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Cancer Protective Properties of Cocoa: A Review of the Epidemiologic Evidence

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Due to their high concentration of catechins and procyanidins, bioactive compounds with distinct properties, cocoa and chocolate products may have beneficial health effects against oxidative stress and chronic inflammation, risk factors for cancer and other chronic diseases. This review focuses on the epidemiologic evidence for protective effects against cancer and overall mortality. The very small number of observational epidemiologic studies offers weak support for a reduction in mortality and little data related to cancer, whereas several intervention studies, despite their short duration, have reported some favorable changes in biomarkers assessing antioxidant status but very few findings related to inflammatory markers. In moderation, cocoa products may offer strong antioxidant effects in combination with a pleasurable eating experience. The benign profile of its fatty acids in combination with the low content of sugar of dark chocolate should lessen concerns about the adverse effects of cocoa products. Future nutritional trials need to assess a larger number of biomarkers that may be relevant for cancer risk, whereas epidemiologic studies require valid dietary assessment methods to examine the association of cocoa products with cancer risk in larger populations and to distinguish possible cancer protective effects of cocoa products from those due to other polyphenolic compounds.

INTRODUCTION

Chocolate is an ancient fermented product, just like wine, and contains catechin and epicatechin, readily water soluble flavonoids classified as flavan-3-ols (1,2). Because flavan-3-ols, as many polyphenolic compounds, are reducing agents, one of their functions is protection against oxidative stress (3), an imbalance between the concentrations of reactive oxygen species (ROS) and the defense mechanisms of the body (4). An interesting aspect of cocoa products as compared to other flavonoid containing foods is their high concentration of procyanidins, polymeric condensation products of catechins (dimers to decamers) formed during fermentation (5). These bioactive compounds constitute 60% of the total polyphenol content in cocoa products and confer a strong antioxidant capacity (6,7).

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Chocolate has properties of foods and drugs and was considered medicinal in ancient times (8,9). Starting from the Olmec, Mayans, and Aztecs, the Spanish and other Europeans identified many medicinal uses including stimulation of the nervous system and improvement of digestion (10). In combination with the increasing knowledge about health effects of flavan-3-ols, this raises the possibility that chocolate may have beneficial health effects (1,11). Many epidemiologic studies have explored the beneficial effects of green tea, soy beans, and other foods containing polyphenols (2,12–14), whereas the epidemiology of chocolate is in its infancy. The majority of human studies on cocoa products and disease risk have reported on cardiovascular disease and its risk factors, in particular, high blood pressure and platelet aggregation (15,16). This review focuses on the epidemiologic evidence that supports protective effects of cocoa and chocolate against cancer risk and overall mortality. The emphasis is on two related mechanisms of action that may lower cancer risk and have been examined in humans: anti-inflammatory effects and antioxidant activity (17–19). A relation between chronic inflammation and cancer is supported by signs of inflammation at the cancer site, the fact that inflammatory conditions predispose to cancer, and an association of polymorphisms of cytokine genes with cancer (20). Inflammatory cells produce an attractive environment for tumor growth, facilitate genomic instability, and promote angiogenesis (17). Markers of inflammation, such as interleukin (IL)-6, C-reactive protein (CRP), tumor necrosis factor (TNF)- α , and cytotoxic activity of peripheral-blood lymphocytes have been associated with cancer risk in prospective studies (18,19). As part of inflammatory processes, ROS are produced and result in oxidative stress and a variety of chronic conditions unless a sufficient supply of antioxidants counteracts their effects (4,21). In an experimental setting, catechins and procyanidins influenced the immune response by modulating the activation of NF κ B, a transcription factor involved in the regulation of cytokine production, inflammatory responses, cellular proliferation, and cell adhesion (22).

Polyphenol Content of Cocoa and Chocolate

Fermentation and drying of cacao beans are the keys to flavan-3-ol content and chocolate flavor. Among the many chemical reactions that take place, oxidation is an important one and

causes the color to darken, whereas the polyphenols are oxidized by means of polyphenol oxidases (23). The nib, that is, the center of the cocoa bean, is ground to liquor or mass with a fat content of 55–58%. For cocoa powder, some of the cocoa butter is removed until the butter content is 22–23% or less (24). A press produces a cake that is then broken and sieved to produce cocoa powder. The common treatment with alkali (introduced by Van Houten) improves dispersability of the powder but reduces the polyphenol content (24). To produce plain chocolate, nib or mass is mixed with sugar and additional cocoa butter to enable the chocolate to be molded.

Measuring procyanidins poses challenges over measuring catechins because of their oligomer structure (25). For example, of the 1,636 mg/100 g procyanidins in baking chocolate, just 199 mg are monomers (25). Only during recent years has it been possible to assess procyanidin content of foods (5,7). The most recent USDA database for flavonoids provides information for flavan-3-ols in 6 categories of products (26) derived from 6 different reports (27–32) but did not include a more recent analysis (7). Catechin content of cacao beans was reported as 1.9 mg/g (30), and for dark chocolate candies, the value is 0.5 mg/g (27). It appears that the estimates for catechin content are in good agreement as long as the type of chocolate, for example, unsweetened baking chocolate, is known. A separate USDA database has combined levels of procyanidins in a number of foods, primarily cocoa products, fruits, and vegetables (33). Baking chocolate and cocoa powder had by far the highest concentration of total procyanidins with respective values of 1,637 mg/100 g and 1,374 mg/100 g (33).

A comparison of cocoa and chocolate products from different brands showed the highest catechin and procyanidin content in natural cocoa powders (2.9–3.5 mg/g and 32–49 mg/g, respectively) (7). The respective ranges for unsweetened chocolate were (1.5–2.8 mg/g and 9–25 mg/g); for dark chocolate, they were 0.8 to 1.6 mg/g and 9 to 10 mg/g, with lower levels for chocolate chips, milk chocolate, and Dutched powder. On average, one third of the procyanidin were monomers through trimers, which appear to have higher bioavailability and stronger antioxidant activity than those with a higher number of epicatechin units (7). Overall, a strong correlation between procyanidin content of cocoa products and their antioxidant capacity was demonstrated using oxygen radical absorbance capacity (ORAC) assays (7). In a study that measured the total concentration of redox compounds in 1,113 different foods, of the 50 foods with the highest antioxidant capacity, 5 were chocolate based (34). Unsweetened baking chocolate, as 11 out of 50, had an antioxidant content of 8.9 nmol/100 g (range 1–126 nmol/100 g); only nuts and spices had a higher antioxidant content per weight.

Dietary Assessment

With improved lab methodology and the availability of data bases (26,33), a number of studies have assessed regular intakes of flavan-3-ols in different populations (26). For the United

States, the mean intake was estimated at 58 mg per day or more, much higher than the estimated intake of monomers of other flavonoids (25). The contribution of chocolate to total procyanidin intake was calculated at 18%. A cross-sectional investigation in The Netherlands showed that chocolate consumption contributed 2 to 5 mg of daily catechin intake (depending on age) out of an estimated total of 50 mg per day (35). For the Spanish diet, it was estimated that cocoa products account for 10% of the total antioxidant capacity of dietary intake (36).

As to dietary assessment methods, a Dutch cohort study estimated total intake of cocoa based on 24 cocoa-containing foods collected by a dietary history method (37). The correlations of sugar confectionary consumption at baseline and after 3 and 12 mo were 0.72 and 0.76, respectively. The baseline cocoa intake was also significantly associated with cocoa intake after 5 and 10 yr. Only a few epidemiologic investigations have assessed the intake of chocolate products as part of a food frequency questionnaire (FFQ) (38,39), whereas other studies have simply asked about chocolate intake as part of a lifestyle questionnaire (40,41). The Iowa Women's Study reported correlations of 0.45 to 0.83 between the FFQ and 28-day food records for chocolate-containing foods (38). The European Prospective Investigation into Cancer and Nutrition asked participants about intake of a standard dose (20 g) of chocolate, type, and frequency (39). A more recently developed FFQ assessing human flavonoid intake showed correlation coefficients of 0.75 between FFQ and 4-day food records, although the estimated intake by food diary was considerably higher than by FFQ (42). Challenges to valid estimates of chocolate include the sporadic and seasonal intakes of cocoa products. Furthermore, nutritional flavonoid databases do not yet include values for the large variety of cocoa products that may vary widely in their catechin and procyanidin content due to the strong influence of fermentation on flavan-3-ol content (43).

Observational Studies

Based on their ability to confirm causality, epidemiologic studies will be reviewed starting with cross-sectional studies and ending with interventions. A 2005 review of the epidemiologic literature on polyphenols summarized some of the limited research in the area of catechin intake and cancer risk (13).

Ecologic and cross-sectional studies. In 1944, a report about the Kuna Indians in Panama noted the low blood pressure in this population (44). After the high cocoa intake contributing more than 900 mg flavan-3-ols per day had been described, the health status of the Kuna was examined repeatedly with the underlying hypothesis that the flavan-3-ols sustain nitric oxide synthesis activation, which maintains a low blood pressure (45). When causes of deaths for Kuna Indians living in the San Blas islands were compared with the population in mainland Panama who did not consume cocoa drinks, lower mortality rates for cancer and other chronic diseases were found among islanders

than in mainland Panama, but possible confounding by other lifestyle factors was not considered (46). The 10-fold higher intake of cocoa-containing beverages was also confirmed by the 6 times higher urinary flavan-3-ol metabolite excretion in island inhabitants than among mainlanders (47).

In a large Italian cohort, the levels of CRP, a marker of chronic inflammation, were compared between 1,317 subjects with no chocolate intake and 824 subjects who ate dark chocolate regularly, with a mean of 5.7 g (range = 0.7–20 g) per day (39). Even after adjustment for lifestyle factors and other nutrients, serum CRP concentrations remained significantly lower in chocolate consumers than nonconsumers. In a J-shaped distribution, the lowest CRP concentrations were observed in consumers of up to 1 serving (20 g) of dark chocolate every 3 days. In the same report, chocolate consumers also had a significantly lower mean body mass index than nonconsumers (39).

Case-control studies. Although intake of catechins and procyanidins has been associated with a reduced risk for several cancers (13,48,49), these studies did not specify the nutritional source for these compounds. Only two case-control studies specifically investigated chocolate and cocoa intake in relation to cancer (13). A high chocolate dietary pattern, identified through cluster analysis in a French study, showed no significant association with any stage of colorectal disease ranging from polyps, to adenomas, and colorectal cancer (50). In an earlier report from the same study, chocolate was identified as a risk factor for colorectal cancer (51). An adenoma study in North Carolina observed a nonsignificantly lower prevalence of adenomatous polyps that showed a dose-response relation associated with consumption of chocolate candy (52).

Cohort studies. Four prospective cohorts included questions on cocoa and chocolate as part of their nutritional assessment and reported mortality and/or cancer outcomes: the Zutphen Elderly study from the Netherlands (37), the Iowa women's study (38), the Harvard Alumni study (40), and the Leisure World Cohort study (41).

The Zutphen study is a prospective cohort study of 806 men aged 65 to 84 yr at baseline (53). This study measured 6 catechins in 120 foods. The mean catechin intake at cohort entry was estimated at 72 ± 47.8 mg. Chocolate contributed 3% of the total catechin intake, tea 87%, and apples 8%. After 15 yr of follow-up, the adjusted relative risk for overall mortality among men in the highest tertile (>2.5 g/day) was 0.53 when compared to the lowest tertile (<0.5 g/day) of cocoa intake (54). After 10 yr of follow-up, 96 incident epithelial cancers were recorded, including 42 cases of lung cancer (37). Catechin intake was not associated with epithelial cancer or lung cancer when the models were adjusted for confounders. Catechins from chocolate were nonsignificantly inversely associated with lung cancer incidence and all epithelial cancers; the respective risk estimates were 0.76 and 0.89, but the confidence intervals included 1.

In the Iowa Women's study, mean intake of catechins was 25 ± 32 mg/day at baseline (38). Tea contributed 56% of catechins, apples and pears 26%, but the percentage derived

from chocolate was not reported separately. A significant inverse association was observed between flavonoids and total mortality among subjects in this cohort; the risk of dying was 12% lower for women in the high-intake group (55). Individual flavonoid-rich foods that contributed to mortality reduction included chocolate; the association with cardiovascular mortality was borderline significant ($P = 0.06$). Although catechin intake was weakly protective against rectal cancer among women of this cohort, no separate risks were shown for chocolate (56).

Mortality, not cancer incidence, was studied in the other two investigations (40,41). In the Harvard Alumni study, subjects who reported consuming candy 1 to 3 times per mo had a 27% lower risk of mortality (40). In the Leisure World Cohort Study (41), the subjects with the most frequent chocolate consumption did not experience a reduction in mortality, but those who reported occasional chocolate intake had a statistically significant lower mortality by 6%.

Human Intervention Studies

A large number of cardiovascular biomarkers have been investigated in trials with cocoa products (11,16). Despite the short duration, the small number of subjects, and the varying doses and products, intriguing effects of cocoa products on blood pressure, vascular environment, and heart disease risk have been reported (15,16). Also, the bioavailability of catechins and procyanidins from chocolate has been investigated repeatedly and has shown an excellent dose response relation between intake of cocoa products and epicatechin measured in serum (57). On average, serum levels increased by 200 nmol/l for 100 mg catechins from cocoa products when measured 2 h after intake, the peak time.

Quite a few trials with cocoa products, primarily short-term, have assessed markers of oxidative stress and inflammation as outcome (Table 1). The trials presented here applied three methods to assess oxidative stress:

1. Concentrations of lipid peroxidation products: malondialdehyde (MDA), an end product of peroxidation (21), plasma thiobarbituric acid-reactive substances (TBARS), and F_2 isoprostanes. The latter are produced by free radical-induced peroxidation of arachidonic acid, released into the circulation before excretion in the urine, and are a relatively good measure for oxidative stress because they are stable and specific (58).
2. Oxidative potency of a biologic fluid: Antioxidant capacity is designed to measure the overall antioxidant status of a plasma sample by measuring the capacity to scavenge or trap oxygen radicals (ORAC), total peroxy radical-trapping antioxidant potential (TRAP), trolox equivalents (TEAC), or ferric reducing ability (FRAP) (4).
3. Susceptibility of body fluids to ex vivo oxidation (4): resistance of low density lipoprotein (LDL) to oxidation or increase in lag time, a biomarker of oxidative defense that was suppressed by micromolar concentrations of (-)-epicatechin

TABLE 1
Human intervention studies with cocoa products investigating antioxidant or inflammatory markers^a

Lead Author, Yr	N	Treatment	Duration	Control	Epicatechin at 2 h	Outcomes
Rein, 2000 (62)	13	80 g chocolate (557 mg procyanidins, 137 mg epicatechin)	1 day	Vanilla milk chips	257 nmol/l	TEAC ↑ TBARS ↓
Wang, 2000 (63)	20	27, 58, 80 g chocolate (186, 365, 551 mg procyanidins)	1 day	Bread alone	133, 258, 355 nmol/l	TEAC (↑); 8-Isoprostane ø TBARS (↓)
Wan, 2001 (70)	23	22 g cocoa & 16 g chocolate (466 mg procyanidins, 111 mg catechins)	4 wk	Controlled diet (cross-over)	36 nmol/l	TEAC ↑ LDL oxidation lag ↑
Mathur, 2002 (61)	25	37 g dark chocolate & 31 g cocoa (651 mg procyanidins)	6 wk	Self	NA	LDL oxidation lag ↑ ORAC ø; F ₂ -Isoprostane ø CRP, TNF α , IL-6, IL1 β ø
Murphy, 2003 (66)	32	234 mg flavan-3-ol tablets	28 days	Placebo	116 nmol/l	TRAP ø; F ₂ -Isoprostane ø TBARS ø FRAP ↑
Serafini, 2003 (65)	12	100 g dark chocolate (500 mg polyphenols)	1 day	100 g milk chocolate	↑	ORAC ø; 8-Isoprostane ø LDL oxidation ø
Engler, 2004 (67)	21	46 g chocolate (213 mg procyanidins, 46 mg epicatechin)	14 days	46 g low flavonol chocolate	204 nmol/l	TEAC ø; F ₂ -isoprostanes ↓ MDA (↓)
Wiswedel, 2004 (68)	20	100 ml cocoa drink (187 mg flavan-3-ols)	2 days	100 ml low flavanol cocoa drink	144 nmol/l	TRAP ø; F ₂ -isoprostanes ↓ MDA (↓)
Mursu, 2004 (75)	45	75 g dark chocolate or drink (274 or 418 mg procyanidins)	3 wk	75 g white chocolate	NA	TRAP ø; F ₂ -isoprostanes ø LDL oxidation (↓)
Fraga, 2005 (70)	28	105 g chocolate (168 mg flavan-3-ols)	14 days	Cocoa butter chocolate	NA	TRAP ø MDA ↓ CRP ø
Kurlandsky, 2006 (74)	49	41 g dark chocolate	6 wk	Almonds/no dietary change	NA	CRP ø
Taubert, 2007 (72)	44	6.3 g dark chocolate (30 mg flavan-3-ols)	18 wk	6.3 g white chocolate	12.1 nmol/l (3.5 ng/ml)	8-Isoprostane ø
Heiss, 2007 (71)	11	3 × 100 ml drink (918 mg flavanols)	1 wk	Low phenolic cocoa drink	NA	TEAC ø; MDA ø
Crews, 2008 (73)	101	37 g chocolate and 237 ml cocoa drink (754 procyanidins)	6 wk	Placebo products	NA	CRP ø

^aAbbreviations are as follows: TEAC, trolox equivalent antioxidant capacity; TBARS, thiobarbituric acid reactive substances; LDL, low density lipoprotein; NA, not assessed; ORAC, oxygen radical absorbance capacity; CRP, C-reactive protein; TNF α , tumor necrosis factor α ; IL6, interleukin 6; IL1 β , interleukin 1 β ; TRAP, total radical trapping parameter; FRAP, ferric reducing ability; MDA, malondialdehyde; ↑ or ↓, significant increase or decrease; (↑ or ↓), nonsignificant increase or decrease; ø, no change.

(59). As to inflammatory markers, CRP and cytokines were endpoints in a small number of studies (60).

With the exception of one study summarized in Table 1 (61), the number of biomarkers measured was 3 or less per trial; markers of antioxidant status were more commonly measured than markers of inflammation. Of the 11 studies that assessed antioxidant capacity, 5 used the TEAC, 3 the TRAP, two the ORAC, and one the FRAP method. Four studies showed an increase in antioxidant capacity (62–65), but 7 did not (61,66–71). TBARS decreased in two interventions (62,63) but not in another one (66). Also, MDA decreased in two studies (68,70) and not in another one (71). Isoprostanes decreased only in one study (68) but not in 6 other investigations (61,63,66,67,69,72). Three studies measured CRP and observed no change during the interventions (61,73,74). Neither were the levels of the cytokines, IL6, IL1 β , and TNF α modified during 6 wk of intervention with cocoa and dark chocolate (61). LDL oxidation had more promising results; the LDL oxidation lag increased in studies (61,64), LDL oxidation decreased in one study (69), but no change was observed in another trial (67).

CONCLUSIONS

Based on a limited body of literature, a few interesting effects of cocoa products on endpoints that may be related to cancer risk have emerged. The observational studies have offered weak support for a reduction in mortality and little data related to cancer, which is not a surprise given the lack of FFQs that assess the intake of cocoa products. Several intervention studies, despite their short duration, have reported some favorable changes in biomarkers assessing antioxidant status but produced hardly any findings related to inflammatory status.

Cocoa products deserve further investigation for a number of reasons. Although soy beans, green tea, and wine contain bioactive compounds with similar properties as those in cocoa products, the high concentration of procyanidins is a distinctive property of cocoa products. Few foods have procyanidin concentrations as high as cocoa powder and dark chocolate (33). Procyanidins have been examined in experimental systems for a variety of potential anticancer effects including inhibition of breast cancer cell proliferation, local antioxidant activity in the gastrointestinal tract, regulation of signal transduction pathways, suppression of oncogenes, induction of apoptosis, modulation of enzyme activity related to detoxification, stimulation of the immune system, angiogenesis, and regulation of hormone metabolism (1,6,7,75); but those mechanisms have not yet been investigated in human studies.

The pleasurable eating experience in combination with strong antioxidant effects is another distinct property of cocoa products. Although the bioactive components in chocolate may be similar to those reported for wine, green tea (14), and soy isoflavones (76), the sensory reward of the melting sweetness, apparently the predominant factor in chocolate cravings (77), are exclusive to cocoa products. Looking at the meaning of eating in French

culture, it can be seen that moderation, focus on quality, and emphasis on the joys of eating contribute to a healthier lifestyle and allow the consumption of modest amounts of “unhealthy” foods (78). As discussed in the context of the French paradox (79), when eaten in moderation, the sugar, fat, and energy content of chocolate products do not need to be a barrier to intake (78). The profile of the primary fatty acid in cocoa butter, that is, stearic acid, is cholesterol neutral (80), and the sugar content of one bar of very dark chocolate (100 g) can be below 20 g, that is, less than the sugar contained in a yogurt or a can of soda (30–40 g) (81). Observable effects due to chocolate products may occur at very low doses. In a German trial (72), a significant decrease in blood pressure was observed with one piece of bittersweet chocolate per day (6 g with 33 kcal).

Cocoa is one of the foods with the highest concentration of flavonoids; over 10% of the weight of cocoa powder is flavonoids (5), and the antioxidant capacity is higher than for many other foods (34). For many individuals, cocoa products constitute a larger proportion of the diet than green tea, wine, or soy beans (6,35,36). Therefore, it would be important to include dietary assessment of cocoa products in nutritional studies that examine the effects of polyphenol on different health outcomes using valid FFQs. This does not only apply to epidemiologic investigations but also to trials with soy beans, green tea, or other foods rich in flavan-3-ols.

Because the exposure to flavan-3-ols in population-based studies is relatively low as compared to interventions that administer products with high concentrations of catechins and procyanidins, nutritional trials may be able to provide insights into the potential effects of cocoa products that cannot be obtained from observational studies. For future interventions, longer trials with well-defined exposure and control products, good compliance measures, valid markers of inflammation and antioxidant status, refined nutrient databases that include a variety of cocoa products, and at-risk populations to observe substantial effects are essential (16,82). Because of the dependence of assay results on oxidant and target probe species, reactive mechanisms, and reaction conditions, it was recommended to apply several assays measuring different aspects of the behavior of antioxidants (4,83). The same is true for inflammatory markers for which a panel of different cytokines may be needed to capture the inflammatory status (84). As to epidemiologic studies, only with high-quality data based on FFQs that provide valid estimates of catechin and procyanidin intake will it be possible to examine the association of cocoa products with cancer risk and to distinguish them from effects due to other polyphenolic compounds.

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