The Emerging Role of Flavonoid-Rich Cocoa and Chocolate in Cardiovascular Health and Disease

a report by

Mary B Engler PhD

University of California, San Francisco, CA, Department of Physiological Nursing, Laboratory of Cardiovascular Physiology

Introduction

Dietary flavonoids and their potential role in the prevention of cardiovascular disease have gained recent scientific and medical interest due to their antioxidant properties.1 Oxidative stress due to excess free radicals or reactive oxygen species (ROS) is associated with a number of cardiovascular risk factors, i.e., hypertension, dyslipidemias, diabetes, smoking. Cellular DNA, proteins, and lipids are susceptible to ROS attack which can result in damage to cell membranes and organelles. Tissue damage and pathophysiological processes eventually ensue. The oxidative modification of low density lipoprotein (LDL) due to oxidative stress is believed to be a major contributing factor in atherosclerosis. Thus, dietary flavonoids due to their antioxidant properties may be beneficial in cardiovascular health and disease.

Epidemiological studies suggest flavonoid-rich diets high in fruits and/or vegetables reduce the risk of coronary heart disease.2-4 A recent meta-analysis of seven prospective cohort studies with 105,000 individuals indicated that high dietary intake of flavonoids from a small number of fruits and vegetables, tea and red wine are inversely associated with coronary heart disease risk.5 The antioxidant properties of flavonoids are related to their structure, two aromatic rings on the ends bound by an oxygenated heterocycle in the middle which promote free radical scavenging. The flavonoids as a subclass of polyphenols, are ubiquitous micronutrients derived from plants, primarily fruits and vegetables. There are more than 5,000 flavonoids identified and the six major flavonoid categories include: flavanols, flavanones, flavones, isoflavones, flavonols, and anthocyanidins.6

The various subclasses are listed below and include typical foods or beverages with a substantial content of flavonoids: flavanol (catechin, epicatechin–chocolate, tea, red wine, beans, apricot, cherry, grape, peach, blackberry, apple), flavanones (hesperetin, naringenin, eriodictyol–citrus fruits and juices), flavones (apigenin, luteolin–parsley, celery), isoflavones (daidzein, genistein–soy products), flavonols (queretin, kaempferol, myricetin–onions, kale, broccoli, tomato, blueberry, apples, tea, red wine), anthocyanidins (cyanidin, pelargonidin, peonidin, delphinidin, malvidin–blueberry, black grape, cherry, blackberry, black currant, rhubarb, strawberry, red wine, plum, red cabbage).7-9 Interestingly, cocoa and chocolate contain both a high quantity and quality of antioxidant flavonoids, even exceeding black and green tea as well as red wine.9,10 Dark chocolate ranks the highest of top antioxidant foods as indicated by the oxygen radical absorbance capacity (ORAC) measurement (Figure 1).11,12

The high antioxidant capacity of cocoa and chocolate are attributed to their significant amount of procyanidins, the oligomeric form of the flavanol monomeric units, (-)-epicatechin and (+)-catechin. These monomers, mainly (-)-epicatechin, provide most of the total procyanidin content in chocolate, however; dimers (two monomer units) and up to 10 monomer units are also present. Cocoa and chocolate, especially dark, have only recently been identified as rich sources of flavonoids due to advances in technology and analytical methods used in the detection of flavonoids. It is important to note that the amount of flavonoids in chocolate is not only dependent on the cacao bean, but also on the processing steps involved in its manufacture, e.g., excess heat and alkalization (“Dutch” process) can significantly reduce the amount of flavonoids. Typically, dark chocolate contains 2–3 times as many cocoa flavonoids as milk chocolate.

History

The cacao tree, Theobroma cacao or “food of the gods”, was first cultivated in 250-900 A.D. by the ancient Maya civilization in the Mesoamerican (Mexico to Central America) region.13,14 A typical football-shaped fruit pod of the cacao (pronounced “Kah-Kow”) tree contains approximately 25 to 75 cocoa beans. The Aztec elite civilization in the twelfth to sixteenth centuries drank chocolate derived from the cocoa beans in combination with spices and it was used as a nourishing staple beverage. Both Maya and Aztec royal and religious events had offerings of chocolate to the Aztec god, Quetzalcoatl, who by legend brought heavenly cacao down to earth. The beans were used as currency, e.g., 4 beans for one pumpkin, and for medicinal purposes to fight fatigue and gastrointestinal distress.15,16

Reference Section

Mary B Engler, PhD, RN, MS, FAHA, is a Professor and Director of the Cardiovascular and Genomics Graduate Programs in the Department of Physiological Nursing at the University of California, San Francisco. Her research has focused on nutritional interventions and vascular biology in the prevention and treatment of cardiovascular disease. Her work has been published in a number of prestigious national and international journals including the European Journal of Pharmacology, Canadian Journal of Physiology and Pharmacology, British Journal of Pharmacology, Nutrient Research, The Asia Pacific Heart Journal, American Journal of Hypertension, Lipids, and Circulation, Journal of the American Heart Association (AHA). Dr Engler has received numerous awards including the Clinical Research Award from the AHA, the First Independent Research Support and Transition Award; and the Mentored Research Scientist Development Award, both from the National Institutes of Health. She is recognized as a Fellow in the American Heart Association. Dr Engler has also been a member of many professional organizations including the American Physiological Society, International Society for the Study of Fatty Acids and Lipids, The AHA’s Council on Arteriosclerosis, Thrombosis and Vascular Biology, Council on Cardiovascular Nursing, the Functional Genomics Interdisciplinary Working Group, and the Council on Nutrition, Metabolism and Physical Activity.

Mary B Engler, PhD, RN, MS, FAHA, is a Professor and Director of the Cardiovascular and Genomics Graduate Programs in the Department of Physiological Nursing at the University of California, San Francisco. Her research has focused on nutritional interventions and vascular biology in the prevention and treatment of cardiovascular disease. Her work has been published in a number of prestigious national and international journals including the European Journal of Pharmacology, Canadian Journal of Physiology and Pharmacology, British Journal of Pharmacology, Nutrient Research, The Asia Pacific Heart Journal, American Journal of Hypertension, Lipids, and Circulation, Journal of the American Heart Association (AHA). Dr Engler has received numerous awards including the Clinical Research Award from the AHA, the First Independent Research Support and Transition Award; and the Mentored Research Scientist Development Award, both from the National Institutes of Health. She is recognized as a Fellow in the American Heart Association. Dr Engler has also been a member of many professional organizations including the American Physiological Society, International Society for the Study of Fatty Acids and Lipids, The AHA’s Council on Arteriosclerosis, Thrombosis and Vascular Biology, Council on Cardiovascular Nursing, the Functional Genomics Interdisciplinary Working Group, and the Council on Nutrition, Metabolism and Physical Activity.
Following the Spanish conquest of Mexico by Hernán Cortés, the cocoa beans were brought back to Spain in 1528. Over the next 100 years, chocolate as a fad swept across Europe. Cane sugar, vanilla, cinnamon, and aniseed were added to the bitter chocolate drink in favor of peppers and other native herbs used by the Aztecs. Sweet, hot chocolate was thus born. It was such a status symbol that in France, only the royal courts were allowed to drink chocolate. In 1657, the first chocolate house opened in London, England. However; chocolate was not readily available in the United States until the mid 1800s due to the high duties on imports of cocoa beans and sugar. It was during World War I that chocolate was provided as rations to U.S. servicemen in Europe. Interestingly, the chocolate was resistant to spoilage, now believed to be due to the natural antioxidant flavonoids it contains.

**Antioxidant Activity**

Decreased susceptibility of low density lipoprotein (LDL) oxidation has recently been ascribed to the cocoa flavonoids. The antioxidant capacity and diminished production of oxidative products in plasma is related to increased concentrations of the cocoa and chocolate flavonoid, (-)-epicatechin. A study on the plasma kinetics of epicatechin showed significant increases in epicatechin at 2 hours after chocolate consumption. Plasma epicatechin levels reach 0.7 µmol/l following acute ingestion of 80 g of dark chocolate (164 mg of epicatechin) and 0.2 µmol/l with 2 week daily consumption of 46 g (46 mg of epicatechin) dark chocolate. Table 1 lists the studies which have investigated the effects of cocoa and chocolate on oxidation. Various plasma measurements including: total antioxidant capacity, LDL oxidation susceptibility or lag time,
oxygen radical absorbance capacity (ORAC), 8-isoprostanes, 2-thiobarbituric acid reactive substances (TBARS), were examined in these investigations. Overall, favorable changes in oxidative measurements and increases in plasma epicatechin concentrations following cocoa or chocolate consumption were found. In the studies with negative findings related to improvement in oxidative measurements, this may be attributed to a number of factors including the differences in the subjects’ baseline epicatechin concentrations and the magnitude of increase in these levels seen after consumption. This may be due, in part, to differences in baseline diets or in the detection sensitivity of low concentrations of plasma epicatechin.22,27 It is also interesting that the presence of milk with chocolate consumption appears to diminish an increase in total antioxidant capacity and epicatechin concentrations27; although, a separate study reported by Schroeter et al28 found no differences in 12 healthy volunteers under similar conditions.

Vasodilation

Endothelial dysfunction is recognized as an early event in the development of atherosclerosis and is associated with decreased bioavailability of the vasodilator, nitric oxide. Karim et al in 2000 was the first to show that cocoa extracts induce endothelium-dependent relaxation and activate endothelial nitric oxide synthase in isolated rabbit aortic rings.26 Oligomeric forms of the monomeric units, (-)-epicatechin and (+)-catechin, such as tetramers and higher, were associated with these effects. Additionally, a favorable balance in eicosanoid synthesis has been reported in cultured human aortic endothelial cells exposed to cocoa flavonoids and in human plasma samples from subjects at 2 hours following consumption of high flavanol chocolate (37g).27 A decrease in the plasma leukotriene-prostacyclin ratio was found which would result in more vasodilation, less platelet aggregation, and an anti-inflammatory profile. A significant rise in plasma epicatechin was also noted at the 2 hour time-point following chocolate consumption.

Other recent studies (Table 2) in healthy subjects following 4 days to 2 weeks daily consumption of a cocoa beverage or flavonoid-rich dark chocolate bar reported increased vasodilation or improvement in endothelial function.22,28 Participants who had at least one cardiovascular risk factor, including hypertension, hyperlipidemia, diabetes, smoking, or history of coronary artery disease also demonstrated a reversal of endothelial dysfunction with just a single dose of a cocoa beverage.29 An increase in nitric oxide bioactivity was seen in this study29 and further increases in vasodilation were reversed with the nitric oxide synthase inhibitor, N\textsuperscript{G} -nitro-L-arginine methyl ester (L-NAME), given intravenously in the study by Fisher et al.30 Plasma epicatechin concentrations were also significantly increased following cocoa or chocolate consumption in several of these studies.

As seen in Figure 2, a possible mechanism for the vasodilatory effect of cocoa and chocolate may be related to increases in plasma epicatechin concentrations which signal the release of vasoactive substances from the endothelium including nitric oxide (NO) and prostacyclin. The above studies provide evidence for increased NO synthesis and beneficial changes in the eicosanoid ratio. Moreover, several of the studies27,28,29 measured endothelium-dependent flow-mediated dilation, which reflects an increase in flow and shear stress after reactive hyperemia, and is mediated by endothelium-derived nitric oxide.30

Blood Pressure Effects

The studies on the effects of cocoa and chocolate on blood pressure are limited and show no effect in healthy subjects.19,22,28,31 Only one randomized crossover trial in untreated stage 1 mild isolated systolic hypertensives has shown a blood pressure lowering effect (−5.1 mmHg systolic and −1.8 mmHg diastolic) following 14 day consumption of 100g dark chocolate (500mg flavonoids) (32). Of note, a recent report suggests cocoa flavonols may lower blood pressure by acting as an angiotensin I converting enzyme (ACE) inhibitor, which also has antioxidant properties and can modulate NO production.32

Platelet Function Effects

A suppressive effect on platelet reactivity and platelet-related primary hemostasis has been demonstrated in many studies even after a single chocolate dose (Table 3).26,28 The antiplatelet effects of cocoa and chocolate may be due to increased production of nitric oxide, which not only cause vasodilation as previously discussed, but also inhibits platelet aggregation. Increased plasma epicatechin concentrations were reported in the studies by Pearson et al (2002) and Murphy et al (2003) which may signal increased NO synthesis in both the endothelial cells and platelets. Increased production of prostacyclin, an inhibitor of platelet aggregation, has also been proposed as a possible mechanism.28 These platelet inhibitory effects by cocoa and chocolate may be beneficial due to the pathophysiological role of platelets in atherosclerosis and thrombotic events.

Inflammation and Immune Function Effects

It is now widely accepted that atherosclerosis is a chronic inflammatory disease.33 Inflammation as well
as increased oxidative stress promote endothelial dysfunction and atherogenesis.\textsuperscript{23} Nitric oxide normally inhibits nuclear transcription factor (NFκB), which binds to the promoter regions of genes coding for proinflammatory proteins, such as cytokines and adhesion molecules. In endothelial dysfunction which is manifested by decreased bioavailability of NO, this inhibition is lost. Excess intracellular ROS in oxidative stress also activate NFκB. Cocoa flavonoids may prevent activation of NFκB and subsequent cytokine transcription by diminishing intracellular ROS.

In experimental studies, the expression of proinflammatory cytokines, interleukin-1β (IL-1β) and interleukin-6, is modulated by the cocoa flavonoids. Specifically, interleukin-1β (IL-1β) expression in phytohemagglutinin-stimulated peripheral blood mononuclear cells are reduced by purified monomer to tetramer cocoa flavonoids and IL-2 mRNA expression of and IL-2 secretion by T cells have also been shown to be inhibited with cocoa treatment.\textsuperscript{41,42} Cocoa flavonoids are also incorporated into Jurkat T cells with pretreatment and inhibit PMA-induced NFκB activation.\textsuperscript{18} Mathur et al\textsuperscript{19} recently reported no effect on markers of inflammation (whole-blood cytokines, IL-1β, IL-6, TNF-α, high sensitivity C-reactive protein and P-Selectin).

The healthy subjects in this study consumed the cocoa and chocolate supplementation (651 mg of cocoa flavonoids) for 6 weeks. Epicatechin was not detected in the subjects’ plasma and the author’s attribute the lack of effect on inflammatory markers to the short half-life of cocoa flavonoids. It is known that epicatechin peaks in the plasma at 2 hours after cocoa or chocolate consumption and is cleared approximately 8 hours later.

### Table 2: Effects of Cocoa and Chocolate on Vasodilation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type</th>
<th>Cocoa Flavonoids Amount</th>
<th>Model</th>
<th>Endothelium-dependent relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karim 2000</td>
<td>Cocoa extracts</td>
<td>10-7 to 10-5 mol/L</td>
<td>Isolated rabbit aorta</td>
<td>+</td>
</tr>
<tr>
<td>Fisher 2003</td>
<td>Cocoa beverage</td>
<td>821 mg/day</td>
<td>Healthy adults (n=27)</td>
<td>+</td>
</tr>
<tr>
<td>Heiss 2003</td>
<td>Cocoa beverage</td>
<td>176 mg/day</td>
<td>Adults with 1 cardiovascular risk factor or history of CAD (n=26)</td>
<td>+</td>
</tr>
<tr>
<td>Engler 2004</td>
<td>Dark chocolate bars</td>
<td>259mg/day</td>
<td>Healthy adults (n=21)</td>
<td>+</td>
</tr>
</tbody>
</table>

**Conclusion**

The investigations on the antioxidant, vasodilatory, blood-pressure lowering, anti-platelet, and anti-inflammatory effects of cocoa and chocolate provide exciting new evidence into the potential cardiovascular benefits of flavonoids-rich foods. Balance and moderation are also important in a healthy diet and must be considered for food, such as chocolate, that is high in calories and fat. Interestingly, the fat in chocolate (cocoa butter) contains approximately 35% oleic acid, the monounsaturated fat found in olive oil, and 60% saturated fat (35% stearic acid, 25% palmitic acid). Palmitic acid has cholesterol-raising effects, however; it is believed to be offset by the neutral cholesterol effects of stearic acid and the slightly cholesterol-lowering effect of oleic acid. Stearic acid...
Nutritional therapy with flavonoid-rich foods, especially with those that raise plasma epicatechin concentrations, may prove beneficial in reducing or preventing oxidative stress and endothelial dysfunction. As illustrated in Figure 3, the cocoa flavonoids may inhibit both pathophysiological processes that lead to atherosclerosis and eventual cardiovascular events. Based on the existing literature, it would be practical to advise consumption of wide-range of flavonoids-rich foods and beverages, especially, those that contain substantial amounts of the same flavonoids (flavanols) found in cocoa and dark chocolate including: green and black tea, especially Ceylon, red wine, cherries (sweet), apples, purple grapes, blackberries, raspberries, and broadbeans. And in moderation with a healthy and active lifestyle, small amounts of dark chocolate may also be good for your heart!

Table 3: Effects of Cocoa and Chocolate on Platelet Function

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type</th>
<th>Cocoa Flavonoids Amount</th>
<th>Subjects</th>
<th>Platelet function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rein 2000 37</td>
<td>Cocoa beverage 300ml single dose</td>
<td>897 mg</td>
<td>Healthy adults (n=10)</td>
<td>Platelet activation</td>
</tr>
<tr>
<td>Rein 2000 38</td>
<td>Cocoa beverage 300ml single dose</td>
<td>897 mg</td>
<td>Healthy adults (n=30)</td>
<td>Platelet activation &amp; microparticle formation Aspirin-like effect on primary hemostasis</td>
</tr>
<tr>
<td>Pearson 2002</td>
<td>Cocoa beverage 300ml single dose</td>
<td>897 mg</td>
<td>Healthy adults (n=16)</td>
<td>Platelet activation &amp; induced platelet plug formation</td>
</tr>
<tr>
<td>Holt 2002</td>
<td>Semisweet chocolate chips 25g single dose</td>
<td>220 mg</td>
<td>Healthy adults (n=18)</td>
<td>Platelet-related primary hemostasis</td>
</tr>
<tr>
<td>Murphy 2003</td>
<td>Cocoa tablets 6 tablets/day for 28 days</td>
<td>234 mg</td>
<td>Healthy adults (n=32)</td>
<td>Platelet activation &amp; induced aggregation</td>
</tr>
</tbody>
</table>

References

The Emerging Role of Flavonoid-Rich Cocoa and Chocolate


