

Review

Chocolate: Food as Medicine/Medicine as Food

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Cocoa and chocolate products have been delicacies for hundreds of years. Only recently have they been recognized as significant sources of phytochemicals with healthful effects. These foods are among the most concentrated sources of the procyanidin flavonoids, catechin and epicatechin. Recent studies have shown that these polyphenols are absorbed from the intestine of animals and humans with epicatechin absorbed much more than catechin. These flavonoids have potent antioxidant and antiplatelet activities following consumption of cocoa or chocolate.

Key teaching points:

- Cocoa and chocolate products have the highest concentration of flavonoids among commonly consumed foods
- The predominant flavonoids are catechin and epicatechin, which are found as polymers
- These flavonoids have potent antioxidant effects *in vitro* and *in vivo*, and spare antioxidant vitamins
- Cocoa-derived polyphenols also inhibit platelet reactivity
- Multiple mechanisms are described by which phytochemicals from cocoa may exert healthful effects

Why study chocolate and health? Why study chocolate and cocoa in a serious scientific way? What is the bioavailability of some of the chocolate flavonoids? What are the effects of chocolate on flavonoids? What are the effects of these flavonoids on antioxidant status in subjects? What are the effects of cocoa on platelet activation? What are some of the effects of chocolate on eicosanoid synthesis?

The idea that chocolate or cocoa may have some health benefits is not necessarily a novel concept. When Cortez first visited Central America, one of his first observations was the routine use of chocolate, particularly by the high priest. Most intriguing was reference to chocolate primarily as a medicine. Historical documents in Europe refer to chocolate's medicinal value. By the 1600s and 1700s, chocolate and cocoa were viewed not just as a beverage with a pleasurable taste, but primarily as a food to treat a number of disorders, including angina and heart pain. The concept that cocoa beverages may provide some health benefits was widely accepted up until about the 1850s and into the early 1900s. Only in the past 30 or 40 years have perceptions of chocolate changed from its being

a medicinal food to a confectionery with no health benefits or possible negative effects on one's health.

Our rationale for studying chocolate stems from the recognition that chocolate *per se* is a food rich in flavonoids. Numerous epidemiological studies indicate a strong inverse correlation between ischemic heart disease and consumption of wine [1], other alcoholic beverages [2], and flavonoids and other polyphenols in wine, fruits and vegetables [3]. Because cocoa is a polyphenol-rich food containing primarily procyanidins, as well as methylxanthines, one can argue that intake of cocoa may also be associated with reduced risk for cardiovascular disease.

Cocoa is one of the richest flavonoid-containing foods available. Over 10 percent of the weight of cocoa powder, which is used to make beverages, is flavonoids [4]. Chocolate also contains a small amount of theobromine (1.2%). Contrary to common belief, chocolate is not a rich source of caffeine. Typically, the cocoa beverage used in most studies contains less caffeine than found in a cup of decaffeinated coffee [5].

Chocolate and cocoa are unique in the type of flavonoids

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present. The flavonoids in cocoa/chocolate, which are principally catechin and epicatechin, exist in long polymers. The procyanidins contain two, three, or up to ten of the catechin or epicatechin units linked, which is fairly distinctive [6]. Most flavonoid-rich foods tend to have more catechins and epicatechins in dimers or trimers, not pentamers, hexamers, etc. Also intriguing is the chemistry of flavonoids in cocoa which are really R-oligomers. The structures of catechin and epicatechin would lead one to immediately predict their antioxidant properties. Catechin and epicatechin have been identified in red wine, as well as in green tea. In fact, these cocoa flavonoids are thought to be responsible for some of the cardioprotective effects attributed to cocoa. Indeed, *in vitro* studies have demonstrated that cocoa flavonoids inhibit endothelial cell mediated LDL oxidation, similar to that initiated with copper *in vitro* [7]. Whether or not the antioxidant effect of cocoa flavonoids is structure specific remains to be determined. Cocoa compounds of long oligomeric structures may be more effective than compounds with shorter oligomeric fractions [8]. Findings indicate that flavonoids in cocoa are relatively potent and inhibit oxidation at a low concentration of about one μM . A wealth of *in vitro* data indicates that approximately one-half to one μM inhibits COX-2.

Research demonstrates that flavonoids isolated from cocoa have potent antioxidant effects *in vitro*. The real question, however, is whether similar effects are observed in humans following consumption of cocoa or chocolate. To answer this question, researchers measured plasma levels of epicatechin and catechin in humans at zero, one, two, and six hours following consumption of a single meal of chocolate [9]. Epicatechin and catechin plasma levels peaked at about one hour. However, by six hours these flavonoids almost disappeared from the plasma. Much less catechin is absorbed than epicatechin. These cocoa flavonoids are absorbed and cleared from circulation relatively quickly. This observation is supported by a number of clinical trials with chocolate and other flavonoid-rich foods [10]. One could argue that this rapid turnover of flavonoids supports current dietary recommendations to consume five servings a day of fruits and vegetables. Spacing intake of flavonoid-rich foods throughout the day would help to provide a continuous supply of flavonoids.

A number of studies has examined the antioxidant effects of cocoa flavonoids in humans. According to these studies, no age or gender effects have been found. In one study, subjects were asked to refrain from taking vitamin supplements or eating foods rich in flavonoids for 24 hours [11]. The subjects were then given three different doses of chocolate (i.e., semi-sweet chocolate mini baking bits)—35, 70 or 105 grams—and a small amount of white bread. The chocolate contained 5.3 mg/g procyanidins of which 1.3 mg/g was epicatechin. Blood was drawn at baseline and again at two and six hours after chocolate consumption. A dose-response increase in plasma epicatechin occurred following chocolate intake. This finding that cocoa/

chocolate intake in humans increases antioxidants in the blood provides evidence that epicatechin is absorbed.

The next question is whether consumption of chocolate has antioxidant effects in humans. Using the TRAP assay to assess total antioxidant potential, researchers found a positive correlation between intake of procyanidins and antioxidant potential. But does increasing the antioxidant capacity of plasma have any physiological significance? To answer this question, investigators measured plasma 2-thiobarbituric acid reactive substances (TBARS), a marker of lipid oxidation. With increasing plasma levels of epicatechin, TBARS quickly decreased, independent of subjects' total lipid levels [9].

Based on studies which have examined epicatechin and catechin uptake in the plasma of close to 60 subjects, it can be concluded that epicatechin, as well as catechin, is rapidly absorbed. Because there is about six to seven times more epicatechin than catechin in cocoa/chocolate, most attention has focused on epicatechin. Consistent with *in vitro* studies, human studies indicate that small doses of epicatechin (i.e., about one μM) are effective. Beneficial effects of similar concentrations of other flavonoid-rich foods such as green tea and black teas have been demonstrated. There is a statistically significant increase in plasma antioxidant capacity and reduction in lipid peroxides following cocoa/chocolate consumption. Researchers hypothesize that the observed improvement in the ability of plasma to protect itself against oxidative damage and inhibit lipid peroxidation following cocoa/chocolate intake is likely due to flavonoids such as epicatechin, although other possible components cannot be ruled out.

What is the mechanism(s) for flavonoids' antioxidant effect? Epicatechin and other flavonoids may not only have a direct antioxidant effect, but they may also have a sparing effect on other antioxidants such as vitamins C and E. Typically, plasma levels of vitamin C decrease very quickly, vitamin E decreases slowly, and TBARS increase. However, when epicatechin is consumed at a concentration of one micromolar, there is a sparing effect on vitamins C and E [12].

In addition to its influence on oxidative defense mechanisms, are there other mechanisms by which cocoa/chocolate could benefit cardiovascular health? To help answer this question, researchers examined platelet reactivity. A modest decrease in platelet reactivity can be of value because it reduces the probability of clotting. This is one reason why daily aspirin intake (81 mg/day) is often recommended for individuals after the age of 40. Another reason to study platelet reactivity is because some reports indicate that flavonoid-rich foods affect platelet reactivity. For example, one study found that grape juice, but not orange or grapefruit juice, inhibits platelet activity in dogs and Rhesus monkeys [13]. Researchers have performed a series of studies to assess the effect of cocoa consumption on platelet reactivity. Several markers of platelet activation (e.g., surface proteins, platelet function, microparticles) have been used and flavonoid-rich test beverages (e.g., dealcoholized red wine) have been compared to appropriate

controls. In one study to determine whether cocoa inhibits platelet activation and function, subjects were given either water or 300 mL of cocoa that provided a very high amount (approximately 900 mg) of polyphenols [14]. A couple of surface marker proteins were measured. As platelets become activated, P-selectin and PAC1 increased. Using flow cytometry to search for these particular surface markers, investigators can determine if a cell has gone from an inactive to an activated state. During the six hour time period following intake of cocoa, there was a reduction in P-selectin, suggestive of less reactive platelets. This is the same response that one would expect to see following intake of antiplatelet agents such as aspirin. Both a high and low level of ADP stimulation has been demonstrated to reduce P-selectin. The same effect occurred with PAC1, although it was not statistically significant in unstimulated platelets. However, in platelets stimulated with epinephrine, a modest reduction in PAC1 was found.

Another way to evaluate the effect of cocoa consumption on coagulation is to examine the formation of platelet microparticles. These are hemostatically active phospholipid-rich microvesicles which fragment off when platelets start getting larger. Platelet microparticles correlate positively with thrombotic disorders. The higher the concentration of platelet microparticles, the greater the risk for thrombosis. A reduction in microparticle formation occurs following consumption of cocoa beverages [15]. This finding is consistent with that seen for surface protein markers.

Is the cocoa-induced change in microparticle formation functionally significant? To help answer this question, researchers employed platelet function analysis [15]. This test measures primary platelet related hemostasis as closure time of a capillary (in seconds). Basically, blood is placed in a small capillary and the time it takes for the blood to coagulate is recorded. This test is used routinely in blood banks to determine whether individuals have taken too much aspirin. Following consumption of a phenol-rich cocoa beverage, a modest, consistent increase in closure time results, although the increase is less than that typically seen with aspirin. This finding indicates that consumption of cocoa, at least the type of cocoa used in the study, is associated with a transitory reduction in platelet reactivity, which in turn may reduce the risk for formation of clots.

It is important to recognize that not all chocolates are equal in terms of their flavonoid content. Some alcoholized chocolate and many types of cocoa mixes, which are alcoholized, contain virtually no flavonoids. Researchers recently investigated whether or not an interaction exists between aspirin and cocoa [15]. No strong synergism was found in their effects on closure time, although modest additive effects were observed on microparticle formation and on some of the surface proteins. Use of a procyanidin low cocoa may have contributed, at least in part, to the findings of this study. The lack of or limited synergism was disappointing given that perhaps a combination of a very low dose aspirin with flavonoid-rich cocoa or other

flavonoids might allow individuals who have difficulty taking aspirin to reduce the dose.

Recent investigations have examined the effect of cocoa/chocolate consumption on eicosanoid synthesis *in vivo* [16]. The finding from this research parallels that observed in *in vitro* studies. The cardioprotective effect of cocoa/chocolate may be explained by this food's effect on prostacyclins. Prostacyclins, as well as their stable analogs, inhibit platelet aggregation and reduce the risk for thrombosis, vasoconstriction and, importantly, the entry of low-density LDLs into the arterial wall. Because of these effects, a number of different drugs are now being designed to increase prostacyclin concentration. In addition to prostacyclin, researchers have examined the influence of cocoa/chocolate on leukotrienes for just the reverse reason. Typically, drugs are developed to try to reduce leukotriene production. A high level of leukotrienes is associated with an increased risk of some disorders such as asthma, ischemia and shock. Interestingly, the old literature on chocolate suggests that this food is particularly beneficial for asthmatics.

To examine the effect of cocoa/chocolate on leukotriene production, 10 healthy adults were recruited for a two week study [16]. During the first week, six of the subjects received a procyanidin low (3.3 mg/bar) chocolate bar and four received a procyanidin high (147 mg/bar) chocolate bar. During the second week, the treatments were reversed. Following an overnight fast, blood was drawn immediately (baseline) and again at two and six hours. In the vast majority of subjects who consumed the high procyanidin bars, plasma TBARS were reduced. This finding supports the antioxidant activity of the polyphenolic fraction. Also, over the period of the study prostacyclin increased and leukotrienes decreased in the majority of the subjects. These changes were within the same range seen with pharmacologic agents. The leukotriene/prostacyclin ratio dropped in every subject. To the researchers' knowledge, this was the first demonstration of this type of rapid effect with a food product.

CONCLUSION

Based on scientific findings to date, acute doses of chocolate-derived procyanidins can induce transient cardioprotective effects in humans. Whether or not similar effects would result from chronic intakes, or large amounts of cocoa/chocolate for multiple weeks, is unknown. It also remains to be established whether the effects become attenuated or amplified with time. More research is needed to identify the amount and specific class or composition of flavonoids in cocoa/chocolate that exhibit beneficial health effects. For example, specific oligomer fractions in cocoa may be more beneficial than others. Researchers speculate that the inverse association between consumption of flavonoid-rich foods and risk for cardiovascular disease is due to multiple factors including flavonoid-induced

changes in oxidant defense, vascular reactivity, and platelet reactivity.

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