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Abstract—Epidemiological data demonstrate that regular dietary intake of plant-derived foods and beverages reduces the risk of coronary heart disease and stroke. Among many ingredients, cocoa might be an important mediator. Indeed, recent research demonstrates a beneficial effect of cocoa on blood pressure, insulin resistance, and vascular and platelet function. Although still debated, a range of potential mechanisms through which cocoa might exert its benefits on cardiovascular health have been proposed, including activation of nitric oxide and antioxidant and antiinflammatory effects. This review summarizes the available data on the cardiovascular effects of cocoa, outlines potential mechanisms involved in the response to cocoa, and highlights the potential clinical implications associated with its consumption. **(*Circulation*. 2009;119:1433-1441.)**

Key Words: cocoa ■ endothelium ■ hypertension ■ platelets

For centuries, cocoa-rich chocolate has been known not only for its good taste but also for its proposed health effects. Indeed, the Incas considered it the drink of gods, an association that gave rise to the scientific name of the cocoa tree, *Theobroma cacao*, from the Greek words theo (god) and broma (drink). The first hints of cocoa consumption date back to 1600 BC. In Honduras, archeologists uncovered elaborately designed bowls of this period that are believed to have been used by the Aztecs to drink liquid cocoa thousands of years ago.[1] In the 16th century, Aztec Emperor Montezuma was a keen admirer of cocoa, calling it a "divine drink, which builds up resistance and fights fatigue. A cup of this precious drink permits a man to walk for a whole day without food" (Hernán Cortés, 1519). In the language of the Aztecs, this drink was called chocolatl. With the discovery of the New World, cocoa came to Europe in the 16th century.[2] Since then, the modern chocolate industry has developed, and cocoa seeds are now processed in different ways.

Several supposed health effects of cocoa have been considered, including improved heart function and relief of angina pectoris, stimulation of the nervous system, facilitated digestion, and improved kidney and bowel function. In addition, cocoa has been used to treat anemia, mental fatigue, tuberculosis, fever, gout, kidney stones, and even poor sexual appetite.[2] In the 19th century, chocolate became a luxury item; hence, its consumption was a sin rather than a remedy. Nowadays, chocolate is associated with caries, obesity, high blood pressure, and diabetes. Therefore, many physicians currently tend to warn patients about the potential health hazards of consuming large amount of chocolate-based nutrients. However, the recent discovery of biologically active phenolic compounds in cocoa has changed this perception[3] and stimulated research on its effects in ageing, blood pressure regulation, and atherosclerosis.

Here, we review the clinically relevant cardiovascular effects of cocoa, focusing on potential mechanisms involved in the response to cocoa and the potential clinical implications associated with its consumption. It is important to strictly differentiate between the natural product *cacao* and the processed product *chocolate*, which refers to the combination of cocoa, sugar, and eventually milk and other ingredients into a solid food product. Many of the health effects of cocoa and its contents discussed here may not be applicable to chocolate.

Epidemiological Evidence

Epidemiological data demonstrate that regular dietary intake of plant-derived foods and beverages reduces the risk of coronary heart disease[4–7] and stroke[8] and is inversely associated with the risk of cardiovascular disease.[5,7]

First evidence of a similar effect of cocoa was obtained in Kuna Indians, a native population living on islands off the coast of Panama. The Kuna belong to one of the few cultures that are protected against the age-dependent increase in blood pressure and the development of arterial hypertension. Interestingly, the Kunas consume enormous amounts of cocoa daily, sometimes even enriched with salt.[9] Clinical studies revealed that the Kunas indeed have lower blood pressure values[9] and no age-dependent decline in kidney function.[10] Moreover, in this native population, mortality resulting from cardiovascular events is markedly lower compared with other Pan-American citizens (9.2±3.1 versus 83.4±0.7 age-adjusted

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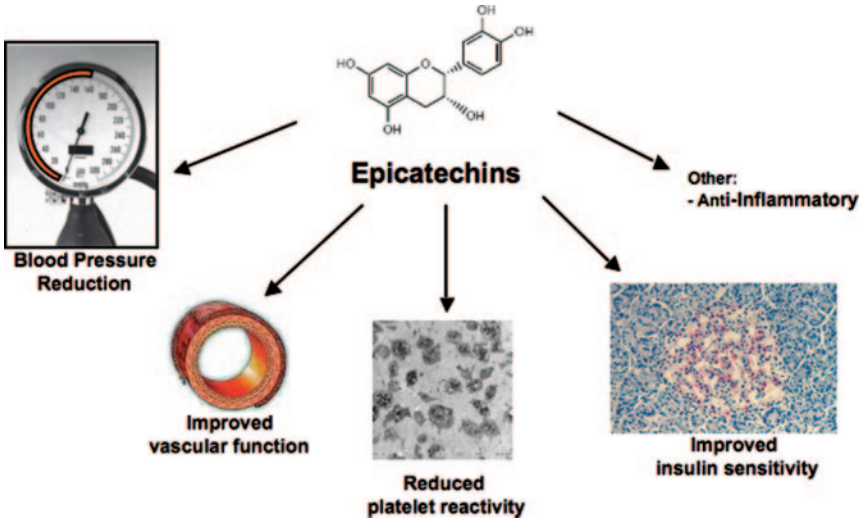

Figure 1. Health-relevant effect of epicatechins.

deaths per 100′000).[11] The factors involved are clearly environmental rather than genetic because this protection is lost on migration to urban Panama City, where the home-prepared cocoa is replaced by other food with a lower flavanol content.[9]

A prospective study in 34 489 postmenopausal women with a 16-year follow-up in the Iowa Women's Health Study who were free of cardiovascular disease found that foods rich in flavonoids were associated with a decreased risk of death caused by coronary heart disease. Furthermore, a borderline significant inverse association between chocolate intake and cardiovascular mortality after multivariate adjustment was observed.[12] The Dutch Zutphen Study provided important further data. In a cross-sectional analysis, cocoa intake was inversely related to blood pressure, and in a prospective analysis, intake was associated with a reduction of cardiovascular and all-cause mortality.[13] Indeed, the Zutphen Elderly Study, involving 470 elderly men free of chronic disease, highlighted the protective effects of cocoa intake. After adjustment for age, body mass index, lifestyle factors, drug use, food, and caloric intake, the risk of cardiovascular mortality for men in the highest tertile of cocoa intake was reduced by 50% compared with the lowest tertile ($P=0.004$). The adjusted relative risk for all-cause mortality was 0.53 (95% CI, 0.39 to 0.72; $P\leq0.001$).

Cocoa Polyphenols

These epidemiological observations led to the hypothesis that such health benefits might be linked, at least in part, to plant-derived flavonoids, a large subgroup of the heterogeneous group of polyphenols. All flavonoids share a common chemical structure: C6-C3-C6 (Figure 1). Flavonoids can be further distinguished: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols.[14] Flavanols (also called flavan-3-ols) have attracted particular interest because they can be found in high concentrations in certain fruits and vegetables. In the context of human nutrition, certain teas, grape juice, wine, various berries, and especially cocoa represent noteworthy sources (Table 1). Flavanols occur as the monomers epicatechin and catechin, which are the main flavanols in fruits.[15] These monomers can form links between C4 and C8, allowing them to assemble as dimers, oligomers, and polymers of catechins, the so-called procyanidins.[16] Procyanidins are also known as condensed tannins, which, through the formation of complexes with salivary proteins, are responsible for the bitterness of cacao.[14]

After oral intake of cocoa, both the flavanol content and the total antioxidant capacity in plasma increase. These effects appear to be markedly reduced when cocoa is consumed with milk or if cocoa is ingested as milk chocolate[17]; however, this finding is controversial.[18,19] The highest plasma peak concentrations of flavanols are obtained 2 to 3 hours after ingestion in a dose-dependent manner[17,20] and are still measurable after 8 hours.[21] In addition, molecular size matters; ie, the smaller the polyphenol is, the higher the concentration in blood is. However, there is a large interpersonal variation in absorption. Therefore, a single measurement of plasma levels at 2 hours cannot be considered a measurement of bioavailability but rather a check for compliance, limiting the usefulness of this measure.[22]

Beside molecular size, there are other important factors modulating the in vivo efficacy of polyphenols that must be considered, eg, their metabolic conversion in intestinal cells, liver, and other tissues; their binding to proteins; their accumulation in cells; and the urinary elimination rate.[23]

Table 1. Catechin/Epicatechin Concentrations Found in Food[14]

Source	Flavanol Content, mg/kg or mg/L
Chocolate	460–610
Beans	350–550
Apricots	100–250
Cherries	50–220
Peaches	50–140
Blackberries	130
Apples	20–120
Green tea	100–800
Black tea	60–500
Red wine	80–300
Cider	40

Figure 2. The fruit of the theobroma cocoa tree.

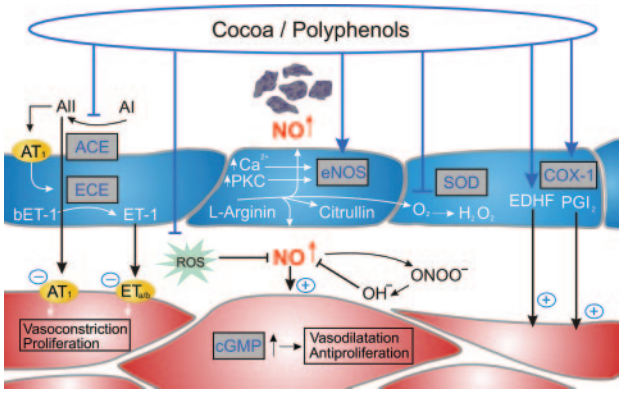

Figure 3. Endothelium-dependent effect of cocoa polyphenols. NO is released from endothelial cells mainly in response to shear stress elicited by the circulating blood or receptor-operated substances such as acetylcholine, bradykinin, or serotonin. NO is synthesized by eNOS from L-arginine in the presence of the cofactor thetrahydrobiopterin. The activation may be due to an increase in Ca^{2+} or a phosphorylation of eNOS by the PI3-kinase/Akt pathway. Cocoa also lowers vascular arginase activity in human endothelial cell in vitro, thus augmenting the local levels of L-arginine. Once released, NO increases intracellular cGMP concentrations and, in turn, induces a relaxation of vascular smooth muscle cells. NO not only leads to vasodilation but also prevents leukocyte adhesion and migration, smooth muscle cell proliferation, and platelet adhesion and aggregation. Other NO-mediated mechanisms are discussed. Antioxidant effects may reduce the production of reactive oxidant species, thus contributing to an enhanced endothelial function. Cocoa polyphenols may activate endothelium-derived hyperpolarizing factor (EDHF), increase endothelial prostacyclin release, or inhibit the synthesis of endothelin-1 (ET). Moreover, polyphenols may directly inhibit angiotensin-converting enzyme (ACE). AII indicates angiotensin II; AI, angiotensin I; PKC, protein kinase C; SOD, superoxide dismutase; PGI_2, prostacyclin; ACE, angiotensin-converting enzyme; ECE, endothelin-converting enzyme; AT_1, angiotensin receptor; ET-1, endothelin 1; bET-1, big endothelin 1; $ET_{a/b}$, endothelin receptor a and b; cGMP, cyclic guanosine monophosphate; and ROS, reactive oxygen species.

Therefore, careful distinction between in vitro and in vivo effects of flavanols is mandatory. For example, although procyanidins are biologically active in vitro, they are hardly absorbed in the intestine and thus are largely inactive in vivo.

An important point is that, during the conventional chocolate manufacturing process from fresh cocoa seeds (Figure 2) to the final product, the concentration of flavanols markedly decreases.[24] In particular, food processing methods such as fermentation and roasting have a detrimental impact on the final flavanol content of foods. Furthermore, flavanol concentrations may depend on the agricultural origin of the raw cocoa.[25] In particular, milk chocolate has the lowest flavanol content compared with cocoa powder and dark chocolate.[26]

Although still debated, a range of potential mechanisms through which flavanols and cocoa might exert their benefits on cardiovascular health have been proposed (Figure 3): activation of nitric oxide (NO) and antioxidant, antiinflammatory, and antiplatelet effects, which in turn might improve endothelial function, lipid levels, blood pressure, insulin resistance, and eventually clinical outcome.[27]

Possible Mechanisms of the Protective Effects of Cocoa

Endothelial Function and NO

The endothelium is a continuous, smooth, nonthrombogenic surface of all blood vessels that exhibits a highly selective permeability in its healthy state. It synthesizes and releases a broad range of vasoactive substances. Functional impairment of the vascular endothelium in response to injury occurs long before the development of structural atherosclerotic changes. NO, synthesized by endothelial NO synthase (eNOS) from L-arginine[28] in the presence of the cofactor thetrahydrobiopterin, is released from endothelial cells mainly in response to shear stress elicited by the circulating blood or receptor-operated substances such as acetylcholine, bradykinin, or serotonin.[29] NO has an in vivo half-life of only a few seconds and rapidly crosses biological membranes. After diffusion from endothelial to vascular smooth muscle cells, NO increases intracellular cGMP concentrations and, in turn, induces a relaxation of vascular smooth muscle cells.[30,31] However, NO not only leads to vasodilation but also prevents leukocyte adhesion and migration, smooth muscle cell proliferation, and platelet adhesion and aggregation. Reduced eNOS expression and/or NO bioavailability is associated with endothelial dysfunction and eventually atherosclerotic disease.[32] Indeed, endothelial dysfunction is associated with cardiovascular disease. Endothelial dysfunction in the forearm circulation correlates with coronary vascular dysfunction and is predictive of future coronary events.[33–36]

In patients with coronary artery disease, eating food rich in flavanols, particularly short- and long-term consumption of black tea[37] and red wine,[38,39] mostly improves endothelial function. In healthy smokers, green tea exerts similar effects.[40] In line with these findings, cocoa induces NO-dependent vasodilation in the rat aorta[41] and in the finger or forearm circulation of healthy humans[42–44] or patients with cardiovascular risk factors, including diabetes (Table 2).[45–49]

The underlying mechanisms are still elusive. In cultured endothelial cells[50,51] and rat aorta,[52] plant extracts rich in flavonoids increase eNOS activity. Incubation of endothelial cells with flavonoid-rich red wine upregulates eNOS mRNA and protein expression, most likely via stabilization of eNOS mRNA. Furthermore, endothelial cells produce up to 3 times more bioactive NO than control cells under such conditions.[53] Cocoa also lowers vascular arginase activity in human endothelial cells in vitro, thus

Table 2. Studies Investigating Cocoa and Endothelial Function

Authors	Year	n	Animals/Patients	Duration	Intervention	Outcome
Karim et al[41]	2000	5	Aortic rings from rats	Immediately	Procyanidins derived from cocoa	Endothelium-derived relaxation mediated by activation of NOS
Fisher et al[42]	2003	27	Healthy people	5 d	Flavanol-rich cocoa (821 mg/d)	Peripheral vasodilatation, improvement in vasodilator response to ischemia assessed by pulse-wave amplitude on the finger
Engeler et al[43]	2004	21	Healthy subjects	2 wk	High-flavonoid chocolate (213 mg procyanidins, 46 mg epicatechin) vs low-flavonoid chocolate	Improvement in flow-mediated vasodilatation of the brachial artery, increase in epicatechin concentrations
Schroeter et al[44]	2006	16	Healthy subjects, isolated rabbit rings		Drink with high flavonoid content	Improvement in flow-mediated vasodilatation paralleled the appearance of flavanols in plasma; concentrations in plasma enough to mediate ex vivo vasodilatation; pure epicatecins mimic vascular effects of cocoa; high-flavanol diet is associated with high urinary excretion of NO metabolites
Heiss et al[45]	2003	26	Patients with at least 1 cardiovascular risk factor	2 h (crossover)	Flavanol-rich cocoa drink (100 mL)	Improvement in flow-mediated vasodilatation, increase in levels of nitrosated and nitrosylated species
Hermann et al[46]	2006	20	Healthy smokers	2 h	40 g commercially available dark chocolate vs white chocolate	Increase in flow-mediated vasodilatation of the brachial artery, improvement in antioxidant status and platelet function
Grassi et al[47]	2005	20	Patients with untreated essential hypertension	15 d (crossover)	100 g dark chocolate (21.91 mg catechin, 65.97 mg epicatechins) vs flavanol free white chocolate	Increase in flow-mediated vasodilatation of the brachial artery, decrease in blood pressure and LDL cholesterol, increase in insulin sensitivity
Heiss et al[48]	2005	11	Smokers	2 h (crossover)	100 mL cocoa drink with high (176–18 mg) or low (<11 mg) flavanol content	Increase in flow-mediated vasodilatation and circulating NO pool, increase in flavanol metabolites
Balzer et al[49]	2008	41	Diabetes	4 wk	Drink with high flavanol content	Improvement in flow-mediated vasodilatation (acute and chronic)
Flammer et al[66]	2007	22	Heart transplant recipients	2 h	40 g commercially available dark chocolate vs flavonoid-free placebo chocolate	Induction of coronary vasodilation, improvement in coronary endothelial function and platelet function
Grassi et al[89]	2008	19	Hypertensives, impaired glucose tolerance patients	2 wk	Flavanol-rich dark chocolate	Improvement in flow-mediated vasodilatation, insulin sensitivity, β-cell function, and blood pressure
Shiina et al[100]	2008	39	Healthy subjects	2 wk	45 g commercially available dark chocolate vs white chocolate	Improvement in coronary circulation as measured by coronary velocity flow reserve

augmenting the local levels of L-arginine.[54] Importantly, cocoa-derived flavanols induce NOS in vitro.[55]

In vivo, in patients with cardiovascular risk factors, including smoking, a cocoa drink high in flavonol content (176 to 185 mg) rapidly enhances the circulating pool of bioactive NO by more than a third and, in turn, augments flow-mediated vasodilation.[45,48] Moreover, infusion of N^G-monomethyl-L-arginine, an inhibitor of NO synthesis, reverses the increase in NO and the augmentation in endothelial function associated with cocoa intake, whereas infusion of ascorbic acid has no effect.[48] Similarly, in isolated aortic rings, concentrations of flavanols comparable to those occurring in plasma after cocoa intake induce endothelium-dependent relaxations. Chronic consumption of a high-flavanol diet is associated with a high urinary excretion of NO metabolites, consistent with an augmented NO production or diminished degradation.[44] Finally, in humans, epicatechins closely mimic the vascular effects of flavanol-rich cocoa, suggesting that they represent the primary mediator of the beneficial effect of cocoa flavanols on vascular function.[44]

At the molecular level, it appears in endothelial cells that the short-term effects of epicatechin are due mainly to diminished inactivation of NO by free radicals through inhibition of NADPH oxidase by epicatechin metabolites, whereas an increased generation of NO as a consequence of higher protein eNOS expression is involved in the long-term

effects.[23,56] Furthermore, pure epicatechin ingestion not only augments NO bioavailability but also acutely reduces the plasma levels of endothelin-1, a potent endothelium-derived vasoconstrictor in healthy men.[57]

Of interest, drinking a flavonoid-enriched cocoa beverage results in regional changes in cerebral blood flow and an overall increased blood flow to gray matter for up to 3 hours as assessed by functional magnetic resonance imaging.[58] In addition, in elderly humans, cerebral blood flow velocity in the middle cerebral artery increases, as measured by transcranial Doppler ultrasound,[59] suggesting that cocoa flavanols may protect against dementia and stroke.

Antioxidant Properties

Besides their direct effects on eNOS expression and activity, cocoa flavanols and procyanidins exert strong antioxidant effects in vitro. First evidence came from an experiment in which extracted polyphenols from commercial cocoa delayed low-density lipoprotein (LDL) oxidation.[60] Other studies showed a reduction in the production of reactive oxygen species in activated leukocytes[61] and an inhibition of ultraviolet-induced DNA oxidation.[62] In humans, flavanol-rich cocoa counteracts lipid peroxidation and therefore lowers the plasma level of F_2-isoprostanes, markers of in vivo lipid peroxidation,[63] and plasma levels of oxidized LDL in hypercholesterolemic patients[64] and increases overall antioxidant capacity.[16,20] In young healthy smokers, commercially available dark chocolate (74% cocoa), but not white chocolate, markedly improves flow-mediated vasodilation and improves plasma antioxidant status, suggesting that induction of eNOS and, in turn, elevated NO levels and a reduction in the production of reactive oxidant species contribute to the enhanced endothelial function under these conditions.[46] Indeed, antioxidants may prevent NO transformation into peroxynitrite and protect against vasoconstriction and vascular damage.[65] Oxidative stress and reduced antioxidant defense play a crucial role in the pathogenesis of atherosclerosis, particularly transplant vasculopathy. We therefore recently assessed in a double-blind, randomized study the effect of flavonoid-rich dark chocolate compared with cocoa-free control chocolate on coronary vasomotion in cardiac transplant recipients. Interestingly, consumption of 40 g dark chocolate induced coronary vasodilation, improved coronary vascular function, and decreased platelet adhesion. These beneficial effects were again paralleled by a reduction in serum oxidative stress as assessed by plasma isoprostanes and were positively related to serum epicatechin concentrations.[66]

However, the antioxidative effects of cocoa have been disputed recently. Indeed, Sies[67] cautioned that fruits and vegetables contain many macronutrients and micronutrients in addition to flavanols that may directly or through their metabolites affect the total antioxidative capacity of plasma. The large increase in plasma total antioxidative capacity observed after the consumption of flavanol-rich food is probably not due to flavanols but more likely is a consequence of the increased uric acid levels resulting from fructose metabolism.[68]

Platelet Function

Platelet dysfunction is another hallmark of atherosclerotic vascular disease.[69] Interestingly, in addition to providing antioxidant vitamins, certain fruits and vegetables may also protect against thrombosis because of their high flavanol content.[70] Several studies have demonstrated platelet inhibitory properties of cocoa.[71–73] Cocoa reduces ADP/collagen-activated, platelet-related primary hemostasis within hours of ingestion. These effects were explained, at least in part, by a reduction in the ADP-induced expression of the activated conformation of glycoprotein IIb/IIIa surface proteins. Furthermore, similar to low-dose aspirin, ex vivo catechin and epicatechin reduce glycoprotein IIb/IIIa expression, thereby exerting antiplatelet effects.[72] In healthy volunteers, consuming 100 g dark chocolate reduced platelet aggregation, an effect not seen after ingestion of white chocolate or milk chocolate.[74] Cocoa decreases not only platelet aggregation but also adhesion. In young healthy smokers, dark chocolate reduces platelet adhesion as assessed by a shear stress–dependent platelet test.[46] Similarly, stearic acid, a saturated fat commonly found in chocolate, reduces mean platelet volume, an index of platelet activation, in humans.[75,76]

Antihypertensive Effects of Cocoa

Besides the initial observations in Kuna Indians, epidemiological support for the blood pressure–lowering capacity of chocolate comes from the Zutphen Elderly Study. In this cohort of 470 men, cocoa intake was inversely related to blood pressure.[13] Even after multivariate adjustment, mean systolic blood pressure was 3.8 mm Hg lower in the highest tertile of cocoa intake compared with the lowest tertile. Another study evaluated the association between chocolate consumption and new-onset hypertension in a cohort of university graduates; however, no protection of cocoa was observed.[77]

More evidence on potential antihypertensive properties of cocoa comes from a recently published interventional study that compared the long-term effect of dark compared with white chocolate consumption in patients with prehypertension or stage I hypertension. A small amount of dark chocolate daily (6 g) in the evening significantly reduced mean systolic blood pressure by 2.9 ± 1.6 mm Hg and diastolic blood pressure by 1.9 ± 1.0 mm Hg with no changes in body weight, plasma lipid levels, glucose, and 8-isoprostane.[78] However, serum levels of *S*-nitrosoglutathione, which is produced by unstable NO reacting with thiol groups to form a stable product,[79] were increased in the dark chocolate group. Although preliminary in nature, these changes indicate an increase in NO production as a potential mechanism of the small reduction in blood pressure seen with dark chocolate consumption.[80]

Besides increased eNOS activity, other mechanisms may contribute to the antihypertensive effect of cocoa-rich food. Indeed, either isolated or food-derived flavanols inhibit angiotensin-converting enzyme activity in vitro.[81] Whether such angiotensin-converting enzyme inhibition also occurs in vivo needs to be evaluated further. Finally, stearic acid or theobromine[82] may contribute to these effects. Indeed, a cross-sectional linear regression analysis within the Multiple Risk

Factor Intervention Trial found that stearic acid levels are inversely associated with diastolic blood pressure.[83]

No matter what mechanism is responsible, several independent, albeit small, studies indicate that ingestion of cocoa-rich chocolate has blood pressure–lowering effects. One study reported reductions in systolic and diastolic blood pressures in hypertensive elderly subjects,[84] and another study noted a decrease in daytime and nighttime blood pressures, as assessed by ambulatory 24-hour measurements, after intake of 100 g flavonoid-rich dark chocolate daily for 2 weeks.[47] In the latter study, systolic blood pressure decreased after consumption of dark chocolate by 12 mm Hg, whereas white chocolate had no effect. However, other studies showed no effect on blood pressure.[42,43] Because these studies were performed in a relatively small number of normotensive individuals and with a lower chocolate intake of shorter duration, an antihypertensive effect may have been missed as a result of their study design.[42]

A recent meta-analysis of randomized controlled studies of cocoa administration (173 subjects; mean duration, 2 weeks) confirmed a significant reduction in pressure: mean systolic and diastolic blood pressures were reduced by 4.7 mm Hg (95% CI, 7.6 to 1.8; $P=0.002$) and 2.8 mm Hg (95% CI, 4.8 to 0.8; $P=0.006$), respectively.[85] This finding is remarkable in that the blood pressure–lowering effects of currently used antihypertensive drugs are in the same range.

Considering the small number of subjects studied so far and the variable dose of flavanols and/or chocolate used, a large, well-controlled, interventional study appears warranted.

Since the demonstration that treatment of prehypertensive subjects with candesartan reduced the risk of incident hypertension, there has been ongoing discussion about the therapeutic need in this large population.[86] Therefore, the response to flavanol-rich cocoa in subjects with prehypertensive (Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure VII, 120 to 139 mm Hg) or high-normal (European Society of Cardiology, 130 to 139 mm Hg) blood pressure would be particularly interesting because it could have the potential to delay the onset of hypertension in this population.

Cocoa and Other Antiatherogenic Effects

Insulin Resistance

Insulin sensitivity is partly dependent on insulin-mediated NO release.[87] Thus, flavanols and dietary antioxidants may decrease insulin resistance by ameliorating NO bioavailability. In line with this concept, Grassi et al[47,88] reported reduced insulin resistance in patients with essential hypertension after a 15-day diet with 100 g flavonoid-rich chocolate daily. Moreover, in hypertensive patients with impaired glucose tolerance, flavonoid-rich dark chocolate not only reduced blood pressure and improved endothelial function but also ameliorated insulin sensitivity and β-cell function.[89] Because intravenous infusion of ascorbic acid improves not only flow-mediated dilation but also insulin sensitivity in subjects with impaired glucose tolerance and in smokers,[90] the antioxidant properties of flavanols might contribute to these beneficial effects of coca on insulin sensitivity.

However, because studies with cocoa in diabetics are scarce and because diabetics tend to be obese, recommending cocoa or flavonoid-rich chocolate consumption to such patients should be done cautiously. Nevertheless, experimental evidence in obese diabetic mice suggests that cocoa dose dependently prevents hyperglycemia.[91]

Blood Lipids

Cocoa butter, a fat derived from cocoa plants and found predominantly in dark chocolate, contains an average of 33% monounsaturated oleic acid and 33% stearic acid. In general, plant stearic acid neither lowers high-density lipoprotein (HDL) nor increases LDL or total cholesterol.[92,93] Interestingly, in a study involving young healthy subjects, consumption of a milk chocolate bar (46 g) instead of a high-carbohydrate snack increased HDL cholesterol and decreased plasma triglycerides but did not affect LDL despite an increase in total fat in the diet.[94] In hypertensive patients, daily consumption of 100 g flavonoid rich chocolate over 2 weeks led to a significant 12% reduction of serum total and LDL cholesterol levels.[47] Moreover, cocoa appears to inhibit LDL oxidation.[95] In healthy subjects, daily consumption of 75 g polyphenol-rich dark chocolate over 3 weeks increases HDL cholesterol by up to 14% and inhibits lipid peroxidation.[96] A recent Japanese study demonstrated that, in hypercholesterolemic patients, flavanol-rich cocoa lowers plasma levels of LDL and oxidized LDL and increases HDL serum concentrations.[64,97]

Overall, the effects of chocolate and its various components on lipid levels are not conclusive, strongly suggesting that a larger well-controlled study appears mandatory. Importantly, however, despite its high fat content, cocoa itself does not seem to exert untoward effects on lipid metabolism. It must be stressed that many chocolate products contain milk or processed fats, eg, palm oils. The effect of processed chocolate on blood lipids is not known and may indeed be less favorable.

Precautions and Limitations

Although many positive effects of chocolate and its ingredients have been documented in the cardiovascular system, precautions in its use are mandatory. Indeed, the high caloric load of commercially available chocolate (about 500 kcal/100 g) may induce weight gain, a risk factor for hypertension, dyslipidemia, and diabetes. Surprisingly, a study in 49 healthy women showed no weight gain after daily consumption of 41 g chocolate, 60 g almonds, or almonds and chocolate together for 6 weeks.[98] Thus, weight gain may occur only with higher amounts of daily chocolate and/or its prolonged use. Furthermore, the occasionally high sugar and fat content of commercially available chocolate has to be considered. Because high sugar intake is associated with obesity, caries, and diabetes, cocoa-based products with no or low sugar content are certainly preferred. On the other hand, cocoa itself, unlike chocolate, can be recommended without hesitation because it is low in sugar and fat.

Although further research is required, current evidence suggests that the beneficial effects of cocoa are attributed mainly to its flavanol content, especially epicatechin. There-

fore, direct dietary supplementation with flavanols instead of chocolate consumption deserves further study. Indeed, protocols using epicatechin or other flavanoids specifically are now feasible and should clarify this question. At this point, recommending dietary supplementation with flavanols, similar to vitamins, appears problematic because potential prooxidative effects of large quantities cannot be excluded.[99]

Because of the limitations of the data available so far, future studies should provide detailed information about the chocolate product used; the exact content in polyphenols, especially flavanols; and most importantly, the flavanol plasma concentrations achieved. Furthermore, it has to be taken into account that cocoa contains many other potentially active substances, eg, theobromine or magnesium, substances not discussed in this review.

Finally, to definitively clarify the protective effects of cacao on cardiovascular health, larger studies with a placebo-controlled prospective design focusing initially on surrogate end points such as carotid atherosclerosis and eventually morbidity and mortality are needed.

Conclusions

For many centuries, cocoa has been known for its good taste and its beneficial effects on health. Recent research revealed that cocoa does indeed exert beneficial cardiovascular effects, probably mediated mainly by its polyphenols, a heterogeneous group of molecules found primarily in fruits and vegetables. The beneficial effects of cacao are most likely due to an increased bioavailability of NO. This may explain the improvement in endothelial function, the reduction in platelet function, and the potentially beneficial effects on blood pressure, insulin resistance, and blood lipids.

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Disclosures

Drs Lüscher and Hollenberg have been consultants for MARS Inc. The other authors report no conflicts.

References

1. Henderson JS, Joyce RA, Hall GR, Hurst WJ, McGovern PE. Chemical and archaeological evidence for the earliest cacao beverages. *Proc Natl Acad Sci U S A*. 2007;104:18937–18940.
2. Dillinger TL, Barriga P, Escarcega S, Jimenez M, Salazar Lowe D, Grivetti LE. Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *J Nutr*. 2000;130: 2057S–2072S.
3. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev*. 1998;56:317–333.
4. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med*. 1995;155:381–386.
5. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet*. 1993;342:1007–1011.
6. Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ*. 1996;312:478–481.
7. Joshipura KJ, Hu FB, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Colditz G, Ascherio A, Rosner B, Spiegelman D, Willett WC. The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann Intern Med*. 2001;134:1106–1114.
8. Keli SO, Hertog MG, Feskens EJ, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Arch Intern Med*. 1996;156:637–642.
9. Hollenberg NK, Martinez G, McCullough M, Meinking T, Passan D, Preston M, Rivera A, Taplin D, Vicaria-Clement M. Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension*. 1997; 29:171–176.
10. Hollenberg NK, Rivera A, Meinking T, Martinez G, McCullough M, Passan D, Preston M, Taplin D, Vicaria-Clement M. Age, renal perfusion and function in island-dwelling indigenous Kuna Amerinds of Panama. *Nephron*. 1999;82:131–138.
11. Bayard V, Chamorro F, Motta J, Hollenberg NK. Does flavanol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, stroke, diabetes mellitus, and cancer in Panama. *Int J Med Sci*. 2007;4:53–58.
12. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *Am J Clin Nutr*. 2007;85: 895–909.
13. Buijsse B, Feskens EJ, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. *Arch Intern Med*. 2006;166:411–417.
14. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr*. 2004;79:727–747.
15. Arts IC, van de Putte B, Hollman PC. Catechin contents of foods commonly consumed in The Netherlands, 1: fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem*. 2000;48:1746–1751.
16. Adamson GE, Lazarus SA, Mitchell AE, Prior RL, Cao G, Jacobs PH, Kremers BG, Hammerstone JF, Rucker RB, Ritter KA, Schmitz HH. HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. *J Agric Food Chem*. 1999;47:4184–4188.
17. Serafini M, Bugianesi R, Maiani G, Valtuena S, De Santis S, Crozier A. Plasma antioxidants from chocolate. *Nature*. 2003;424:1013.
18. Roura E, Andres-Lacueva C, Estruch R, Mata-Bilbao ML, Izquierdo-Pulido M, Waterhouse AL, Lamuela-Raventos RM. Milk does not affect the bioavailability of cocoa powder flavonoid in healthy human. *Ann Nutr Metab*. 2007;51:493–498.
19. Schroeter H, Holt RR, Orozco TJ, Schmitz HH, Keen CL. Nutrition: milk and absorption of dietary flavanols. *Nature*. 2003;426:787–788.
20. Rein D, Lotito S, Holt RR, Keen CL, Schmitz HH, Fraga CG. Epicatechin in human plasma: in vivo determination and effect of chocolate consumption on plasma oxidation status. *J Nutr*. 2000;130: 2109S–2114S.
21. Richelle M, Tavazzi I, Enslen M, Offord EA. Plasma kinetics in man of epicatechin from black chocolate. *Eur J Clin Nutr*. 1999;53:22–26.
22. Cooper KA, Donovan JL, Waterhouse AL, Williamson G. Cocoa and health: a decade of research. *Br J Nutr*. 2008;99:1–11.
23. Schewe T, Steffen Y, Sies H. How do dietary flavanols improve vascular function? A position paper. *Arch Biochem Biophys*. 2008;476:102–106.
24. Andres-Lacueva C, Monagas M, Khan N, Izquierdo-Pulido M, Urpi-Sarda M, Permanyer J, Lamuela-Raventos RM. Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. *J Agric Food Chem*. 2008;56:3111–3117.
25. Counet C, Ouwerx C, Rosoux D, Collin S. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. *J Agric Food Chem*. 2004;52:6243–6249.
26. Miller KB, Stuart DA, Smith NL, Lee CY, McHale NL, Flanagan JA, Ou B, Hurst WJ. Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. *J Agric Food Chem*. 2006;54: 4062–4068.
27. Ding EL, Hutfless SM, Ding X, Girotra S. Chocolate and prevention of cardiovascular disease: a systematic review. *Nutr Metab (Lond)*. 2006;3:2.
28. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988;333:664–666.
29. Boulanger C, Luscher TF. Release of endothelin from the porcine aorta: inhibition by endothelium-derived nitric oxide. *J Clin Invest*. 1990;85: 587–590.
30. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Luscher TF. Nitric oxide is responsible for flow-dependent dilatation of

human peripheral conduit arteries in vivo. *Circulation*. 1995;91:1314–1319.
31. Joannides R, Richard V, Haefeli WE, Linder L, Luscher TF, Thuillez C. Role of basal and stimulated release of nitric oxide in the regulation of radial artery caliber in humans. *Hypertension*. 1995;26:327–331.
32. Oemar BS, Tschudi MR, Godoy N, Brovkovich V, Malinski T, Luscher TF. Reduced endothelial nitric oxide synthase expression and production in human atherosclerosis. *Circulation*. 1998;97:2494–2498.
33. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, Selwyn AP. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235–1241.
34. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol*. 1994;24:1468–1474.
35. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101:1899–1906.
36. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101:948–954.
37. Duffy SJ, Keaney JF Jr, Holbrook M, Gokce N, Swerdloff PL, Frei B, Vita JA. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation*. 2001;104:151–156.
38. Karatzi K, Papamichael C, Aznaouridis K, Karatzis E, Lekakis J, Matsouka C, Boskou G, Chiou A, Sitara M, Feliou G, Kontoyiannis D, Zampelas A, Mavrikakis M. Constituents of red wine other than alcohol improve endothelial function in patients with coronary artery disease. *Coron Artery Dis*. 2004;15:485–490.
39. Whelan AP, Sutherland WH, McCormick MP, Yeoman DJ, de Jong SA, Williams MJ. Effects of white and red wine on endothelial function in subjects with coronary artery disease. *Intern Med J*. 2004;34:224–228.
40. Nagaya N, Yamamoto H, Uematsu M, Itoh T, Nakagawa K, Miyazawa T, Kangawa K, Miyatake K. Green tea reverses endothelial dysfunction in healthy smokers. *Heart*. 2004;90:1485–1486.
41. Karim M, McCormick K, Kappagoda CT. Effects of cocoa extracts on endothelium-dependent relaxation. *J Nutr*. 2000;130:2105S–2108S.
42. Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens*. 2003;21:2281–2286.
43. Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, Kwak HK, Milbury P, Paul SM, Blumberg J, Mietus-Snyder ML. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J Am Coll Nutr*. 2004;23:197–204.
44. Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Uribe C, Schmitz HH, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Natl Acad Sci U S A*. 2006;103:1024–1029.
45. Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effects of cocoa rich in flavan-3-ols. *JAMA*. 2003;290:1030–1031.
46. Hermann F, Spieker LE, Ruschitzka F, Sudano I, Hermann M, Binggeli C, Luscher TF, Riesen W, Noll G, Corti R. Dark chocolate improves endothelial and platelet function. *Heart*. 2006;92:119–120.
47. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, Desideri G, Blumberg JB, Ferri C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension*. 2005;46:398–405.
48. Heiss C, Kleinbongard P, Dejam A, Perre S, Schroeter H, Sies H, Kelm M. Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *J Am Coll Cardiol*. 2005;46:1276–1283.
49. Balzer J, Rassaf T, Heiss C, Kleinbongard P, Lauer T, Merx M, Heussen N, Gross HB, Keen CL, Schroeter H, Kelm M. Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial. *J Am Coll Cardiol*. 2008;51:2141–2149.
50. Choi YJ, Kang JS, Park JH, Lee YJ, Choi JS, Kang YH. Polyphenolic flavonoids differ in their antiapoptotic efficacy in hydrogen peroxide-treated human vascular endothelial cells. *J Nutr*. 2003;133:985–991.
51. Leikert JF, Rathel TR, Wohlfart P, Cheynier V, Vollmar AM, Dirsch VM. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation*. 2002;106:1614–1617.
52. Benito S, Lopez D, Saiz MP, Buxaderas S, Sanchez J, Puig-Parellada P, Mitjavila MT. A flavonoid-rich diet increases nitric oxide production in rat aorta. *Br J Pharmacol*. 2002;135:910–916.
53. Wallerath T, Poleo D, Li H, Forstermann U. Red wine increases the expression of human endothelial nitric oxide synthase: a mechanism that may contribute to its beneficial cardiovascular effects. *J Am Coll Cardiol*. 2003;41:471–478.
54. Schnorr O, Brossette T, Momma TY, Kleinbongard P, Keen CL, Schroeter H, Sies H. Cocoa flavanols lower vascular arginase activity in human endothelial cells in vitro and in erythrocytes in vivo. *Arch Biochem Biophys*. 2008;476:211–215.
55. Vinson JA, Proch J, Zubik L. Phenol antioxidant quantity and quality in foods: cocoa, dark chocolate, and milk chocolate. *J Agric Food Chem*. 1999;47:4821–4824.
56. Steffen Y, Schewe T, Sies H. (-)-Epicatechin elevates nitric oxide in endothelial cells via inhibition of NADPH oxidase. *Biochem Biophys Res Commun*. 2007;359:828–833.
57. Loke WM, Hodgson JM, Proudfoot JM, McKinley AJ, Puddey IB, Croft KD. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr*. 2008;88:1018–1025.
58. Francis ST, Head K, Morris PG, Macdonald IA. The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J Cardiovasc Pharmacol*. 2006;47(suppl 2):S215–S220.
59. Sorond FA, Lipsitz LA, Hollenberg NK, Fisher ND. Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. *Neuropsychiatr Dis Treat*. 2008;4:433–440.
60. Waterhouse AL, Shirley JR, Donovan JL. Antioxidants in chocolate. *Lancet*. 1996;348:834.
61. Sanbongi C, Suzuki N, Sakane T. Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans in vitro. *Cell Immunol*. 1997;177:129–136.
62. Ottaviani JI, Carrasquedo F, Keen CL, Lazarus SA, Schmitz HH, Fraga CG. Influence of flavan-3-ols and procyanidins on UVC-mediated formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine in isolated DNA. *Arch Biochem Biophys*. 2002;406:203–208.
63. Wiswedel I, Hirsch D, Kropf S, Gruening M, Pfister E, Schewe T, Sies H. Flavanol-rich cocoa drink lowers plasma F(2)-isoprostane concentrations in humans. *Free Radic Biol Med*. 2004;37:411–421.
64. Baba S, Natsume M, Yasuda A, Nakamura Y, Tamura T, Osakabe N, Kanegae M, Kondo K. Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo- and hypercholesterolemic humans after intake of different levels of cocoa powder. *J Nutr*. 2007;137:1436–1441.
65. Wever RM, Luscher TF, Cosentino F, Rabelink TJ. Atherosclerosis and the two faces of endothelial nitric oxide synthase. *Circulation*. 1998;97:108–112.
66. Flammer AJ, Hermann F, Sudano I, Spieker L, Hermann M, Cooper KA, Serafini M, Luscher TF, Ruschitzka F, Noll G, Corti R. Dark chocolate improves coronary vasomotion and reduces platelet reactivity. *Circulation*. 2007;116:2376–2382.
67. Sies H. Total antioxidant capacity: appraisal of a concept. *J Nutr*. 2007;137:1493–1495.
68. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radic Biol Med*. 2006;41:1727–1746.
69. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque, part I: evolving concepts. *J Am Coll Cardiol*. 2005;46:937–954.
70. Keen CL, Holt RR, Oteiza PI, Fraga CG, Schmitz HH. Cocoa antioxidants and cardiovascular health. *Am J Clin Nutr*. 2005;81:298S–303S.
71. Holt RR, Schramm DD, Keen CL, Lazarus SA, Schmitz HH. Chocolate consumption and platelet function. *JAMA*. 2002;287:2212–2213.
72. Pearson DA, Paglieroni TG, Rein D, Wun T, Schramm DD, Wang JF, Holt RR, Gosselin R, Schmitz HH, Keen CL. The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thromb Res*. 2002;106:191–197.
73. Rein D, Paglieroni TG, Wun T, Pearson DA, Schmitz HH, Gosselin R, Keen CL. Cocoa inhibits platelet activation and function. *Am J Clin Nutr*. 2000;72:30–35.

74. Innes AJ, Kennedy G, McLaren M, Bancroft AJ, Belch JJ. Dark chocolate inhibits platelet aggregation in healthy volunteers. *Platelets*. 2003;14:325–327.
75. Kelly FD, Sinclair AJ, Mann NJ, Turner AH, Abedin L, Li D. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. *Eur J Clin Nutr*. 2001;55:88–96.
76. Kelly FD, Sinclair AJ, Mann NJ, Turner AH, Raffin FL, Blandford MV, Pike MJ. Short-term diets enriched in stearic or palmitic acids do not alter plasma lipids, platelet aggregation or platelet activation status. *Eur J Clin Nutr*. 2002;56:490–499.
77. Alonso A, de la Fuente C, Beunza JJ, Sanchez-Villegas A, Martinez-Gonzalez MA. Chocolate consumption and incidence of hypertension. *Hypertension.* 2005;46:e21–e22.
78. Taubert D, Roesen R, Lehmann C, Jung N, Schomig E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. *JAMA*. 2007;298:49–60.
79. Stamler JS. S-nitrosothiols in the blood: roles, amounts, and methods of analysis. *Circ Res*. 2004;94:414–417.
80. Foster MW, Pawloski JR, Singel DJ, Stamler JS. Role of circulating S-nitrosothiols in control of blood pressure. *Hypertension*. 2005;45: 15–17.
81. Actis-Goretta L, Ottaviani JI, Fraga CG. Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. *J Agric Food Chem*. 2006;54:229–234.
82. Kelly CJ. Effects of theobromine should be considered in future studies. *Am J Clin Nutr*. 2005;82:486–487.
83. Simon JA, Fong J, Bernert JT Jr. Serum fatty acids and blood pressure. *Hypertension*. 1996;27:303–307.
84. Taubert D, Berkels R, Roesen R, Klaus W. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *JAMA*. 2003;290:1029–1030.
85. Taubert D, Roesen R, Schomig E. Effect of cocoa and tea intake on blood pressure: a meta-analysis. *Arch Intern Med*. 2007;167:626–634.
86. Julius S, Nesbitt SD, Egan BM, Weber MA, Michelson EL, Kaciroti N, Black HR, Grimm RH Jr, Messerli FH, Oparil S, Schork MA. Feasibility of treating prehypertension with an angiotensin-receptor blocker. *N Engl J Med*. 2006;354:1685–1697.
87. Zeng G, Nystrom FH, Ravichandran LV, Cong LN, Kirby M, Mostowski H, Quon MJ. Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation*. 2000;101:1539–1545.
88. Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am J Clin Nutr*. 2005;81:611–614.
89. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr*. 2008;138: 1671–1676.
90. Hirai N, Kawano H, Hirashima O, Motoyama T, Moriyama Y, Sakamoto T, Kugiyama K, Ogawa H, Nakao K, Yasue H. Insulin resistance and endothelial dysfunction in smokers: effects of vitamin C. *Am J Physiol Heart Circ Physiol*. 2000;279:H1172–H1178.
91. Tomaru M, Takano H, Osakabe N, Yasuda A, Inoue K, Yanagisawa R, Ohwatari T, Uematsu H. Dietary supplementation with cacao liquor proanthocyanidins prevents elevation of blood glucose levels in diabetic obese mice. *Nutrition*. 2007;23:351–355.
92. Bonanome A, Grundy SM. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N Engl J Med*. 1988;318:1244–1248.
93. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr*. 2003;77:1146–1155.
94. Kris-Etherton PM, Derr JA, Mustad VA, Seligson FH, Pearson TA. Effects of a milk chocolate bar per day substituted for a high-carbohydrate snack in young men on an NCEP/AHA Step 1 Diet. *Am J Clin Nutr*. 1994;60:1037S–1042S.
95. Kondo K, Hirano R, Matsumoto A, Igarashi O, Itakura H. Inhibition of LDL oxidation by cocoa. *Lancet*. 1996;348:1514.
96. Mursu J, Voutilainen S, Nurmi T, Rissanen TH, Virtanen JK, Kaikkonen J, Nyyssonen K, Salonen JT. Dark chocolate consumption increases HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans. *Free Radic Biol Med*. 2004;37: 1351–1359.
97. Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, Fukuda K, Muto Y, Kondo K. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. *Am J Clin Nutr*. 2007;85:709–717.
98. Kurlandsky SB, Stote KS. Cardioprotective effects of chocolate and almond consumption in healthy women. *Nutr Res*. 2006;26:509–516.
99. Halliwell B. Dietary polyphenols: good, bad, or indifferent for your health? *Cardiovasc Res*. 2007;73:341–347.
100. Shiina Y, Funabashi N, Lee K, Murayama T, Nakamura K, Wakatsuki Y, Daimon M, Komuro I. Acute effect of oral flavonoid-rich dark chocolate intake on coronary circulation, as compared with non-flavonoid white chocolate, by transthoracic Doppler echocardiography in healthy adults. *Int J Cardiol*. 2009;131:424–429.