26 g) daily for 4 weeks on the lipid panel in normocholesterolemic and mildly hypercholesterolemic individuals (14). Although none of the polyphenol-containing cocoa treatments differed significantly from the placebo cocoa, all three significantly improved LDL and HDL levels from baseline in subjects with high LDL at the start of the study. However, these improvements were small. Baba *et al.* observed a greater increase in HDL (24%) after daily ingestion of 12 g sugar plus 26 g cocoa powder for 12 weeks. This increase was significantly different than the small increase (5%) observed in the control group, which only received 12 g sugar daily (15). At least three studies found no effect of cocoa beverage consumption on blood lipids (45, 178, 188), including one which tested a combination of liquid cocoa and solid dark chocolate (45).

Because of their high flavonoid content, the cocoa solids present in chocolate are typically hypothesized to affect measures of cardiovascular health, whereas other components of chocolate products (*e.g.*, cocoa butter) are thought to have little or no effect. Given this reasonable assumption, the results of a study by Kris-Etherton *et al.* are surprising (135). In their study, consumption of milk chocolate as a substitute for a high-carbohydrate snack bar improved levels of serum HDL and triglycerides. This improvement occurred despite the fact that the milk chocolate bar increased the total fat and saturated fat in the diet. Because milk chocolate contains a relatively small proportion of cocoa solids, this finding might suggest that a component in chocolate other than flavonoids (possibly stearic acid) was responsible. However, in another trial, HDL increased by 11.4% and 13.7% when subjects consumed dark chocolate and polyphenol-enriched dark chocolate, respectively, but not when they consumed white chocolate (181). One possible explanation for the results of Kris-Etherton *et al.* is that the chocolate bar's displacement of a high-carbohydrate snack, rather than some active compound in the chocolate bar itself, led to the change in lipid levels.

A 2010 meta-analysis of eight trials summarized the short-term impact of cocoa consumption on blood lipids (119). The data from these trials indicate that cocoa may significantly reduce LDL cholesterol, and may also reduce total cholesterol when consumed in low doses by individuals with cardiovascular risk factors. This meta-analysis supports a lipid-lowering effect of cocoa, but it is limited by the small total sample ($n = 215$), the paucity of well-designed trials, and the heterogeneity of the studies included. Whether cocoa products substantially improve lipid levels in the blood remains unclear. There is, however, convincing evidence that con-

sumption of chocolate in most forms has at worst, a neutral effect on the lipid profile. This evidence should allay fears that the high saturated fat content of chocolate would negate the effects of its other health-promoting compounds. Further, although cocoa may not appreciably change the quantity of lipids in the blood, it may change their quality, that is, their ability to cause blood vessel damage.

Lipids, n-6 fatty acids in particular, are susceptible to peroxidation by reactive oxygen species (ROS) produced as a result of normal metabolic processes or pathological events. To a certain extent, cells can compensate for the activity of ROS with endogenous antioxidant defenses, but when prooxidants exceed antioxidants, cells experience oxidative stress (287). Exogenous antioxidants from food sources are therefore important in maintaining a favorable balance between ROS and the antioxidants that can inactivate them (287).

Levels of oxidized LDL in the blood have been shown to predict CAD better than total cholesterol, triglycerides, HDL, and LDL (63, 110). In general, intervention studies suggest that cocoa can inhibit LDL oxidation. Decreased levels of plasma-oxidized LDL have been observed in subjects after long-term daily consumption of cocoa powder (14, 15) and dark chocolate (267). These effects might be attributed to epicatechin, which attenuates LDL oxidation and protects the endothelium from the actions of oxidized LDL (246). However, some studies suggest that consumption of chocolate low in flavonoids may also be beneficial. In two studies, milk chocolate (135) and white chocolate (181) inhibited LDL oxidation. In the latter study, a marker of lipid peroxidation decreased 11.9% after consumption of white chocolate, dark chocolate, or dark chocolate enriched with polyphenols (181). These results indicate that the fatty acids in chocolate may play an important role in LDL oxidation.

Although one study failed to find an effect of either flavonoid-rich dark chocolate or low-flavonoid chocolate on LDL oxidation (65), most available evidence supports the hypothesis that consumption of cocoa or chocolate protects lipids from oxidation.

E. Effects on BP

A relationship between cocoa consumption and reduced BP was first observed in the Zutphen Elderly Study (29). Subsequent randomized, controlled trials have confirmed the association. A meta-analysis of five such trials found that cocoa consumption was associated with significant reductions in systolic and diastolic BPs of 4.7 and 2.0 mm Hg, respectively. Other intervention studies have had similar findings (6, 66, 84) but a few have found no effect of cocoa on BP (45, 65, 178, 180). A 2010 study found that a daily dose of 1052 mg cocoa flavanols was required to reduce 24-h ambulatory BP. There was no effect on seated BP or on either measure at lower doses of flavanols (48). Another study found that a dark chocolate bar containing 500 mg polyphenols lowered BP as effectively as a bar containing 1000 mg (6). Crews *et al.* did not observe any change in BP among healthy men and women over the age of 60 who consumed both a cocoa beverage and dark chocolate daily for 6 weeks (providing a total of 754.71 mg proanthocyanins), relative to those consuming low-flavanol control products (45). However, Berry and colleagues demonstrated that consumption of a high-flavanol (701 mg) cocoa beverage could significantly

improve BP response to exercise compared to a low-flavanol (22 mg) beverage (21).

Although some studies have not observed significant changes in BP associated with consumption of cocoa products containing average levels of flavanols, the majority of the research conducted in this area indicates that regular cocoa/chocolate ingestion can reduce BP. A meta-analysis of 10 trials published in 2010 confirmed the findings of the previous 2007 meta-analysis. Mean change in systolic and diastolic BP across the trials was -4.5 ± 1.35 and -2.5 ± 1.36 mm Hg, respectively. There was a wide range of flavanol intake across these trials, with epicatechin intake as low as 5 mg/day or as high as 174 mg/day (56). Of note, in the trial reporting the lowest level of flavanols, subjects, nevertheless, experienced significant improvement in BP (256). Similar to the trials of flow-mediated dilatation, Hooper and colleagues' meta-analysis of flavonoid-rich food trials highlighted cocoa as the sole food capable of significantly reducing BP in human studies (107). In addition to a reduction in risk for MI and stroke, Buijse *et al.* reported significantly lower systolic ($p=0.0008$) and diastolic ($p<0.0001$) BPs among individuals consuming higher amounts of chocolate (30).

A number of mechanisms have been proposed to explain cocoa's effects on BP (83). Because of their importance in BP maintenance, the improvements in nitric oxide availability and endothelial function associated with cocoa consumption may explain much, if not all, of its antihypertensive effects (183). However, there is some evidence that flavanols and flavanol-rich foods, including cocoa, can inhibit angiotensin-converting enzyme (ACE) activity *in vitro* (2, 3). Angiotensin-converting enzyme regulates the renin-angiotensin system; it cleaves angiotensin-I into angiotensin-II, which stimulates the release of vasopressin or aldosterone and antidiuretic hormone, increasing sodium and water retention. It also inactivates vasodilators bradykinin and kallidin (138). Whether ACE inhibition mediates the antihypertensive activity of cocoa flavanols in humans is not yet known.

F. Cardiovascular effects: conclusion

Rimbach *et al.* noted that beneficial effects on BP, FMD, and platelet aggregation have not been found in all human trials (67, 73). Further, improvements are often small when they are observed (165, 256). The authors also raise the concern about chocolate's caloric density and its potential to contribute to weight gain, which may counteract any small benefits of cocoa's polyphenol content. This is a legitimate concern, as evidenced by a study by Taubert *et al.*, in which daily dark chocolate consumption providing an additional 480 kcal per day decreased systolic BP by 5 mm Hg (255). However, in a subsequent study, Taubert and colleagues found that a mere 30 kcal per day of dark chocolate for 18 weeks reduced systolic BP by 2.9 mm Hg (256). Their argument also ignores the fact that the bioactive components of cocoa (*i.e.*, flavanols) are found in the nonfat portion of the cocoa bean and can be readily isolated in a low-energy-dense form, that is, cocoa powder. For example, in their study in CAD patients, Heiss *et al.* used an active treatment that provided a substantial daily dose of 375 mg flavanols but only 50 kcal (94).

Rimbach *et al.* also questioned the rigor of cocoa feeding trials, noting their small sample sizes and acknowledging the need for randomized, placebo-controlled, and cross-over

studies. However, the majority of the endothelial function studies discussed in this review, including those from our lab, are randomized and placebo-controlled, and many are cross-over designs and/or double-blinded. It is true that most cocoa and chocolate studies have used small sample sizes, increasing variance and risk of type 2 error, biasing toward the null. Nevertheless, when they detect significant differences in cardiovascular measures that favor treatment, this is a testament to the strength of the observed effect. Table 5 summarizes results from these human trials investigating cardiovascular effects of cocoa or chocolate.

IV. Effects on Insulin Resistance

There is reason to believe that the flavanols in cocoa may ameliorate insulin resistance by reducing oxidative stress, improving endothelial function, and/or altering glucose metabolism. Ceriello and Motz have proposed that oxidative stress is the underlying mechanism for both insulin resistance and cardiovascular disease (35). This hypothesis is supported by the observation that many antidiabetic drugs and drugs used to treat cardiovascular disease demonstrate antioxidant effects. This effect may be direct—as in the case of calcium channel blockers, statins, ACE inhibitors, and AT-1 receptor antagonists—or indirect—as in acarbose and glinides, which prevent oxidative stress caused by postprandial hyperglycemia (35). If this hypothesis is correct, the demonstrated antioxidant activity of cocoa flavanols (170) could theoretically also protect against insulin resistance. However, better evidence exists for an insulin-sensitizing effect mediated by changes in endothelial function and/or glucose metabolism.

Many polyphenols, including catechin and epicatechin, have been found to alter glucose metabolism in animal and *in vitro* studies (90). In studies, catechin has inhibited alpha-glucosidase activity (114) and inhibited absorption of glucose from the intestine (121, 273). Studies in diabetic rats support an insulin-sensitizing effect of cocoa. In two such studies, epicatechin regenerated pancreatic β -cells (37) and increased insulin secretion (37, 100). In a 2005 study, supplementation of diabetic rats with cocoa extract for 4 weeks was dose-dependently associated with reduced serum glucose and LDL, and increased HDL (213). A similar study by Jalil and colleagues found that cocoa extract reduced postprandial hyperglycemia, plasma free fatty acids, and 8-isoprostane, a biomarker of oxidative stress (115). However, no change was observed in fasting glucose or insulin.

The well-documented effects of cocoa on endothelial function also point to a possible effect on insulin sensitivity. The relationship between endothelial function and insulin resistance is a reciprocal one. Increased insulin sensitivity improves endothelial function; conversely, improvement in endothelial function can increase insulin sensitivity (131). In healthy individuals, insulin increases blood flow to skeletal muscles and glucose uptake by muscle cells through vasodilation. In contrast, in insulin-resistant individuals, insulin-mediated vasodilation is impaired and glucose disposal is inhibited. Insulin resistance has been associated with reduced activity of endothelium-derived NO synthase and with increased plasma levels of asymmetric dimethylarginine, an endogenous NOS inhibitor (254). Thus, the availability of NO likely plays a role in mediating cells' response to insulin. Because of cocoa's ability to increase bioavailability of NO, the

possible relationship between cocoa consumption and insulin resistance has been investigated.

Although low rates of hypertension may be the most striking of the Kuna population's unique characteristics (102), their comparatively low rates of diabetes are also intriguing (103). Grassi and colleagues reported that ingestion of 100 g of flavonoid-rich dark chocolate for 15 days was associated with not only reduced BP and improved endothelial function, but also improved insulin sensitivity in hypertensive patients (84). These results have also been observed in healthy individuals and in hypertensives with impaired glucose tolerance (84). In the former study, subjects received oral glucose tolerance tests (OGTT) after consuming either dark chocolate or white chocolate for 15 days. Compared with white chocolate, ingestion of dark chocolate was associated with higher homeostasis model insulin resistance (HOMA-IR) values, and lower quantitative insulin-sensitivity check index values. In the latter study, hypertensive, glucose-intolerant subjects also received OGTT after 15 days of daily consumption of either flavanol-rich dark chocolate or white chocolate. Dark chocolate decreased HOMA-IR, increased insulin sensitivity, and increased β -cell function compared to white chocolate.

Insulin sensitivity improved significantly in overweight and obese adults consuming high-flavanol cocoa (902 mg flavanols) for 12 weeks compared to low-flavanol cocoa (49). Cocoa's effects on insulin resistance may be dependent on its continual consumption over a long period of time. In one study, daily cocoa (900 mg flavanols) ingestion for 2 weeks was not sufficient to improve measures of insulin resistance, though it did improve insulin-mediated vasodilation (178). Almoosawi *et al.* found in their study, that fasting capillary whole blood glucose was significantly reduced after 2 weeks of daily dark chocolate (500 or 1000 mg polyphenols) consumption (6).

The evidence from these studies suggests that cocoa may be useful in ameliorating insulin resistance in metabolic syndrome and slowing the progression to type 2 diabetes. Results from other studies indicate that cocoa may also have therapeutic potential in preventing cardiovascular complications in diabetic patients. In one such study, flavanol-rich cocoa consumption three times daily for 30 days increased flow-mediated dilatation by 30% in medicated diabetics. Further, acute improvement in FMD after a single dose of cocoa was found to be dose-dependent (17). Although studies in humans with diabetes are lacking, a number of animal studies support beneficial effects of cocoa on glucose control (115, 213). Additional studies to confirm these effects in humans are needed, though there is biologic plausibility and preliminary evidence for an insulin-sensitizing effect of cocoa.

In summary, there are plausible mechanisms for the antioxidant effects of cocoa polyphenols to directly influence insulin resistance and, in turn, reduce risk for diabetes. Cocoa may induce pancreatic β -cell regeneration and stimulate insulin secretion, have a hypoglycemic effect, and improve glucose tolerance. The vasodilatory effects of cocoa can also improve insulin sensitivity mediated by endothelial function. Sustained consumption of cocoa over long periods may affect insulin resistance to a greater degree than single doses of cocoa products.

V. Effects on Immune Function and Carcinogenesis

There are no clinical trials documenting immune effects of cocoa or cocoa extracts in humans. However, preclinical evi-

dence in mice and rats demonstrates that cocoa may exhibit some immunomodulatory effects. Ramiro-Puig *et al.* (202) demonstrated that a diet consisting of 10% cocoa can enhance antioxidant defenses in the thymus and influences differentiation of thymocytes. Other studies have found that 3 weeks of a 10% cocoa diet increases the percentage of B cells and decreases the percentage of T-helper cells in the spleen of young rats (200, 201). Further, gut-associated lymphoid tissue is also affected by cocoa intake—specifically, Peyer's patches and mesenteric lymph nodes that show changes in lymphocyte composition and T-helper cell percentages. It is believed that flavonoids most likely represent the active ingredients responsible for immunomodulatory effects (127).

In vitro, cocoa has been found to reduce secretion of TNF- α , monocyte chemoattractant protein-1, and NO by lipopolysaccharide-stimulated macrophages (127), with differential effects, based on fraction size, on IL-5 secretion in peripheral blood mononuclear cells (152). Cocoa procyanidins have also been shown to affect signaling pathways of polymorphonuclear cells, white blood cells involved in inflammation and injury (127). Kenny *et al.* demonstrated that flavanol fractions can enhance secretion of the cytokines TNF- α , IL-1, IL-6, and IL-10 from stimulated human peripheral blood mononuclear cells (126). Cocoa procyanidins decrease IL-2 at the transcriptional level. Ramiro *et al.* demonstrated that IL-4 release can be enhanced in the presence of cocoa flavonoids, thus downregulating T-lymphocyte activation and the acquired immune response (199).

Chronic inflammation and oxidative stress are significant contributing factors to carcinogenesis. Reactive oxygen (ROI) and nitrogen intermediates (RNI) can damage DNA or interfere with DNA repair, leading to mutations necessary for cells to avoid controls on growth and replication. Inflammation increases the production of ROI and RNI stimulated by cytokines and chemokines, and angiogenesis (69). Cocoa constituents, such as procyanidins and catechins, influence immune responses by modulating activation of the transcription factor NF- κ B, involved in inflammatory responses, cellular proliferation, cell adhesion, and regulating cytokine production (149).

The antioxidant activity of cocoa flavanols is of particular interest for its potential influence on the initiation stage of carcinogenesis. In a study examining the radical scavenging capacity of 10 beverages containing antioxidants, cocoa mix ranked fifth, behind coffee, prune juice, and green tea, but ahead of grape juice and other types of tea (137). In another study, dark chocolate consumption significantly improved DNA resistance to oxidative stress in the short-term. In this study, Spadafranca *et al.* (239) assigned 10 healthy subjects to consume 45 g of dark chocolate or white chocolate for 14 days. Oxidative damage to DNA was reduced in the dark chocolate group 2 h after consumption; 22 h later the effects were not seen (239).

It has been suggested that foods containing antioxidant polyphenols, including cocoa, may have anticancer properties (271). In particular, the polyphenols epicatechin gallate and EGCG in green tea appear to have significant anticancer effects (38, 197, 283). Intake of soy, an important source of the isoflavone genistein, has been associated with up to 49% reduced risk of endometrial and ovarian cancers in women (182). Epidemiologic studies of cocoa intake and cancer risk are few, and those assessing overall mortality provide only

TABLE 5. HUMAN TRIALS INVESTIGATING CARDIOVASCULAR EFFECTS OF COCOA OR CHOCOLATE

Author, year	Population	N	Treatment	Duration	Control	Outcomes
Balzer, 2008	Medicated diabetics	41	High-flavanol cocoa (321 mg flavanols per dose), three times daily	30 days	Low-flavanol cocoa (25 mg per dose), three times daily	FMD ↑ BP Ø HR Ø glycemic control Ø FMD Ø SAC Ø Soluble CAM Ø FBF Ø All treatments: FMD ↑ BP ↓
Farouque, 2006	CAD patients	40	High-flavanol chocolate bar and cocoa (444 mg total flavanols)	6 weeks	Low-flavanol chocolate bar and cocoa (19.6 mg total flavanols)	
Faridi, 2008	Overweight adults	45	High-flavanol dark chocolate (821 mg), sugar-free cocoa (805 mg), and sugared cocoa (805 mg)	Single dose	Flavanol-free white chocolate bar or white cocoa	
Fisher, 2003	Healthy individuals	27	High-flavanol cocoa (205 mg flavanols per dose), four times/day	5 days	Low-flavanol cocoa (<10.25 mg per dose); single day pre-post evaluation in seven subjects	FPWA ↑
Fisher, 2006	Older (>50) and younger (<50) healthy individuals	24	High-flavanol cocoa (205 mg flavanols per dose), four times/day	4–6 days	No control	FPWA ↑ ^a
Engler, 2004	Healthy adults	21	High-flavanol dark chocolate (259 mg)	2 weeks	Low-flavanol dark chocolate (trace)	FMD ↑ LDL oxidation Ø TAC Ø 8-isoprostanes Ø BP Ø Serum lipids Ø BMI Ø CAD ↑ EDCV ↑ Platelet adhesion ↓
Flammer, 2007	Heart transplant recipients	22	High-flavanol dark chocolate (624 mg)	Single dose	Flavanol-free chocolate	FMD ↑ SBP ↓ DBP ↓ Total chol ↓ LDL ↓
Grassi, 2008	Hypertensive, glucose intolerant individuals	19	High-flavanol dark chocolate (1008 mg total phenols)	15 days	Flavanol-free white chocolate	FMD ↑ Ambulatory BP ↓ LDL ↓
Grassi, 2005	Hypertensive patients	20	High-flavanol dark chocolate (88 mg flavanols)	15 days	Flavanol-free white chocolate	

(continued)

TABLE 5. (CONTINUED)

Author, year	Population	N	Treatment	Duration	Control	Outcomes
Heiss, 2003	Outpatients with ≥ 1 cardiovascular risk factor	20	High-flavanol cocoa (176 mg flavanols)	2 days	Low-flavanol cocoa (<10 mg flavanols)	FMD \uparrow RNO \uparrow Plasma nitrite \emptyset Plasma nitrate \emptyset
Heiss, 2007	Individuals with smoking-related endothelial dysfunction	6	High-flavanol cocoa (306 mg per dose), three times/day	7 days	Low-flavanol cocoa (12 mg per dose), three times/day	FMD \uparrow Plasma nitrite \uparrow Plasma nitrate \emptyset
Heiss, 2010	Patients with CAD	16	High-flavanol cocoa (375 mg)	30 days	Low-flavanol cocoa (9 mg)	FMD \uparrow CACs \uparrow CAC functions \emptyset SBP \downarrow Plasma nitrite \uparrow FMD \uparrow RNO \uparrow FMD \uparrow SSDPF \downarrow
Heiss, 2005	Smokers	11	High-flavanol cocoa (176–185 mg)	Single dose	Low-flavanol cocoa (<11 mg)	Both treatments: FMD \uparrow BP \emptyset
Herrmann, 2006	Male smokers	20	40g dark chocolate	Single dose	40g white chocolate	Total chol, HDL, LDL, TG \emptyset CRP \emptyset
Njike, 2009	Overweight adults	44	Sugar-free cocoa (805 mg), and sugared cocoa (805 mg)	6 weeks	Flavanol-free white cocoa	LDL oxidation \emptyset Lipid hydroperoxide \emptyset Endothelin \emptyset
Schroeter, 2006	Healthy men	16	High-flavanol cocoa (917 mg)	Single dose	Low-flavanol cocoa (37 mg)	FMD \uparrow RNO \uparrow PAT \uparrow
Shiina, 2009	Healthy men	39	High-flavanol dark chocolate (550 mg polyphenols)	2 weeks	Flavanol-free white chocolate	CFVR \uparrow BP \emptyset HR \emptyset
						Total chol, HDL, LDL, TG \emptyset

Unless otherwise specified, treatment refers to daily dose.

^aFPWA increased to a greater extent in older subjects.

HR, heart rate; SAC, systemic arterial compliance; CAM, cellular adhesion molecules; FBF, forearm blood flow; FPWA, finger pulse wave amplitude; TAC, total antioxidant capacity; CAD, coronary artery disease; EDCV, endothelium-dependent coronary vasomotion; RNO, sum of nitrosylated and nitrosated species; CAC, circulating angiogenic cell; SSDPF, shear stress dependent platelet function; PAT, peripheral arterial tonometry index; CFVR, coronary flow velocity reserve.

weak support for a benefit of cocoa. However, human intervention trials indicate that cocoa favorably affects intermediary factors in cancer progression—specifically, markers of antioxidant status (155). *In vitro* and *ex vivo* studies support an anti-inflammatory effect of cocoa flavanols, but these effects have not been universally replicated *in vivo* (225). One study, a cross-over feeding trial in men and women at high risk for atherosclerosis, found that cocoa consumption was associated with reduced expression of the adhesion molecules VLA-4, CD40, and CD36 on monocyte surfaces and reduced circulating levels of the inflammatory markers soluble P-selectin and ICAM-1. However, other biomarkers of inflammation, including IL-6 and high sensitivity-CRP, were unaffected (172). The observed decrease in adhesion molecules is consistent with a previous cocoa study in postmenopausal hypercholesterolemic women (269).

Cocoa polyphenols have also been found to inhibit the mutagenic activity of heterocyclic amines *in vitro* and *ex vivo* (157). Kim *et al.* found that a cocoa polyphenol extract inhibited TNF- α (a pro-inflammatory cytokine involved in the pathogenesis of cancer) as well as VEGF expression in JB6 mouse epidermal cells. This extract reduced TNF- α -induced upregulation of VEGF by directly inhibiting phosphoinositide 3-kinase and mitogen-activated protein kinase kinase-1 activities (132).

There is growing evidence that polyphenols may play a role in regulating apoptosis (203). Apoptosis may be triggered intrinsically, through the mitochondrial pathway (118) or extrinsically by death ligands and receptors (99). It is the external pathway that may potentially be modulated by bioactive food components. Numerous dietary agents have been found to induce apoptosis in cancer cells *in vitro*; they include compounds in green tea, cruciferous vegetables, red pepper, grapes, and many other fruits, vegetables, herbs, and spices (130). Flavanols found in cocoa have also exhibited pro-apoptotic effects. Proanthocyanadins inhibited growth of human lung cancer cells *in vitro* and *in vivo* (230), and epicatechin synergistically enhanced apoptosis in lung cancer cells treated with EGCG (252). Phenol-rich cocoa extracts were found to prevent apoptosis in MLP29 liver cells induced by Celecoxib, suggesting that upstream components of the apoptotic pathway (such as the *Bax* gene) are targets of cocoa phytochemicals (11).

In summary, cocoa exhibits regulatory effects on immune cells involved in innate and acquired immunity. Cocoa is a rich source of flavonoid antioxidants, which might promote changes in redox-sensitive signaling pathways involved in the expression of many genes and consequently in several cell functions, such as the immune response. Procyanidins and catechins may prevent inflammatory and oxidative damage to DNA and thus affect carcinogenesis. No human trials demonstrate benefit in cancer treatment, though cocoa has consistently demonstrated an ability to increase serum antioxidant status, and enhance apoptosis of cancer cells, thus theoretically reducing cancer risk.

VI. Effects on the Central Nervous System

Flavonoids in cocoa have demonstrated a variety of effects in central nervous processes, and there is promising preliminary evidence for protection from neurodegradation, increased perfusion, decreased neuroinflammation, and

modulation of neuronal function through interaction with a number of signaling pathways.

The pathogenesis of Parkinson's disease involves the death of neurons in the substantia nigra. This process is believed to be mediated by the formation of the endogenous neurotoxin 5-S-cysteinyldopamine (5-S-cys-DA) and its oxidation product, dihydrobenzothiazine, that may be produced through a mechanism involving ROS (242). Neuronal injury by 5-S-cys-DA is attenuated by a number of derivatives of catechin and epicatechin, including quercetin, hesperetin, and caffeic acid (242).

Neuroinflammation is involved in the pathogenesis of Parkinson's disease, Alzheimer's disease, and neuronal injury associated with stroke (111). Chronic responses in glial cells, occupying the majority of brain volume (133), can lead to progressive neuronal degeneration (5). Flavonoids may modulate this response; quercetin structurally resembles a number of kinase inhibitors that have anti-inflammatory effects in glial cells (240). Further, flavonoids interact with a variety of neuronal protein kinase and lipid kinase signaling cascades (240), and can possibly prevent excitotoxic death in neurons (122).

The consumption of flavonoid-enriched cocoa affects cerebral blood flow; functional magnetic resonance imaging studies demonstrate increased blood flow to gray matter 3 h after consuming cocoa as well as other changes to regional blood flow (77). Increased blood flow to the cerebral gray matter induces angiogenesis and new nerve cell growth in the hippocampus, a key region involved in the processing of memory (242). Increased blood flow velocity in the middle cerebral artery has been demonstrated, providing insights to possible protective effects against dementia and stroke (238).

Epicatechin induces both extracellular signal regulated kinase and cyclic AMP response element binding protein (CREB) activation in cortical neurons increasing CREB-regulated gene expression (221) involved in the formation of long-term memory (24). Flavonoids and their metabolites may interact within MAPK signaling pathways involved in mitogenesis, differentiation, apoptosis, and various forms of cellular plasticity (241), as well as, serine/threonine kinase, one of the key downstream effectors of phosphatidylinositol-3 kinase involved in neuronal survival (243).

In summation, the antioxidant properties of catechin and epicatechin derivatives can protect from neuronal injury and neuroinflammation implicated in the pathogenesis a number of neurological conditions. Cocoa can also increase cerebral blood flow, which can be neuroprotective. Further, epicatechin is involved in the processes of formation of long-term memory. These results are preliminary; clinical trials are necessary to confirm cocoa's neuroprotective effects in humans.

VII. Effects on Skin

Cocoa butter is a common ingredient in skin moisturizers, but cocoa's beneficial effects on skin may extend beyond its use as a topical agent. A number of studies have identified a role of cocoa flavanols in protecting skin from damage from UV light, believed to be mediated through factors, such as generation of ROS and inflammatory markers such as prostaglandins, NO, leukotrienes, and histamine (276). Twelve weeks of high-flavanol cocoa consumption decreased erythema induced by UV light by 25% in one study (91) and

specially produced high-flavanol chocolate (>600 mg flavanols and >10 000 ORAC units per 20 g portion) more than doubled the dose of UV light necessary to produce erythema in another. However, commercially available chocolate did not have this effect (276). The antioxidant actions of cocoa flavanols are one possible mechanism by which skin protection could be conferred. There is also some evidence that their vasodilatory effects may be important in nutrient delivery, and thermoregulation is dependent on cutaneous microcirculation (22). In support of this hypothesis is a study that found that consumption of high-flavanol cocoa for 12 weeks increased dermal blood flow and oxygen saturation by 70% and 80%, respectively (187). Though the volume of research in this area is limited, there is reason to believe that skin protection may be another possible health benefit of cocoa.

VIII. Effects on Obesity

Although chocolate may be perceived as an indulgent and fattening food because of its energy density, randomized trials have not found an increase in weight after sustained consumption of small amounts of cocoa (17, 49) or dark chocolate (256). In one study, a daily dose of 25 g (125 kcal) of dark chocolate slightly increased body weight after 3 months, but a 6 g dose (30 kcal) was not associated with any weight change. In both doses, the 24-h mean ambulatory BP decreased (55).

Nitric oxide (NO) has been shown to increase uptake of glucose, increase oxidation of fatty acids and glucose, inhibit fat synthesis, and enhance lipolysis in adipose tissue (120). Cocoa has been found to decrease visceral adipose tissue in rats, possibly by changing the expression of genes for enzymes and transport molecules involved in fatty acid synthesis and thermogenesis in liver and white adipose tissue (157).

Massolt and colleagues (156) demonstrated that the smell of chocolate could suppress appetite in humans. In their study, 12 females were given chocolate to eat and then randomized to either smell chocolate or to serve as a control (no eating or smelling). At the start of these sessions, insulin, glucagon-like peptide-1 (GLP-1), and cholecystokinin (CCK), but not glucose, were found to correlate with appetite levels. They also found that ghrelin levels correlated inversely with appetite. Chocolate eating and smelling both resulted in appetite suppression with no relationship seen between appetite levels and insulin, GLP-1, or CCK levels. Of note, the smell of dark chocolate (85% cocoa) resulted in a satiation response that was inversely correlated with ghrelin levels. As higher levels of ghrelin increase food intake and increase fat mass (257), these provocative findings suggest that regular cocoa and chocolate consumption may reduce appetite and possibly weight gain.

Although research to date has not specifically addressed whether cocoa may actually reduce adiposity, there is reason to believe that cocoa consumption may induce favorable metabolic changes through its effects on NO availability or lipid metabolism. The olfactory properties of cocoa may increase satiety and thus reduce appetite. Given the potential beneficial effects of cocoa on conditions frequently associated with obesity and the negligible risk of weight gain associated with effective doses of chocolate, moderate chocolate consumption appears to have a favorable risk/benefit profile for obese individuals.

IX. Psychoactive Effects

A. Chocolate craving

In addition to the wide range of somatic effects of cocoa, possible psychological effects on mood, cravings, and cognitive function have also been suggested. Much of the research on cocoa's effects on psychological variables has focused on explaining chocolate cravings, likely because chocolate is such a widely craved food. In one study, chocolate accounted for 49% of all food cravings reported by a sample of 25 healthy women (101). Greater chocolate craving has been reported among women than among men (193, 212, 289) particularly during the perimenstrual period (27, 212, 288), and a significant but small decline in cravings may occur after menopause (108). However, this association may be at least partially explained by cultural factors, as suggested by the work of Zellner *et al.* (288). They found a large and significant difference between the proportions of American and Spanish women reporting perimenstrual chocolate cravings when asked an open-ended question about timing of cravings (40% *vs.* 4%) or when asked directly if they experienced perimenstrual cravings (60% *vs.* 24%).

Cravings or increases in chocolate consumption also appear to be associated with negative mood (208). In a cross-sectional study, chocolate consumption was associated with higher depression scores (indicating more depressive symptoms), but whether the relationship is a causal one is still unclear (208). Further, individuals classified as "emotional eaters" have reported greater craving for and consumption of chocolate (148). Willner and colleagues investigated the relationship between depressed mood and chocolate craving in an experimental study (278). In this trial, 120 female college students who liked chocolate were randomly assigned to hear music intended to induce either elated or depressed mood. Before and after listening to the music, subjects completed questionnaires measuring mood and attitudes to chocolate. They were then given the opportunity to complete a computer task to earn reinforcers of chocolate or carob. Afterward, subjects completed the questionnaires once more. Subjects in the depressed condition rated their craving for chocolate significantly higher than those in the elated condition and provided significantly more responses in the computer task to receive more reinforcers when chocolate, but not carob was given. The results of this study suggest that depressed mood may increase craving for chocolate. However, the reason that chocolate cravings occur during times of emotional distress is still not clear (278).

Parker *et al.* reviewed the psychoactive properties and mood effects of cocoa in 2006, with some noteworthy conclusions (195). They contend that a psychoactive component of cocoa is not likely to be the cause of chocolate cravings because milk chocolate, which contains a lower proportion of cocoa solids than dark chocolate or cocoa powder, is typically preferred. Rather, the unique orosensory properties of chocolate, which are responsible for its pleasurable taste, are the most likely explanation (195).

The findings of Rozin and colleagues provide strong evidence that it is not the bioactive compounds in cocoa solids, but rather the particular taste and mouth-feel of solid chocolate products that satisfy chocolate cravings (167). In their 1993 study, a total of 34 individuals who reported experiencing chocolate cravings at least once per week were given six

sequentially numbered opaque boxes and instructed to open one at each occasion of a craving, in order. The boxes contained the following treatments, in random order: (i) a 44 g milk chocolate bar; (ii) six opaque capsules containing 3.8 g cocoa powder; (iii) six opaque capsules containing 4.5 g white flour; (iv) 42.7 g white chocolate; (v) 39.9 g white chocolate plus six cocoa-containing capsules; and (vi) nothing. The boxes also contained craving rating scales, which the subjects were asked to complete before, immediately after, and 90 min after consuming the given treatment. The doses of the treatments were varied according to reported usual quantity of chocolate intake. Subjects who reported typically eating one chocolate bar when they experienced a craving received the doses described above, and those who reported eating more than one chocolate bar to satisfy a craving received double the amounts for all treatments. Milk chocolate was associated with the greatest reduction in craving (61 points on the 70-point scale), whereas white chocolate and white chocolate plus cocoa capsules were associated with significantly smaller decreases (42 and 43 points, respectively). The cocoa capsules had only a minimal decrease (16 points), which was similar to the reductions observed after consumption of flour capsules and no treatment. The results of this study strongly suggest that sensory properties are responsible for the satiation of chocolate cravings.

B. Mood and cognitive function

Two studies by Macht have also supported the view that chocolate's psychoactive properties are solely taste-related (146, 147). These studies found that chocolate consumption can improve a negative mood state in the short-term, but that the improvement is likely attributable to chocolate's palatability (146, 147). In Macht's 2007 study, ingestion of a palatable chocolate improved mood significantly more than a nonpalatable chocolate, whose effects were not significantly different from the control condition (nothing eaten) (147). In a 2006 study, Macht found that a chocolate bar elevated mood and elicited joy to a greater extent than an apple, but these effects were most pronounced at 5 and 30 min after consumption. This suggests that the sensory experience of eating chocolate improved mood, rather than neurochemical effects, which would be expected to manifest later (146).

On the other hand, there is some evidence to support a psychoactive effect of flavanols or methylxanthine compounds in cocoa—effects that may extend beyond mood to cognitive function. Scholey and colleagues conducted a randomized, controlled, double-blind crossover trial in 30 healthy adults to investigate the effects of cocoa flavanol ingestion on cognitive performance, state anxiety, and mental fatigue (218). The three treatments tested were cocoa beverages containing 46 mg (control), 520 mg, and 994 mg of total flavanols. Both of the flavanol-rich preparations significantly increased cognitive performance and reduced mental fatigue relative to the control beverage. Smit *et al.* looked to cocoa's methylxanthine compounds, caffeine and theobromine, as potential mood-altering agents (236) in two studies. The first was designed to compare the effects on mood, alertness, and cognitive performance of encapsulated cocoa powder (CP) equivalent to a 50 g chocolate bar, isolated caffeine and theobromine (CA + TB) equivalent to a 50 g chocolate bar, and a placebo. In a second study, the treatments were 60 g portions of chocolate prepared to look identical, but contain differing amounts of methylxanthines

(zero, low-, and high-methylxanthines [MX]). Cocoa powder and CA + TB both significantly decreased reaction time and increased energetic arousal compared to placebo. CA + TB also significantly increased scores on a rapid visual information processing (RVIP) task and increased hedonic tone (a measure of positive mood) compared to placebo, whereas CP was associated with a nonsignificant increase. The chocolate treatments of varying methylxanthine levels produced similar results. High MX chocolate significantly decreased reaction time, whereas both low and high MX chocolates increased RVIP scores relative to placebo. Both MX chocolates increased energetic arousal and hedonic tone more than placebo, but these differences were not significant. In a follow-up study, fondness for a novel drink increased over repeated exposures when paired with theobromine and caffeine, but not when paired with a placebo, suggesting that the methylxanthines in cocoa may also contribute to partiality for chocolate (235). In all three of these studies, the effect of chocolate's orosensory characteristics was controlled for.

In a randomized, double-blind, placebo-controlled trial that investigated the effects of cocoa and dark chocolate on BP, blood lipids, and C-reactive protein (all null), Crews and colleagues also assessed neuropsychological functioning in a healthy, older (≥ 60 years) population (45). Ninety participants completed the trial, which was 6 weeks in duration. During the intervention period, subjects consumed either a polyphenol-rich artificially sweetened cocoa beverage and a dark chocolate bar (754.71 mg total proanthocyanidins) or nutrient-matched placebo cocoa and chocolate daily. Eight neuropsychological tests were administered as was a self-report questionnaire to assess subjects' perceptions of changes in their cognitive functioning. There were no significant differences in any of the cognitive test measures between the intervention and placebo groups.

Martin *et al.* conducted a comprehensive assessment of the metabolic impact of dark chocolate consumption in 30 subjects who consumed 40 g of dark chocolate daily for 2 weeks (154). They found that dark chocolate had significant effects only in subjects who reported higher anxiety at the beginning of the study. In these individuals, chocolate consumption reduced urine cortisol and catecholamines and partially normalized other metabolic parameters associated with the high-anxiety trait.

More research is needed to make sense of the inconsistencies in the current literature on cocoa's psychoactive effects. Nevertheless, cocoa products are unique among potentially health-promoting foods, and they are generally considered pleasurable and may positively affect mood. Currently, research suggests that any mood improvement is likely short-lived and due to chocolate's palatability, rather than its flavanol content, concentration of methylxanthines, or other bioactive compound.

X. Bioavailability of Cocoa Polyphenols

A. Metabolism of cocoa polyphenols

Because little is known about the absorption and metabolism of polyphenols, there is increasing interest and much speculation in this area (46, 150, 234). Cocoa has a high concentration of proanthocyanidins, which is large relative to that of other polyphenols (150) and can be detected in serum 30 min after consumption of cocoa (106). A dose-response

effect has been shown between cocoa intake and serum epicatechin levels where 100 mg catechins increased serum levels increased by 200 nM 2 h after consuming cocoa products (151). Wang *et al.* found a similar dose–response effect in plasma epicatechin associated with dose–response increases in plasma antioxidant capacity and decreases in plasma lipid oxidation products (268).

Some studies have indicated that oligomers larger than trimers may not be able to be absorbed in the small intestine (16, 54, 62). Although it is possible that procyanidin oligomers are broken down into monomers and dimers, which may be absorbed, this does not appear to occur in the stomach (207). Therefore, the effects of proanthocyanidins in cocoa on plasma concentrations are much lower than those of epicatechin, the predominant monomeric flavanol in cocoa (106). However, the antioxidant activity of proanthocyanidins may be beneficial in the colon (150).

Urpi-Sarda *et al.* found that epicatechin metabolites from cocoa accumulated in the thymus, testicles, liver, lymph nodes, and spleen in Wistar rats fed a high cocoa diet compared to control rats, suggesting that these sites are important sites of oxidation (264).

B. Effects of food processing on flavonoids

The caveat of consuming chocolate for its health benefits is the requirement that the chocolate or cocoa contains an effective dose of the active components. Flavonoid content varies by the proportion of cocoa liquor present in different types of chocolate (white, milk, and dark), but even within categories there may be significant variation due to differences in cocoa bean processing (Table 2). For example, cocoa liquor that has undergone more fermentation has higher concentrations of flavonoids (164). The extent to which cocoa beans are roasted also has a substantial impact on the antioxidant capacity of the cocoa's phenolic compounds; longer roasting time is associated with reduced antioxidant activity (191). However, radical-scavenging Maillard reaction products such as melanoidins are also produced during roasting, partially replacing the lost antioxidant activity of cocoa polyphenols (191).

Variation in antioxidant capacity can also be seen in cocoa powder (169). Cocoa powder is often treated with alkali to neutralize the natural acetic acid in cocoa in a process called "Dutching." This process substantially reduces the flavanol content of the cocoa. In one study of commercially available cocoa powders, the flavanol content of heavily alkalized cocoas was 78.5% lower than that of natural cocoas (Fig. 9) (164, 169). With the exception of products that have undergone Dutching, most cocoa and chocolate products will contain amounts of polyphenols that are reflective of the proportion of nonfat cocoa solids present (170).

With the assurance that a particular cocoa product contains a biologically relevant dose of flavanols, concern about the bioavailability may remain. The addition of milk, sugar, and other substances to cocoa powder or cocoa liquor in the production of commercial cocoa drink mixes and chocolate candy may impact the bioavailability of cocoa flavanols if not their concentration. A number of studies have sought to determine whether milk affects flavanol metabolism, with somewhat mixed results (129, 176, 210, 211, 227). In 2003, Serafini and colleagues found that consumption of dark chocolate with

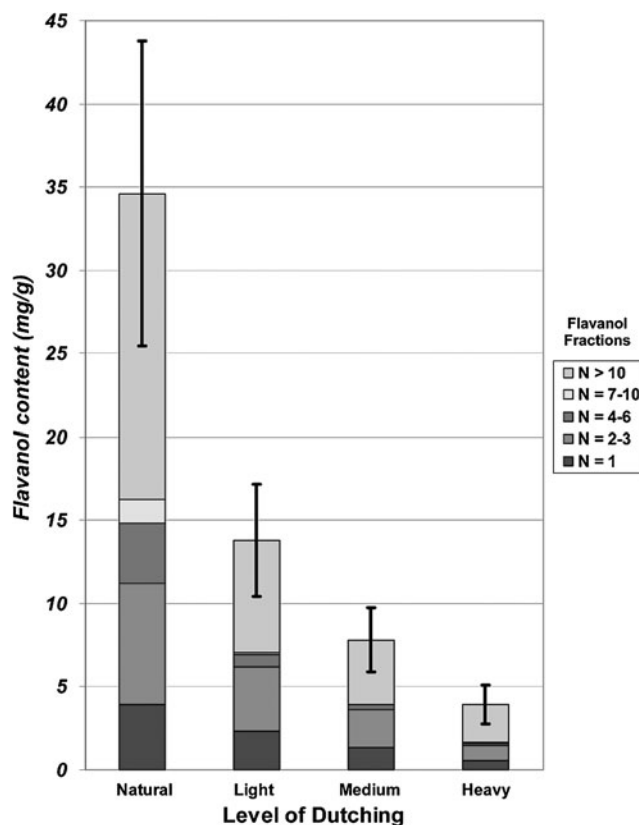


FIG. 9. Average flavanol content of natural cocoa powders and cocoa powders undergoing light, medium, or heavy alkalization. Reprinted with permission from Miller *et al.* (169).

200 ml of milk attenuated the increase in plasma antioxidant capacity observed after consumption of dark chocolate alone (227). They hypothesized that proteins in the milk may bind to cocoa flavanols, reducing their absorption. This study was followed by a string of other studies, which found no effect of milk on the bioavailability of cocoa flavanols (129, 210, 211). In a 2009 study, however, consumption of 250 ml of commercial cocoa with milk was associated with reduced urinary epicatechin metabolites, though plasma metabolites were not substantially affected (176). Cooper *et al.* found that (–)-catechin is the most affected by manufacturing conditions and cocoa origin compared to other cocoa polyphenols (40). Belscak-Cvitanović *et al.* found that cocoa powder mixtures containing lower fat (10%–12%) had higher total polyphenol, total flavonoid, flavan-3-ol, and proanthocyanidin contents compared to cocoa prepared from higher fat content (16%–18%) cocoa powder (20).

The impact of sugar on the biological effects of cocoa has not been investigated to a great extent. Studies in humans (219) and rats (186) indicate that the consumption of cocoa with carbohydrates, including sugar, may actually increase the bioavailability of cocoa flavanols. Whether the potential negative effects of over-consumption of sugar—namely, endothelial dysfunction (140), insulin resistance, and weight gain (109)—negate any benefit of increased plasma flavanol concentrations is not yet understood. In one trial, a sugar-free cocoa beverage produced greater improvements in BP and

endothelial function than a sugar-sweetened cocoa; however, the differences were not statistically significant (188). If artificially sweetened cocoa is determined to have greater benefit than cocoa with sugar, palatability of such products will be an important consideration. Interestingly, subjects in another study reported preferring the taste of cocoa mixtures sweetened with nonnutritive sweeteners (aspartame/acesulfame K and stevia extract) to those sweetened with sugar or glucose, suggesting that sugar-free cocoas are acceptable to consumers (20).

Concurrent milk consumption can affect the metabolism of phenolic acid constituents of cocoa. Urpi-Sarda and colleagues found that urinary excretion of vanillic acid and phenylacetic acid increased after milk and cocoa were consumed together, whereas urinary excretion of other components (3,4-dihydroxyphenylacetic acid, protocatechuic acid, 4-hydroxybenzoic acid, 4-hydroxyhippuric acid, and hippuric acid) was reduced (263).

Often cocoa products are not consumed as purchased, but rather used to make other foods, beverages, or confections. In one study, polyphenol content of cocoa powder was not substantially affected by preparation techniques used to make chocolate frosting, hot cocoa beverage, or chocolate cookies; however, after undergoing baking in a chocolate cake, natural cocoa powder retained only 5% of its epicatechin content and 54% of its antioxidant activity measured by ORAC. The reason for this is likely the increase in pH caused by the addition of baking soda (244).

XI. Detrimental Health Effects

Chocolate has been implicated in conditions, such as acne (47) and heart burn or gastroesophageal reflux disease (GERD) (179, 282). There is also a common perception that chocolate can be a trigger for migraines (279). As discussed elsewhere in this review, weight gain is also a concern when energy-dense solid chocolate is consumed regularly.

Some population-based studies suggest that dairy intake may be positively associated with acne, and clinical trials indicate that a high glycemic index or glycemic load diet may also play a role in acne severity and duration (25, 70). Currently, evidence does not support a link between chocolate or any other specific food and acne, though few well-designed studies have been undertaken (47).

The American College of Gastroenterology names chocolate as one of the foods that may contribute to GERD symptoms, though it acknowledges a lack of rigorous testing of this hypothesis (58). Chocolate has been found to reduce lower esophageal sphincter pressure (179, 282). Yet, no studies have investigated whether eliminating chocolate from the diet can improve symptoms. There is currently not sufficient evidence to suggest that dietary modifications of any kind can improve GERD symptoms or pathological measures (123). Interestingly, one study found that chocolate was the most often cited constipation-causing food among patients with constipation-predominant irritable bowel syndrome (177), but no other studies have investigated this reported effect.

It has been hypothesized that phenylethylamine, present in chocolate, could provoke a migraine (160, 161). The perceived association between chocolate and migraines appears to be a widely held misconception. In one study investigating trigger factors of migraines, the proportion of patients who reported having heard that chocolate could be a migraine trigger (61.7%)

was significantly and substantially greater than the proportion who identified chocolate as a trigger based on their own experience (14.3%, $p < 0.001$) (279). Although one small study in 1991 found that chocolate ingestion was nearly significantly associated with migraine compared with placebo ($p = 0.051$) (82), one trial conclusively found no association (153).

The majority of the research discussed in this review has discussed protective or health-promoting effects of cocoa or chocolate consumption. It is certainly biologically plausible for cocoa to cause detrimental effects on human health. Chocolate consumption may exacerbate symptoms of GERD or trigger migraine headaches in sensitive persons, though human trials establishing a causal relationship are scant and inconclusive. The real possibility of weight gain when overindulging in chocolate or cocoa-based foods is real, but certainly not attributable to the cocoa content.

XII. Conclusions and Implications

Cacao, the quintessential ingredient in all true chocolate and cocoa products, is a highly complex food source. As this review indicates, cacao imparts to chocolate a rich endowment of nutritional properties, from minerals, to antioxidants, to vasoactive, and even psychoactive compounds.

The tendency in Western science when a food appears to have specific health effects is to seek the active ingredient. Indeed, specific effects of chocolate may be attributable to specific constituents, such as flavonoids. However, just as the active ingredient making a food such as spinach or broccoli highly nutritious may be nothing less than spinach or broccoli, the same may be true of chocolate. The active ingredient in chocolate may be chocolate, or at least cacao, with overall health effects representing at least the sum of diverse parts.

There is, however, much interest in, and potential gain from, elucidating those parts and their mechanisms of action. Table 6 begins to suggest an array of mechanistic pathways, enumerating various constituents of chocolate, and their actions at diverse sites. Numerous cytokines and enzyme systems are influenced by various polyphenols in chocolate. Some of these pathways clearly involve direct antioxidant effects, whereas others likely do not. Among the unknowns is which of the many polyphenolic compounds in chocolate exert specific effects, and this area invites further investigation.

The implications of this review—an indication of both how much is known, and how much is unknown about specific metabolic and molecular effects of chocolate—are apt to vary with professional orientation and perspective. For the public health nutritionist, the operative questions are: is there a benefit, of what kind, and for whom? There is a strong body of accumulating evidence pointing to a net health benefit from routine consumption of dark chocolate, but further epidemiologic study before such evidence is widely acknowledged as truly decisive.

For the clinician/nutritionist dealing with patients individually, the operative questions are apt to be: what dose do I prescribe, what frequency, and for whom? The notion that chocolate can serve, quite literally, as “medicine” has been espoused in the peer-reviewed literature (78). Before such an assertion can migrate from postulate to practice, however, the prescriptive details are required. Short-term intervention studies utilizing such outcomes as endothelial function as a

TABLE 6. EFFECTS OF COCOA POLYPHENOLS ON ENZYME SYSTEMS AND MOLECULAR TARGETS

Compound	Target	Function	Reference
Catechin	↓ a-glucosidase	Catalyze hydrolysis of polysaccharides	114
Epicatechin	↓ Xanthine oxidase	Catalyzes the oxidation of hypoxanthine to xanthine, generates ROS	64, 222
Epicatechin	↓ NADPH oxidase	Scavenges NO	216, 247
Epicatechin	↑ cGMP	Activates intracellular protein kinases	216
Epicatechin	↑ endothelial NO• synthase (eNOS)	Vasodilating factor	216, 222, 277
Epicatechin	↑ extracellular signal regulated kinase and cyclic AMP response element binding protein activation in cortical neurons	Involved in long-term memory formation	221
Epicatechin	↓ plasma levels of endothelin-1		
Procyanidins	↓ lipoxigenases		
Procyanidins	↑ IL-2 transcription		
Quercetin, hesperetin, and caffeic acid	↓ 5-S-cysteinyldopamine and its oxidation product, dihydrobenzothiazine	Constrict blood vessels; raise blood pressure	144
Cocoa polyphenols (unspecified)	↑ prostacyclin	Arachidonic acid metabolism and the biosynthesis of leukotrienes (inflammatory pathways)	220, 233
Cocoa polyphenols (unspecified)	↑ endothelium-derived hyperpolarizing factor		
Cocoa polyphenols (unspecified)	↓ endothelin-1		
Cocoa polyphenols (unspecified)	↓ VEGF	Cytokine immune system signaling molecule	199
Cocoa polyphenols (unspecified)	↓ NF-κB activity	Endogenous neurotoxin	242
Cocoa polyphenols (unspecified)	↓ angiotensin-converting enzyme		
Cocoa polyphenols (unspecified)	↓ 8-isoprostane		
Cocoa polyphenols (unspecified)	↓ monocyte chemoattractant protein-1		
Cocoa polyphenols (unspecified)	↓ TNF-α in lipopolysaccharide-stimulated macrophages		
Cocoa polyphenols (unspecified)	↑ IL-1 secretion from stimulated human peripheral blood mononuclear cells		
Cocoa polyphenols (unspecified)	↑ IL-6 secretion from stimulated human peripheral blood mononuclear cells		
Cocoa polyphenols (unspecified)	↑ IL-10 secretion from stimulated human peripheral blood mononuclear cells		
Cocoa polyphenols (unspecified)	↑ IL-4 release		
Cocoa polyphenols (unspecified)	↓ expression of CD40 on monocyte surfaces		
Cocoa polyphenols (unspecified)	↓ expression of VLA-4 on monocyte surfaces		
Cocoa polyphenols (unspecified)	↓ expression of CD36 on monocyte surfaces		
Cocoa polyphenols (unspecified)	↓ circulating levels of P-selectin		
Cocoa polyphenols (unspecified)	↓ circulating levels of ICAM-1		
Cocoa polyphenols (unspecified)	↓ phosphoinositide 3-kinase		
Cocoa polyphenols (unspecified)	↓ mitogen-activated protein kinase kinase-1		
Cocoa polyphenols (unspecified)	serine/threonine kinase		
Cocoa polyphenols (unspecified)		Regulates renin-angiotensin system	2, 3
Cocoa polyphenols (unspecified)		Biomarker of oxidative stress	115
Cocoa polyphenols (unspecified)		Recruitment of monocytes and lymphocytes to sites of cellular immune reactions	127
Cocoa polyphenols (unspecified)		Cytokine involved in systemic inflammation	127
Cocoa polyphenols (unspecified)		Cytokine, involved in regulation of immune responses	126
Cocoa polyphenols (unspecified)		Pro-inflammatory and anti-inflammatory cytokine	126
Cocoa polyphenols (unspecified)		Anti-inflammatory cytokine	126
Cocoa polyphenols (unspecified)		Acquired immune response	199
Cocoa polyphenols (unspecified)		Adhesion molecule	172
Cocoa polyphenols (unspecified)		Adhesion molecule	172
Cocoa polyphenols (unspecified)		Adhesion molecule	172
Cocoa polyphenols (unspecified)		Inflammatory marker	
Cocoa polyphenols (unspecified)		Inflammatory marker	
Cocoa polyphenols (unspecified)		Upregulates VEGF, mediated through TNF-α	132
Cocoa polyphenols (unspecified)		Upregulates VEGF, mediated through TNF-α	132
Cocoa polyphenols (unspecified)		Involved in neuronal survival	243

VEGF, vascular endothelial growth factor.

proxy for likely effects on cardiovascular endpoints are eminently feasible, and needed to clarify how the potentially medicinal effects of dark chocolate might best be translated into clinical counseling.

For the nutritional biochemist, a likely focus is: what are the specific constituents of chocolate responsible for observed effects *in vivo* and *in vitro*? As suggested by Table 6, diverse constituents of chocolate are known to exert a wide array of metabolic effects of potential clinical importance. Among the compounds in chocolate with promising effects, epicatechin stands out to some degree. Although molecular, metabolic, and overall medical effects of chocolate may owe something more to the whole than to any given part, food science would, nonetheless, benefit from further study of specific parts, or compounds, both in isolation and in various combination. Insights generated might have implications for fortification and functional foods, and help food scientists answer the questions most germane to their efforts: what additions, deletions, and/or modifications enhance or attenuate favorable health effects of chocolate?

Finally, for molecular biologists, and perhaps the dedicated readers of this journal, the focus may be less on the active compounds in chocolate, than on their actions. Namely, a question of general interest is apt to be: what are the mechanistic pathways involving oxidation and reduction that mediate effects of chocolate? As noted (see pp. 9–11), the means by which chocolate ingestion results in even as well-studied an effect as enhanced endothelial function is far from certain. Polyphenols, and in particular epicatechin, are known to reduce levels of endothelin, and increase levels of nitric oxide. However, these compounds also influence gene expression, and may help preserve eNOS. Which of these effects predominates, and which has the most direct clinical significance is unknown. Molecular and genetic studies are required to elucidate further the mechanisms of chocolate's diverse metabolic effects.

In general, this review invites the question: is dark chocolate legitimately considered a "health food"? The weight of evidence reviewed here would seem to support a qualified "yes." The qualification relates both to what is known, and what is not. What is known is that chocolate, unlike spinach, carries with it the liabilities of processing, added ingredients (notably sugar), and energy density. In Western societies prone to obesity, a concentrated source of calories represents at least a potential health threat.

Thus, the benefits of chocolate consumption must be weighed against the at-least theoretical risk of weight gain. Little actual evidence of that harm exists, however, at least for dark chocolate *per se*.

What is not known is how best to make use of chocolate for purposes of health promotion *per se*. While specific quantities of fruits and vegetables may be recommended, and even alcohol prescribed with some degree of confidence, a health-enhancing dose of chocolate remains a matter of conjecture. How much of what kind of chocolate is ideal for overall health? What frequency is optimal? Are there populations in which chocolate is of particular benefit, and populations in which it is less so- or even detrimental? The differential effects of chocolate observed in those with and without established coronary disease is an area clearly warranting further examination. Perhaps constituents of chocolate can help forestall, but not treat, coronary atherosclerosis. This is as yet unknown.

What is known, however, is that apparent health benefits of chocolate consumption relate to diverse constituents of chocolate and pertain to a wide array of health outcomes. While the case is strongest for cardiovascular benefits, the immunomodulating effects of chocolate hint at potential benefits in both infectious disease and cancer. Metabolic and psychological benefits are also strongly suggested, and mechanistically plausible.

The voluminous literature addressing health effects of cocoa and chocolate, only a portion of which is represented here, is overall quite compelling. The probability of a net health benefit for most people from habitual intake of dark chocolate is high. The translation of this general theme in the literature into specific public health guidance would seem the next great challenge for the research community. The recommendations, of course, must consider the potential for weight gain and increased cardiovascular risk by consuming chocolate products containing high amounts of sugar, possibly countering some of the documented benefits of cocoa. If a dose range for alcohol intake can be established, doing so for chocolate would seem perfectly feasible. Food can be medicine, and chocolate may be a noteworthy example. However, potential in this area will not be fully realized until or unless parameters for prescription are established.

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Abbreviations Used

BP = blood pressure
 CAC = circulating angiogenic cell
 CAD = coronary artery disease
 CCK = cholecystokinin
 CFVR = coronary flow velocity reserve
 CP = cocoa powder
 CREB = cyclic AMP response element binding protein
 CRP = C-reactive protein
 EGCG = epigallocatechin-3-gallate
 FMD = flow-mediated dilatation
 GERD = gastroesophageal reflux disease
 GLP-1 = glucagons-like peptide-1
 HOMA-IR = homeostasis model insulin resistance
 MX = methylxanthines
 OGTT = oral glucose tolerance test
 PAT = peripheral arterial tonometry
 RDA = recommended dietary allowance
 RNI = reactive nitrogen intermediates
 RNO = nitrosylated and nitrosated species
 ROI = reactive oxygen intermediates
 RVIP = rapid visual information processing
 TB = theobromine
 VEGF = vascular endothelial growth factor

