MINIREVIEW

Chemopreventive Effects of Cocoa Polyphenols on Chronic Diseases[1]

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We have explored the causes of the major chronic diseases prevailing in the world and the relevant mechanisms as a sound basis for recommendations for their prevention. Research shows that the cocoa bean, and tasty products derived from the cocoa bean such as chocolate, and the beverage cocoa, popular with many people worldwide, is rich in specific antioxidants, with the basic structure of catechins and epicatechin, and especially the polymers procyanidins, polyphenols similar to those found in vegetables and tea. Metabolic epidemiological studies indicate that regular intake of such products increases the plasma level of antioxidants, a desirable attribute as a defense against reactive oxygen species (ROS). The antioxidants in cocoa can prevent the oxidation of LDL-cholesterol, related to the mechanism of protection in heart disease. Likewise, a few studies show that ROS associated with the carcinogenic processes is also inhibited, although there have not been many studies on a possible lower risk of various types of cancer either in humans or in animal models consuming cocoa butter or chocolates. Based on the knowledge acquired thus far, it would seem reasonable to suggest inhibition of the several phases of the complex processes leading to cancer, as a function of quantitative intake of antioxidants, including those from cocoa and chocolates. Cocoa and chocolate also contain fats from cocoa butter. These are mainly stearic triglycerides (C18:0) that are less well absorbed than other fats, and are excreted in the feces. Thus, cocoa butter is less bioavailable and has minimal effect on serum cholesterol. [Exp Biol Med Vol. 226(10):891–897, 2001]

Key words: cocoa; chocolate; polyphenols; reactive oxygen species; carcinogens; genotoxic; epigenetic; fats; stearate; cancer; heart diseases

In the Western world, coronary heart disease and many specific types of cancer associated with tobacco use and improper nutritional habits represent major diseases (1–7). There is a high cost and often poor prognosis connected with the clinical management of these diseases. Therefore, for economic and ethical reasons, approaches to their effective prevention constitute a key goal. Accrued knowledge through worldwide medical and scientific research provides a basis for hope that realistic preventive measures can be implemented in the near future, mainly through adjustment of lifestyle.

Research on the etiological factors bearing on heart disease show that a key reaction is the oxidation of LDL cholesterol (8–15). In the cancer field, we distinguish between genotoxic carcinogens that are DNA reactive and mutagenic, and nongenotoxic agents that act by epigenetic promoting mechanisms (16). Virtually all human cancers involve the initial effects of genotoxic carcinogens. In turn, such carcinogens are often metabolized by oxidative reactions to DNA-reactive components, sometime after additional secondary activation reactions. The metabolic formation of active oxygen, reactive oxygen species (ROS), superoxide anion, hydrogen peroxide and peroxyl radicals, hydroxy radicals, and the like, also leads to modified DNA or oxidized LDL-cholesterol (17–23). The risk of ROS formation is particularly high in the cellular energy-generating particles, the mitochondria, where oxygen serves to generate essential biochemical reactants such as ATP. The mitochondria need, therefore, special protection against the generation of ROS through the availability in those, and indeed in all cellular systems, of effective antioxidants (24).

It is clear that the prevention or decrease of the key oxidation reactions could be an important means of lowering the risk of coronary heart disease and of many types of cancer affecting millions of people. Research has demonstrated that antioxidants in natural products, vegetables, fruits, soy products, black or green tea, cocoa, and choco-

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late, can decrease these oxidations reactions (25–28). In the field of a lower risk of coronary heart disease, it has been shown, through studies in humans as well as animal models, that the intake or use of foods containing antioxidants is beneficial.

This paper will summarize recent interesting and relevant findings of active antioxidants in chocolate or cocoa as nutritional components to lower the adverse effects of ROS. Also, the major fatty acid in cocoa is stearic acid, which displays the interesting attribute that unlike some other fats, it may not have a hypercholesterolemic action. Results of research with antioxidants polyphenols in green or black tea lead to the conclusion that tea is a beverage capable of promoting good health and prevention diseases in humans, in animal models, and in experimental approaches (20, 25). We will make a parallel to these documented findings with those established or potentially applicable to cocoa and chocolate.

Cocoa and Chocolate

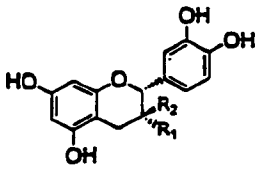
Background. Cocoa is the seed of the cocoa tree, *Theobroma cacao*, which is an evergreen reaching a height of 6 to 12 meters. It grows best at altitudes of 30 to 300 meters in areas where temperatures are moderate, ranging from about 18° to 32°C, and it needs adequate moisture, with rainfall of 1 to 5 liters/m^2 per year.

The cocoa tree has an old history; it is thought it was cultivated over 3,000 years ago by the original inhabitants of Central America and Northern South America. The inhabitants knew how to prepare a beverage from the cocoa bean and occasionally it was used on formal occasions as a ceremonial beverage. It is said that the cocoa bean was used as a currency. The production of beverages from the cocoa beans is quite similar to that associated with the management of tea leaves to yield tea. The beans undergo fermentation involving bacterial oxidative processes. The resulting product is roasted and then ground into a powder. The original discoverer of the Americas, Columbus, was made aware of the cocoa plant and bean. After his fourth voyage and return to Spain about the year 1502, he introduced the cocoa bean to Spain. The Spanish that landed in Mexico about the year 1519 were made familiar with the chocolate beverages through the Aztecs. From Spain, the cocoa tradition reached Italy and France some hundred years later, and in the middle of the 17th century, shops selling cocoa were opened in the United Kingdom. In the middle of the 18th century, chocolate manufacturing was introduced in Massachusetts with cocoa from the West Indies and Central America. A Dutchman, Van Houten, obtained a patent in 1828 to produce chocolate powder by pressing "cocoa butter" from the roasted and ground cocoa beans, and in the middle of the 19th century, a London firm added sugar to the chocolate liquor and cocoa butter to produce commercial chocolates. Finally, in 1876, a Swiss producer added dry milk to market milk chocolate. Cultivation of the cocoa plants spread to West Africa, which currently produces about 70% of the World's total production, the remainder coming from Central and South America, the West Indies, and small amounts in tropical areas of Asia. A major set of reviews on cocoa and chocolate has appeared (29).

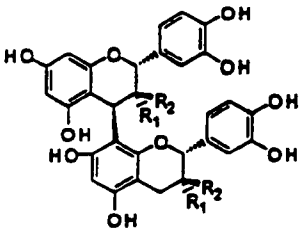
Antioxidants in Cocoa and Chocolate. Being a natural product, the cocoa bean contains many different types of physiologically active constituents. We will emphasize here only those relevant to the discussion of the health effects of cocoa and chocolates, which act as effective antioxidants through specific constituents belonging to the epicatechin oligomer class. Whereas the coffee bean and tea leaf are sources of caffeine, the cocoa bean contains little caffeine. The main methylxanthine compound in cocoa is theobromine, about 2% to 3% by weight, and small amounts of caffeine, 0.2%. Theobromine has little stimulating effect on the central nervous system, unlike caffeine. Thus, cocoa beverages and chocolates can be offered to children without fear of inducing hyperactivity or sleeplessness. Also of great importance is the presence of a number of antioxidants belonging to a class of polyphenols, namely procyanidins. The monomeric epigallocatechin gallate from green tea yields the monomeric theaflavin, but more so polymeric thearubigins during the oxidative conversion through action of polyphenol oxidase of the green tea polyphenols to the typical black tea polyphenols. In a similar fashion, during the processing of an extract of the cocoa beans, the polyphenol oxidase-mediated oxidation of the cocoa polyphenols forms a series of polymeric procyanidins (Fig. 1). The results of a cooperative study by four laboratories using purified oligomers from Brazilian cocoa beans, polymers up to decamers have been observed, although the main polymers were the dimers to the hexamers (Table I). These have been resolved by high-performance liquid chromatography and their structure has been explored by mass spectrometry. The basic monomer has the structure of (−)-epicatechin, similar to that in tea, which, however, often is (+)-epicatechin. Details of the identification have been described (30–33).

Based on their structure, the procyanidins would be expected to be excellent antioxidants (Fig. 1). This attribute has been demonstrated and explored in relation to parameters associated with their effects in health promotion.

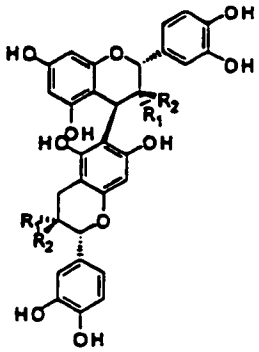
Coronary Heart Disease. It has been known for over 40 years that there is an association between serum cholesterol levels and risk of coronary heart disease. A series of lipoproteins associated with cholesterol were identified. The key lipoprotein associated with heart disease is the low-density lipoprotein, LDL-cholesterol. It is now known that this product can undergo biochemical oxidation to an oxo or peroxo derivative, which is actually the atherogenic principle (10). There is good epidemiological evidence worldwide that populations such as vegetarians in the United States and Europe, or people in the Mediterranean region who traditionally consume vegetables as part of the dietary tradition, have a lower risk of heart disease. This is also true for people such as those in Asia that regularly drink appreciable amounts of tea or consume soy foods.



Flavan-3-ol Monomer



Procyanidin (4β→8)-Dimers



Procyanidin (4β→6)-Dimers

Figure 1. The flavan-3-ol monomer is the fundamental structure of both the polyphenols in cocoa where R1 is H, R2 is OH, representing (−)-catechin, the building block for the procyanidins shown as dimers found in cocoa. The (+)-epicatechin is the building block for the polyphenols in the tea leaf in which R1 is OH and R2 is H. However, the stereochemistry of the polyphenols in cocoa and tea is not absolute and the reverse structure is found in cocoa and in tea, but in smaller amounts (see Ref. 46).

Table I. Percent Composition of Polyphenols in Brazilian Cocoa Beans

Monomer	9.8
Dimer	13.3
Trimer	9.9
Tetramer	10.5
Pentamer	10.5
Hexamer	12.7
Heptamer	8.0
Octamer	8.6
Nonamer	11.6
Decamer	5.4

Note. This information is from Ref. 33. The analyses were coordinated in four different laboratories of a standard product derived from Brazilian cocoa beans. Data in percentage of each isomeric fraction to total.

Table II. Antioxidant Properties of Cocoa or Chocolate Polyphenols

Product	Phenols (μmol/g)	IC_{50} (μM)	Antioxidant index × 10^3
Cocoa	224 ± 66.4	0.32 ± 0.07	710 ± 213
Dark Chocolate	126 ± 17.4	0.25 ± 0.03	500 ± 20.4
Milk Chocolate	52.2 ± 20.4	0.41 ± 0.04	136 ± 36.3
Hot Cocoa Mixes	8.2 ± 2.9		

Note. This information is summarized from Ref. 28. For milk chocolate, data are mean total phenols of five samples; for dark chocolate, mean of six samples, and for cocoa, mean of four samples. IC_{50} is the concentration of polyphenols inhibiting the oxidation by cupric ions of LDL plus VLDL by 50%, and tea antioxidant index, devised by Dr. Vinson, is the ratio of total phenols to the IC_{50}.

Table III. Concentration of Total Polyphenols, Catechins, and Epicatechin in Extracts of Cacao Liquor from Countries

	Total polyphenols (%)	Catechins (%)	Epicatechin (%)
Colombia	11.4	0.43	1.22
Ecuador	9.2	0.49	1.02
Ivory Coast	6.7	0.31	0.35
Brazil	3.0	0.41	1.68
Ghana	9.7	0.31	0.82

Note. This information was obtained from Ref. 34. The data generated is from analysis of extracts from the countries listed. A reviewer noted that the information depends on "the diversity of cultivars and post-harvesting practices affecting flavanoids content." As is true with any plant extract, there are differences as a function of many variables such as soil, growth rates, seasons, and manufacturing practices. Yet the results of Osakabe et al. (34) demonstrate orders of magnitude of factors analyzed.

These results are based on substantial intakes of foods containing antioxidants of various types, mainly of a polyphenol nature (1, 2, 33). Cocoa and chocolate are quite rich in such antioxidants, to the order of 224 ± 66.4 μmol/g for cocoa, 126 ± 7.4 μmol/g for dark chocolate, and 52.2 ± 2.04 μmol/g for milk chocolate (28) (Tables II and III). Nonetheless, there is less antioxidant availability from cocoa as a beverage or chocolate as a food, even calculating the antioxidants for a 100 g serving, compared with the antioxidants in the recommend 5 to 10 vegetables or soy foods, or 5 to 10 cups of tea. Cocoa and chocolate have a highly valued taste, and their intake provides additional antioxidant defenses against ROS (33, 34). Indeed, it can be calculated that eight 150-ml cups of tea, with the usual 2.25 g of black or green tea, provide 5.4 g of tea polyphenols. In contrast, an entire 7-oz. (198 g) bar of black chocolate contains only 1.7 g of procyanidins. Most people would not consume such a bar at once, and might eat it in two portions after lunch and dinner. Likewise, it seems unlikely that a person would consume eight cups of cocoa per day because of the caloric content, whereas eight cups of tea provides fluid without calories. For that reason, it would be difficult to assess the protective effect of the antioxidants in cocoa and chocolates through epidemiological approaches, in view of the more limited daily or weekly intake. We will, therefore, provide the basic experimental background on which such a protective effect can be reasonably surmised, and we will include chocolate and cocoa in the class of foods that would add to the overall beneficial nutritional habits associated with foods rich in antioxidants.

The oxidation of LDL has been studied through *in vitro* approaches utilizing a specific chemical to initiate the oxidative process and measuring the lag time of the LDL oxidation spectrophotometrically (35–40). Cocoa did display such an effect, and it seems to be of a similar order of magnitude as that shown by red wine or tea. Similar results

were obtained with a classic test measuring oxygen radical absorbance capacity (ORAC) (21, 33). A linear association between ORAC values and analysis of procyanidins monomers to decamers was obtained. There is a suggestion that the lower polymers, from 2 to 5, have a slightly better effect than either the monomer or the higher polymers, from 6 to 10. The explanation may be one of inherent effectiveness and transport through cell membranes and thus, absorption and presence in adequate concentrations at the target site (41–46). The maximum effectiveness observed in the 3 to 5 polymers may rest on their decreased rate of conjugation and excretion, and their absorption across membranes and availability as antioxidants in critical cellular sites. The plasma of people eating chocolate shows a rapid increase of epicatechin, and an equally rapid decay (Table IV) (45). Along these lines, the antioxidants after procyanidin intake rises to a peak at 2 hr, at the same time as the significant indicators of plasma 2-thiobarbituric acid reactive products (TBARS) declined (Table V) (43). In rats fed a vitamin E-deficient diet, the lipid peroxide levels, measured as TBARS, increased in several organs, including liver, kidney, heart, and brain, and an extract of cocoa liquor rich in polyphenols counteracted this increase (47).

Neoplastic Diseases. Cancer represents a large number of diseases with distinct etiological factors such as tobacco use and smoking, excess alcohol intake, and above all, locally prevailing nutritional traditions. These lifestyle-associated causes involve genotoxic carcinogens that are the actual initiating agents modifying DNA and the genome through mutational events. In most instances, promoting and accelerating factors that are not genotoxic play enhancing roles (16). Fundamental mechanisms are associated with the involvement of ROS, as is true for the oxidation of LDL-cholesterol, discussed above. For that reason, irrespective of the basic mechanism of carcinogenesis, population studies have demonstrated that people with a regular intake of foods containing antioxidants such as vegetables, fruits, tea, or soy products display a lower incidence of various types of cancer (1, 2, 25–27). It can be postulated, therefore, that consumption of cocoa or chocolate, adding to the available antioxidant load, would also be beneficial in the decreasing the burden and effectiveness of genotoxic and epigenetic carcinogens. The regular intake of antioxidants in cocoa and chocolate, even by those who love this tasty food, is likely insufficient by itself to provide human population data, as discussed above, and the presumption of benefit is based on laboratory research in humans, laboratory animals, and *in vitro* investigations, in analogy with demonstrated benefits in chronic disease control in tea drinkers (15, 20, 25).

Genotoxic carcinogens can be detected and measured readily by the mutagenicity assays in *Salmonella typhimurium* developed by Ames (16). There are few studies that suggest that extracts of cocoa also display an effect in decreasing the mutagenicity of carcinogens, but there has been a chronic study of dietary doses as high as 5% of cocoa powder in rats to detect adverse toxic effects, including cancer, and none was found (48). Cocoa polyphenols block the effects of peroxynitrite, an endogenous product formed in inflammatory cells from superoxide and nitric oxide. As noted above, the effect of superoxide and other ROS is inhibited by antioxidants such as those in cocoa (49). Thus, cocoa itself, and by implication, the procyanidins, is perfectly safe and they emphasize beneficial effects through their antioxidant actions.

Irrespective of the mechanisms involved in carcinogenesis, it is clear that both the initiation and developmental aspects of cancer also involve ROS. For that reason, epidemiological information, geographic pathology, and laboratory experiments have shown that synthetic and naturally occurring antioxidants are protective. It can be expected that the antioxidants in cocoa likewise could display an inhibiting effect in the ROS associated with phases of carcinogenesis. This has been demonstrated in a number of instances, as discussed above.

Theobromine occurring in cocoa is a purine and it has been shown to act as an antioxidant, just as caffeine does (50, 51). The contribution of theobromine to the overall effect is relatively small, because the amounts of theobromine are small compared with the other antioxidants in cocoa, the procyanidins, as already noted.

Lipids in Cocoa. Although not directly related to the overall topic of this paper, namely the antioxidants in cocoa

Table IV. Concentration of Epicatechin in Plasma of Subjects Fed Different Amounts of Procyanidin-Rich Chocolate

Time (hr)	Procyanidin-rich chocolate food consumed (g)			
	nmol/L			
	0	27	53	80
0	1 ± 1 (9)[a]	2 ± 2 (13)[a]	4 ± 2 (13)[a]	4 ± 3 (10)[a]
2	19 ± 14 (9)[a]	133 ± 27 (13)[b]	258 ± 29 (13)[b]	355 ± 49 (10)[b]
6	1 ± 1 (9)[a]	26 ± 8 (13)[c]	66 ± 8 (13)[c]	103 ± 16 (10)[c]

Note. The procyanidin-rich chocolate was a commercial product of Mars, Inc. (Hackettstown, NJ), namely M&M semi-sweet chocolate mini-baking bits made with cocoapro cocoa. There were 186 mg of procyandadins in the 27 g sample. Values are expressed as means ± SE. Values in parentheses indicate number of subjects. Means within a column not sharing a common superscript letter are significantly different at $P < 0.05$. Reprinted with permission from Ref. 45.

Table V. Oxidative Stress Parameters Evaluated before and after the Consumption of a Procyanidin-Rich Dark Chocolate or Low-Procyanidin Food

Parameter	Hours after chocolate consumption		
	0	2	6
Epicatechin (nmol/L)			
Procyanidin-rich meal[a]	22 ± 4	257 ± 66[b]	153 ± 69
Low-procyanidin meal	11 ± 10	12 ± 11	11 ± 9
Antioxidant capacity (s)			
Procyanidin-rich meal	389 ± 39	510 ± 43[b]	306 ± 34
Low-procyanidin meal	344 ± 41	291 ± 23	257 ± 30
TBARS (μmol/mmol triglycerides)			
Procyanidin-rich meal	3.14 ± 0.52	1.87 ± 0.26[b]	2.20 ± 0.38[b]
Low-procyanidin meal	2.03 ± 0.25	2.09 ± 0.20	2.04 ± 0.30

[a] The procyanidin-rich meal was 80 g of a procyanidin-rich chocolate as 80 g of M&M semi-sweet chocolate (Table IV), providing 557 mg of total procyanidins. The control low-procyanidin meal consisted of in isocaloric amounts, vanilla milk chips from Guittard Chocolate Company (Burlingame, CA).
[b] Values are means ± SE ($n = 10$ for the group fed the procyanidin-rich dark chocolate and $n = 3$ for the control group fed the low-procyanidin vanilla milk chips). Comparison of 2- and 6-h values with a 0-h baseline by paired t test, $P < 0.01$; reprinted with permission from Ref. 43.

and chocolate, it is relevant to consider the role of lipids in cocoa in connection to the overall effect of cocoa and chocolate in their action on health promotion (4). It has been demonstrated that most saturated fats present a high risk of atherosclerosis, and thus of heart disease. Of even greater relevance is the fact that the total fat intake, as a percentage of calories, plays a key role in cancer development. The Western level of total fat intake used to be 40% to 45% of calories. Public health efforts and recommendations have decreased the total fat intake to about 35% of calories, but geographic pathology research and laboratory approaches have demonstrated that about 25% of calories in fat, or better, even less, 15% to 20%, are the recommended levels in a new dietary tradition of lower risk of both heart disease and cancer, with emphasis on the use of monounsaturated oils such as canola or olive oils (52).

The important finding was made that the main lipid in cocoa and in chocolate is stearate, a saturated fat (53, 54). Yet, this lipid, for several reasons, does not seem to augment the risk of heart disease where this was demonstrated, and most likely also of the nutritionally linked cancers. The underlying reason appears to be one of limited absorption from the intestinal tract, in contrast to most other lipids that are readily absorbed. A series of articles reviewed this field (53–57). However, Connor (58) disagrees and suggests stearic acid represents a risk for heart disease, as do other saturated fats. He accepts that stearic acid does not seem to increase plasma cholesterol. One point is that stearic acid is desaturated in the liver to yield lipoproteins with oleic acid. Yet appreciable intakes of stearate may control other elements associated with heart disease risk. Thus, we conclude that moderation is important. Any low risk from an individual food could be disregarded through maintenance of a health-promoting overall nutritional tradition rich in antioxidants from vegetables, fruits, soy, tea, and cocoa. As noted above, a daily addition of 100 to 150 g of dark chocolate to such a nutritional scheme permits the discounting of any possible action of stearate, irrespective of the specific mechanisms underlying its effect, especially if food calories match energy needs so as to avoid obesity. Kelly *et al.* (59) have recently provided evidence that a diet with appreciable amounts of stearate in the fat component lowers the risk of atherogenesis. The addition of 2.25% calcium carbonate to dark chocolate increased the fecal excretion of palmitic, and more so of stearic acid over a 2-week period, which also decreased the serum LDL-cholesterol (60).

Discussion

Worldwide international research in geographic pathology and epidemiology as well as laboratory investigations demonstrated that major chronic diseases are associated with ROS, which lead to damage in various essential cells in the body and destroy or inhibit their ability to function. For that reason, there is need for nutritional elements that provide antioxidants to limit the effects of ROS. These concepts have led to recommendations to consume vegetables and fruits rich in antioxidants (61). Extensive research has demonstrated the presence in these foods of antioxidants of a polyphenol nature that are more effective than the antioxidant vitamins (17, 18).

The cocoa bean and chocolates derived therefrom contain important classes of antioxidants, monomeric catechins, and the polymeric procyanidins. In some respects these antioxidants are similar to those in tea, but they have distinct chemical structures and, therefore, distinct metabolic functions and attributes. In most instances the polymers representing a 2 to 5 polymerization state seem to be optimal, probably because the monomer is metabolized too rapidly and excreted, whereas the higher 6 to 10 unit polymers may suffer from difficulty in penetrating cellular membranes and may be poorly absorbed.

The mechanisms of action of the tea polyphenols are better known than those associated with cocoa. The tea polyphenols can act as powerful antioxidants; inducers of detoxifying phase II enzymes systems; effective cellular growth control elements, especially for neoplastic cells; and

as modifiers of the intestinal microflora, favoring the outgrowth of health-promoting bacteria (25). With the cocoa and chocolate products, only the first item, the antioxidant properties of procyanidins, has been explored and demonstrated to some extent. The other three topics require future research.

Nonetheless, what is known so far about the actions of chocolate and cocoa products suggests that they can be considered part of a wholesome health-promoting nutritional scheme in that they add to the available antioxidants associated with other components of a disease-preventing nutritional tradition. The reader is referred to a historic, semipopular treatise on cocoa and chocolate as an interesting introduction to the field (62).

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